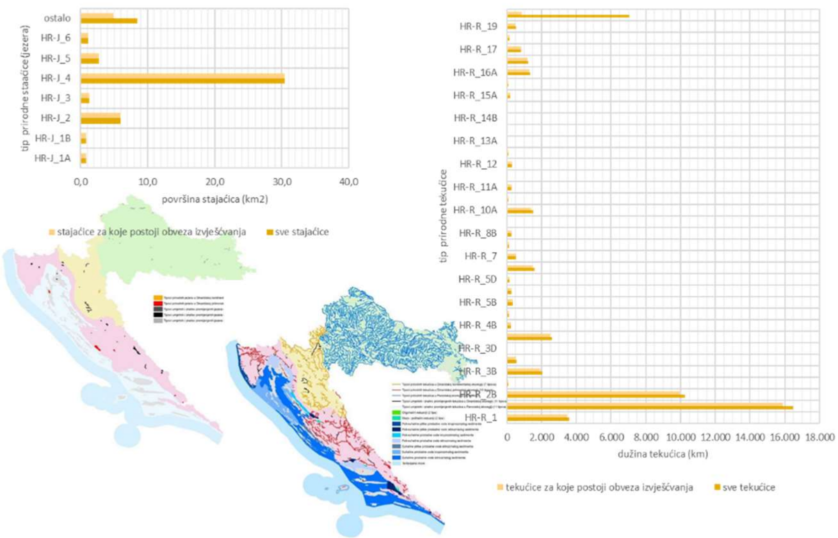
HRVATSKE VODE

ZAVOD ZA VODNO GOSPODARSTVO

2022.



PLAN UPRAVLJANJA VODNIM PODRUČJIMA 2022. – 2027.
Prateća dokumentacija

IZVJEŠTAJI O PROVEDENIM INTERKALIBRACIJSKIM POSTUPCIMA

Podaci o dokumentu

Naslov:	Izvještaji o provedenim interkalibracijskim postupcima
Izdanje:	Hrvatske vode
Datum:	ožujak 2022.
Autor:	Hrvatske vode, Sektor razvitka i Zavod za vodno gospodarstvo
Objava:	Hrvatske vode, Zavod za vodno gospodarstvo

	Ime i prezime
Autor:	Mr.sc. Valerija Musić, dipl. ing.biol. Marija Šikoronja, dipl.ing.biol.
Recenzent/Revident	-
Voditelj:	mr.sc. Sanja Barbalić, dipl.ing.građ.

Sadržaj

1 POLAZIŠTE I PRAVNA OSNOVA

Plan upravljanja vodnim područjima (2022. - 2027.) izrađen je na temelju Zakona o vodama (Narodne novine, br. 66/19 i 84/21) kojima su propisani: Planski dokumenti upravljanja vodama (članak 37.), Plan upravljanja vodnim područjima (članak 39.) i Plan upravljanja rizicima od poplava (članak 127.). Dokument je novela drugog Plana upravljanja vodnim područjima (Narodne novine, broj 66/16) kojeg je Vlada Republike Hrvatske donijela 6. lipnja 2016. godine za plansko razdoblje od 2016. do 2021. godine.

Struktura dokumenta usklađena je s odredbom iz članka 127. Zakona o vodama kojom je propisano da je sastavni dio Plana upravljanja vodnim područjima i Plan upravljanja rizicima od poplava, te s odredbama Pravilnika o sadržaju plana upravljanja vodnim područjima (Narodne novine, br. 74/13, 53/16 i 64/18). S time u svezi Plan upravljanja vodnim područjima 2022. - 2027. se sastoji od dvije komponente upravljanja vodnim područjima:

- **Upravljanje stanjem voda**, sadržajno usklađena s odredbama članka 39. Zakona o vodama, odnosno odredbama članka 13. i dodatka VII. Direktive 2000/60/EZ Europskog parlamenta i Vijeća od 23. listopada 2000. o uspostavi okvira za djelovanje Zajednice u području vodne politike (SL L 327, 22. 12. 2000.) - Poglavlje B.
- **Upravljanje rizicima od poplava**, sadržajno usklađena s odredbama članka 127. Zakona o vodama, odnosno odredbama članka 7. i Dodatka Direktive 2007/60/EZ Europskoga parlamenta i Vijeća od 23. listopada 2007. o procjeni i upravljanju poplavnim rizicima (Tekst značajan za EGP) (SL L 288, 6.11.2007.) - Poglavlje C.

Nacrt plana upravljanja vodnim područjima 2022. - 2027. su izradile Hrvatske vode u suradnji s mnogim znanstvenim i stručnim institucijama i specijaliziranim tvrtkama koje su pripremale stručne podloge, polazeći od drugog Plana upravljanja vodnim područjima (2016. - 2021. godina), strateških odrednica iz Strategije upravljanja vodama (Narodne novine, broj 91/08), te zaključaka sa četiri bilateralna sastanka predstavnika hrvatskih nadležnih institucija s predstavnicima Europske komisije i komunikacije s Europskom komisijom putem takozvanog „Pilot sustava“.

Ograničen opseg podataka prilikom pripreme Plana upravljanja vodnim područjima 2016. - 2021. dijelom je utjecao na smanjenje pouzdanosti tadašnje procjene stanja voda, analize opterećenja i utjecaja, te praćenja učinka provedenih mjera. Radi toga je u razdoblju od 2016. do 2021. godine intenziviran monitoring stanja voda prema Programu usklađenja monitoringa objavljenom u travnju 2016. godine, do razine neophodne za učinkovito i vjerodostojno upravljanje vodama te je intenziviran rad na daljnjoj pripremi znanstvenih i stručnih podloga, sve sa ciljem osiguranja što kvalitetnije podatkovne osnovice za pripremu Plana upravljanja vodnim područjima 2022. - 2027. Programom usklađenja monitoringa je predviđeno unaprjeđenje organizacije provedbe monitoringa s tendencijom jačanja laboratorijskih kapaciteta uz dodatna ulaganja u prostor, opremu i kadrove, što se postupno provodi.

Veliki doprinos kvaliteti Plana upravljanja vodnim područjima 2022. - 2027. je provedena interkalibracija klasifikacijskih sustava površinskih kopnenih, prijelaznih i priobalnih voda koja je kroz suradnju hrvatskih biologa s recenzentima određenim od strane Europske komisije dovršena potkraj 2021. godine.

U ovom dokumentu navode se svi izvještaji o uspješno provedenim interkalibracijskom postupcima.

2 IZVJEŠTAJI O PROVEDENIM INTERKALIBRACIJSKIM POSTUPCIMA

Report on fitting the Croatian classification method for phytobenthos in rivers to the results of the completed intercalibration of the Mediterranean GIG (R-M1, R-M2 and R-M5)

Working Group:

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Hrvatske vode

Zagreb, October 09th 2020

Report on fitting the Croatian classification method for phytobenthos in rivers to the results of the completed intercalibration of the Mediterranean GIG (R-M1, R-M2 and R-M5)

1. INTRODUCTION

The official intercalibration exercise of phytobenthos-based methods of ecological status assessment of rivers within the Mediterranean GIG was successfully finalized in 2012 (Milestone 6 Report 2012). The results of the first phase were included in the first Commission Decision (COM DEC 2008/915/EC). In the second phase the exercise was repeated using larger datasets in order to be fully compliant with the requirements of the new guidance, and its results were included in the second Commission Decision (COM DEC 2013/480/EC). Croatia did not participate in any rounds of the intercalibration exercise with data for Phytobenthos.
The goal of this report is to declare that the present Croatian assessment method of ecological status of Mediterranean rivers of the IC types (R-M1, R-M2 and R-M5) based on benthic diatoms is compliant with the WFD normative definitions and its class boundaries are in accordance with the results of the completed intercalibration exercise.
In particular, the classification method was verified for WFD compliance and IC feasibility and the class boundaries were compared with agreed boundaries from the Med-GIG intercalibration exercise following the instructions of the CIS Guidance Document n°30: "Procedure to fit new or updated classification methods to the results of a completed intercalibration exercise" (Willby et al. 2014).

Typology
Within the Med GIG five common IC river types were considered for intercalibration of phytobenthos (*Table 1*). Types R-M1, R-M2 and R-M4 were treated together following the same principles throughout the intercalibration process of phytobenthos, whilst R-M5 was treated separately. Type R-M3 was not intercalibrated due to the lack of comparability between MS methods and insufficient number of reference sites.

Table 1. Common IC river types in the Mediterranean GIG from the Milestone 6 report (Milestone 6 Report 2012).

IC type	Type characteristics	MS sharing IC common type
RM1	catchment <100 km^2; mixed geology (except non-siliceous); highly seasonal	SP, FR, IT, PT, SI, HR
RM2	catchment 100-1000 km^2; mixed geology (except non-siliceous); highly seasonal	SP, IT, PT, SI, HR
RM3	catchment 1000-10000 km^2; mixed geology (except siliceous); highly seasonal	This type cannot be intercalibrated due to the lack of comparability between MS methods and insufficient number of reference sites.
RM4	non-siliceous streams; highly seasonal	IT, CY, SP, FR
RM5	temporary rivers	SP, IT, PT, CY, SI, HR

The Croatian rivers included in this report belonging to 9 national types with pertaining sub-types (HR-R_11A, HR-R_11B, HR-R_12, HR-R_13A, HR-R_13B, HR-R_14A, HR-R_14B, HR-R_14C, HR-R_15A, HR-R_15B, HR-R_16A, HR-R_16B, HR-R_17, HR-R_18 and HR-R_19), as part of the Dinaric Western Balkan ecoregion (ER5; sensu Illies 1978), are grouped into 3 common IC MED-river GIG types: R-M1, R-M2 and

R-M5 (*Table 2*). They are all part of the Adriatic drainage system located along the Adriatic coastline stretching in the northwest-southeast direction. The rivers are influenced by mostly Mediterranean and partly contintental climate with significant seasonal flow fluctuations.

Table 2. Croatian river types included into common IC river types of the Mediterranean GIG, together with the reference and poorest values of Croatian Trophic Diatom Index (TDI_{HR}) of each national river type.

<table>
<tr><th colspan="2">ECOREGION</th><th colspan="2">NATIONAL TYPE NAME</th><th>NATIONAL TYPE</th><th>NATIONAL INDEX</th><th>Reference value</th><th>Poorest value</th><th>IC TYPE</th></tr>
<tr><td rowspan="17">DINARIC ECOREGION (5. DINARIC WESTERN BALKAN)</td><td rowspan="14">DINARIC COASTAL SUB-ECOREGION</td><td colspan="2" rowspan="2">Small lowland and upland rivers</td><td>HR-R_11A</td><td rowspan="2">TDI_{HR}</td><td rowspan="2">1,90</td><td rowspan="2">4,58</td><td rowspan="2">M1</td></tr>
<tr><td>HR-R_11B</td></tr>
<tr><td colspan="2">Medium and large upland rivers</td><td>HR-R_12</td><td>TDI_{HR}</td><td>1,83</td><td>4,58</td><td>M2</td></tr>
<tr><td colspan="2" rowspan="2">Medium and large lowland rivers</td><td>HR-R_13A</td><td rowspan="2">TDI_{HR}</td><td rowspan="2">1,83</td><td rowspan="2">4,58</td><td rowspan="2">M2</td></tr>
<tr><td>HR-R_13B</td></tr>
<tr><td colspan="2">Small short-flow lowland rivers with a channel drop >5 ‰</td><td>HR-R_14A</td><td>TDI_{HR}</td><td>1,90</td><td>4,58</td><td>M1</td></tr>
<tr><td colspan="2">Medium short-flow lowland rivers with a channel drop >5 ‰</td><td>HR-R_14B</td><td>TDI_{HR}</td><td>1,83</td><td>4,58</td><td>M2</td></tr>
<tr><td colspan="2">Large short-flow lowland rivers with a channel drop >5 ‰</td><td>HR-R_14C</td><td>TDI_{HR}</td><td>1,83</td><td>4,58</td><td>M2</td></tr>
<tr><td colspan="2">Small and medium rivers in karst fields</td><td>HR-R_15A</td><td>TDI_{HR}</td><td>1,90</td><td>4,58</td><td>M1</td></tr>
<tr><td colspan="2">Medium rivers in karst fields</td><td>HR-R_15B</td><td>TDI_{HR}</td><td>1,83</td><td>4,58</td><td>M2</td></tr>
<tr><td rowspan="2">Intermittent rivers</td><td>Small and medium upland intermittent running waters</td><td>HR-R_16A</td><td rowspan="2">TDI_{HR}</td><td rowspan="2">2,45</td><td rowspan="2">4,58</td><td rowspan="2">M5</td></tr>
<tr><td>Small lowland intermittent running waters</td><td>HR-R_16B</td></tr>
<tr><td rowspan="3">DINARIC COASTAL SUB-ECOREGION - ISTRIA</td><td colspan="2">Small lowland and upland rivers in Istria</td><td>HR-R_17</td><td>TDI_{HR}</td><td>1,90</td><td>4,58</td><td>M1</td></tr>
<tr><td colspan="2">Medium lowland rivers in Istria</td><td>HR-R_18</td><td>TDI_{HR}</td><td>1,83</td><td>4,58</td><td>M2</td></tr>
<tr><td colspan="2">Small lowland intermittent rivers in Istria</td><td>HR-R_19</td><td>TDI_{HR}</td><td>2,45</td><td>4,58</td><td>M5</td></tr>
</table>

2. DESCRIPTION OF NATIONAL ASSESSMENT METHODS

The Croatian national method for ecological status assessment of rivers considers benthic diatoms as proxies for phytobenthos. It is compliant with normative definitions of WFD used by other MS and takes intoaccount both taxonomic composition and species' relative abundance of benthic diatom assemblages. Sampling, sample treatment, diatom identification and data processing are based on the European standards EN 13946: 2014 and EN 14407: 2014 (European Committee for Standardization, 2014a, b). Ecological status is evaluated using TDI_{HR} (Croatian Trophic Diatom Index), a diatom metric modified from Rott's Trophic Index (Rott et al. 1999). The complete procedure is described in detail in the "Methodology for sampling, laboratory analyses and determination of ecological quality ratios for biological quality elements" (Official Gazette 96/19).

2.1. SAMPLING AND DATA PROCESSING

Sampling method: Benthic diatoms are scrubbed from hard substrata (minimum of five stones) in the main water current of the river, in the well exposed euphotic zone.

Sampling time and frequency: Sampling is performed once a year, principally in spring time during favourable and stable water level.

Sample treatment/data processing: Diatom samples in the laboratory are treated according to Standard HRN EN 13946:2014, where the hydrochloric acid is used to remove inorganic material, and sulphuric acid or hot hydrogen peroxide are used to remove all the organic material. Permanent slides are prepared by mounting clean diatom suspension with Naphrax on the microscopic slides.

Identification level: Around 400 valves are counted and identified to the lowest taxonomic level possible on each slide using light microscope with Differential Interference Contrast at 1000 x magnification.

2.2. DESCRIPTION OF NATIONAL METHODOLOGY

Metric calculation: Trophic indicator values and weights of all identified diatom species were defined according to the extended Operational list of diatom taxa for rivers included in the "Methodology for sampling, laboratory analyses and determination of ecological quality ratios for biological quality elements" (Official Gazette 96/19). Taxa list of diatoms with assigned indicator values and weights and with corresponding relative abundances is used for calculation of TDI_{HR} by using the modified Zelinka-Marwan equation (1961):

$$TDI_{HR} = \frac{\sum_{i=1}^{n} Ai \times IVi \times IWi}{\sum_{i=1}^{n} Ai \times IWi}$$

Where:
Ai = Total number of cells/valves of a species in the sample, representing the number of a certain species on 400 counted diatoms.
IVi = Indicator value (tolerance) of a species
IWi = Indicator weight (sensitivity) of a species

ES assessment: Ecological status is assessed on the basis of EQR values of TDI_{HR}. EQR_TDI_{HR} is calculated using the formula described in the "Methodology for sampling, laboratory analyses and determination of ecological quality ratios for biological quality elements" (Official Gazette 96/19):

$$EQR\_TDI_{HR} = \frac{Index\ value - Poorest\ value}{Reference\ value - Poorest\ value}$$

2.3. NATIONAL BOUNDARY SETTING

The national dataset utilized for intercalibration comprises data coming from a total of 56 samples coming from 37 streams (*Table 3*). Hydrochemical data, including basic physico-chemical data (total phosphorus, orthophosphates, total nitrogen, nitrates, nitrites, ammonium, oxygen saturation) and land-use data in catchment (artificial areas, intensive and non-intensive agriculture, semi-natural areas), as well as the biological data (TDI_{HR}, diatom taxalist with relative abundances) are available for all samples concerned (*Table 4)*. The pressure gradient is considered sufficient, although the worst part of the gradient is less well represented as no sites with poor and just one site with bad status were present in sampling.

Table 3. List of data available in the national dataset included in the intercalibration.

IC type	Present	Number of samples	Physico-chemical data	Hydro-morphological data	Biological data	Complete dataset	Number of benchmark samples
R-M1	Yes	15	15	15	15	15	6
R-M2	Yes	22	22	22	22	22	10

R-M3	No	-	-	-	-	-	-
R-M4	No	-	-	-	-	-	-
R-M5	Yes	19	19	19	19	19	3

Table 4. Range of values of different environmental variables at river sites included in the intercalibration.

(N=56)	MIN	MAX
P-Total (TP) [mg L^{-1}]	0.0045	0.5498
P-PO_4^{3-} [mg L^{-1}]	0.0015	0.3669
N-NO_3^- [mg L^{-1}]	0.0048	2.6167
N-NH_4^+ [mg L^{-1}]	0.0010	7.1591
N-Total (TN) [mg L^{-1}]	0.3100	18.5167
N-NO_2^- [mg L^{-1}]	0.0005	0.2517
O_2 %	58.1	165.1
Artificial areas (catchment) [%]	0.0	25.7
Intensive agriculture (catchment) [%]	0.0	52.2
Extensive agriculture (catchment) [%]	0.0	38.6
Semi-natural areas (catchment) [%]	21.3	99.1
TDI_{HR}	1.33	4.58

Selection of benchmark sites was based on the Mediterranean GIG-river common benchmark criteria from the MED-GIG Rivers Milestone 6 report: Phytobenthos (2012). The benchmark criteria were selected for both abiotic (water chemistry and land-use) and biotic parameters (TDI_{HR}) to ensure that the intensity of human activities at the selected sites is low and has only very minor impacts on diatom assemblages.
The benchmark criteria of abiotic parameters (land-use and hydrochemical criteria) were adopted from the MED-GIG Intercalibration of diatoms and macrophytes (*Table 5*).

Table 5. Criteria for identifying benchmark sites for the MED-GIG from the Milestone 6 report (2012).

	Benchmarks are accepted if	
Pressure variables	**RM1+RM2+RM4**	**RM5**
General Morphology (Classes 1-3) **General Hydrology** (Classes 1-3) **Riparian Vegetation** (Classes 1-3)	≤ 2	
DO (mg/L) [1]	6.39-13.70	
O_2 (%)	73.72-127.92	60.34-127.92
N-NH_4^+ (mg L^{-1})	≤0.09	
N-NO_3^- (mg L^{-1})	≤1.15	
P-Total (mg L^{-1})	≤0.07	
P-PO_4^{3-} (mg L^{-1})	≤0.06	
% Artificial areas (catchment)	≤1	
% Intensive agriculture (catchment)	≤11	
% Extensive agriculture (catchment)	≤32	
% Semi-natural areas (catchment)	≥68	
% Urbanisation (reach) [2]	≤1	
% Land use (reach) [2]	≤20	
% Agriculture (reach) [2]	≤20	

[1] for macrophytes only, instead of O_2 (%)

[2] for diatoms only, instead of land use in the catchment

Only sites that met land-use and hydro-chemical criteria were included into the boundary setting protocol. From the total of 15 samples in the R-M1 type, six samples met the given benchmark criteria. For the R-M2 type, 10 of the 22 samples were identified as benchmark sites. As for the R-M5 type, three out of the 19 samples complied to the MED-GIG benchmark criteria (*Table 3*). According to the CIS Guidance No. 30 (Willby et al. 2014), implementation of case A1 is plausible when a sufficient number of reference condition or other high quality sites that can be used for benchmarking, i.e. a minimum of three sites, is present in the national dataset.

Setting of reference and poorest values:
The reference values of national types included in the IC were adjusted for further IC process. Therefore, 10th percentile of TDI_{HR} of benchmark sites for corresponding IC type was calculated and set as reference value (*Table 2*).

The poorest value of TDI_{HR} was taken as the lowest value of TDI_{HR} on all samples that were included in the IC Med-river GIG types (*Table 2*).

Setting of EQR boundaries:
The High/Good EQR boundary was derived from EQR variability at available spatial based benchmark sites. The remaining degradation continuum was divided into four equal width classes.

H/G boundary = median of benchmark sites
G/M boundary = H/G * 0.75
M/P boundary = H/G * 0.50
P/B boundary = H/G * 0.25

Summary of the class boundaries for the EQR_TDI_{HR} values are presented in *Table 6*.

Table 6. Summary of the class boundaries for the EQR_TDI_{HR} values in the IC river types.

	R-M1	R-M2	R-M5
Reference TDI_{HR} values	1.90	1.83	2.45
Reference	1.00	1.00	1.00
High/Good Boundary	0.83	0.83	0.85
Good/Moderate Boundary	0.55	0.55	0.59
Moderate/Poor Boundary	0.41	0.41	0.48
Poor/Bad Boundary	0.21	0.21	0.24

EQR_TDI_{HR} is used as the final metric in comparison with the intercalibration common metric (ICM).

2.4. PRESSURES ADDRESSED

The different national methods of the MS of the completed intercalibration exercise were reported to mainly address eutrophication, organic matter and general degradation (MED-GIG Rivers Milestone 6 report 2012). Statistical analyses were performed to explore the responsiveness of the national diatom-based assessment method to various anthropogenic stressors.

The pressure-response relationships were tested via:

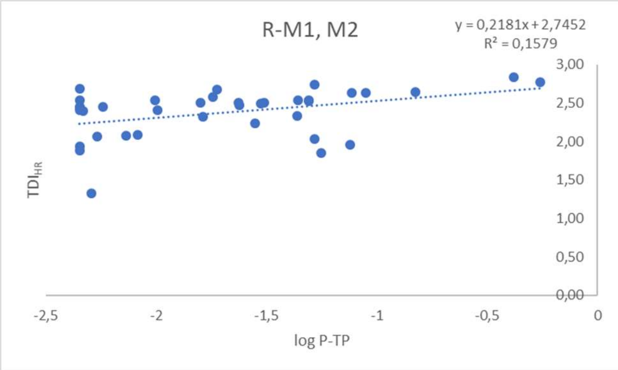
(1) non-parametric Spearman rank correlations of the national diatom metric (TDI_{HR}) with environmental parameters (P-Total (TP), $P\text{-}PO_4^{3-}$, N-Total (TN), $N\text{-}NO_3^-$, $N\text{-}NO_2^-$, $N\text{-}NH_4^+$, oxygen saturation) and general land-use and hydrology parameters

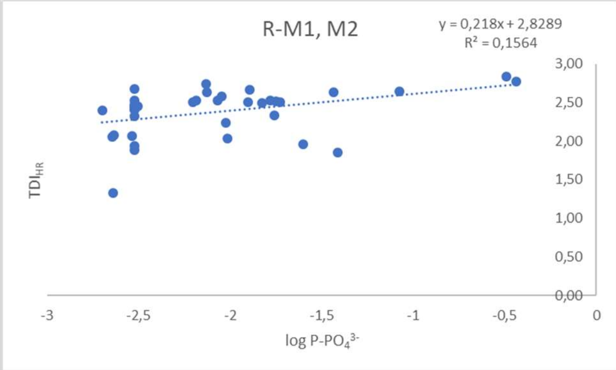
(2) linear regressions of the national diatom metric (TDI_{HR}) with pressure variables.

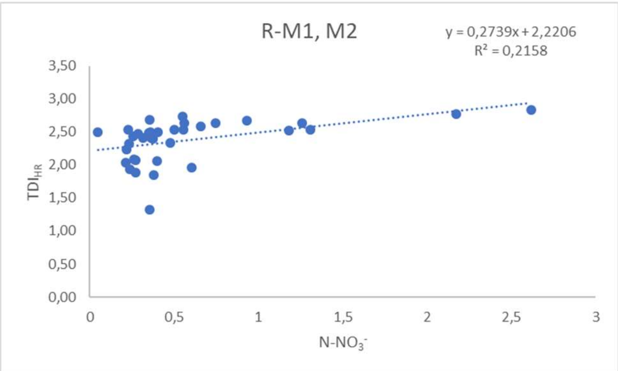
Table 7. Summary of the Spearman correlations of the national diatom metric (TDI_{HR}) with different hydro-chemical, environmental and land-use pressures. Correlations marked in red are significant at p < 0.05.

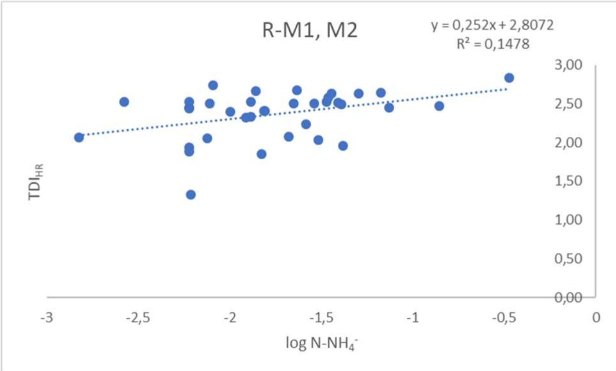
	R-M1, M2	**R-M5**
	TDI_{HR}	**TDI_{HR}**
P-Total (TP)	0.3900 p=0.0170	0.0720 p=0.7696
$P\text{-}PO_4^{3-}$	0.4463 p=0.056	0.0053 p=0.9829
N-Total (TN)	0.4926 p=0.0020	-0.1440 p=0.5565
$N\text{-}NO_3^-$	0.5792 p=0.0002	-0.3126 p=0.1926
$N\text{-}NO_2^-$	0.1936 p=0.2509	-0.0660 p=0.7885
$N\text{-}NH_4^+$	0.4026 p=0.0135	0.01176 p=0.9431
O_2 [%]	-0.4698 p=0.0033	0.5233 p=0.0215
Artificial areas [%]	0.5898 p=0.0001	-0.0466 p=0.8496
Intensive agriculture [%]	0.5094 p=0.0013	0.0395 p=0.8723
Extensive agriculture [%]	-0.0093 p=0.9566	-0.1353 p=0.5809
Semi-natural areas [%]	-0.2894 p=0.0823	-0.0053 p=0.9896
General Morphology (Classes 1-3)	0.3223 p=0.0176	-0.3841 p=0.1044
General Hydrology (Classes 1-3)	0.1733 p=0.0517	0.1839 p=0.4510
Riparian vegetation	0.2014 p=0.2321	-0.1468 p=0.5488

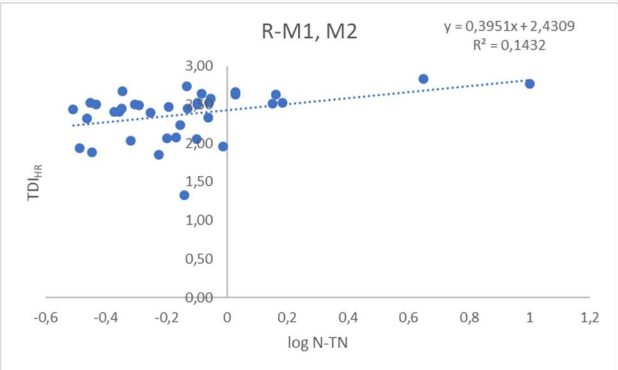
The results of Spearman correlation of TDI_{HR} with pressure variables are shown in *Table 7*. The coefficient showed statistically significant relationships ($p<0.05$) between national metric and several different pressures. The pressures that present the strongest relationships with the national metric are presented in *Figure 1*. Different river types present different responses to pressures. In general, diatom assemblages of Croatian national types which were classified into IC types R-M1 and R-M2 responded well to all nutrient pressures, in particular to total phosphorus (TP), orthophosphates ($P\text{-}PO_4^{-3}$), total nitrogen (TN), nitrates ($N\text{-}NO_3^-$) and ammonium ($N\text{-}NH_4^+$), as well as to oxygen saturation and to land use parameters such as artificial areas and intensive agriculture.

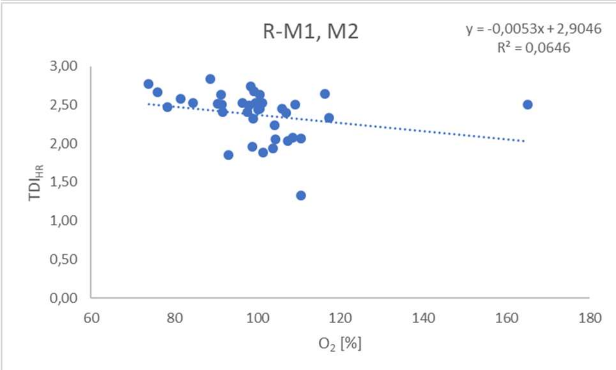

R-M1, M2
y = 0,2181x + 2,7452
R² = 0,1579
TDI_HR
3,00 2,50 2,00 1,50 1,00 0,50 0,00
-2,5 -2 -1,5 -1 -0,5 0
log P-TP

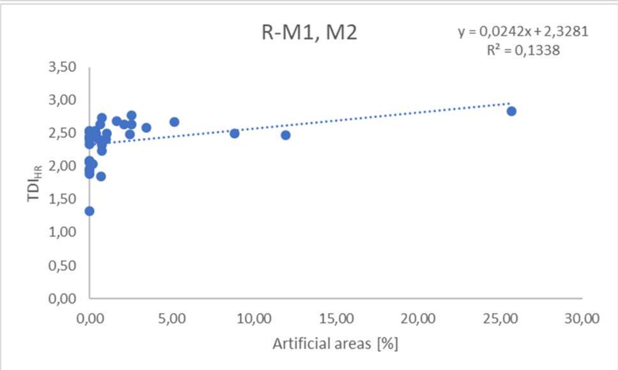

R-M1, M2
y = 0,218x + 2,8289
R² = 0,1564
TDI_HR
3,00 2,50 2,00 1,50 1,00 0,50 0,00
-3 -2,5 -2 -1,5 -1 -0,5 0
log P-PO4 3-

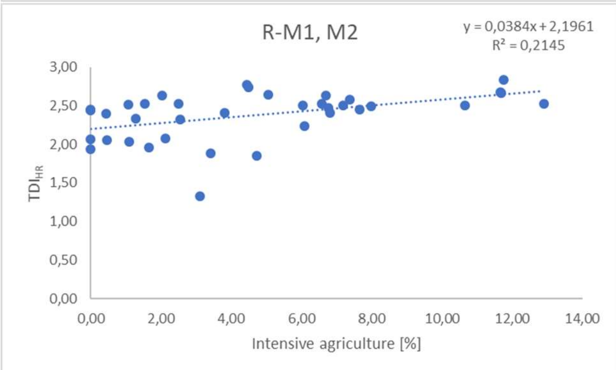

R-M1, M2
y = 0,2739x + 2,2206
R² = 0,2158
TDI_HR
3,50 3,00 2,50 2,00 1,50 1,00 0,50 0,00
0 0,5 1 1,5 2 2,5 3
N-NO3-


R-M1, M2
y = 0,252x + 2,8072
R² = 0,1478
TDI_HR
3,00 2,50 2,00 1,50 1,00 0,50 0,00
-3 -2,5 -2 -1,5 -1 -0,5 0
log N-NH4-


R-M1, M2
y = 0,3951x + 2,4309
R² = 0,1432
TDI_HR
3,00 2,50 2,00 1,50 1,00 0,50 0,00
-0,6 -0,4 -0,2 0 0,2 0,4 0,6 0,8 1 1,2
log N-TN


R-M1, M2
y = -0,0053x + 2,9046
R² = 0,0646
TDI_HR
3,00 2,50 2,00 1,50 1,00 0,50 0,00
60 80 100 120 140 160 180
O2 [%]


R-M1, M2
y = 0,0242x + 2,3281
R² = 0,1338
TDI_HR
3,50 3,00 2,50 2,00 1,50 1,00 0,50 0,00
0,00 5,00 10,00 15,00 20,00 25,00 30,00
Artificial areas [%]


R-M1, M2
y = 0,0384x + 2,1961
R² = 0,2145
TDI_HR
3,00 2,50 2,00 1,50 1,00 0,50 0,00
0,00 2,00 4,00 6,00 8,00 10,00 12,00 14,00
Intensive agriculture [%]

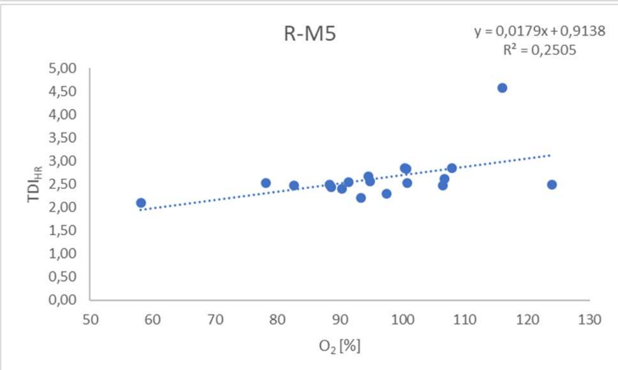

R-M5
y = 0,0179x + 0,9138
R² = 0,2505
TDI_HR
5,00 4,50 4,00 3,50 3,00 2,50 2,00 1,50 1,00 0,50 0,00
50 60 70 80 90 100 110 120 130
O2 [%]

Figure 1. Pressure-response relationship between the most important pressures against the TDI_{HR} in different river types.

3. WFD COMPLIANCE CHECKING

The data acceptance criteria of the MED-GIG as defined in the MED-GIG Rivers Milestone 6 Report are listed in *Table 8*. The Croatian data fulfilled the listed criteria in all aspects except for the number of water quality classes represented in the dataset (last criterion). This criterion, however, is not so strictly relevant for the fit-in procedure. The data set is expected to cover at least three ecological classes, which is fulfilled. The dataset can be therefore considered sufficient for intercalibration.

Table 8. List of the WFD compliance criteria and the WFD compliance checking process and results.

Compliance criteria	Compliance checking
Ecological status is classified by one of **five classes** (high, good, moderate, poor and bad).	Yes
High, good and moderate ecological status are set in line with the WFD's **normative definitions** (**Boundary setting procedure**)	Yes. Equidistant division of the EQR gradient High-good boundary derived from metric variability at near-natural benchmark sites.
All relevant parameters indicative of the biological quality element are covered (see Table 1 in the IC Guidance). A **combination rule** to combine parameter assessment into BQE assessment has to be defined. If parameters are missing, Member States need to demonstrate that the method is sufficiently indicative of the status of the QE as a whole	Yes; both taxonomic composition and species relative abundance are taken into consideration. Diatom metric (TDI_{HR} - Croatian Trophic Diatom Index) - product of species relative abundance × sensitivity × weight value is weighted with product of rel. abundance × weight value
Assessment is adapted to **intercalibration common types** that are defined in line with the typological requirements of the Annex II WFD and approved by WG ECOSTAT	Yes; common MED-river intercalibration types are used: R-M1, R-M2, R-M5
The water body is assessed against **type-specific near-natural reference conditions**	Yes
Assessment results are expressed as **EQRs**	Yes
Sampling procedure allows for **representative** information about water body quality/ecological status **in space and time**	Yes; 1 sampling per year during favourable and stable water level. Using brush/scraper for sampling. Single habitat(s) preferably epilithic phytobenthos – mesolithal (5 stones/cobbles from different points of streamline).
All data relevant for assessing the biological **parameters** specified in the WFD's normative definitions are covered by the **sampling procedure**	Yes; all 5 water quality classes are represented, but class 4 and 5 corresponding to Poor and Bad may be under-represented for most types.
Selected taxonomic level achieves adequate **confidence and precision** in classification	Yes; identification in species level or lower.

4. IC FEASIBILITY CHECKING

The intercalibration process ideally covers all national assessment methods within a GIG. However, the comparison of dissimilar methods ("apples and pears") has clearly to be avoided. Intercalibration exercise is focused on specific type / biological quality element / pressure combinations. The second step of the process introduces an “IC feasibility check” to restrict the actual intercalibration analysis to methods that address the same common type(s) and anthropogenic pressure(s), and follow a similar assessment concept.

4.1. TYPOLOGY

The RM typological system was found to be the most appropriate for describing Croatian rivers. Five types are included in the MED-GIG, of which types R-M1, R-M2 and R-M5 are applicable for Croatia (*Tables 1, 2*). Reference sites for large river types are open to criticism and therefore they are not included in the MED-GIG intercalibration exercise.

4.2. PRESSURES ADDRESSED

Diatom assemblages as summarized by the national metric (TDI_{HR}) respond to nutrient pollution, especially nitrogen compounds, oxygen related pressures, as well as land use related to agriculture and artificial use. These pressures were also found to be significant when correlated with the common metric of the other MS during the intercalibration exercise.

4.3. ASSESSMENT CONCEPT

The national diatom-based assessment system consists of the Croatian Trophic Diatom Index (TDI_{HR}), modified from Rott’s Trophic Index (Rott et al. 1999), which is an indicator of a nutrient load in a given water body, i.e. its trophic degree based on the representation of diatom species. The TDI_{HR} takes into consideration the relative abundances of diatoms present in the assemblage and their assigned tolerances (indicator values) and sensitivities (indicator weights). The index responded to several pressures addressed (see above Section "2.4. Pressures addressed").

4.4. CONCLUSION ON THE INTERCALIBRATION FEASIBILITY

The RM typology was chosen. The Croatian Trophic Diatom Index (TDI_{HR}) takes into consideration tolerance and sensitivity of the species present in the assemblage and their relative abundances. The index addresses various pressures (see above Section "2.4. Pressures addressed"). It is concluded that the fitting of TDI_{HR} to the results of the MED-GIG river intercalibration was feasible.

5. DEMONSTRATING THE COMPLIANCE WITH THE COMPLETED INTERCALIBRATION EXERCISE

Following Figure 1 in the CIS Guidance No. 30 (Willby et al. 2014), case A1 (Option 2) is applied for fitting the HR assessment method using phytobenthos to the results of the River MED-GIG type R-M1, R-M2 and R-M5.

The requirements for case A1 are:

- *Full details of the common metric*

  The ICM applied in the MED-GIG is composed of two diatom metrics (according to Kelly et al. 2009):
  - IPS (Coste in CEMAGREF, 1982): this metric measures ‘general water quality’, with low values corresponding to high pressure levels and, therefore, low EQRs
  - TI (Rott *et al.* 1999): a trophic index which needs to be adjusted so that high values represent high EQR values

  ICM=(EQR-IPS + EQR-TI)/2

- *A suitable site x biology dataset covering a range of environmental quality from which the national EQR and common metric can be calculated*

A total of 56 samples were available covering 4 classes of ecological status (see Section "2.3. National boundary setting")

- *Accompanying pressure data in the same format as that used in the completed exercise.*
  All accompanying pressure data are available (see *Table 3*).

- *Information on the specific thresholds already used in the completed exercise to define reference or alternative benchmark sites*
  The benchmark criteria of abiotic parameters (land-use and hydrochemical criteria) were adopted from the MED-GIG Intercalibration of diatoms and macrophytes (see *Table 5*).

- *Details of exactly how benchmarking was undertaken in the complete exercise. If the completed exercise concluded that benchmarking was not necessary the mean value of the benchmark sites from each country must be provided so that the joining Member State can also judge the need to benchmark its own method.*
  Given benchmark criteria were applied by each MS in order to identify benchmark sites within each national dataset. Median values of TDI_{HR} of the national benchmark dataset were used for calculation of common metric EQR (EQR_TDI_{HR}). Linear regression was established between the values of the national method and the ICM so that the national boundaries could be translated to ICM using the equation.
  If the number of national benchmark sites turns out insufficient, then the global median of all participating MS has to be used.

- *Values of the global mean view of the HG and GM boundaries on the common metric scale for Member States who participated in the completed exercise.*
  Mean H/G (relevant for R-M1, R-M2): 0.896
  Mean G/M (relevant for R-M1, R-M2): 0.688

  Mean H/G (relevant for R-M5): 0.914
  Mean G/M (relevant for R-M5): 0.688

The process of fitting the HR method to the completed IC exercise:
According to the Willby et al. (2014), the following steps should be followed:

i. Calculate the common metric (CM) on the national dataset.

The ICM applied in the MED-GIG is composed of two diatom metrics (according to Kelly et al. 2009):

- IPS (Coste in CEMAGREF, 1982): this metric measures 'general water quality', with high pressure levels rendering low values and thus low EQRs:
  EQR_IPS = Observed value / reference value

- TI (Rott et al. 1999): a trophic index, with higher eutrophication levels rendering high values and thus needs to be adjusted so that high values represent high EQRs:
  EQR_TI = (4-observed value) / (4-reference value)

ICM = (EQR-IPS + EQR-TI)/2

ii. Use the associated pressure data to identify sites in the national dataset that meet the criteria established by the GIG for the selection of benchmark or reference sites.

Benchmark sites have been identified based on environmental pressures above (see Section "2.3. National boundary setting") *iii. Standardize the common metric (CM_bm) against the benchmark according to the approach used in the completed exercise.*

The common metric was calculated for the benchmark sites in the national dataset. For the IC river types R-M1 and R-M2 the mean *CM_bm* was CM_M1,M2=1.00, whilst for the R-M5 the mean *CM_bm* was CM_ M5=0.97. These values were inside the range of the mean values of the MS who took part in the intercalibration exercise, therefore, no standardization is required.

iv. *Use OLS regression to establish the relationship between CM_bm (y) and the EQR of the joining method (x).*

Relationship between EQR_TDI_{HR} and ICM for each IC river type and for combined types R-M1, R-M2 and R-M5 are shown (*Table 9, Figure 2*).

Table 9. OLS equations for the relationship between ICM and national EQR.

IC River type	No of samples	No of sites	Linear regression	R^2
R-M1	15	15	$ICM = 0.4672\ EQR\_TDI_{HR} + 0.5644$	0.182
R-M2	22	22	$ICM = 0.8528\ EQR\_TDI_{HR} + 0.2114$	0.432
R-M5	19	19	$ICM = 0.5844\ EQR\_TDI_{HR} + 0.3889$	0.588
R-M1, M2	37	37	$ICM = 0.7718\ EQR\_TDI_{HR} + 0.2875$	0.388

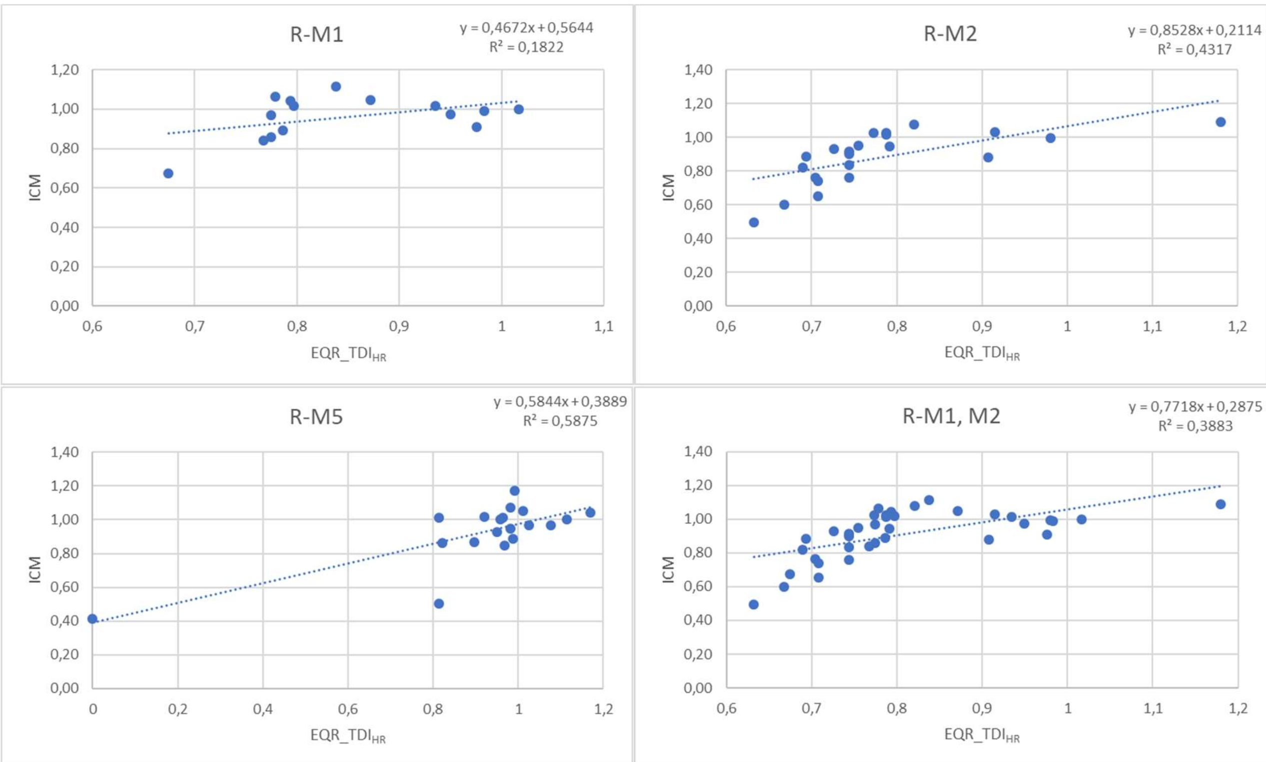


Figure 2. OLS regressions to establish the relationship between ICM and the EQR for each IC river type and combined RM1, M2 river types.

Types R-M1 and R-M2 were treated together, therefore for the translation of reference and boundary positions of the national method onto the ICM scale the linear regression equation of combined R-M1, M2 river types was used. R-M5 was treated separately due to its distinct hydrological conditions.

v. *Predict the position of the national class boundaries (MP, GM, HG and reference) on the CM_bm scale.*

The prediction of the class boundaries on the CM scale was made using the OLS equations of the relationship between the national and the common metric (*Tables 10-12*).

Table 10. Translation of the reference and boundary positions of the national method on the basis of OLS regression (see *Figure 2, Table 9*) into ICM.

IC Type	R-M1, M2		R-M5	
Boundary	EQR	Predicted boundaries on ICM scale	EQR	Predicted boundaries on ICM scale
Reference	1.00	1.06	1.00	0.97
High / good	0.83	0.93	0.96	0.94
Good / moderate	0.62	0.77	0.72	0.79
Moderate / poor	0.41	0.61	0.48	0.64
Poor / bad	0.21	0.45	0.24	0.49

Table 11. Reference values and High/Good class boundary of the ICM values derived from the OLS regression (*Figure 2*) for each IC river type.

	R-M1	R-M2	R-M5
HIGH Max (maximum of national EQR)	**1.198**	**1.198**	**0.967**
H/G Boundary + 0.25H	0.995	0.995	0.948
H/G Boundary (for MS)	**0.927**	**0.927**	**0.941**
H/G Boundary - 0.25H	0.887	0.887	0.903
H/G MedGIG Global mean	**0.896**	**0.896**	**0.914**
H/G quarter (+)	0.068	0.068	0.007
H/G quarter (-)	0.040	0.040	0.038

Table 12. Good/Moderate class boundary of the ICM values derived from the OLS regression (*Figure 2*) for each IC river type.

	R-M1	R-M2	R-M5
Good/Moderate Max	**0.927**	**0.927**	**0.941**
G/M+0.25H	0.807	0.807	0.827
G/M Boundary (for MS)	**0.767**	**0.767**	**0.789**
G/M Boundary - 0.25H	0.727	0.727	0.751
M/P M in	0.607	0.607	0.637
G/M MedGIG Global mean	**0.688**	**0.688**	**0.688**
G/M quarter (+)	0.040	0.040	0.038
G/M quarter (-)	0.040	0.040	0.038

The comparison of H/G and G/M original boundaries values for the types R-M1, R-M2 and R-M5 with the other MS of the MED-GIG is presented in *Figures 3-6*. The explanation of the typological codes used is given in *Table 13*.

Table 13. Typological codes used in the boundary bias analysis.

Code	*MS Type*	*Code*	*MS Type*
PT-Type 1	N1≤100	SP1-Type 3	IBMWP R-M4
PT-Type 2	N2	SP1-Type 4	SP1 R-M5
PT-Type 3	N3	SP2-Type 1	IMM R-M1
PT-Type 4	N1≥100	SP2-Type 2	IMM R-M2
PT-Type 5	S1<100	SP2-Type 3	IMM R-M4
PT-Type 6	S3	SP2-Type 4	SP2 R-M5
FR-Type 1	FR R-M1	SI-Type 1	SL R-M1
FR-Type 2	FR R-M2	SI-Type 2	SL R-M2
FR-Type 3	FR R-M4	SI-Type 3	SI R-M5
IT-Type 1	IT R-M1	CY-Type 1	CY R-M4
IT-Type 2	IT R-M2	CY-Type 2	CY R-M5
IT-Type 3	IT R-M4	HR-Type 1	HR R-M1
IT-Type 4	IT R-M5	HR-Type 2	HR R-M2
SP1-Type 1	IBMWP R-M1	HR-Type 3	HR R-M5
SP1-Type 2	IBMWP R-M2		

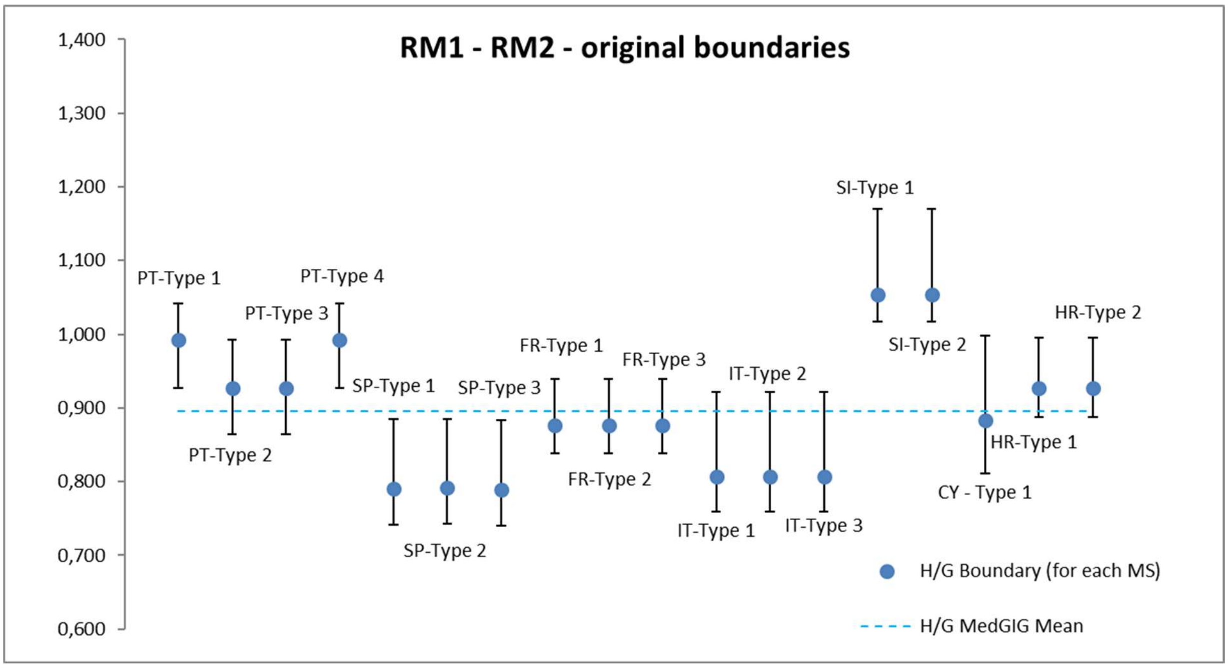


Figure 3. Comparison of H/G original boundaries for the types R-M1 and R-M2 with other MS.

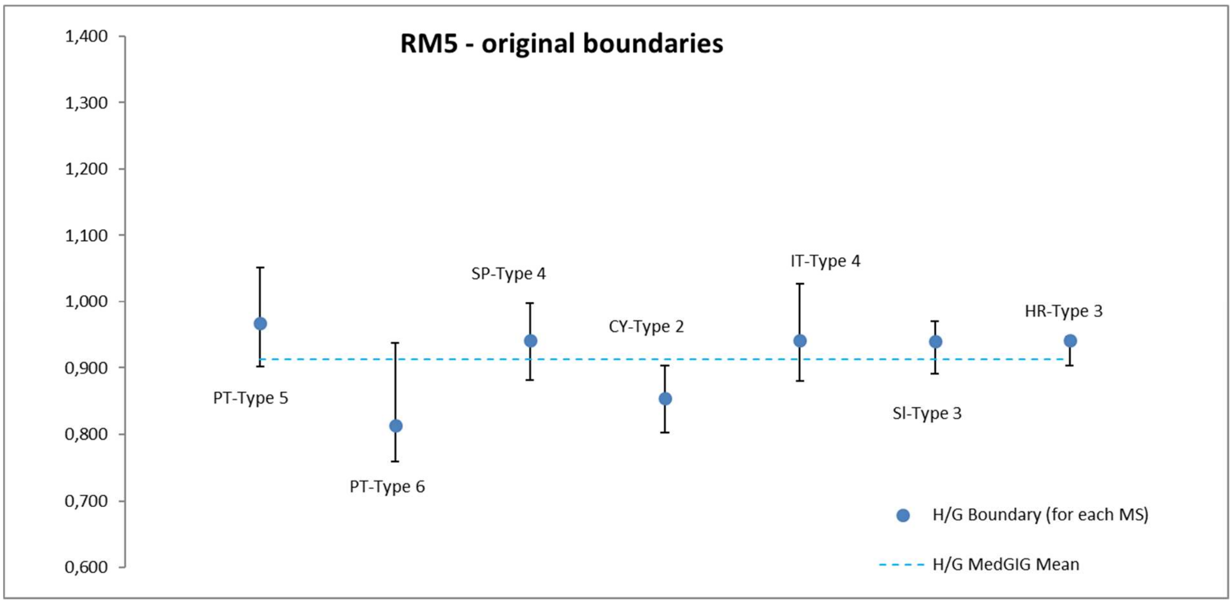


Figure 4. Comparison of H/G original boundaries for the type R-M5 with other MS.

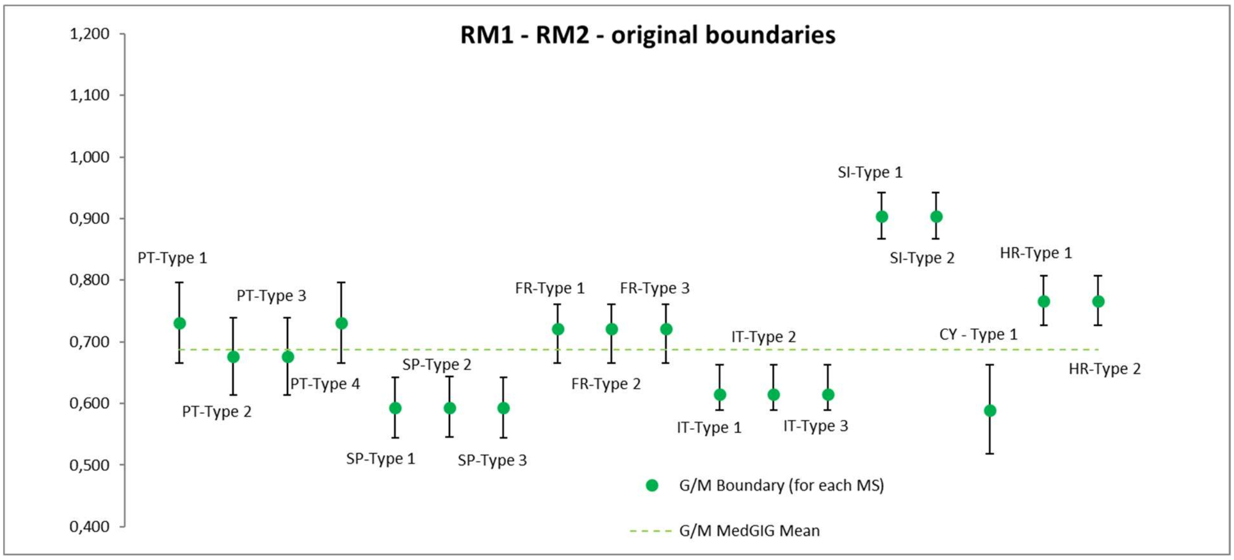


Figure 5. Comparison of G/M original boundaries for the types R-M1 and R-M2 with other MS.

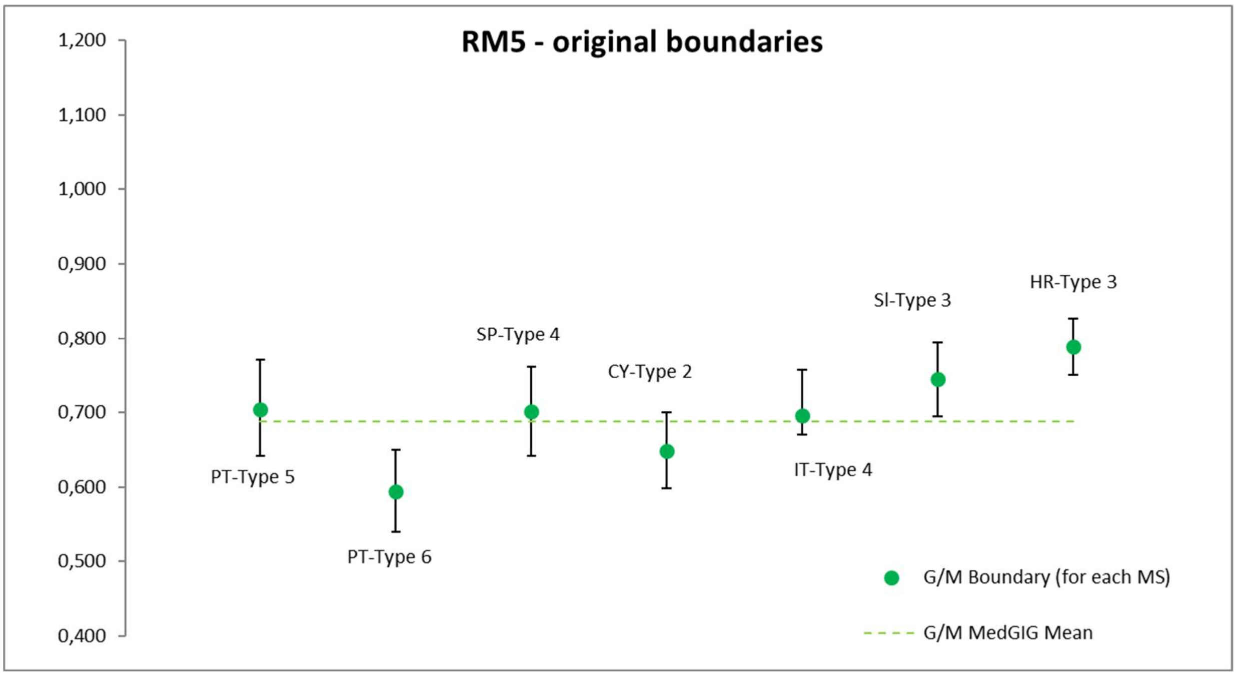


Figure 6. Comparison of H/G original boundaries for the type R-M5 with other MS.

vi. Apply the comparability criteria as summarized in Chapter 6.

The adjustment of the boundaries follows the fit according to the guidance of chapter 6 (Willby et al. 2014). The main principle is that H/G or G/M statistic must not be >|0.25|. The H/G boundary bias in R-M1 and R-M2 river types was within the |0.25| range, so it needed no further adjustment. The G/M boundary bias in R-M1 and R-M2 river types was >0.25, so the adjustment was required (*Table 14* in red) until the appropriate limit was reached (*Tables 15-17*, *Figures 7* and *9*). As for the R-M5 river type, the H/G boundary bias was within the |0.25| range, but since the unchanged value was considered too high and too strict the adjustment was performed within the |0.25| range to lower the value. The G/M boundary bias for the R-M5 river type was >0.25 (*Table 14* in red) and thus adjustment was required by lowering a value until it reached the appropriate limit (*Tables 15-17*, *Figures 8* and 10). The final boundaries adopted after the harmonization are presented in *Table 18*.

Table 14. H/G and G/M statistic bias for each IC river type. Red color represents statistic bias >|0.25|.

Boundary	**R-M1**	**R-M2**	**R-M5**
H/G	0.200	0.200	0.178
G/M	0.487	0.487	0.665

Table 15. Harmonized High/Good class boundary for each IC river type.

	R-M1	**R-M2**	**R-M5**
High Max (maximum of national EQR)	**1.198**	**1.198**	1.075
H/G Boundary + 0.25H	0.996	0.996	0.918
H/G Boundary (for MS)	**0.928**	**0.928**	**0.872**
H/G Boundary - 0.25H	0.875	0.875	0.826
H/G MedGIG Global Mean	**0.871**	**0.871**	**0.917**
H/G quarter (+)	0.067	0.067	0.052
H/G quarter (-)	0.053	0.053	0.040

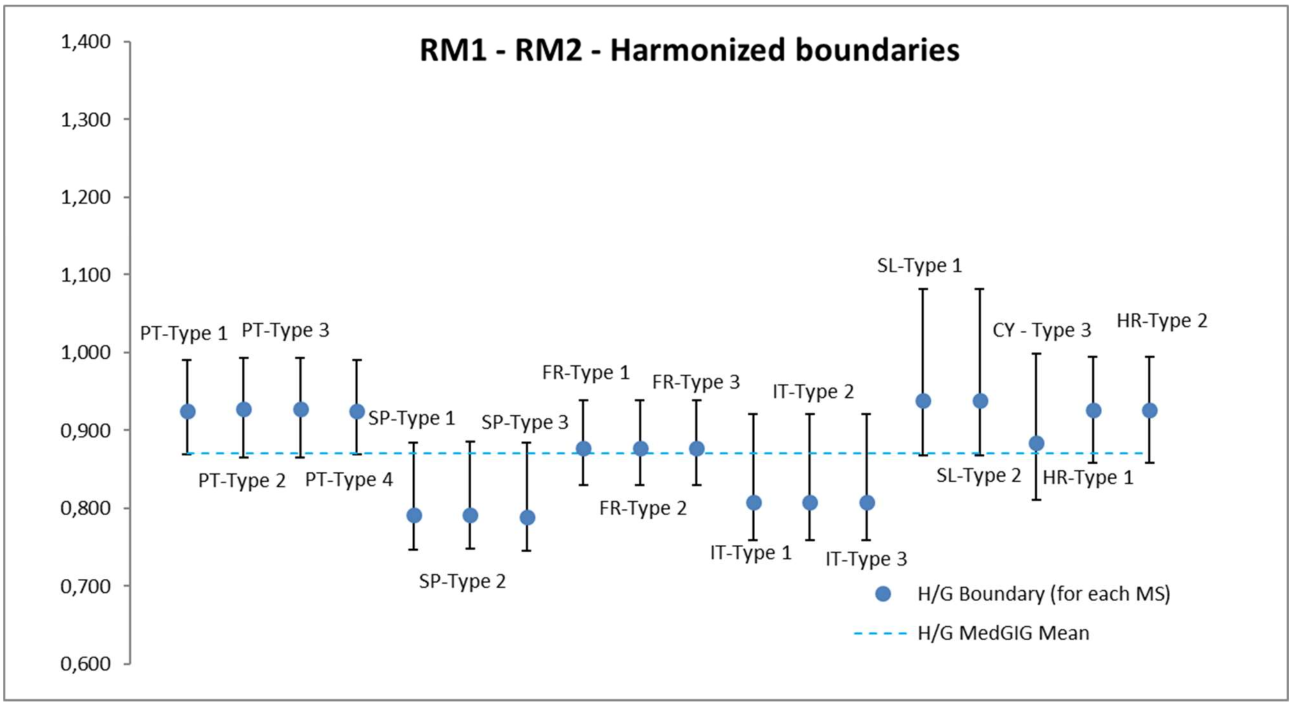


Figure 7. Comparison of H/G harmonized boundaries for the types R-M1 and R-M2 with other MS.

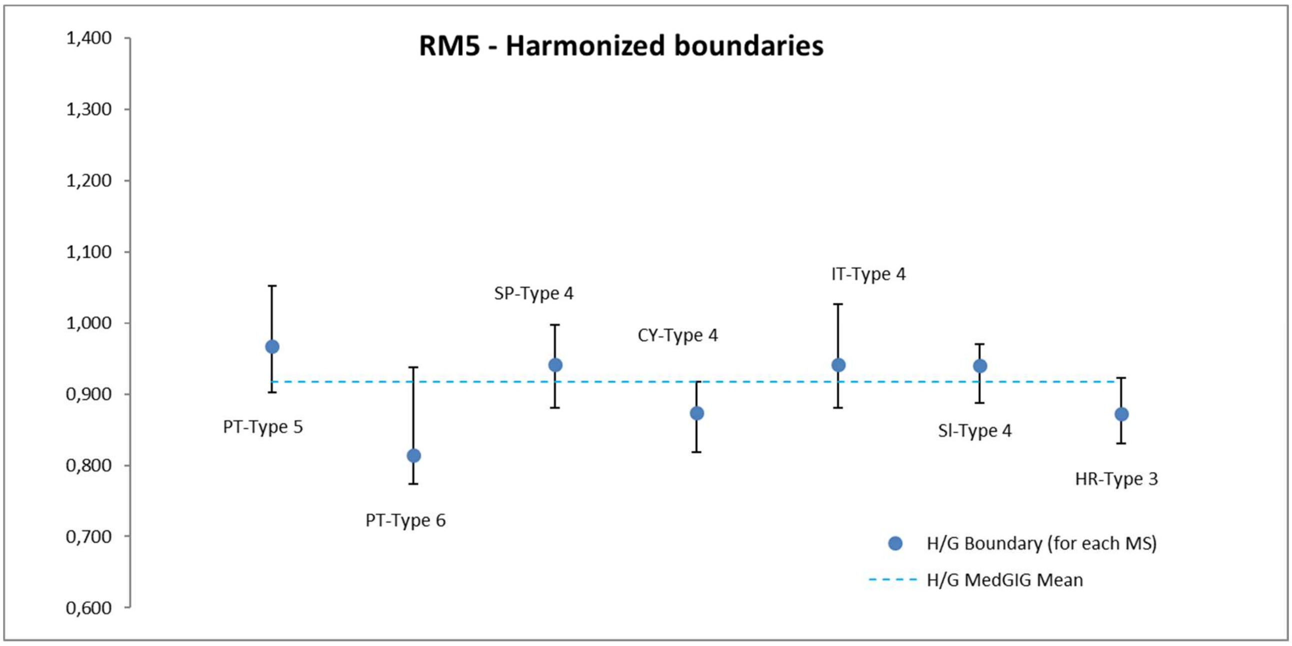


Figure 8. Comparison of H/G harmonized boundaries for the R-M5 with other MS.

Table 16. Harmonized Good/Moderate class boundary for each IC river type.

	R-M1	R-M2	R-M5
Good/Moderate Max	**0.927**	**0.927**	**0.872**
G/M+0.25H	0.722	0.722	0.747
G/M Boundary (for MS)	**0.716**	**0.716**	**0.704**
G/M Boundary - 0.25H	0.642	0.642	0.687
M/P Min	0.607	0.607	0.637
G/M MedGIG Global mean	**0.642**	**0.642**	**0.691**
G/M quarter (+)	0.068	0.068	0.042
G/M quarter (-)	0.012	0.012	0.017

Table 17. H/G and G/M statistic bias for each IC river type after the harmonization process.

Boundary	R-M1	R-M2	R-M5
H/G	0.150	0.150	-0.249
G/M	0.238	0.238	0.241

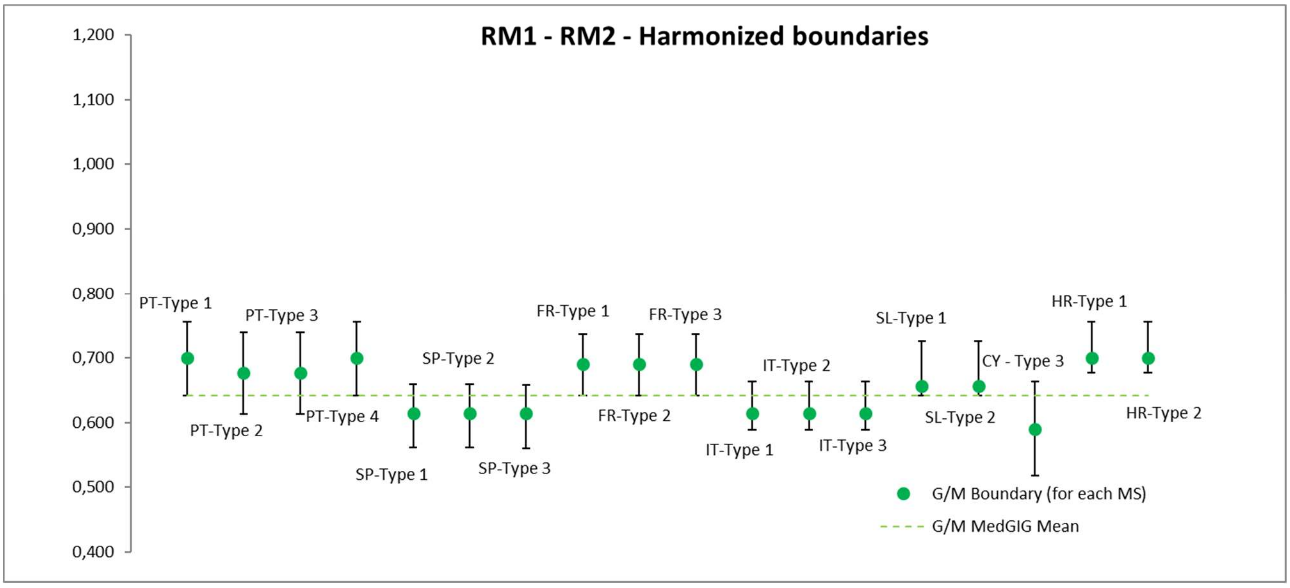


Figure 9. Comparison of G/M harmonized boundaries for the types R-M1 and R-M2 with other MS.

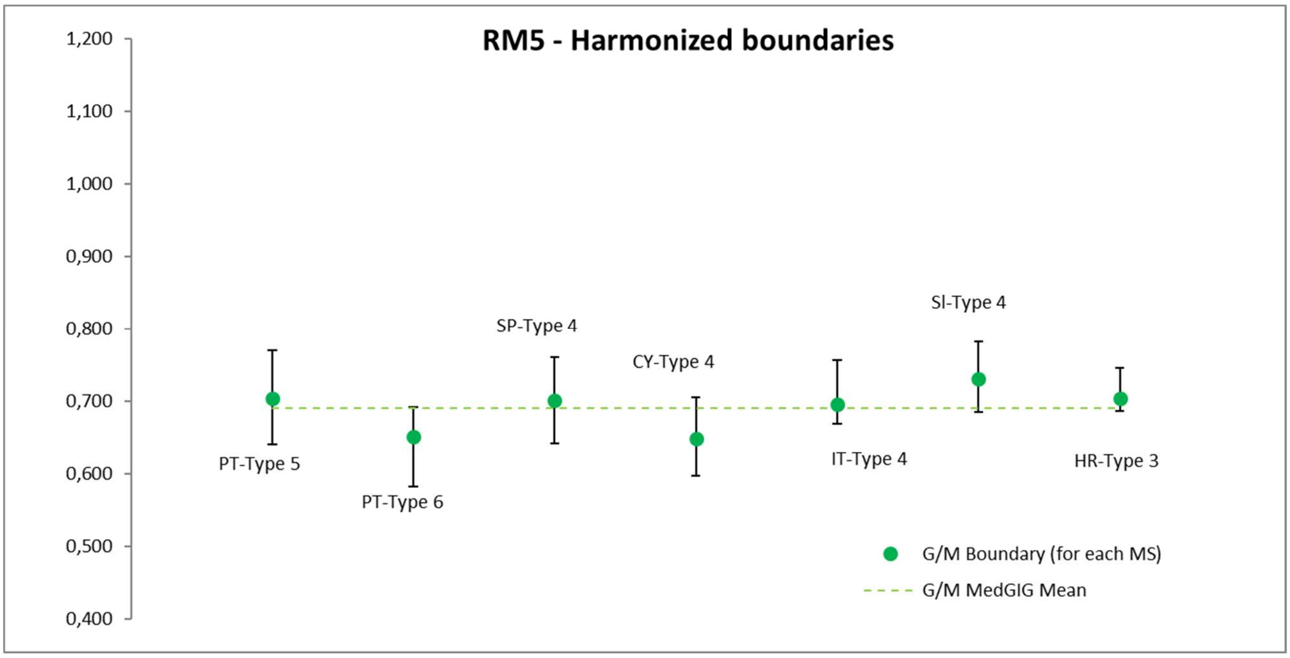


Figure 10. Comparison of G/M harmonized boundaries for the R-M5 with other MS.

Table 18. Final class boundaries adopted for the national metric and the ICM (- not applicable; * no further adjustments needed).

	Boundary	ICM Original	ICM Harmonized	National Original	National Harmonized
HR-Type 1	Reference	1.059	-	1.000	-
	H/G	0.927	*	0.829	*
	G/M	0.767	0.716	0.622	0.555
HR-Type 2	Reference	1.059	-	1.000	-
	H/G	0.927	*	0.829	*
	G/M	0.767	0.716	0.622	0.555
HR-Type 3	Reference	0.967	-	1.000	-
	H/G	0.941	0.872	0.959	0.850
	G/M	0.789	0.704	0.719	0.585

Concluson

This report documents the fitting procedure of the Croatian phytobenthos-based assessment method for the river types R-M1, R-M2 and R-M5 to the results of the completed Mediterranean rivers' intercalibration exercise.

We documented IC feasibility and compliance of the presented assessment method and reported sufficient pressure-response relationships. Following the criteria and steps defined in the fit-in-procedure of Willby et al. (2014), the high-good boundary in the river types R-M1, M2 showed to lie within the acceptable harmonization band. The good-moderate boundary in the river types R-M1, M2 required adjustments (*Table 18).* As for the river type R-M5, both high-good and good-moderate boundary required adjustments (*Table 18*). After adjustment of the aforementioned boundaries, the national assessment method is considered comparable with the already intercalibrated methods and meets the comparability criteria. It is recommended to submit the method to the ECOSTAT group for official approval.

6. DESCRIPTION OF THE BIOLOGICAL COMMUNITIES

Diatom communities dissimilarity in different ecological status conditions was evaluated similarly to the MED-GIG intercalibration exercise. The SIMPER analysis (log transformation of abundance data, Bray-Curtis similarity; up to 90% of contribution to av. similarity, Primer v7) was used to determine the diatom species contributing the most (up to 90% of cumulative contribution) to the average dissimilarity between the sites classified as high and good and to the average similarity of the different status classes.

One or two species are contributing the most in the observed similarity, while the rest significantly contributing species presented a low contribution (*Table 19*). Group similarities were relatively low, indicating a high within ecological status level variability. *Achnanthidium minutissimum* (Kützing) Czarnecki was mainly responsible for the within group similarity for high and good ecological status, and to some extent *Navicula cryptotenella* Lange-Bertalot and *Cocconeis placentula* Ehrenberg. The two groups differed by the contribution of *Achnanthidium biasolettianum* (Grunow) Bukhtiyarova, *Encyonopsis minuta* Krammer & E.Reichardt and *Cymbella parva* (W.Smith) Kirchner to high status group, and *Gomphonema* sp. Ehrenberg contributing to good status group. The contribution of *A. minutissimum* has been also reported from other MS during the intercalibration exercise.

Table 19. Species contribution to similarity within and dissimilarity between ecological status levels. The four most contributing species are presented.

Group High Status Average similarity: 30.51 **Species**	**Average Abundance**	**Contribution %**	**Cumulative contribution %**
Achnanthidium minutissimum	4.40	25.51	25.51
Navicula cryptotenella	2.11	8.98	34.49
Achnanthidium biasolettianum	1.86	5.26	39.76
Cocconeis placentula	1.42	4.02	43.77

Group Good Status Average similarity:28.98 **Species**	**Average Abundance**	**Contribution %**	**Cumulative contribution %**
Achnanthidium minutissimum	4.47	26.25	26.25
Navicula cryptotenella	1.83	8.32	34.57
Cocconeis placentula	1.81	6.53	41.10
Gomphonema sp.	1.39	4.50	45.60

Groups High & Good Status Average dissimilarity = 69.81 **Species**	**Group Good Average Abundance**	**Group High Average Abundance**	**Contribution %**	**Cumulative contribution %**
Achnanthidium biasolettianum	1.86	1.24	3.18	3.18
Encyonopsis minuta	1.60	0.99	2.64	5.83
Cocconeis placentula	1.42	1.81	2.35	8.18
Denticula tenuis	1.29	1.03	2.28	10.46
Cymbella parva	1.23	0.88	2.21	12.67
Achnanthidium minutissimum	4.40	4.47	2.12	14.79
Gomphonema sp.	1.04	1.39	2.03	16.82
Encyonema ventricosum	1.10	1.10	1.95	18.77
Amphora pediculus	1.13	1.18	1.87	20.64
Cymbella excisa	1.01	0.98	1.83	22.47
Navicula cryptotenella	2.11	1.83	1.82	24.29
Gomphonema pumilum	0.93	1.15	1.72	26.01
Gomphonema minutum	1.12	1.10	1.71	27.72
Navicula veneta	0.65	0.94	1.69	29.42
Cocconeis pediculus	0.35	1.07	1.64	31.06
Nitzschia sp.	1.03	0.86	1.63	32.69
Nitzschia dissipata	1.03	1.01	1.62	34.31

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Report on fitting the Croatian classification method for phytobenthos in rivers to the results of the completed intercalibration of the Eastern-Continental GIG (R-E2, R-E3, R-EX5, R-EX6, R-EX7 and R-EX8)

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Report on fitting the Croatian classification method for phytobenthos in rivers to the results of the completed intercalibration of the Eastern-Continental GIG (R-E2, R-E3, R-EX5, R-EX6, R-EX7 and R-EX8)

1. INTRODUCTION

The official intercalibration exercise of phytobenthos-based methods of ecological status assessment of rivers within the Eastern Continental GIG was successfully finalized in 2011 (Opatrilova 2011). The Eastern Continental GIG did not complete the phytobenthos intercalibration in the first round, and no results were included in the first Commission Decision on intercalibration (COM DEC 2008/915/EC). For the second intercalibration round the GIG has filled that gap, following the updated procedures included in the new intercalibration guidance. Croatia (HR) participated in the second round of the intercalibration exercise, but since the HR method was still under development the data were excluded from further analysis.

The goal of this report is to declare that the present Croatian assessment method of ecological status of Eastern Continental rivers of the IC types (R-E2, R-E3, R-EX5, R-EX6, R-EX7 and R-EX8) based on benthic diatoms is compliant with the WFD normative definitions and its class boundaries are in accordance with the results of the completed intercalibration exercise.

In particular, the classification method was verified for WFD compliance and IC feasibility and the class boundaries were compared with agreed boundaries from the EC GIG intercalibration exercise following the instructions of the CIS Guidance Document n°30: "Procedure to fit new or updated classification methods to the results of a completed intercalibration exercise" (Willby et al. 2014).

Typology

Typology within the EC GIG was set based on macroinvertebrates, which was adopted for phytobenthos. A total of 6 common IC river types and 5 additional IC river types were considered for intercalibration of phytobenthos (*Table 1*). Four additional types were defined for the Balkan ecoregion (Illies region 5) – R-EX1, R-EX2, R-EX3 nd R-EX9, but all these types can be found in Croatia and are not shared with any of the other countries participating in the EC GIG, thus no intercalibration for these types was possible.

Table 1. Overview of common intercalibration types in the Eastern Continental rivers GIG (Opatrilova 2011).

Common IC type	MS sharing IC common type	Common IC type	MS sharing IC common type
Main IC types		Additional IC types	
R-E1a	BG, CZ, RO, SK	R-EX1	HR
R-E1b	BG, CZ, RO, SK	R-EX2	HR
R-E2	BG, CZ, HR, HU, RO, SK, HR	R-EX3	HR
R-E3	BG, CZ, HR, HU, RO, SK, HR	R-EX4	CZ, RO, SK
R-E4	AT, BG, RO, SI, SK	R-EX5	BG, CZ, HU, RO, SI, SK, HR
R-E6	HU, RO, SK	R-EX6	BG, RO, SI, HR
		R-EX7	SI, HR
		R-EX8	BG, HU, SI, HR
		R-EX9	HR

The Croatian rivers included in this report belonging to 8 national types with pertaining sub-types (HR-R_1, HR-R_2A, HR-R_2B, HR-R_3A, HR-R_3B, HR-R_3C, HR-R_3D, HR-R4A, HR-R4B, HR-R4C, HR-R_6, HR-R_7, HR-R_8A and HR-R_9), as part of the Pannonian (ER11; sensu Illies 1978) and Dinaric Western

Balkan ecoregion (ER5; sensu Illies 1978), are grouped into 6 common IC EC-river GIG types: R-E2, R-E3, R-EX5, R-EX6, R-EX7 and R-EX8 (*Table 2*).

Table 2. Croatian river types included into common IC river types of the Eastern Continental GIG, together with the reference and poorest values of Croatian Trophic Diatom Index (TDI$_{HR}$) of each national river type.

ECOREGION	*SUBREGION*	*NATIONAL TYPE NAME*		*NATIONAL TYPE*	*NATIONAL INDEX*	*Reference value*	*Poorest value*	*IC TYPE*
PANNONIAN ECOREGION (11 HUNGARIAN LOWLANDS)		Small mountain and mid-altitude rivers		*HR-R_1*	**TID$_{RH}$**	1,5	4	*EX6*
		Small lowland rivers	Small lowland rivers with clay and sand substrate	*HR-R_2A*	**TID$_{RH}$**	1,7	4,6	*EX5*
			Small lowland rivers with gravel and pebble substrate	*HR-R_2B*	**TID$_{RH}$**	1,7	4,6	
		Lowland alluvial rivers	Small lowland alluvial rivers with gravel and pebble substrate	*HR-R_3A*	**TID$_{RH}$**	1,8	4,8	*EX5*
			Small lowland alluvial rivers with clay and sand substrate	*HR-R_3B*	**TID$_{RH}$**	1,8	4,8	
			Medium lowland alluvial rivers with clay and sand substrate	*HR-R_3C*	**TID$_{RH}$**	1,8	4,8	*E2*
			Large lowland alluvial rivers with clay and sand substrate	*HR-R_3D*	**TID$_{RH}$**	1,8	4,8	*E3*
		Medium and large lowland rivers	Medium lowland rivers	*HR-R_4A*	**TID$_{RH}$**	1,9	5	*E2*
			Large lowland rivers	*HR-R_4B*	**TID$_{RH}$**	1,9	5	*E3*
			Large lowland rivers with spring in Dinaric Western Balkan	*HR-R_4C*	**TID$_{RH}$**	1,9	5	
DINARIC ECOREGION (5 DINARIC WESTERN BALKAN)	DINARIC CONTINENTAL SUB-ECOREGION	Small mountain and mid-altitude rivers		*HR-R_6*	**TID$_{RH}$**	1,5	4	*EX7*
		Medium and large mountain and mid-altitude rivers		*HR-R_7*	**TID$_{RH}$**	1,9	5	*EX8*
		Medium and large lowland rivers		*HR-R_8A*	**TID$_{RH}$**	1,8	4,8	*EX8*
		Medium mountain and mid-altitude rivers in karst field		*HR-R_9*	**TID$_{RH}$**	1,8	4,8	*EX8*

2. DESCRIPTION OF NATIONAL ASSESSMENT METHODS

The Croatian national method for ecological status assessment of rivers considers benthic diatoms as proxies for phytobenthos. It is compliant with normative definitions of WFD used by other MS and takes into account both taxonomic composition and species' relative abundance of benthic diatom assemblages. Sampling, sample treatment, diatom identification and data processing are based on the European standards EN 13946: 2014 and EN 14407: 2014 (European Committee for Standardization, 2014a, b). Ecological status is evaluated using TDI_{HR} (Croatian Trophic Diatom Index), a diatom metric modified from Rott's Trophic Index (Rott et al. 1999). The complete procedure is described in detail in the "Methodology for sampling, laboratory analyses and determination of ecological quality ratios for biological quality elements" (Official Gazette 96/19).

2.1. SAMPLING AND DATA PROCESSING

Sampling method: Benthic diatoms are scrubbed from hard substrata (minimum of five stones) in the main water current of the river, in the well exposed euphotic zone.

Sampling time and frequency: Sampling is performed once a year, principally in spring time during favourable and stable water level.

Sample treatment/data processing: Diatom samples in the laboratory are treated according to Standard HRN EN 13946:2014, where the hydrochloric acid is used to remove inorganic material, and sulphuric acid or hot hydrogen peroxide are used to remove all the organic material. Permanent slides are prepared by mounting clean diatom suspension with Naphrax on the microscopic slides.

Identification level: Around 400 valves are counted and identified to the lowest taxonomic level possible on each slide using light microscope with Differential Interference Contrast at 1000 x magnification.

2.2. DESCRIPTION OF NATIONAL METHODOLOGY

Metric calculation: Trophic indicator values and weights of all identified diatom species were defined according to the extended Operational list of diatom taxa for rivers included in the "Methodology for sampling, laboratory analyses and determination of ecological quality ratios for biological quality elements" (Official Gazette 96/19). Taxa list of diatoms with assigned indicator values and weights and with corresponding relative abundances is used for calculation of TDI_{HR} by using the modified Zelinka-Marwan equation (1961):

$$TDI_{HR} = \frac{\sum_{i=1}^{n} Ai \times IVi \times IWi}{\sum_{i=1}^{n} Ai \times IWi}$$

Where:
Ai = Total number of cells/valves of a species in the sample, representing the number of a certain species on 400 counted diatoms.
IVi = Indicator value (tolerance) of a species
IWi = Indicator weight (sensitivity) of a species

ES assessment: Ecological status is assessed on the basis of EQR values of TDI_{HR}. EQR_TDI_{HR} is calculated using the formula described in the "Methodology for sampling, laboratory analyses and determination of ecological quality ratios for biological quality elements" (Official Gazette 96/19):

$$EQR\_TDI_{HR} = \frac{Index\ value - Poorest\ value}{Reference\ value - Poorest\ value}$$

2.3. NATIONAL BOUNDARY SETTING

The national dataset utilized for intercalibration comprises data coming from a total of 218 samples with the availability of pressure variables (*Table 3*). Hydrochemical data, including basic physico-chemical data (total phosphorus, total nitrogen, nitrites, ammonium, orthophosphates, nitrates, BOD_5, conductivity) and land-use data in catchment (Land Use Index - LUI), as well as biological data (TDI_{HR}, diatom taxalist with relative abundances) are available for the samples concerned (*Table 4)*. The pressure gradient is considered sufficient.

Table 3. List of data available in the national dataset included in the intercalibration

IC type	Number of samples	Physico-chemical data	Hydro-morphological data	Biological data	Complete dataset	Number of benchmark samples
R-E2	35	35	35	35	35	17
R-E3	19	19	19	19	19	11
R-EX5	106	106	106	106	106	23
R-EX6	17	17	17	17	17	4
R-EX7	22	22	22	22	22	11
R-EX8	19	19	19	19	19	19

Table 4. Range of values of different environmental variables at river sites from the national dataset included in the intercalibration.

(N=200)	MAX	MIN
P-Total (TP) [mg L^{-1}]	2.6990	0.0057
N-Total (TN) [mg L^{-1}]	20.1800	0.1362
N-NO_2^- [mg L^{-1}]	6.8033	0.0005
N-NH_4^+ [mg L^{-1}]	15.5144	0.0010
P-PO_4^{3-} [mg L^{-1}]	2.1046	0.0015
N-NO_3^- [mg L^{-1}]	31.5836	0.0100
BOD_5 [mg L^{-1}]	55.2546	0.2500
Conductivity [µS cm^{-1}]	1045.36	90.96
Land Use Index [%]	240.72	0.00
TDI_{HR}	4.30	1.58

Selection of benchmark sites was based on the Eastern Continental GIG-river common benchmark criteria from the EC-rivers GIG Milestone report (Opatrilova 2011). The benchmark criteria were selected for both abiotic (water chemistry and land-use) and biotic parameters (TDI_{HR}) to ensure that the intensity of human activities at the selected sites is low and has only very minor impacts on diatom assemblages.
The benchmark criteria of abiotic parameters (land-use and hydrochemical criteria) were adopted from the EC-GIG Intercalibration of diatoms (*Table 5*).

Table 5. Criteria for identifying benchmark sites for the EC-GIG from the Milestone report (Opatrilova 2011).

Stressor	R-E2	R-E3	R-EX5	R-EX6	R-EX7	R-EX8
Land Use Index [%]	175	175	130	130	130	130
Conductivity [µS cm^{-1}]	1000	1000	600	600	500	500
BOD_5 [mg L^{-1}]	4.1	4.1	2.9	2.5	2.5	2.5
P-PO_4^{3-} [mg L^{-1}]	0.2	0.2	0.2	0.1	0.05	0.05
N-NO_3^- [mg L^{-1}]	4.0	4.0	1.9	1.4	1.1	1.1

Only sites that met both land-use and hydro-chemical criteria were included into the boundary setting protocol. Further selection of sites included checking if the biotic criteria (national EQR values) met at least the good status.

Setting of new reference and poorest values:
The old reference values of national types (see *Table 2*) included in the IC were adjusted for further IC process. Since some national types don't have enough stations that qualify for benchmarking process, i.e. a minimum of three sites following the CIS Guidance No. 30 (Willby et al. 2014), national types were grouped on the basis of hydro-geomorphologic similarities into four groups (*Table 6*). This grouping allows setting of relevant and stronger reference values, whilst keeping the national typology and incorporating it into the appropriate IC EC-river GIG typology. The 10th percentile of TDI_{HR} of the benchmark sites was calculated and set as reference value for each group.

The poorest value of TDI_{HR} was taken as the lowest value of TDI_{HR} of all 8 national water types that were included in the IC EC-river GIG types (*Table 4*).

Table 6. Grouping of national types for setting the reference and poorest values of TDI_{HR}.

NATIONAL TYPE	GROUP	Reference value of TDI_{HR}	Poorest value of TDI_{HR}	Number of benchmark samples per group
HR-R_1	1	1.97	4.30	4
HR-R_2A HR-R_2B	2	2.00	4.30	22
HR-R_3A HR-R_3B HR-R_3C HR-R_3D HR-R_4A HR-R_4B HR-R_4C	3	1.74	4.30	25
HR-R_6 HR-R_7 HR-R_8A HR-R_9	4	1.85	4.30	34

Setting of EQR boundaries for both indices:
The High/Good EQR boundary was derived from EQR variability at all available spatial based benchmark sites. The remaining degradation continuum was divided into four equal width classes.

H/G boundary = median of all benchmark sites
G/M boundary = H/G * 0.75
M/P boundary = H/G * 0.50
P/B boundary = H/G * 0.25

Summary of the class boundaries for the EQR_TDI_{HR} values are presented in *Table 7*.

Table 7. Summary of the class boundaries for the EQR_TDI_{HR} values in the EC-GIG river types.

	R-E2, R-E3, R-EX5, R-EX6, R-EX7, R-EX8
Upper High EQR Value	1.11
High/Good Boundary	0.86
Good/Moderate Boundary	0.60
Moderate/Poor Boundary	0.38
Poor/Bad Boundary	0.22

EQR_ TDI_{HR} is used as the final metric in comparison with the intercalibration common metric (ICM).

2.4. PRESSURES ADDRESSED

Different national methods of the MS of the completed intercalibration exercise were reported to address eutrophication and pollution by organic matter. The harmonization between MS has been done on diatom modules/methods (Opatrilova 2011). Statistical analyses were performed to explore the responsiveness of the national diatom-based assessment method to various anthropogenic stressors.

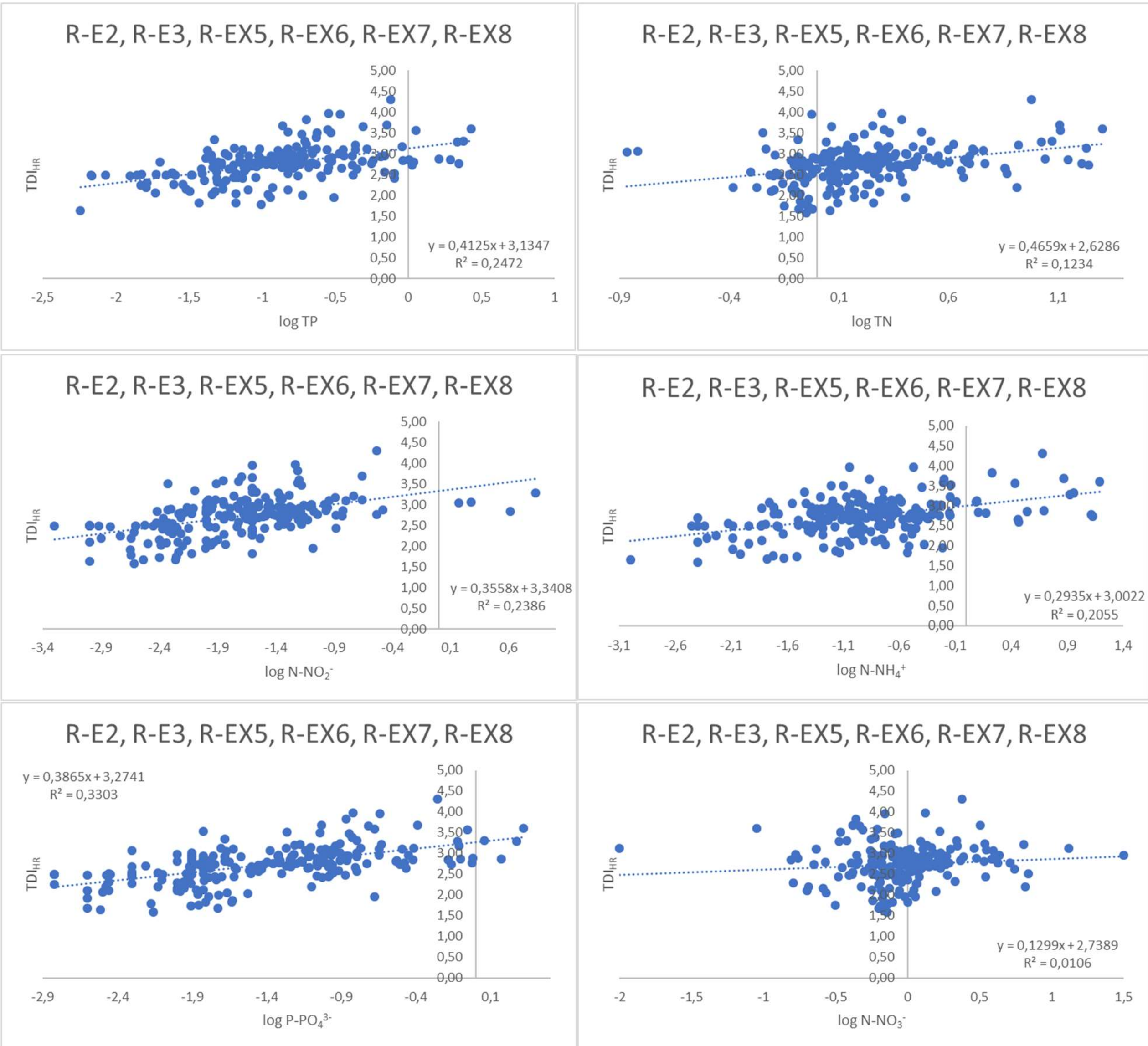
The pressure-response relationships were tested via:
(1) non-parametric Spearman rank correlations of the national diatom metric (TDI_{HR}) with environmental parameters (P-TP, N-TN, N-NO_2^-, N-NH_4^+, P-PO_4^{3-}, N-NO_3^-, BOD_5, conductivity) and land-use parameter (LUI)

(2) linear regressions of the national diatom metric (TDI_{HR}) with pressure variables.

Table 8. Summary of the Spearman correlations of the national diatom metric (TDI_{HR}) with different hydro-chemical, environmental pressures and land-use index. Correlations marked in red are significant at $p < 0.05$.

	R-E2, R-E3, R-EX5, R-EX6, R-EX7, R-EX8
	TDI_{HR}
TP	0.5074 $p=0.5858*10^{-13}$
TN	0.3841 $p=0.4485*10^{-8}$
N-NO_2^-	0.5050 $p=0.1647*10^{-14}$
N-NH_4^+	0.36888 $p=0.1972*10^{-7}$
P-PO_4^{3-}	0.5851 $p=0.2049*10^{-20}$
N-NO_3^-	0.1640 p=0.0153
BOD_5	0.4828 $p=0.1031*10^{-16}$
Conductivity	0.4897 $p=0.1515*10^{-13}$
Land Use Index [%]	0.5021 $p=0.23515*10^{-14}$

The results of Spearman correlation of TDI_{HR} with pressure variables are shown in *Table 8*. The coefficient showed statistically significant relationships ($p<0.05$) between national metric and all tested pressures. All of the selected pressures present strong relationships with the national metric and are presented in *Figure 1*. In general, diatom assemblages of Croatian national types which were classified into EC-river types R-E2, R-E3, R-EX5, R-EX6, R-EX7 and R-EX8 responded well to all selected nutrient pressures, in particular to total phosphorus (TP), total nitrogen (TN), nitrites ($N\text{-}NO_2^-$), ammonium ($N\text{-}NH4^+$) and orthophosphates ($P\text{-}PO_4^{3-}$), as well as to BOD_5, conductivity and Land Use Index.



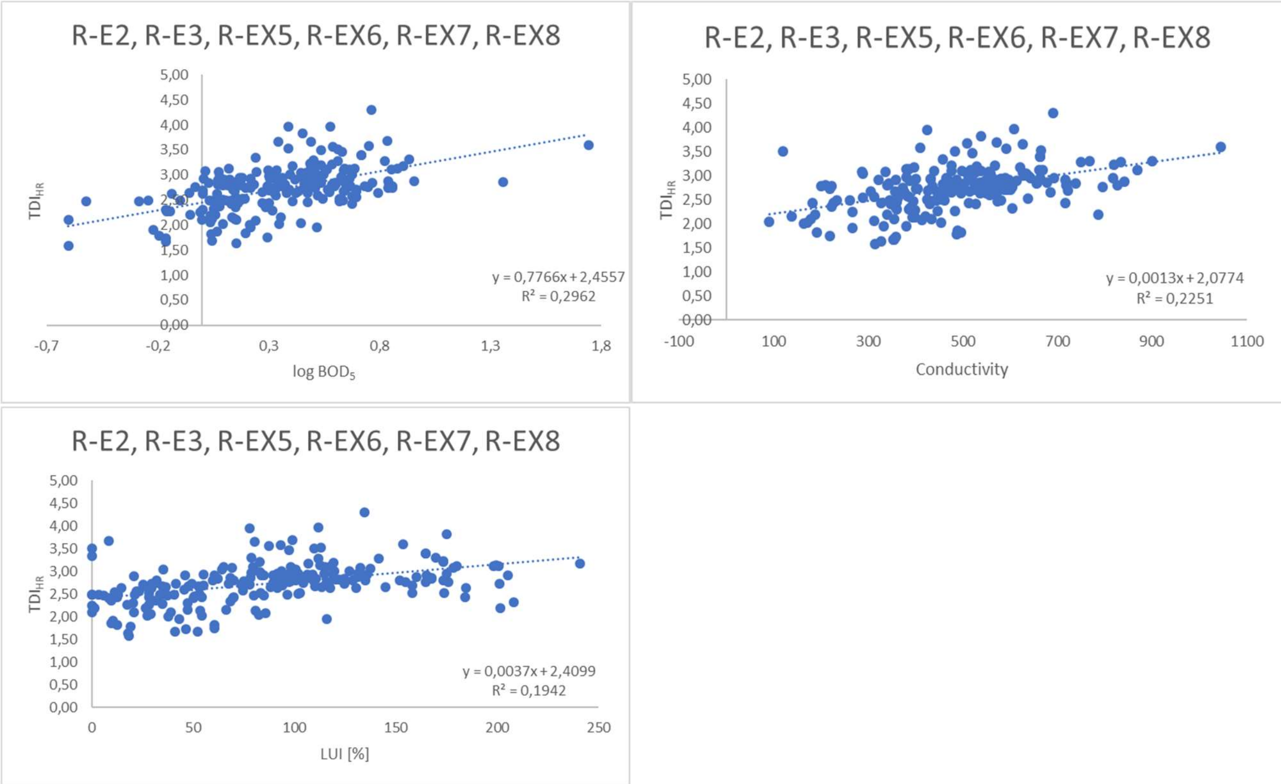


Figure 1. Pressure-response relationship between the most important pressures against the TDI_{HR} in Croatian EC river types.

3. WFD COMPLIANCE CHECKING

The data acceptance criteria of the EC GIG as defined in the EC-rivers GIG Milestone 6 Report (Opatrilova 2011) are listed in *Table 9*. The Croatian data fulfilled the listed criteria in all aspects, and can therefore be considered sufficient for intercalibration.

Table 9. List of the WFD compliance criteria and the WFD compliance checking process and results

Compliance criteria	Compliance checking
Ecological status is classified by one of **five classes** (high, good, moderate, poor and bad).	Yes
High, good and moderate ecological status are set in line with the WFD's **normative definitions** (**Boundary setting procedure**)	Yes. Equidistant division of the EQR gradient. High-good boundary derived from metric variability at near-natural benchmark sites.
All relevant parameters indicative of the biological quality element are covered (see Table 1 in the IC Guidance). A **combination rule** to combine parameter assessment into BQE assessment has to be defined. If parameters are missing, Member States need to demonstrate that the method is sufficiently indicative of the status of the QE as a whole	Yes; both taxonomic composition and species relative abundance are taken into consideration. Diatom metric (TDI_{HR} - Croatian Trophic Diatom Index) - product of species relative abundance × sensitivity × weight value is weighted with product of rel. abundance × weight value
Assessment is adapted to **intercalibration common types** that are defined in line with the typological requirements of the Annex II WFD and approved by WG ECOSTAT	Yes; common EC-river intercalibration types are used: R-E2, R-E3, R-EX5, R-EX6, R-EX7, R-EX8.
The water body is assessed against **type-specific near-natural reference conditions**	Yes

Assessment results are expressed as **EQRs**	Yes
Sampling procedure allows for **representative** information about water body quality/ecological status **in space and time**	Yes; 1 sampling per year during favourable and stable water level. Using brush/scraper for sampling. Single habitat(s) preferably epilithic phytobenthos – mesolithal (5 stones/cobbles from different points of streamline).
All data relevant for assessing the biological **parameters** specified in the WFD's normative definitions are covered by the **sampling procedure**	Yes; all 5 water quality classes are represented.
Selected taxonomic level achieves adequate **confidence and precision** in classification	Yes; identification in species level or lower.

4. IC FEASIBILITY CHECKING

The intercalibration process ideally covers all national assessment methods within a GIG. However, the comparison of dissimilar methods ("apples and pears") has clearly to be avoided. Intercalibration exercise is focused on specific type / biological quality element / pressure combinations. The second step of the process introduces an "IC feasibility check" to restrict the actual intercalibration analysis to methods that address the same common type(s) and anthropogenic pressure(s), and follow a similar assessment concept.

4.1. TYPOLOGY

The RM typological system was found to be the most appropriate for describing Croatian rivers. Six common and nine additional intercalibration types are included in the EC-GIG, of which types R-E2, R-E3, R-EX5, R-EX6, R-EX7 and R-EX8 are applicable for Croatia (*Tables 1, 2*). Reference sites for large river types are open to criticism and therefore they are not included in the EC-GIG intercalibration exercise.

4.2. PRESSURES ADDRESSED

Diatom assemblages as summarized by the national metric (TDI_{HR}) respond well to nutrient pollution, especially to total phosphorus (TP), total nitrogen (TN), nitrites ($N\text{-}NO_2^-$), ammonium ($N\text{-}NH4^+$) and orthophosphates ($P\text{-}PO_4^{3-}$), as well as to BOD_5, conductivity and to Land Use Index. These pressures were also found to be significant when correlated with the common metric of the other MS during the intercalibration exercise.

4.3. ASSESSMENT CONCEPT

The national diatom-based assessment system consists of the Croatian Trophic Diatom Index (TDI_{HR}), modified from Rott's Trophic Index (Rott et al. 1999), which is an indicator of a nutrient load in a given water body, i.e. its trophic degree based on the representation of diatom species. The TDI_{HR} takes into consideration the relative abundances of diatoms present in the assemblage and their assigned tolerances (indicator values) and sensitivities (indicator weights). The index responded to several pressures addressed (see above Section "2.4. Pressures addressed").

4.4. CONCLUSION ON THE INTERCALIBRATION FEASIBILITY

The RM typology was chosen. The Croatian Trophic Diatom Index (TDI_{HR}) takes into consideration tolerance and sensitivity of the species present in the assemblage and their relative abundances. The index addresses various pressures (see above Section "2.4. Pressures addressed"). It is concluded that the fitting of TDI_{HR} to the results of the EC-GIG river intercalibration was feasible.

5. DEMONSTRATING THE COMPLIANCE WITH THE COMPLETED INTERCALIBRATION EXERCISE

Following Figure 1 in the CIS Guidance No. 30 (Willby et al. 2014), case A1 (Option 2) is applied for fitting the HR assessment method using phytobenthos to the results of the River EC GIG type R-E2, R-E3, R-EX5, R-EX6, R-EX7 and R-EX8.
The requirements for case A1 are:

- *Full details of the common metric*

  The ICM applied in the MED-GIG is composed of two diatom metrics (according to Kelly et al. 2009):
  - IPS (Coste in CEMAGREF, 1982): this metric measures 'general water quality', with low values corresponding to high pressure levels and, therefore, low EQRs
  - TI (Rott et al. 1999): a trophic index which needs to be adjusted so that high values represent high EQR values

  ICM=(EQR-IPS + EQR-TI)/2

- *A suitable site x biology dataset covering a range of environmental quality from which the national EQR and common metric can be calculated*

  A total of 218 samples were available covering all 5 classes of ecological status (see Section "2.3. National boundary setting")

- *Accompanying pressure data in the same format as that used in the completed exercise.*

  All accompanying pressure data are available (see *Table 3*).

- *Information on the specific thresholds already used in the completed exercise to define reference or alternative benchmark sites*

  The benchmark criteria of abiotic parameters (land-use and hydrochemical criteria) were adopted from the EC-GIG Intercalibration of diatoms (see *Table 5*).

- *Details of exactly how benchmarking was undertaken in the complete exercise. If the completed exercise concluded that benchmarking was not necessary the mean value of the benchmark sites from each country must be provided so that the joining Member State can also judge the need to benchmark its own method.*

  Given benchmark criteria were applied by each MS in order to identify benchmark sites within each national dataset. Median values of TDI_{HR} of the national benchmark dataset were used for calculation of common metric EQR (EQR_TDI_{HR}). Linear regression was established between the values of the national method and the ICM so that the national boundaries could be translated to ICM using the equation.
  If the number of national benchmark sites turns out insufficient, then the global median of all participating MS has to be used.

- *Values of the global mean view of the HG and GM boundaries on the common metric scale for Member States who participated in the completed exercise.*

  Mean H/G: 1.0588
  Mean G/M: 0.8734

The process of fitting the HR method to the completed IC exercise:
According to the Willby et al. (2014), the following steps should be followed:

i. *Calculate the common metric (CM) on the national dataset.*

The ICM applied in the EC-GIG is composed of two diatom metrics (according to Kelly et al. 2009):

- IPS (Coste in CEMAGREF, 1982): this metric measures 'general water quality', with high pressure levels rendering low values and thus low EQRs:

EQR_IPS = Observed value / reference value

- TI (Rott et al. 1999): a trophic index, with higher eutrophication levels rendering high values and thus needs to be adjusted so that high values represent high EQRs:
  EQR_TI = (4-observed value) / (4-reference value)

ICM = (EQR-IPS + EQR-TI)/2

ii. Use the associated pressure data to identify sites in the national dataset that meet the criteria established by the GIG for the selection of benchmark or reference sites.

Benchmark sites have been identified based on environmental pressures above (see Section "2.3 National boundary setting")

iii. Standardize the common metric (CM_bm) against the benchmark according to the approach used in the completed exercise.

The common metric was calculated for the benchmark sites in the national dataset. For the IC river types R-E2, R-E3, R-EX5, R-EX6, R-EX7 and R-EX8 the median was ICM_E2,E3,EX5,EX6,EX7,EX8 = 1.008. These values were inside the range of the median values of the MS who took part in the intercalibration exercise.

iv. *Use OLS regression to establish the relationship between CM_bm (y) and the EQR of the joining method (x).*

Relationship between EQR_TDI_{HR} and ICM for the IC river types R-E2, R-E3, R-EX5, R-EX6, R-EX7 and R-EX8 is presented (*Table 10, Figure 2*).

Table 10. OLS equations for the relationship between ICM and national EQR (EQR_TDI_{HR}).

IC River type	No of samples	Linear regression	R^2
R-E2, R-E3, R-EX5, R-EX6, R-EX7, R-EX8	218	ICM = 0.691 EQR_TDI_{HR} + 0.4212	0.2995

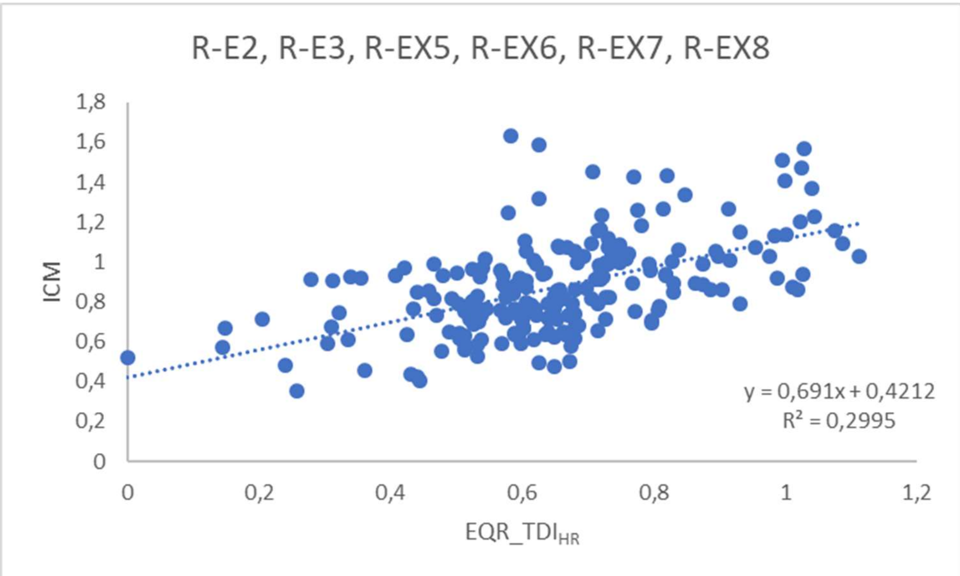


Figure 2. OLS regressions to establish the relationship between ICM and the EQR for IC river types R-E2, R-E3, R-EX5, R-EX6, R-EX7 and R-EX8.

v. Predict the position of the national class boundaries (MP, GM, HG and reference) on the CM_bm scale.

The prediction of the class boundaries on the CM scale was made using the OLS equations of the relationship between the national and the common metric (*Tables 11-13*).

Table 11. Translation of the reference and boundary positions of the national method on the basis of OLS regression (see *Figure 2, Table 10*) into ICM.

IC Type	**R-E2, R-E3, R-EX5, R-EX6, R-EX7, R-EX8**	
Boundary	EQR	Predicted boundaries on ICM scale
Reference	1.00	1.112
Upper high value	1.11	1.189
High / good	0.74	0.932
Good / moderate	0.55	0.804
Moderate / poor	0.37	0.677
Poor / bad	0.18	0.549

Table 12. Reference values and High/Good class boundary of the ICM values derived from the OLS regression (*Figure 2*) for Croatian EC river types.

	R-E2, R-E3, R-EX5, R-EX6, R-EX7, R-EX8
HIGH Max (maximum of national EQR)	**1.189**
H/G Boundary + 0.25H	0.996
H/G Boundary (for MS)	**0.932**
H/G Boundary - 0.25H	0.900
H/G EC-GIG Global mean	1.059
H/G quarter (+)	0.064
H/G quarter (-)	0.032

Table 13. Good/Moderate class boundary of the ICM values derived from the OLS regression (*Figure 2*) for Croatian EC river types.

	R-E2, R-E3, R-EX5, R-EX6, R-EX7, R-EX8
Good/Moderate Max	**0.932**
G/M+0.25H	0.836
G/M Boundary (for MS)	**0.804**
G/M Boundary - 0.25H	0.773
M/P M in	0.677
G/M EC-GIG Global mean	**0.873**
G/M quarter (+)	0.032
G/M quarter (-)	0.032

vi. Apply the comparability criteria as summarized in Chapter 6.

The adjustment of the boundaries follows the fit according to the guidance of chapter 6 (Willby et al. 2014). The main principle is that H/G or G/M statistic must not be >|0.25|. Both H/G and G/M boundary biases in Croatian R-E2, R-E3, R-EX5, R-EX6, R-EX7 and R-EX8 EC river types were >|0.25|, and thus adjustment in these boundaries was required by adding a value to the respective H/G and

G/M boundaries until they reached the appropriate limit (*Tables 14-17*). The final boundaries adopted after the harmonization are presented in *Table 18*.

Table 14. H/G and G/M statistic bias for each IC river type. Red colour represents statistic bias >|0.25|.

Boundary	R-E2, R-E3, R-EX5, R-EX6, R-EX7, R-EX8
H/G	-0.493
G/M	-0.540

Table 15. Harmonized High/Good class boundary for each IC river type.

	R-E2, R-E3, R-EX5, R-EX6, R-EX7, R-EX8
High Max (maximum of national EQR)	**1.189**
H/G Boundary + 0.25H	1.060
H/G Boundary (for MS)	**1.017**
H/G Boundary - 0.25H	0.972
H/G EC-GIG Global Mean	**1.059**
H/G quarter (+)	0.043
H/G quarter (-)	0.045

Table 16. Harmonized Good/Moderate class boundary for each IC river type.

	R-E2, R-E3, R-EX5, R-EX6, R-EX7, R-EX8
Good/Moderate Max	**1.017**
G/M+0.25H	0.881
G/M Boundary (for MS)	**0.836**
G/M Boundary - 0.25H	0.796
M/P M in	0.677
G/M EC-GIG Global mean	**0.873**
G/M quarter (+)	0.045
G/M quarter (-)	0.040

Table 17. H/G and G/M statistic bias for each IC river type.

Boundary	R-E2, R-E3, R-EX5, R-EX6, R-EX7, R-EX8
H/G	-0.244
G/M	-0.208

Table 18. Final class boundaries adopted for the Croatian national metric and the ICM.

	Boundary	ICM Original	ICM Harmonized	National Original	National Harmonized
R-E2, R-E3, R-EX5, R-EX6, R-EX7, R-EX8	H/G	0.932	1.017	0.739	0.862
	G/M	0.804	0.836	0.555	0.600

Concluson

This report documents the fitting procedure of the Croatian phytobenthos-based assessment method for the river types R-E2, R-E3, R-EX5, R-EX6, R-EX7 and R-EX8 to the results of the completed Eastern Continental rivers intercalibration exercise.

We documented IC feasibility and compliance of the presented assessment method and reported sufficient pressure-response relationships. Following the criteria and steps defined in the fit-in-procedure of Willby et al. (2014), both the high-good boundary and the good-moderate boundary in the Croatian EC river types required adjustments (*Table 18*). After adjustment of the aforementioned boundaries, the national assessment method is considered comparable with the already intercalibrated methods and meets the comparability criteria. It is recommended to submit the method to the ECOSTAT group for official approval.

6. DESCRIPTION OF THE BIOLOGICAL COMMUNITIES

Diatom communities dissimilarity in different ecological status conditions was evaluated similarly to the EC-GIG intercalibration exercise. The SIMPER analysis (log transformation of abundance data, Bray-Curtis similarity; up to 90% of contribution to av. similarity, Primer v7) was used to determine the diatom species contributing the most (up to 90% of cumulative contribution) to the average dissimilarity between the sites classified as high, good, moderate and poor and to the average similarity of the different status classes.

One or two species are contributing the most in the observed similarity, while the rest significantly contributing species presented a low contribution (*Table 19*). Group similarities were relatively low, indicating a high within ecological status level variability. *Achnanthidium minutissimum* (Kützing) Czarnecki, *Cocconeis placentula* Ehrenberg and *Amphora pediculus* (Kützing) Grunow were mainly responsible for the within group similarity for high, good and moderate ecological status, and to some extent *Cocconeis placentula*.
Dissimilarity between different ecological groups is also presented (*Table* 20). The high and good groups differed by the contribution of *Achnanthidium pyrenaicum* (Hustedt) H.Kobayasi, *Achnanthidium* spp. Kützing and *Gomphonema* sp. Ehrenberg contributing to high status group, and *Nitzschia palea* (Kützing) W.Smith, *Cocconeis pediculus* Ehrenberg and *Navicula gregaria* Donkin to good status group. The contribution of *A. minutissimum* has been also reported from other MS during the intercalibration exercise. The high and moderate status groups differed by the contribution of mainly *Achnanthidium* genera (*A. minutissimum*, *A. pyrenaicum* and *Achnanthidium* spp.) to high status group, and *Cyclotella meneghiniana* Kützing, *Navicula gregaria* and *Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot to moderate status group. Contribution of *Achnanthidium* group (*A. minutissimum*, *A. pyrenaicum* and *Achnanthidium* spp.) to high status group was also the main cause for the dissimilarity between the high and poor status groups, whilst *Eolimna subminuscula* (Manguin) Gerd Moser, Lange-Bertalot & Metzeltin, *Nitzschia palea and Navicula gregaria* in return contributed to poor status group. The higher contribution of species *Amphora pediculus, Nitzschia palea*, *Cyclotella meneghiniana* and *Planothidium frequentissimum* to the moderate group was the main contributing cause for the dissimilarity between good and moderate status groups. The good and poor status groups differed by the contribution of *Achnanthidium pyrenaicum, Cocconeis placentula, Navicula cryptotenella* Lange-Bertalot and *Navicula tripunctata* (O.F.Müller) Bory contributing to good status group, and *Eolimna subminuscula* and *Nitzschia palea* to poor status group. The moderate and poor status groups differed by the contribution of *Amphora pediculus, Nitzschia dissipata* (Kützing) Rabenhorst and *Navicula cryptotenella* having higher contribution in the good status group, and *Eolimna subminuscula, Navicula veneta* Kützing and *Mayamaea permitis* (Hustedt) K.Bruder & Medlin with higher contribution to poor status group.

Table 19. Species contribution to similarity within and dissimilarity between ecological status levels. The six most contributing species are presented.

Group High Status Average similarity: 32.07 **Species**	**Average Abundance**	**Contribution %**	**Cumulative contribution %**
Achnanthidium minutissimum	4.11	21.96	21.96
Amphora pediculus	2.02	8.68	30.64
Gomphonema sp.	2.10	8.01	38.65
Achnanthidium pyrenaicum	2.33	7.01	45.66
Achnanthidium spp.	2.28	6.53	52.19
Cocconeis placentula	1.60	6.49	58.68

Group Good Status Average similarity: 27.13 **Species**	**Average Abundance**	**Contribution %**	**Cumulative contribution %**
Achnanthidium minutissimum	3.81	20.33	20.33
Cocconeis placentula	2.03	7.53	27.86
Amphora pediculus	2.02	7.00	34.84
Navicula cryptotenella	1.60	5.15	40.01
Navicula tripunctata	1.51	4.84	44.85
Nitzschia dissipata	1.38	3.86	48.71

Group Moderate Status Average similarity: 27.61 **Species**	**Average Abundance**	**Contribution %**	**Cumulative contribution %**
Amphora pediculus	2.70	9.10	9.10
Nitzschia palea	2.14	7.28	16.37
Achnanthidium minutissimum	2.14	7.19	23.56
Navicula cryptotenella	1.60	5.28	28.84
Nitzschia dissipata	1.58	5.03	33.87
Cocconeis placentula	1.60	4.56	38.43

Group Poor Status Average similarity: 27.57 **Species**	**Average Abundance**	**Contribution %**	**Cumulative contribution %**
Eolimna subminuscula	4.00	30.45	30.45
Achnanthidium minutissimum	1.81	9.98	40.44
Nitzschia palea	2.49	9.62	50.05
Cyclotella meneghiniana	1.72	6.59	56.64
Gomphonema parvulum	1.74	5.80	62.45
Planothidium frequentissimum	0.96	4.70	67.14

Table 20. Species contribution to dissimilarity between ecological status levels. The 15 most contributing species are presented.

Groups High & Good Status Average dissimilarity = 75.32 **Species**	**Group High Average Abundance**	**Group Good Average Abundance**	**Contribution %**	**Cumulative contribution %**
Achnanthidium pyrenaicum	2.33	0.14	3.06	3.06
Achnanthidium spp.	2.28	0.39	2.79	5.84
Gomphonema sp.	2.10	0.83	2.34	8.18
Achnanthidium minutissimum	4.11	3.81	2.05	10.23
Amphora pediculus	2.02	2.02	1.92	12.16
Cocconeis placentula	1.60	2.03	1.90	14.06
Navicula cryptotenella	1.23	1.60	1.76	15.82
Nitzschia sp.	1.34	0.92	1.70	17.52
Gomphonema pumilum	1.20	0.79	1.60	19.11
Navicula tripunctata	0.89	1.51	1.59	20.70
Denticula tenuis	1.08	0.40	1.54	22.24
Nitzschia dissipata	0.89	1.38	1.52	23.75
Nitzschia palea	0.51	1.34	1.48	25.24
Cocconeis pediculus	0.52	1.11	1.43	26.67
Navicula gregaria	0.47	1.22	1.40	28.07

Groups High & Moderate Status Average dissimilarity = 79.40 **Species**	**Group High** **Average Abundance**	**Group Moderate** **Average Abundance**	**Contribution %**	**Cumulative contribution %**
Achnanthidium minutissimum	4.11	2.14	2.79	2.79
Achnanthidium pyrenaicum	2.33	0.10	2.78	5.57
Achnanthidium spp.	2.28	0.13	2.55	8.13
Nitzschia palea	0.51	2.14	2.22	10.34
Amphora pediculus	2.02	2.70	2.20	12.54
Gomphonema sp.	2.10	0.65	2.19	14.73
Cyclotella meneghiniana	0.03	1.49	1.79	16.52
Navicula gregaria	0.47	1.64	1.68	18.20
Cocconeis placentula	1.60	1.60	1.63	19.84
Nitzschia sp.	1.34	0.92	1.57	21.40
Planothidium frequentissimum	0.35	1.49	1.57	22.97
Navicula cryptotenella	1.23	1.60	1.53	24.50
Nitzschia dissipata	0.89	1.58	1.49	25.99
Gomphonema parvulum	0.47	1.25	1.43	27.42
Gomphonema pumilum	1.20	0.53	1.40	28.83

Groups High & Poor Status Average dissimilarity = 75.32 **Species**	**Group High** **Average Abundance**	**Group Poor** **Average Abundance**	**Contribution %**	**Cumulative contribution %**
Eolimna subminuscula	0.11	4.00	5.13	5.13
Achnanthidium minutissimum	4.11	1.81	3.39	8.52
Achnanthidium pyrenaicum	2.33	0.00	3.26	11.78
Navicula gregaria	0.47	1.98	3.12	14.90
Achnanthidium spp.	2.28	0.00	2.94	17.84
Nitzschia palea	0.51	2.49	2.90	20.74
Amphora pediculus	2.02	1.36	2.47	23.21
Gomphonema sp.	2.10	0.64	2.44	25.65
Cyclotella meneghiniana	0.03	1.72	2.27	27.92
Gomphonema parvulum	0.47	1.74	2.04	29.97
Navicula minima var. *minima*	0.45	1.67	2.03	31.99
Nitzschia sp.	1.34	1.33	1.78	33.77
Cocconeis placentula	1.60	0.81	1.71	35.49
Navicula veneta	0.11	1.45	1.67	37.16
Gomphonema pumilum	1.20	0.00	1.63	38.78

Groups Good & Moderate Status Average dissimilarity = 74.44 **Species**	**Group Good** **Average Abundance**	**Group Moderate** **Average Abundance**	**Contribution %**	**Cumulative contribution %**
Achnanthidium minutissimum	3.81	2.14	2.79	2.79
Amphora pediculus	2.02	2.70	2.33	5.13
Nitzschia palea	1.34	2.14	1.96	7.09
Cocconeis placentula	2.03	1.60	1.90	8.99
Cyclotella meneghiniana	0.79	1.49	1.82	10.81
Navicula gregaria	1.22	1.64	1.75	12.56

Navicula cryptotenella	1.60	1.60	1.61	14.17
Nitzschia dissipata	1.38	1.58	1.61	15.78
Planothidium frequentissimum	0.84	1.49	1.52	17.29
Gomphonema parvulum	1.00	1.25	1.51	18.80
Navicula tripunctata	1.51	1.10	1.48	20.28
Cocconeis pediculus	1.11	0.85	1.41	21.70
Nitzschia sp.	0.92	0.92	1.41	23.11
Navicula antonii	1.06	1.24	1.39	24.49
Rhoicosphenia abbreviata	1.01	0.92	1.35	25.84

Groups Good & Poor Status
Average dissimilarity = 80.43

Species	**Group Good Average Abundance**	**Group Poor Average Abundance**	**Contribution %**	**Cumulative contribution %**
Eolimna subminuscula	0.40	4.00	4.60	4.60
Achnanthidium minutissimum	3.81	1.81	3.25	7.85
Navicula gregaria	1.22	1.98	3.07	10.92
Nitzschia palea	1.34	2.49	2.47	13.39
Amphora pediculus	2.02	1.36	2.42	15.80
Cocconeis placentula	2.03	0.81	2.11	17.91
Cyclotella meneghiniana	0.79	1.72	2.06	19.97
Gomphonema parvulum	1.00	1.74	1.94	21.91
Navicula minima var. *minima*	0.29	1.67	1.89	23.80
Navicula cryptotenella	1.60	0.53	1.80	25.59
Navicula tripunctata	1.51	0.14	1.72	27.31
Nitzschia sp.	0.92	1.33	1.70	29.01
Navicula veneta	0.26	1.45	1.64	30.66
Mayamaea permitis	0.43	1.36	1.61	32.26
Nitzschia dissipata	1.38	0.60	1.59	33.85

Groups Moderate & Poor Status
Average dissimilarity = 77.38

Species	**Group Moderate Average Abundance**	**Group Poor Average Abundance**	**Contribution %**	**Cumulative contribution %**
Eolimna subminuscula	0.71	4.00	4.17	4.17
Navicula gregaria	1.64	1.98	3.13	7.30
Amphora pediculus	2.70	1.36	2.92	10.22
Nitzschia palea	2.14	2.49	2.48	12.69
Cyclotella meneghiniana	1.49	1.72	2.23	14.92
Navicula minima var. *minima*	0.93	1.67	2.13	17.05
Gomphonema parvulum	1.25	1.74	2.04	19.09
Achnanthidium minutissimum	2.14	1.81	1.84	20.94
Nitzschia dissipata	1.58	0.60	1.80	22.73
Navicula cryptotenella	1.60	0.53	1.77	24.50
Cocconeis placentula	1.60	0.81	1.71	26.21
Nitzschia sp.	0.92	1.33	1.69	27.90
Navicula veneta	0.56	1.45	1.68	29.59
Mayamaea permitis	0.55	1.36	1.58	31.17
Planothidium frequentissimum	1.49	0.96	1.46	32.63

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Supplement Table 1. Mean values for stressors for benchmark sites, i.e. sites which meet the 10th percentile criterion for TDI_{HR}.

Code	IC Type	National type	Year	TN mg L^{-1}	$N\text{-}NH_4^+$ mg L^{-1}	$N\text{-}NO_3^-$ mg L^{-1}	TP mg L^{-1}	Land Use Index (%)	Conduc -tivity µS cm^{-1}	BOD_5 mg L^{-1}	$P\text{-}PO_4^{3-}$ mg L^{-1}	$N\text{-}NO_3^-$ mg L^{-1}
15478	R-E2	HR-R_4	2016	1.0826	0.1234	0.0143	0.0559	93.55	555.17	3.5442	0.0125	0.5700
15590	R-E2	HR-R_4	2016	2.5487	0.5365	0.1035	0.2204	127.14	631.08	2.4908	0.1600	1.7024
15591	R-E2	HR-R_4	2016	2.2598	0.1566	0.0456	0.1925	109.00	580.92	2.3033	0.1356	1.8728
16110	R-E2	HR-R_4	2016	0.7117	0.0188	0.0056	N/A	60.42	218.83	1.9708	0.0140	0.3150
16224	R-E2	HR-R_4	2010	0.8710	0.0552	0.0223	0.1370	47.59	446.00	1.4067	0.0050	0.6660
16225	R-E2	HR-R_4	2016	1.2103	0.2176	0.0349	0.1443	64.29	289.25	2.7958	0.0958	0.8102
16342	R-E2	HR-R_4	2016	1.2902	0.0941	0.0038	0.0701	62.27	343.58	1.0750	0.0169	0.9166
17010	R-E2	HR-R_4	2016	1.1293	0.0209	0.0065	0.0498	59.51	209.33	1.7225	0.0208	1.0413
17013	R-E2	HR-R_4	2016	1.5168	0.0605	0.0209	0.0461	94.81	458.25	4.0392	0.0125	1.0375
17103	R-E2	HR-R_4	2016	1.7326	0.1982	0.0651	0.1433	105.69	582.00	3.9492	0.0125	1.0308
18001	R-E2	HR-R_4	2015	1.1625	0.0508	0.0078	0.0958	84.71	590.75	1.2567	0.0268	0.8592
18002	R-E2	HR-R_4	2015	1.3300	0.0435	0.0131	0.0964	31.86	583.18	1.6545	0.0438	0.9582
18005	R-E2	HR-R_4	2016	1.7133	0.1407	0.0283	N/A	101.73	643.67	1.5167	0.0773	1.0708
21012	R-E2	HR-R_4	2015	1.4942	0.1082	0.0224	0.2793	97.99	439.42	3.6583	0.0983	1.0475
21077	R-E2	HR-R_4	2016	1.3750	0.1154	0.0325	0.0883	97.91	557.67	1.2417	0.0400	0.6542
21085	R-E2	HR-R_4	2015	1.6008	0.1153	0.0310	0.1678	73.73	523.75	2.9083	0.0372	1.1158
13001	R-E3	HR-R_4	2017	1.8182	0.1774	0.0542	N/A	83.52	488.18	3.0909	0.0755	1.3064
14001	R-E3	HR-R_4	2017	0.8242	0.0193	0.0069	N/A	55.01	432.25	1.0167	0.0175	0.6150
14002	R-E3	HR-R_4	2017	0.8208	0.0040	0.0061	N/A	55.21	426.75	1.0083	0.0061	0.5642
14005	R-E3	HR-R_4	2017	0.7525	0.0040	0.0046	N/A	40.63	442.17	1.1750	0.0080	0.5767
15223	R-E3	HR-R_4	2017	1.3783	0.0851	0.0174	N/A	82.66	476.08	3.8417	0.0663	0.7725
15352	R-E3	HR-R_4	2017	2.6150	0.3208	0.0653	N/A	115.83	582.00	3.8583	0.1873	1.5100
16223	R-E3	HR-R_4	2016	1.1309	0.2063	0.0190	0.0759	74.56	382.50	2.1525	0.0304	0.6933
16003	R-E3	HR-R_5A	2016	0.8840	0.0231	0.0052	N/A	47.24	360.30	0.6900	0.0110	0.6460
16004	R-E3	HR-R_5A	2016	0.9158	0.0308	0.0040	N/A	46.38	359.58	0.6875	0.0124	0.6450
16010	R-E3	HR-R_5A	2016	0.8270	0.0166	0.0029	N/A	40.91	352.50	0.6850	0.0025	0.6300
16202	R-E3	HR-R_5A	2016	0.9590	0.0234	0.0055	N/A	52.04	355.60	1.1100	0.0187	0.6570
15496	R-EX5	HR-R_2A	2017	0.9405	0.2727	0.0142	0.0783	36.37	546.83	1.8683	0.0244	0.3520
16105	R-EX5	HR-R_2A	2016	1.3164	0.0497	0.0324	0.1200	41.61	220.00	2.1767	0.0573	1.1538
16107	R-EX5	HR-R_2A	2016	0.6988	0.3253	0.0142	0.0536	34.69	399.13	2.0750	0.0100	0.1603
16228	R-EX5	HR-R_2A	2016	1.4852	0.1846	0.0447	0.1919	94.81	471.75	2.8875	0.1233	1.1485
16230	R-EX5	HR-R_2A	2016	0.8388	0.1876	0.0148	0.0660	82.22	90.96	2.8013	0.0216	0.2716
16233	R-EX5	HR-R_2A	2016	0.6213	0.1551	0.0118	0.0476	39.15	178.88	1.0000	0.0100	0.2016
16234	R-EX5	HR-R_2A	2017	0.8029	0.2262	0.0058	0.0722	76.87	435.22	1.6611	0.0224	0.2687
16236	R-EX5	HR-R_2A	2016	0.7708	0.1729	0.0126	0.0536	66.21	137.91	2.2575	0.0100	0.2585
16239	R-EX5	HR-R_2A	2017	0.7861	0.2177	0.0164	0.0638	49.16	486.44	2.0911	0.0179	0.2250
16745	R-EX5	HR-R_2A	2016	1.2440	0.2978	0.0075	0.0663	60.49	192.10	1.5667	0.0231	0.7873
16746	R-EX5	HR-R_2A	2017	0.9187	0.2241	0.0075	0.0523	91.03	222.75	1.3100	0.0159	0.8824
16747	R-EX5	HR-R_2A	2016	1.3703	0.3150	0.0057	0.0640	54.44	182.18	1.4250	0.0240	0.8835
16748	R-EX5	HR-R_2A	2016	1.1108	0.3058	0.0063	0.1892	37.87	164.92	1.6417	0.0191	0.6806
21081	R-EX5	HR-R_2A	2011	1.9000	0.1620	0.0148	0.1826	75.27	495.08	1.5667	0.0629	1.0942

16050	R-EX5	HR-R_2B	2016	0.4155	0.0083	0.0023	N/A	1.49	188.36	0.8864	0.0074	0.2055
16102	R-EX5	HR-R_2B	2016	1.6534	0.4228	0.0111	0.1101	27.35	171.78	2.2250	0.0297	1.0870
16227	R-EX5	HR-R_2B	2016	1.2398	0.0773	0.0224	0.1483	88.24	477.08	2.6575	0.0768	1.0342
16802	R-EX5	HR-R_2B	2016	1.4908	0.0527	0.0099	0.1139	43.09	381.83	1.6500	0.0163	1.1296
17005	R-EX5	HR-R_2B	2016	1.2508	0.0817	0.0213	0.0837	97.11	561.08	0.9292	0.0438	0.8408
17009	R-EX5	HR-R_2B	2016	1.6900	0.1931	0.0558	0.1537	93.76	588.08	1.9667	0.0903	1.0317
17605	R-EX5	HR-R_2B	2016	1.1545	0.1030	0.0238	0.0767	91.66	570.58	2.4067	0.0125	0.7158
51138	R-EX5	HR-R_2B	2016	2.3963	0.2850	0.0532	0.2515	80.01	344.42	2.5550	0.1343	1.8982
29143	R-EX5	HR-R_3B	2015	0.6429	0.0150	0.0100	0.1500	80.74	393.29	1.1143	0.0150	0.5650
16232	R-EX6	HR-R_1	2017	0.8531	0.1827	0.0071	0.0900	12.55	227.33	2.0001	0.0198	0.3313
17014	R-EX6	HR-R_1	2017	1.3733	0.0389	0.0250	0.0401	9.55	223.76	1.1967	0.0182	1.0078
17403	R-EX6	HR-R_1	2017	0.8433	0.0679	0.0058	N/A	9.72	488.83	1.1667	0.0235	0.5733
21114	R-EX6	HR-R_1	2018	1.1436	0.0597	0.0050	0.0411	20.70	387.73	1.1818	0.0125	1.0145
11076	R-EX7	HR-R_6	2015	0.8917	0.0096	0.0023	0.0980	19.16	487.83	0.6417	0.0067	0.7342
14004	R-EX7	HR-R_6	2017	0.6377	0.0438	0.0107	0.0068	5.87	395.67	0.5211	0.0033	0.3929
16243	R-EX7	HR-R_6	2017	1.7044	0.0911	0.0250	0.0382	14.69	452.22	0.8744	0.0167	1.0822
16346	R-EX7	HR-R_6	2017	1.1451	0.1767	0.0040	0.0300	47.17	421.67	1.3667	0.0111	0.7473
16460	R-EX7	HR-R_6	2017	1.2200	0.0399	0.0010	0.0221	33.53	427.42	1.1242	0.0035	0.9754
16580	R-EX7	HR-R_6	2017	1.2509	0.0972	0.0061	0.0164	20.48	386.75	1.0083	0.0035	0.9132
16587	R-EX7	HR-R_6	2017	1.2912	0.0854	0.0057	0.0319	20.64	355.75	1.4333	0.0035	0.8798
16822	R-EX7	HR-R_6	2016	1.2230	0.0907	0.0041	0.0457	54.29	454.75	1.2417	0.0138	0.8658
16850	R-EX7	HR-R_6	2016	0.8064	0.0040	0.0010	N/A	0.21	434.18	0.2500	0.0025	0.6545
30026	R-EX7	HR-R_6	2016	0.7867	0.0058	0.0018	0.0148	0.24	267.75	0.9792	0.0015	0.6333
51156	R-EX7	HR-R_6	2017	1.8133	0.0733	0.0250	0.0372	12.50	497.22	1.0989	0.0154	1.0089
16334	R-EX8	HR-R_7	2016	1.1622	0.0946	0.0024	0.0496	68.37	390.42	1.4167	0.0152	0.8334
16338	R-EX8	HR-R_7	2016	1.1500	0.0010	0.0010	0.0057	17.96	328.58	1.4279	0.0030	0.6794
16339	R-EX8	HR-R_7	2013	0.8178	0.0209	0.0015	0.0125	37.25	433.44	0.3000	0.0025	0.7244
16453	R-EX8	HR-R_7	2010	0.6778	0.0147	0.0025	0.0331	3.52	356.38	1.0783	0.0050	0.6083
16572	R-EX8	HR-R_7	2010	0.9080	0.0433	0.0050	0.0675	28.06	328.23	1.4546	0.0050	0.6493
16581	R-EX8	HR-R_7	2010	0.8675	0.0082	0.0025	0.0329	21.10	346.62	1.3531	0.0050	0.7468
16583	R-EX8	HR-R_7	2016	0.7967	0.0113	0.0032	0.0186	29.04	312.50	1.0867	0.0032	0.6717
16662	R-EX8	HR-R_7	2017	1.2598	0.0114	0.0010	0.0084	0.00	445.42	0.7983	0.0035	0.9414
16663	R-EX8	HR-R_7	2017	1.2143	0.0478	0.0010	0.0188	53.05	425.08	1.2475	0.0035	0.9058
16753	R-EX8	HR-R_7	2017	1.4094	0.1835	0.0043	0.0741	69.62	347.17	1.9250	0.0122	0.9912
16754	R-EX8	HR-R_7	2017	1.2125	0.1395	0.0048	0.0564	31.72	357.00	1.9417	0.0079	0.8642
30009	R-EX8	HR-R_7	2016	0.9217	0.0083	0.0023	N/A	10.35	266.50	0.6042	0.0025	0.6733
30016	R-EX8	HR-R_7	2016	0.8983	0.0040	0.0024	N/A	18.41	315.33	0.2500	0.0069	0.7242
30020	R-EX8	HR-R_7	2010	0.6483	0.0160	0.0052	0.0243	11.15	289.83	1.4333	0.0050	0.4167
30061	R-EX8	HR-R_7	2017	0.6408	0.0046	0.0012	0.0253	13.02	234.92	1.3783	0.0015	0.5863
30063	R-EX8	HR-R_7	2017	0.6358	0.0035	0.0005	0.0140	0.00	261.83	1.2917	0.0015	0.6042
30064	R-EX8	HR-R_7	2017	0.5325	0.0048	0.0013	0.0162	26.73	339.83	1.0867	0.0033	0.4792
16591	R-EX8	HR-R_8	2018	1.2331	0.1082	0.0043	0.0459	34.83	356.75	2.0750	0.0120	0.8921
30033	R-EX8	HR-R_9	2013	0.7500	0.0150	0.0030	0.0150	101.70	456.00	1.5000	0.0150	0.8045

Report on fitting the Croatian classification method for macrophytes in rivers to the results of the completed intercalibration of the Eastern-Continental GIG (R-E2 and R-E3)

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Report on fitting the Croatian classification method for macrophytes in rivers to the results of the completed intercalibration of the Eastern-Continental GIG (R-E2 and R-E3)

1. INTRODUCTION

- Croatia
- Macrophytes
- Rivers

When the Eastern Continental GIG macrophytes intercalibration exercise was carried out (Birk et al. 2011a, b), Croatia was not yet member of EU.

Extensive data on macrophytes in the rivers of Croatia have been collected starting from 2009. After testing various macrophytes metrics and macrophytes based methods, the reference index (RI) developed for the assessment of ecological status of German medium size lowland rivers (Schaumburg et al. 2006, 2012) has been slightly modified and adapted for assessment of ecological status of rivers in Croatia.

This report aims to compare the class boundaries of Croatian Macrophyte Index for Rivers (RI-HR) with those agreed in Eastern Continental GIG intercalibration exercise, using Croatian rivers data and following the instructions of the CIS Guidance Document n°30: "Procedure to fit new or updated classification methods to the results of a completed intercalibration exercise".

Two common intercalibration types were considered for the macrophyte intercalibration in the completed intercalibration exercise: R-E2 and R-E3.

Baseline for the application of the procedure specified in the intercalibration manual are the results of the completed intercalibration exercise for river macrophytes in the Eastern Continental GIG. These results are documented in the Milestone 6 report of the WFD Intercalibration Phase 2 (BIRK et al., 2011). Central to the fitting procedure applied in this study is the global mean view, i.e. the harmonised position of the high-good and good-moderate class boundary, established by the completed exercise individually for each intercalibration type. This global mean view represents the international standard to which the good status class boundaries of the Croatian method need to comply.

In the following, we present details of the Croatian macrophyte-based assessment system for rivers (RI-HR) including the validation of the pressure-impact relationship, check its compliance with the WFD requirements, and demonstrate the compliance with the completed intercalibration exercise. The analyses performed cover two intercalibration types addressed in the completed exercise, i.e. lowland rivers of the plains (R-E2 and R-E3, catchment area below and above 1000 km2, respectively).

2. DESCRIPTION OF NATIONAL ASSESSMENT METHODS

The national macrophyte-based method – Reference Index Croatia (RI-HR) is established for the assessment of the ecological status of all river types in Croatia. It is modified version of German Reference index (Schaumburg et al. 2006, 2012), and it is compliant with European standardization legislation (EN 14184: 2014, EN 14996: 2006, CEN 230165). The evaluation and the calculation

procedures meet the requirements of the Water Framework Directive (WFD) and are in line with the recommendations of the implementation groups CIS Working Group 2.3 and 2.A (RECFOND, ECOSTAT).
The macrophyte survey is carried out once during the main vegetation period (June-September). In each sampling site, usually a 100 m long section is surveyed. The abundances of a single species are estimated using a five-level scale according to Kohler.
For assessment, the species are designated to three different groups: reference taxa (A), indifferent taxa (B) and degradation indicators (C). The relative share of these different groups decides the ecological class of the investigated site.
Field records also include estimation of four abiotic parameters: flow velocity, shading, substrate type and mean depth. Shading is noted based on five-degree scale (1 – completely sunny, 2 – sunny, 3 – partly overcast, 4 – half shaded, 5 – completely shaded) of Wörlein (1992). The other three parameters are determined after Schaumburg et al. (2004, 2006) in a semi-quantitative way using class scales, to enable a fast and easy application. The velocity of flow is recorded using six-point scale: I – not visible, II – barely visible, III – slowly running, IV – rapidly running (current with moderate turbulences), V – rapidly running (turbulently running), VI – torrential. The substratum conditions at the sampling site are classified in 5% steps according to an eight-point scale: % mud, % clay/loam (<0.063 mm), % (0.063-2.0 mm), % fine/medium gravel (2.0-6.3/6.3-20 mm), % coarse gravel (20-63 mm), % stones (63-200 mm), % boulders (>200 mm) and % organic/peat. The mean depth is noted on a three-degree scale (I – 0-30 cm, II – 30-100 cm and III >100 cm).

Prior to performing any calculations, the nominally scaled values of plant abundance are converted into metric quantities using the following function:
Q (quantity) = A (abundance)^3.
The taxa occurring at the sampling site is assigned to type species group that corresponds to reference (or sensitive) taxa, indifferent taxa and degradation indicators (tolerant species). The quantities of different species are summed up separately for each group and for all species of a sampling site.
RI-HR is calculated according to the following formula:

$$RI = \frac{\sum QAi - \sum QCi}{\sum Qgi} 100$$

RI – Croatian Macrophyte Index for Rivers
QAi – Quantity of the i-th taxon of species group A
QCi – Quantity of the i-th taxon of species group C
Qgi – Quantity of the i-th taxon of all groups
nA – Total number of taxa in group A
nC – Total number of taxa in group C
ng – Total number of taxa in all groups.

The resulting index values range from +100 (only species group A taxa) to -100 (only species group C taxa).
The additional criteria provided below are type-related correcting factors of the RI-HR:
- if Sp or Po communities (for definition of communities see chapter 2.3) have less than three submerse species the RI-HR is reduced by 50

In order to obtain EQR values, the index values must be transformed:

$$EQR = \frac{(RI + 100)*0,5}{100}$$

2.1. METHODS AND REQUIRED BQE PARAMETERS

Table 1. Overview of the metrics included in the national method

HR	Taxonomic composition	Abundance
RI-HR	x	x

Combination rule used in the method: quantity of sensitive species in relation to the quantity of tolerant and indifferent species. Additional criteria are used depending on the number of taxa and the relative quantity of specific taxa.
Conclusion on the WFD compliance: all indicative parameters of the macrophytes (listed in IC Guidance document on the intercalibration process) are included.

2.2. SAMPLING AND DATA PROCESSING

Description of sampling and data processing:

- Sampling time and frequency:
  Sampling takes place once in a year, during summer or early autumn (June-September), when macrophytes are optimally developed. July and August are usually optimal for sampling. For operational monitoring, sampling is done once in three years, while for surveillance monitoring, once in six years.
- Sampling method:
  The sampling location is selected on the stretch of river with no visible disturbances, such as bridges, tributaries, disturbed riverbank etc. and from the ecologic point of view, it should be homogenous. In the case of variability of ecological conditions (riffles, changes of slope, substrates, riparian vegetation or brightness), it is recommended to take several samples on shorter river stretches. The length of the sampled river stretch is approximately 100 m (i.e. 50-100 m for small and medium rivers).

  Macrophytes occurring in the mapped section are investigated by wadding, if possible, against the direction of flow, through the running water. To cover the whole width of the running water, wading should be carried out following a zigzag pattern.
  In shallow rivers, sampling can be done using a water viewer. In deeper water additionally, a boat and a rake should be used.
  Species that are hard to determine in the field are sampled and stored for later identification in the laboratory.
  The assessment of total covering is done using the Kohler scale:
  1 – very rare
  2 – rare
  3 – common
  4 – frequent
  5 – abundant/predominant

- Data processing:
  For data analyses, the macrophyte abundance data is transformed into "plant quantity" using the function $Q = A^3$.

- Identification level:
  Species level for bryophytes, vascular plants and charophytes. Genus level for other macroalgae (preferably also species level).

2.3. NATIONAL REFERENCE CONDITIONS

Reference conditions are established for each national biotic river type, based on expert judgement and least disturbed sites, when they were available. In those conditions, three main macrophyte communities were recognized regardless of the national biotic types (Table 5.) and the intercalibration river types (R-E2 and R-3):

- community dominated by nympheids and vallisnerids (Sp) – i.e. *Nuphar lutea, Potamogeton lucens*, *P. perfoliatus*, *Sparganium emersum*, *Schoenoplectus lacustris, Sagittaria saggitifolia*, *Nymphaea alba* and species of oligotrophic and weakly eutrophic waters: *Callitirche hamulata*, Characeae, *Lemna trisulca*, *P. gramineus*, *Riccia fluitans*, *Utricularia* spp., *Hippuris vulgaris* and others.
  This community is recognizable by patches, sometimes very abundant, of floating leaves of *Nuphar lutea* and submerged "meadows" of *Schoenoplectus lacustris* and *Sparganium emersum*. The first one is much more abundant in Croatian rivers and almost regularly has emergent, easily recognizable steams. The plant diversity in this community is high encompassing different growth forms.
- community dominated by broad leaved *Potamogeton species* (Po) – i.e. *P. lucens*, *P. perfoliatus*, *P. nodosus*, *P. gramineus*.
  This community is similar to previous, but floating leaves of *Potamogeton* species (*P. nodosus* and *P. natanas* are the most widespread) replace *Nuphar lutea*. Beside them, submerged species *P. lucens* and *P. perfoliatus* are also abundant. The overall diversity is lower in comparison to previous community.
- community dominated by myriophilids (My) – i.e. *Myriophyllum spicatum*, *Ranunculus trichophyllus* and other water *Ranunculus* species (*Ranunculus* subgen. *Batrachium*), broad leaved *Potamgeton* species (*P. lucens*, *P. perfoliatus*).
  This community is characterised by lack of species with floating leaves and submerged stands of species with finely divided leaves. The species *M. spicatum* and *Ranunculus trichophillus* are the most common. Submerged species of broad leaved *Potamogeton* species can also be members of this community.

One of these community types were assigned to every monitoring station. In IC type E2 to almost all monitoring station community Sp was assigned. The exceptions are two monitoring stations with naturally steeper banks where rich belt of macrophhytes with floating or emergent leaves cannot be developed. In IC type E3, the community Sp was also the most frequent. Only in largest rivers of this type, with stepper banks and very narrow zone of shallow water, we assumed that Po community is the referent one; due to lack of suitable microhabitats for many species occurring in community type Sp. The community My was assigned also to sites with steeper banks and, in relation to other sites, higher water velocity. This is a very rare type, known from only a few rivers and localities.
In general, macrophyte communities follow national river types, but because national biological typology is based on macrozoobenthos in relation to abiotic types, there is some discrepancies in

comparison to the macrophyte communities. Therefore, we have introduced community types, which allowed us to describe the each monitoring site more precisely regarding the macrophytes.

2.4. NATIONAL BOUNDARY SETTING

Ecological status is classified by one of five classes (high, good, moderate, poor and bad).

The boundaries were set at the zones of distinct changes of the macrophytes assemblage: analysing discontinuities in a pressure-response relationship and adjusting by expert judgment based on changes in type specific reference and tolerant species (Table 2).

Table 2. Classification of the EQR values into the categories of ecological status.

class	Range of EQR	Interpretation
High	>0.79	The HG boundary was assumed as one quarter (0.25) below the median value at which species of Group A are in clear dominance, and species of Group C are completely absent.
Good	0.55-0.79	The GM boundary was the point at which species of Group B (indifferent taxa) are dominant, and species of Group A are still dominant over species of Group C.
Moderate	0.55-0.30	The MP boundary was set as the average where the community is dominated by species of Group C (disturbance indicators). Species of Group A disappear.
Poor	0.01-0.30	The PB boundary is a point at which macrophyte species are extinct due to anthropogenic pressure.
Bad	<0.01	Complete loss of macrophytes due to anthropogenic pressure.

2.5. PRESSURES ADDRESSED

Pressures addressed by the method are eutrophication and general degradation. The national dataset for EC-GIG was used to test the response to different types of pressures (Tables 3 and 4, Figures 1 and 2). It can be concluded that the fitting procedure is feasible in terms of pressures.

Table 3. Correlation coefficients between ERQs and general physical and chemical parameters.

	EQR			EQR	
	Pearson Correlation	Significance		Spearman correlation	Significance
log_t	-.161**	.006	t	-.243**	.000
log_pH	.055	.196	pH	.010	.437
log_conductivity	-.333**	.000	conductivity	-.308**	.000
log_suspended particles	-.408**	.000	suspended particles	-.343**	.000
log_alkalinity	-.244**	.000	alkalinity	-.232**	.000
log_hardiness	-.273**	.000	hardiness	-.241**	.000
log_O2_dissolved	.227**	.000	O2_dissolved	.245**	.000

	EQR			EQR	
	Pearson Correlation	Significance		Spearman correlation	Significance
log_O2_saturation	.221**	.000	O2_saturation	.211**	.000
log_NH3	-.325**	.000	NH3	-.321**	.000
log_NO2	-.373**	.000	NO2	-.315**	.000
log_NO3	-.025	.351	NO3	-.147*	.011
log_N_total	-.222**	.000	N_total	-.278**	.000
log_PO4	-.434**	.000	PO4	-.387**	.000
log_P_total	-.448**	.000	P_total	-.383**	.000

**. Correlation is significant at the 0.01 level.

*. Correlation is significant at the 0.05 level.

Table 4. Correlation coefficients between ERQs and different measures of general degradation (EXT – % extensive agriculture, INT – % intensive agriculture, NAT – % natural and seminatural areas, ART – % of urban and artificial areas).

	Pearson Correlation	Significance	Spearman correlation	Significance
EXT	-.037	.341	-.033	.354
INT	-.352**	.000	-.316**	.000
NAT	-.043	.317	.319**	.000
URB	-.192*	.015	-.267**	.001

**. Correlation is significant at the 0.01 level.

*. Correlation is significant at the 0.05 level.

3. WFD COMPLIANCE CHECKING

In general, RI-HR method fulfils all WFD compliance criteria (Table 5).

Table 5. List of the WFD compliance criteria and the WFD compliance checking process and results

Compliance criteria	Compliance checking
Ecological status is classified by one of **five classes** (high, good, moderate, poor and bad).	YES
High, good and moderate ecological status are set in line with the WFD's **normative definitions** (**Boundary setting procedure**)	YES. The boundaries were set at the zones of distinct changes of the assemblage: analysing discontinuities in pressure-response relationship and adjusting by expert judgement based on changes in type specific reference and tolerant species.
All relevant parameters indicative of the biological quality elements are covered (see Table 1 in the IC Guidance). A **combination rule** to combine parameter assessment into BQE assessment has to be	YES. All relevant parameters of the BQE (i.e. composition and abundance) are covered.

defined. If parameters are missing, Member States need to demonstrate that the method is sufficiently indicative of the status of the QE as a whole	
Assessment is adapted to **intercalibration common types** that are defined in line with the typological requirements of the Annex II WFD and approved by WG ECOSTAT	YES. All assessed rivers were assigned to common river types R-E2 and R-E3.
The water body is assessed against **type-specific near-natural reference conditions**	YES
Assessment results are expressed as **EQRs**	YES
Sampling procedure allows for **representative** information about water body quality/ecological status **in space and time**	YES
All data relevant for assessing the biological **parameters** specified in the WFD's normative definitions are covered by the **sampling procedure**	YES. The biological parameters of the WFD's normative definitions (i.e. composition and abundance) are covered by the sampling procedure.
Selected taxonomic level achieves adequate **confidence and precision** in classification	YES. RI-HR uses species level. This guarantees adequate confidence and precision in classification.

4. IC FEASIBILITY CHECKING

With regard to the stream types addressed by the Romanian assessment system, these match all of the relevant common intercalibration types. Table 6 lists the river types treated in the completed intercalibration exercise of the EC-Rivers-GIG (Birk et al. 2011).

Table 6. Common intercalibration types treated in the Eastern Continental Rivers GIG.

Type name	Common IC type	Type characteristics		MS sharing the IC common type
R-E2 R-E3	Lowland rivers of the plains	Catchment area: and Altitude: Geology: Channel substrate:	100-1000 km² >1000 km² <200 m a.s.l. mixed sand, slit gravel	Bulgaria Hungary Slovakia Slovenia Czech Republic Romania **Croatia**

4.1. TYPOLOGY

Validation of the national sites' allocation to the common IC river types was performed based on mean altitude above sea level, catchment area and from qualitative information concerning hydrological features that were included in the dataset. The established national sites corresponded to two reported common intercalibration Eastern Continental river types – R-E2 and R-E3.

Table 7. Overview of national types and their fitting in EC GIG intercalibration types.

Type	Common intercalibration type	Ecoregion (Illies, 1967)	Catchment area [km2]	Altitude [m]	Geology	Channel substrate	National biotic type
R-E2	Plains: medium-sized, lowland	11,12	100 - 1,000	< 200	mixed	sand and silt	HR-R_3C HR-R_4A
R-E3	Plains: large, lowland	11,12	> 1,000	< 200	mixed	sand, silt and gravel	HR-R_3D HR-R_4B HR-R_4C
R-E4	Plains: medium-sized, mid-altitude	11,12	100 - 1,000	200-500	mixed	sand and gravel	/

The biological typology of running waters in Croatia was initially established in 2011 (Mihaljević et al., 2011), mainly based on expert opinion of type-specific benthic macroinvertebrate communities, due to general lack of all data types: both biological and pressure data. Today, biological data in most types are sufficient, as well as data on pressures such as water chemistry and land use. The data sets are still lacking hydromorphological scoring from many sites as the hydromorphological evaluation of running waters in Croatia began only recently, in 2017. With more data on hydromorphology we wish to fine-tune values for every type. Hence, the typology will remain as initially determined.

4.2. PRESSURES ADDRESSED

Croatian method is based on indicator species responding to eutrophication and general degradation. The method is similar to those of the intercalibrated methods. All classifications are based on indicator species responding to anthropogenic stress, especially eutrophication. The relationships between the index and the environmental factors are presented in chapter 2.5.

4.3. ASSESSMENT CONCEPT

National macrophyte-based method (RI-HR) is based on multihabitat sampling covering the whole river channel and banks. Reference index defines type-specific reference and non-specific disturbance indicating taxa (stonewort, other macroalgae, bryophytes and vascular plants).

Intercalibrated methods in Eastern Continental GIG are also indicator species based methods designed according to the concept of positive, negative and indifferent indicator species to specific pressures. The indication value has been derived from data that showed a correlation between the presence and abundance of the species and the impact value of the pressure. The calculation of EQR differs between the methods, but it is some way of weighted averaging of indicator species abundance. RI-HR is of the same concept.

4.4. CONCLUSION ON THE INTERCALIBRATION FEASIBILITY

We conclude that fitting of Croatian Reference Index (RI-HR) to the results of Eastern Continental GIG intercalibration exercise is feasible.

5. DEMONSTRATING THE COMPLIANCE WITH THE COMPLETED INTERCALIBRATION EXERCISE

5.1. Background

The macrophyte Eastern Continental GIG has finalized intercalibration, but Croatia did not join the group. Thus, Croatia has to perform fit-in-procedure. First, it had to be shown that macrophyte-based national method is compliant with the WFD normative definitions (Chapter 3) and intercalibration is feasible in terms of typology (Chapter 4). In the second step, it has to be proven that the RI-HR class boundaries are in line with the results of the intercalibration exercise.

The completed river macrophyte intercalibration exercise corresponded with Case B2: IC Option 3 using continuous benchmarking (Willby et al. 2014). This means that a common biological metric was not used in the completed exercise (but a so-called 'pseudo-common metric'), and reference or alternative benchmark sites were not commonly available at a national level. Key to successful intercalibration is thus to identify a BRINC (the best-related and intercalibrated national classification method).
For the two common intercalibration types (R-E2 and R-E3), the Slovenian RMI (Kuhar et al. 2011) was identified as BRINC. Due to biogeographical and methodological differences, benchmark standardization has to be applied.

All biological data used in this intercalibration exercise were sampled in the years 2011 to 2017. Representative river stretches were visually inspected during the vegetation season (June to September) by direct collecting, wading, using rake or grapnel or where necessary. Representative sites spanned 100-500 m of river length. Selected measurements of physico-chemical parameters (annual average values matching the years of biological sampling) and parameters of catchment land use (acquired from the CORINE database (European Environmental Agency 2006)) were included in the analyses. The macrophyte survey data were collected following a very similar procedure as the already intercalibrated Slovenian methods RMI. The dataset used in this study complies with the data acceptance criteria of Birk & Willby (2011) and is thus suitable for performing the necessary boundary calculations.

5.2. Description of IC dataset

The data used in the analysis of the lowland rivers (R-E2 and R-E3) covered 67 macrophyte surveys (20 in R-E2 and 54 in R-E3), scattered through their range in Croatia.

5.3. Description of Intercalibration procedure

5.3.1. Identification of Best-Related Intercalibrated National Classification (BRINC)

After inspecting national methods from Eastern Continental GIG, those from neighbouring countries were chosen, i.e. Hungarian Reference Index (RI-HU) and Slovenian River Macrophyte Index (RMI) due to at least partially similar biogeography, as well as methodological similarities. Table 8 shows the results of the correlation analysis of the Croatian method with the Hungarian and Slovenian methods intercalibrated for the lowland rivers (R-E2 and R-E3). However, it was not possible to calculate RI-HU and RMI for all Croatian sites, due to methodological restrictions. They were especially emphasized in Slovenian method, which requires at least three indicator taxa from the list for reliable calculation of RMI (Kuhar et al. 2011).

Due to higher and significant correlation of RI-HR with RMI, Slovenian national classification method was used as BRINC.

Table 8. Results of the correlation analysis of the RI-HR with the Hungarian and Slovenian national classification methods intercalibrated for the lowland rivers (R-E2 and R-E3). R – correlation coefficient, N – number of surveys.

National classification method	Pearsons's R	N
Hungarian Reference Index (RI_HU)	0.199	46
Slovenian River Macrophyte Index (RMI)	0.582*	34

*. Correlation is significant at the 0.01 level.

5.3.2. Benchmark standardization

We tested the pressure-impact relationship of the BRINC for 14 surveys at Slovenian streams (taken from BIRK et al. 2012), and 34 surveys at Croatian streams. Linear mixed modelling (GLMM) was used (see XGIG Large River MZB report, Annex 2, BIRK et al. 2016; Romanian river macrophyte EC report, Pall et al. 2016; Czech river macrophyte report, Opartilova et al. 2016) referring to the following pressure parameters: percent of artificial and urban areas in catchment, percent of intensive agriculture in catchment, percent of extensive agriculture in catchment, percent (near-)natural areas in catchment, biological oxygen demand (BOD), NH_4-N water concentration, NO_3-N water concentration, PO_4-P water concentration and total phosphorus water concentration.
The modelling revealed an offset of 0.071 BRINC-units, i.e. the BRINC needs to be lowered by 0.071 when applied to Croatian data. This results in the benchmark standardised BRINC (BRINC_bm).

5.3.3. Global mean view translated into BRINC

To translate the global mean view of the completed intercalibration exercise into the units of the BRINC (i.e. the Slovene River Macrophyte Index – RMI), we referred to the values of the RMI boundary positions and the boundary-specific class biases documented in Table 8.3 (National class boundaries and boundary bias) of the Milestone 6 report (BIRK et al., 2011). We reconstructed the global mean view in BRINC units according to the formulas:

5.3.3.1. High-good boundary

$0.800^{a} - [(1.000^{b} – 0.800^{a})* -0.02^{c}] = 0.804^{d}$

[a] high-good boundary of the RMI-method,
[b] reference value of the RMI-method,
[c] boundary bias of the high-good boundary,
[d] position of the high-good global mean view in units of the RMI method.

5.3.3.2. Good-moderate boundary

$0.600^{a} - [(0.600^{a} – 0.400^{b})* -0.07^{c}] = 0.586^{d}$

[a] good-moderate boundary of the RMI-method,
[b] moderate-poor boundary of the RMI-method,
[c] boundary bias of the good - moderate boundary,
[d] position of the good-moderate global mean view in units of the RMI method.

5.3.4. Predicting the position of Croatian river method's (RI-HR) class boundaries on the BRINC scale

The ordinary least squares regression (OLS) was calculated to establish the relationship between BRINC_bm and the RI-HR (Figure 1):

BRINC_bm = -0.005 + 1.002*RI-HR

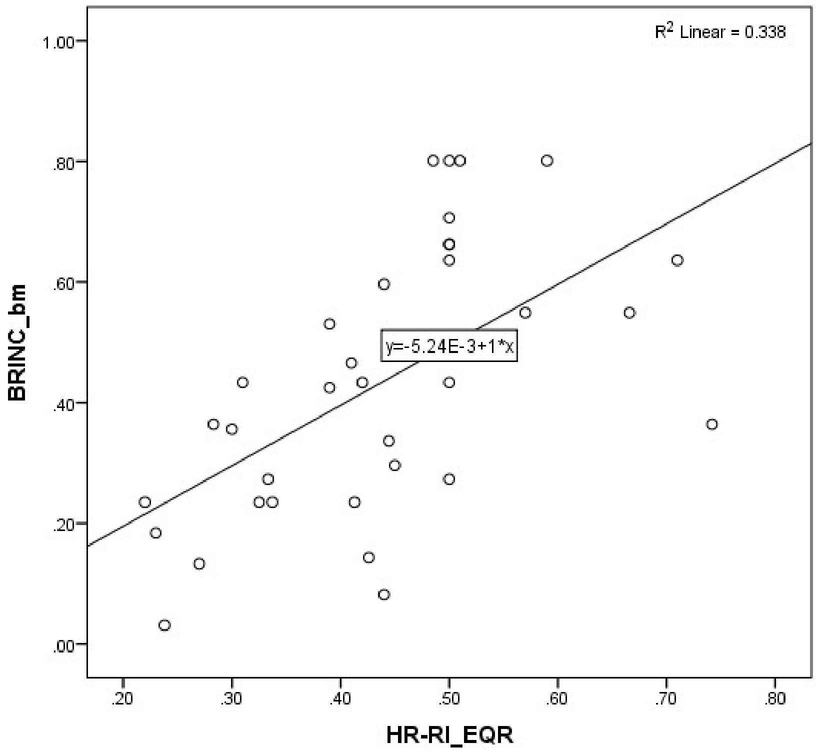

Figure 1. Regression plot of the RI-HR_EQR against the benchmark standardized BRINC for the lowland rivers (R-E2 and R-E3).

The above equation, relating BRINC_bm with RI-HR was used to predict the position of the national class boundaries of RI-HR on the BRINC-bm scale (Table 9).

Table 9. Translation of the reference and boundary position based on regression equation.

	RI-HR_EQR	BRINC_bm
reference	1.00	0.997
high/good	0.79	0.786
good/moderate	0.55	0.546
moderate/poor	0.30	0.295

5.3.5. Calculating the high-good class boundary bias of the Croatian river method

With the global mean view of the high-good boundary at 0.804 BRINC EQR-units and the Croatian method's high-good boundary translated into BRINC EQR-units at 0.786, the Croatian method's high-good boundary is positioned below the global mean view. This means, that the boundary bias needs to be calculated against the class width of the Croatian method's high status class. The boundary bias is calculated according the following formula:

$(0.786^a – 0.804^b) / (0.997^c - 0.786^a) = -0.084^d$

[a] Croatian method's high-good boundary translated into BRINC_bm EQR-units,
[b] global mean view of the high-good boundary,
[c] Croatian method's reference boundary translated into BRINC_bm EQR-units,
[d] high-good class boundary bias of the Croatian method.

A boundary bias of -0.0834 resulted. This means, that the high good boundary of the Croatian method lies in the line with the global mean view (i.e. falls above the threshold of -0.25) and thus complies with the required standard of intercalibration.

5.3.6. Calculating the good-moderate class boundary bias of the Croatian river method

With the global mean view of the good moderate boundary at 0.586 BRINC EQR-units and the Croatian method's good-moderate boundary translated into BRINC EQR-units at 0.54586, the Croatian method's good-moderate boundary is positioned below the global mean view. This means, that the boundary bias needs to be calculated against the class width of the Croatian method's good status class. The boundary bias is calculated according the following formula:

$(0.546^a – 0.586^b) / (0.786^c – 0.546^a) = -0.167^d$

[a] Croatian method's good-moderate boundary translated into BRINC_bm EQR-units,
[b] global mean view of the good-moderate boundary,
[c] Croatian method's high-good boundary translated into BRINC_bm EQR-units,
[d] good-moderate class boundary bias of the Croatian method.

A boundary bias of -0.167 resulted. This means, that the high good boundary of the Croatian method lies in the line with the global mean view (i.e. falls above the threshold of -0.25) and thus complies with the required standard of intercalibration.

5.3.7. Piecewise linear transformation of class boundaries

Class boundaries were transformed into the range 0.20, 0.40, 0.60 and 0.80, using piecewise linear transformation, in order to obtain uniform and common class ranges for all macrophyte communities.

Table 10. Classification of EQR values to ecological status classes and associated transformation equations

Ecological status class	EQR range	Uniform EQR range	Equation
Very good	>0.79	>0.80	0.80+0.20*(OEK-0.79)/0.21
Good	0.55-0.78	0.60-0.79	0.60+0.20*(OEK-0.55)/0.24

Ecological status class	EQR range	Uniform EQR range	Equation
Moderate	0.30-0.54	0.40-0.59	0.40+0.20*(OEK-0.30)/0.25
Bad	0.00-0.29	0.20-0.39	0.20+0.20*(OEK)/0.29
Poor	-	<0.2	-

5. 4. Summary

In this report, we documented the fitting procedure of the Croatian macrophyte-based assessment system for rivers to the results of the completed Eastern Continental Rivers' intercalibration exercise. The Croatian rivers method RI-HR revealed significant pressure-impact relationships and successfully passed the tests of intercalibration feasibility and WFD compliance. The intercalibration analyses against the global mean view of the completed exercise showed that in case of lowland rivers (R-E2 und R-E3) the required standards of intercalibration by the Croatian method were met (Table 11).

Table 11. Results of the Croatian rivers method's RI-HR intercalibration exercise for the intercalibration types R-E2 and R-E3, assigning the discrete EQR scores to the ecological status classes and specifying the ecological status class boundaries.

Ecological status class	R-E2 and R-E3 EQR boundaries	R-E2 and R-E3 class boundaries
high	0.80-1.00	H-G: 0.80
good	0.60-0.79	G-M: 0.60
moderate	0.40-0.59	M-P: 0.40
poor	0.20-0.39	P-B: 0.20
bad	<0.20	B: 0.20

6. DESCRIPTION OF THE BIOLOGICAL COMMUNITIES

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT HIGH STATUS

Type-specific reference species are dominant, pressure indictors are rare.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT GOOD STATUS

In good status, reference species are abundant, degradation indicators occur.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT MODERATE STATUS

In moderate status, degradation indicators dominate over reference species.

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Annex A. List of Croatian type specific indicator species for Sp (nympheid-vallisnerid community), My (myriophyllid community) and Po (community of broad leaved *Potamogeton* species) in R-E2 and R-E3 rivers (A – reference indicators, B – indifferent taxa, C – degradation indicators).

Species	Sp	My	Po
Acorus calamus	B	C	B
Agrostis stolonifera	B	B	B
Alisma lanceolatum	B	C	B
Alysma plantago-aquatica	B	C	C
Amblystegium serpens	B	B	B
Amblystegium varium	B	B	B
Apium repens	B	A	B
Azolla filiculoides	C	C	C
Batrachospermum spp.	A	A	A
Berula erecta	A	A	A
Bolboschoenus maritimus	B	B	B
Bryum pseudotriquetrum	A	A	A
Butomus umbellatus	B	B	B
Caliergonella cuspidata	B	B	B
Callitriche cophocarpa	B	B	B
Callitriche hamulata	A	A	A
Callitriche obtusangula	B	B	B
Callitriche platycarpa	B	B	B
Cardamine amara	B	B	B
Carex acuta	B	B	B
Carex acutiformis	B	B	B
Carex elata	B	B	B
Ceratophyllum demersum	C	C	C
Ceratophyllum submersum	C	C	C
Chara aspera	A	A	A
Chara contraria	A	A	A
Chara globularis	A	A	A
Chara hispida	A	A	A
Chara intermedia	A	A	A
Chara tomentosa	A	A	A
Chara vulgaris	A	A	A
Cinclidotus riparius	A	A	A
Cinclodotus aquaticus	A	A	A
Cinclodotus danubicus	A	A	A
Cinclodotus fontinaloides	A	A	A

Species	Sp	My	Po
Cladophora sp.	C	C	C
Conocephalum conicum	B	A	B
Cratoneuron filicinum	A	A	A
Cyperus longus	B	B	B
Drepanocladus aduncus	A	A	A
Eleocharis palustris	B	C	B
Elodea canadensis	C	C	C
Equisetum arvense	B	C	B
Equisetum palustre	B	C	B
Eurhynchium praelongum	A	A	A
Fontinalis antipyretica	A	A	A
Galium palustre	B	B	B
Glyceria fluitans	B	B	B
Glyceria maxima	B	C	B
Hippuris vulgaris	A	A	A
Holoschoenus vulgaris	B	B	B
Hottonia palustris	A	B	B
Hydrocharis morsus-ranae	B	B	C
Hygroamblystegium tenax	A	A	A
Hygrohypnum luridum	A	A	A
Hymenostylium recurvirostrum	A	A	A
Hyophila involuta	B	B	B
Iris pseudacorus	B	B	B
Juncus articulatus	A	B	B
Juncus bulbosus	A	A	A
Juncus compressus	B	B	B
Juncus inflexus	B	B	B
Jungermannia atrovirens	A	A	A
Lemna gibba	C	C	C
Lemna minor	B	C	C
Lemna trisulca	A	B	A
Leptodyctium riparium	C	C	C
Lycopus europaeus	B	C	B
Lysimachia nummularia	B	C	B
Lysimachia vulgaris	B	C	B
Lythrum salicaria	B	C	B
Marchantia polymorpha	B	B	B
Marsilea quadrifolia	B	B	B
Mentha aquatica	B	B	B
Myosotis scorpioides	B	B	B
Myriophyllum spicatum	B	A	B
Myriophyllum verticillatum	B	A	B
Najas minor	B	B	B
Nasturtium officinale	B	B	B
Nitella spp.	A	A	A
Nitellopsis obtusa	A	A	A
Nuphar lutea	B	C	B

Species	Sp	My	Po
Nymphaea alba	B	B	B
Oenanthe aquatica	B	B	B
Oenanthe cf. fistulosa	B	B	B
Palustriella commutata	A	A	A
Pellia endiviaefolia	A	A	A
Phalaris arundinacea	B	B	B
Phragmites australis	B	C	B
Plagiomnium undulatum	B	B	B
Platyhypnidium riparioides	B	B	B
Pohlia ludwigii	B	B	B
Polygonum amphibium	B	B	B
Polygonum lapathyfolium	B	C	C
Potamogeton berchtoldii	C	C	C
Potamogeton crispus	C	C	C
Potamogeton gramineus	A	A	A
Potamogeton lucens	A	A	A
Potamogeton natans	B	B	B
Potamogeton nodosus	A	B	B
Potamogeton pectinatus	C	C	C
Potamogeton perfoliatus	A	A	A
Potamogeton pusillus	C	C	C
Potamogeton trichoides	C	C	C
Pulicaria dysenterica	C	C	C
Ranunculus aquatilis	B	A	B
Ranunculus circinatus	B	A	B
Ranunculus flammula	A	A	A
Ranunculus fluitans	B	A	B
Ranunculus peltatus	B	A	B
Ranunculus repens	B	C	B
Ranunculus sceleratus	B	C	B
Ranunculus trichophyllus	B	A	B
Riccia fluitans	A	B	A
Ricciocarpus natans	B	C	B
Rorippa amphibia	B	B	B
Rorippa sylvestris	B	B	B
Rumex hydrolapathum	B	C	B
Sagittaria sagittifolia	B	B	C
Salvinia natans	B	C	C
Scirpus lacustris	B	B	B
Sparganium emersum	B	B	B
Sparganium erectum	B	B	B
Spirodella polyrhiza	B	C	B
Spirogyra sp.	C	C	C
Thamnobryum alopecurum	A	A	A
Tolypela spp.	A	A	A
Trapa natans	B	C	C
Typha angustifolia	B	C	C

Species	Sp	My	Po
Typha latifolia	B	B	B
Urtica dioica	C	C	C
Utricularia australis	A	A	A
Utricularia vulgaris	A	A	A
Veronica anagalis-aquatica	B	B	B
Veronica anagalloides	B	B	B
Veronica catenata	B	B	B
Wolfia arrhiza	B	C	C
Zanichellia palustris	C	C	C

Report on fitting the Croatian classification method for macrophytes in rivers to the results of the completed intercalibration of the of the Mediterranean GIG (R-M1 and R-M2)

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Report on fitting the Croatian classification method for macrophytes in rivers to the results of the completed intercalibration of the of the Mediterranean GIG (R-M1 and R-M2)

1. INTRODUCTION

- Croatia
- Macrophytes
- Rivers (R-M1 and RM2)

The official intercalibration of macrophyte-based methods for ecological status assessment within the Mediterranean Geographical Intercalibration Group (MedGIG) was finalized. Croatia did not join the official IC round because it was not the member state of the EU.
The MedGIG of river macrophytes involved seven countries and two assessment methods with similar data acquisition and assessment concept: the Macrophyte Biological Index for Rivers (IBMR) in Cyprus, France, Greece, Italy, Portugal and Spain, and the River Macrophyte Index (RMI) in Slovenia. Later was fitted Reference Index (RI) for Bulgaria (Pall et al. 2016).
The objective of this report is to declare that the present Croatian classification method of the ecological status of Mediterranean river types, based on macrophytes is compliant with the WFD normative definitions and that its class boundaries are in line with the results of the completed intercalibration exercise.
In particular, the classification method (Reference Index Croatia) is an intercalibratable finalized method. The class boundaries were compared with agreed boundaries from the MedGIG intercalibration exercise following the instructions of the CIS Guidance Document n°30: “Procedure to fit new or updated classification methods to the results of a completed intercalibration exercise”.

The biological analysis of macrophytes in MedGIG revealed a poor segregation between types RM1, RM2 and RM4 (Table 1), both for reference sites and for all sites (based on ANOSIM test). Therefore these types were treated together throughout the IC process.

Table 1. Overview of common intercalibration types in the Mediterranean rivers GIG and MS sharing the types.

Common IC Type	Type characteristics	MS sharing IC common type
R-M1	catchment <100 km^2; mixed geology (except non-siliceous); highly seasonal	France, Italy, Portugal, Slovenia, Spain, Bulgaria
R-M2	catchment 100-1000 km^2; mixed geology (except non-siliceous); highly seasonal	France, Italy, Portugal, Slovenia, Spain, Bulgaria

Common IC Type	Type characteristics	MS sharing IC common type
R-M3	catchment 1000-10000 km^2; mixed geology (except non-siliceous); highly seasonal	Greece, Portugal, Spain *This type was not intercalibrated because assessment methods were not fully developed.*
R-M4	non-siliceous streams; highly seasonal	Cyprus, France, Greece, Italy, Spain
R-M5	temporary rivers	Cyprus, Italy, Portugal, Slovenia, Spain (*Subject to a separate dana treatment due to large structural and functional differences*, Aguiar et al., 2010)

2. DESCRIPTION OF NATIONAL ASSESSMENT METHODS

The national macrophyte-based method – Reference Index Croatia (RI-HR) is established for the assessment of the ecological status of all river types in Croatia. It is modified version of German Reference index (Schaumburg et al. 2006, 2012), and it is compliant with European standardization legislation (EN 14184: 2014, EN 14996: 2006, CEN 230165). The evaluation and the calculation procedures meet the requirements of the Water Framework Directive (WFD) and are in line with the recommendations of the implementation groups CIS Working Group 2.3 and 2.A (RECFOND, ECOSTAT).
The macrophyte survey is carried out once during the main vegetation period (June-September). In each sampling site, usually a 100 m long section is surveyed. The abundances of a single species are estimated using a five-level scale according to Kohler.
For assessment, the species are designated to three different groups: reference taxa (A), indifferent taxa (B) and degradation indicators (C). The relative share of these different groups decides the ecological class of the investigated site.
Field records also include estimation of four abiotic parameters: flow velocity, shading, substrate type and mean depth. Shading is noted based on five-degree scale (1 – completely sunny, 2 – sunny, 3 – partly overcast, 4 – half shaded, 5 – completely shaded) of Wörlein (1992). The other three parameters are determined after Schaumburg et al. (2004, 2006) in a semi-quantitative way using class scales, to enable a fast and easy application. The velocity of flow is recorded using six-point scale: I – not visible, II – barely visible, III – slowly running, IV – rapidly running (current with moderate turbulences), V – rapidly running (turbulently running), VI – torrential. The substratum conditions at the sampling site are classified in 5% steps according to an eight-point scale: % mud, % clay/loam (<0.063 mm), % (0.063-2.0 mm), % fine/medium gravel (2.0-6.3/6.3-20 mm), % coarse gravel (20-63 mm), % stones (63-200 mm), % boulders (>200 mm) and % organic/peat. The mean depth is noted on a three-degree scale (I – 0-30 cm, II – 30-100 cm and III >100 cm).

Prior to performing any calculations, the nominally scaled values of plant abundance are converted into metric quantities using the following function:
Q (quantity) = A (abundance)^3.
The taxa occurring at the sampling site is assigned to type species group that corresponds to reference (or sensitive) taxa, indifferent taxa and degradation indicators (tolerant species). The quantities of different species are summed up separately for each group and for all species of a sampling site.
RI-HR is calculated according to the following formula:

$$RI = \frac{\sum QAi - \sum QCi}{\sum Qgi} 100$$

RI – Croatian Macrophyte Index for Rivers
QAi – Quantity of the i-th taxon of species group A
QCi – Quantity of the i-th taxon of species group C
Qgi – Quantity of the i-th taxon of all groups
nA – Total number of taxa in group A
nC – Total number of taxa in group C
ng – Total number of taxa in all groups.

The resulting index values range from +100 (only species group A taxa) to -100 (only species group C taxa).
The additional criteria provided below are type-related correcting factors of the RI-HR:
- if BN community is dominated by helophytes, the RI-HR is reduced by 50
- if BN community does not have any of characteristic species (Group A), the RI-HR is reduced by 60
- if PF community is dominated by helophytes, the RI-HR is reduced by 50

For community definitions see section 2.3.

In order to obtain EQR values, the index values must be transformed:

$$EQR = \frac{(RI + 100)* 0,5}{100}$$

2.1. METHODS AND REQUIRED BQE PARAMETERS

Table 2. Overview of the metrics included in the national method

HR	Taxonomic composition	Abundance
RI-HR	x	x

Combination rule used in the method: quantity of sensitive species in relation to the quantity of tolerant and indifferent species. Additional criteria are used depending on the number of taxa and the relative quantity of specific taxa.
Conclusion on the WFD compliance: all indicative parameters of the macrophytes (listed in IC Guidance document on the intercalibration process) are included.

2.2. SAMPLING AND DATA PROCESSING

Description of sampling and data processing:

- Sampling time and frequency:
  Sampling takes place once in a year, during summer or early autumn (June-September), when macrophytes are optimally developed. July and August are usually optimal for sampling. For operational monitoring, sampling is done once in three years, while for surveillance monitoring, once in six years.
- Sampling method:

The sampling location is selected on the stretch of river with no visible disturbances such as bridges, tributaries, disturbed riverbank etc., and from the ecologic point of view it should be homogenous. In the case of variability of ecological conditions (riffles, changes of slope, substrates, riparian vegetation or brightness), it is recommended to take several samples on shorter river stretches. The length of the sampled river stretch is approximately 100 m (i.e. 50-100 m for small and medium rivers).

Macrophytes occurring in the mapped section are investigated by wadding, if possible, against the direction of flow, through the running water. To cover the whole width of the running water, wading should be carried out following a zigzag pattern.
In shallow rivers, sampling can be done using a water viewer. In deeper water additionally, a boat and a rake should be used.
Species that are hard to determine in the field are sampled and stored for later identification in the laboratory.
The assessment of total covering is done using the Kohler scale:
1 – very rare
2 – rare
3 – common
4 – frequent
5 – abundant/predominant

- Data processing:
  For data analyses, the macrophyte abundance data is transformed into "plant quantity" using the function $Q = A^3$.

- Identification level:
  Species level for bryophytes, vascular plants and charophytes. Genus level for other macroalgae (preferably also species level).

2.3. NATIONAL REFERENCE CONDITIONS

Reference conditions for MedGIG rivers in Croatia are established in accordance with Feio et al. (2014). In those conditions, two macrophyte communities were recognized regardless of the national biotic types (Table 7) and the intercalibration river types (R-M1 and R-M2):

- community dominated by bryophytes (PF)
  This community is clearly distinguished by dominance of bryophytes and (almost) complete lack of vascular plants. Since all rivers in Meditarranean region of Croatia flow over carbonate bedrock, these communities are built up of typical calciphyllous or indifferent species as *Rhynchostegium riparioides*, *Cinclidotus aquaticus*, *C. fontinaloides*, *C. riparius*, *Cratoneuron filicinum*, *Palusstriella commutata*, *Fissidens crassipes*, *F. adianthoides*, *Didymodon tophaceus*, *Eucladium verticillatum*, *Fontinalis antipyretica, Pellia endiviifolia*, *Preissia quadrata* and others.
- community dominated by herbids and other growth forms of vascular plants (miriophyllids and magnopotamids in first order) (BN).
  This community is characterised by almost constant presence of *Berula erecta*. Other common species are *Mentha aquatica*, *Apium repens*, *Veronica anagallis-aquatica*, *Juncus articulatus*, *Agrostis stolonifera*, *Nasturtium officinale*, *Oenanthe fistulosa*, *Hippuris vulgaris*, charophytes and bryophytes.

One of these community types were assigned to every monitoring station. Namely, IC types M1 and M2 in Croatia are typical karst rivers with mosaic of plant communities dominated by mosses or by vascular plants. Occurrence of each community depends on water velocity, presence of waterfalls (natural barriers) and substratum. In conditions where water velocity is higher and the substratum formed of large stones, boulders and rocks, mosses are the main plant group forming communities. On the other hand, if water is slower and the substratum has larger proportion of gravel and sand, than regularly occurs the community with *Berula erecta* and other species of vascular plants.

2.4. NATIONAL BOUNDARY SETTING

One of five classes (high, good, moderate, poor and bad) classifies ecological status.

The boundaries were set at the zones of distinct changes of the macrophytes assemblage: analysing discontinuities in a pressure-response relationship and adjusting by expert judgment based on changes in type specific reference and tolerant species (Table 3).

Table 3. Classification of the EQR values into the categories of ecological status.

class	Community	Range of EQR	Interpretation
High	PF	>0.70	The HG boundary was assumed as one quarter (0.25) below the median value at which species of Group A are in clear dominance, and species of Group C are completely absent.
	BN	>0.65	
Good	PF	0.69-0.50	The GM boundary was the point at which species of Group B (indifferent taxa) are dominant, and species of Group A are still dominant over species of Group C.
	BN	0.64-0.50	
Moderate	PF	0.30-0.49	The MP boundary was set as the average where the community is dominated by species of Group C (disturbance indicators). Species of Group A disappear.
	BN	0.25-0.49	
Poor	PF	0.29-0	The PB boundary is a point at which macrophyte species are extinct due to anthropogenic pressure.
	BN	0.24-0	
Bad	PF	-	Complete loss of macrophytes due to anthropogenic pressure.
	BN	-	

2.5. PRESSURES ADDRESSED

Pressures addressed by the method are eutrophication and general degradation. The national dataset for MedGIG was used to test the response to different types of pressures (Tables 4 and 5, Figures 1 and 2). It can be concluded that the fitting procedure is feasible in terms of pressures.

Table 4. Correlation coefficients between ERQs and general physical and chemical parameters.

	EQR			EQR	
	Pearson Correlation	Significance		Spearman correlation	Significance
log_t	-.241*	.035	t	-.177	.093

	EQR			EQR	
	Pearson Correlation	Significance		Spearman correlation	Significance
log_pH	.079	.279	pH	.101	.228
log_condut	-.085	.266	condut	-.343**	.005
log_susp	-.327**	.007	susp	-.340**	.005
log_alcal	-.266*	.023	alcal	-.371**	.002
log_hard	-.128	.172	hard	-.323**	.007
log_O2_diss	.297*	.012	O2_diss	.318**	.008
log_O2_sat	.220*	.050	O2_sat	.180	.091
log_NH_4^+	-.586**	.000	NH3	-.579**	.000
log_NO_2^-	-.464**	.000	NO2	-.413**	.001
log_NO_3^-	-.300*	.012	NO3	-.265*	.023
log_N_total	-.468**	.000	N_total	-.372**	.002
log_PO_4^{3-}	-.496**	.000	PO4	-.455**	.000

**. Correlation is significant at the 0.01 level.

*. Correlation is significant at the 0.05 level.

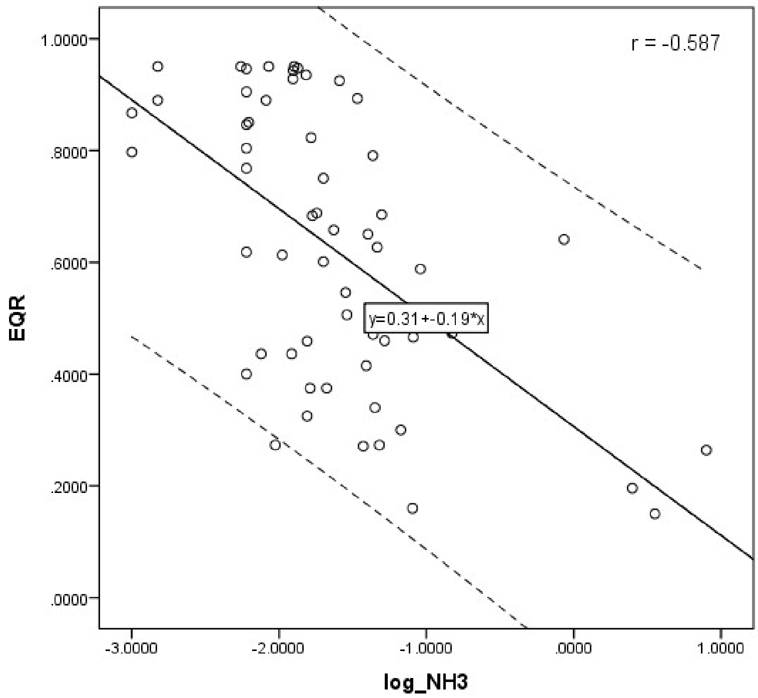

Figure 1. Response of the assessment method to the concentration of ammonium (95% confidence lines are dashed).

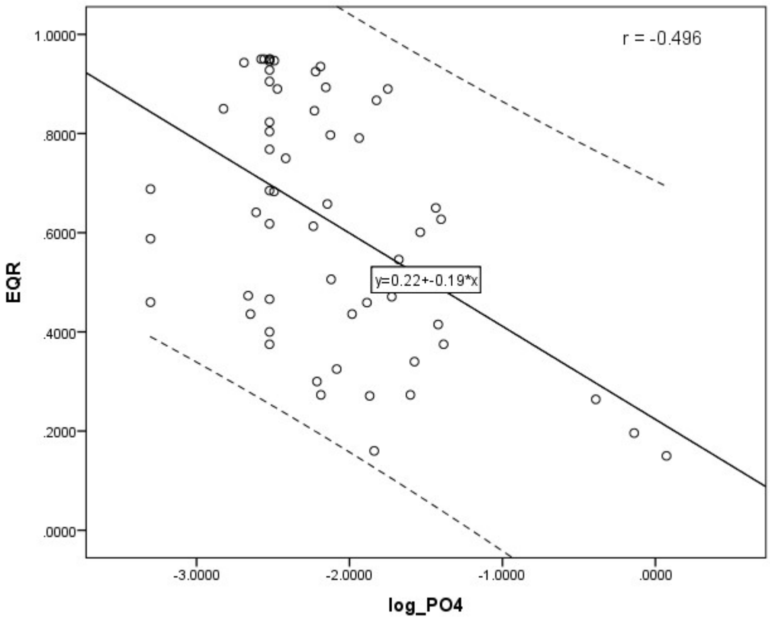

Figure 2. Response of the assessment method to the concentration of orthophosphates (95% confidence lines are dashed).

Table 5. Correlation coefficients between ERQs and different measures of general degradation (PST – catchment area, POE_T – % extensive agriculture, POI_T – % intensive agriculture, PRI_T – % natural and seminatural areas, URB_T – % of urban and artificial areas; long_con – longitudinal connectivity, morph – general morphology, hydro – hydrological degradation, total – total hydromorphological degradation).

	EQR			
	Pearson Correlation	Significance	Spearman Correlation	Significance
PST	-.048	.361	.001	.497
POE_T	-.210	.058	-.193	.075
POI_T	-.084	.266	-.274*	.019
PRI_T	.278*	.018	.333**	.006
URB_T	-.276*	.019	-.208	.061
long_con	-.091	.251	-.083	.270
morph	-.376**	.002	-.353**	.004
hydro	-.204	.064	-.239*	.036
total	-.393**	.001	-.354**	.003
**. Correlation is significant at the 0.01 level.				
*. Correlation is significant at the 0.05 level.				

3. WFD COMPLIANCE CHECKING

The first step in the Intercalibration process requires the checking of national methods considering the following WFD compliance criteria.

Table 6. List of the WFD compliance criteria and the WFD compliance checking process and results

Compliance criteria	Compliance checking
Ecological status is classified by one of **five classes** (high, good, moderate, poor and bad).	YES
High, good and moderate ecological status are set in line with the WFD's **normative definitions** (**Boundary setting procedure**)	YES
All relevant parameters indicative of the biological quality element are covered (see Table 1 in the IC Guidance). A **combination rule** to combine parameter assessment into BQE assessment has to be defined. If parameters are missing, Member States need to demonstrate that the method is sufficiently indicative of the status of the QE as a whole	YES
Assessment is adapted to **intercalibration common types** that are defined in line with the typological requirements of the Annex II WFD and approved by WG ECOSTAT	YES
The water body is assessed against **type-specific near-natural reference conditions**	YES
Assessment results are expressed as **EQRs**	YES
Sampling procedure allows for **representative** information about water body quality/ecological status **in space and time**	YES
All data relevant for assessing the biological **parameters** specified in the WFD's normative definitions are covered by the **sampling procedure**	YES
Selected taxonomic level achieves adequate **confidence and precision** in classification	YES

4. IC FEASIBILITY CHECKING

Here IC feasibility check of the HR-RI is documented in terms of typology, addressed pressure and assessment concept.

4.1. TYPOLOGY

Validation of the national sites' allocation to the common IC river types was performed based on: mean altitude above sea level, catchment area and from qualitative information concerning hydrological features that were included in the dataset. The established national sites corresponded to two of reported common intercalibration Mediterranean river types (Aguiar et al., 2014) – R_M1 and R-M2, since the type R-M5 was not intercalibrated due to large structural and functional differences (Aguiar et al., 2010).

Table 7. Overview of national types and their fitting in MedGIG intercalibration types.

National biotic type	National biotic type	Intercalibration type
Lowland and mid-altitude small river, calcareous geology	HR-R_11A	R-M1
Mid-altitude medium river, calcareous geology	HR-R_12	R-M2
Lowland medium river, calcareous geology	HR-R_13	R-M2
Large lowland rivers with barrage pools	HR-R_13A	R-M2
Lowland short-flow small rivers with >5 ‰ slop, calcareous geology	HR-R_14A	R-M1
Lowland short-flow medium rivers with >5 ‰ slop, calcareous geology	HR-R_14B	R-M2
Small and medium rivers in karst fields	HR-R_15A	R-M1
Medium rivers in karst fields	HR-R_15B	R-M2
Mid-altitude small and medium temporary rivers, calcareous geology	HR-R_16A	R-M5
Lowland small temporary rivers, calcareous and siliceous-calcareous geology	HR-R_16B	R-M5
Lowland and mid altitude small spring rivers of Istria, calcareous-flysch geology	HR-R_17	R-M1
Lowland medium rivers of Istria, calcareous-flysch geology	HR-R_18	R-M2
Temporary small lowland rivers of Istria, calcareous-flysch geology	HR-R_19	R-M5

The biological typology of running waters in Croatia was initially established in 2011 (Mihaljević et al., 2011), mainly based on expert opinion of type-specific benthic macroinvertebrate communities, due to general lack of all data types: both biological and pressure data. Today, biological data in most types are sufficient, as well as data on pressures such as water chemistry and land use. The data sets are still lacking hydromorphological scoring from many sites as the hydromorphological evaluation of running waters in Croatia began only recently, in 2017. With more data on hydromorphology, we wish to fine-tune values for every type. Hence, the typology will remain as initially determined.

4.2. PRESSURES ADDRESSED

Croatian method is based on indicator species responding to eutrophication and general degradation. The method is similar to those of the intercalibrated methods. The relationships between the index and the environmental factors are presented in chapter 2.5.

4.3. ASSESSMENT CONCEPT

National macrophyte-based method (RI-HR) is based on multihabitat sampling covering the whole river channel and banks. Reference index defines type-specific reference and non-specific disturbance indicating taxa (stonewort, other macroalgae, bryophytes and vascular plants).

Intercalibrated methods in MedGIG RMI (Slovenia) and IBMR (Cyprus, Greece, France, Italy, Portugal, Spain), as well as additionally fitted RI-BG (Bulgaria), are also indicator species based methods.

4.4. CONCLUSION ON THE INTERCALIBRATION FEASIBILITY

We conclude that fitting of Croatian Reference Index (RI-HR) to the results of MedGIG intercalibration exercise is feasible.

5. DEMONSTRATING THE COMPLIANCE WITH THE COMPLETED INTERCALIBRATION EXERCISE

5.1. Background

The macrophyte MedGIG has finalized intercalibration, but Croatia did not join the group. Thus, Croatia has to perform fit-in-procedure. First, it had to be shown that macrophyte-based national method is compliant with the WFD normative definitions (Chapter 3) and intercalibration is feasible in terms of typology (Chapter 4). In the second step, it has to be proven that the RI class boundaries are in line with the results of the intercalibration exercise.

The MedGIG Macrophyte group successfully completed intercalibration for rivers in the second round (2013). Seven member states (Portugal, Greece, France, Spain, Cyprus, Slovenia and Italy) were included. With one exception, they all used the French IBMR (Indice Biologique Macrophytique en Rivière (Haury et al. 2006) as the assessment system. Only Slovenia was using another method: the Slovenian RMI (River Macrophyte Index (Kuhar et. al. 2011).

Accordingly, a combination of Option 1 (for all countries using the IBMR-method) and Option 3 (median boundary values of all countries against the Slovenian RMI-method) was used for intercalibration. However, concerning the intercalibration of the Slovenian method, no "real" Option 3 was performed. As the harmonized median values of the H/G boundary and the G/M boundary of the countries intercalibrated previously via Option 1 was used as fixed benchmarks (Aguiar et al. 2013), the way of intercalibration in reality corresponded to a "fit-in procedure" (Pall et al. 2016).

In case Option 3 was applied in the previous finalized intercalibration exercise, the current guidance for the fit-in-procedure "Instruction manual to fit new or revised national classifications to the completed IC exercise" stipulates Case B1 "IC Option 3, using reference/benchmark sites" as to be used for the fit-in procedure. However, as described above, this in reality was not the case in the MedGIG. For this reason, we decided not to follow Case B1. Furthermore, during testing the Slovenian method in preparation of Bulgarian classification fitting it turned out that due to the low number of indicator species, an application of the Slovenian Index for many Bulgarian sites was not possible (Pall et al. 2016). A similar situation was in the MedGIG itself: Besides the Slovenian sites, only some sites from France, Spain and Cyprus could be assessed with the Slovenian method.

For this reason, we decided to follow Case A1 for the fit-in procedure: "**IC Option 1 or 2 using reference/benchmark sites"**.

Benchmark standardization used in the completed MedGIG IC exercise follows Feio et al. (2014), who established thresholds for benchmarks (Table 8).

Table 8. Thresholds for benchmarks in MedGIG after Feio et al. (2014).

Variables	Boundary
Channelization	≤ 2
Bank alternation (1-4)	≤ 2
Local habitat alternation (1-4)	≤ 2
Riparian vegetation (1-4)	≤ 2
Connectivity (1-4)	≤ 2
Stream flow (1-4)	≤ 2

Variables	Boundary
Upstream dam influence (1-4)	≤ 2
Hydropeaking (1-4)	≤ 2
DO (mg/l)	6.39-13.70
N-NH_4^+ (mg/l)	≤ 0.09
N-NO_3^- (mg/l)	≤ 1.15
P-Total (mg/l)	≤ 0.07
P-PO_4^{3-} (mg/l)	≤ 0.06
% artificial areas (catchm)	≤ 1
% intensive agriculture (catchm)	≤ 11
% extensive agriculture (catchm)	≤ 32
% semi-natural areas (catchm)	≥ 68

5.2. Description of IC dataset

The national dataset contains data (both biological and non-biological) for 57 sites from the common intercalibration MedGIG river types – R-M1, R-M2 and R-M5 (Table 9). However, the type R-M5 was excluded from fitting process as it is explained in the introduction, so it remained 42 sites belonging to river types R-M1 and R-M2 for fitting exercise. The fitting exercise is carried out based on qualified national dataset of the national river monitoring in 2017 provided by Hrvatske vode. This dataset covers the whole Mediterranean part of Croatia covered by MedGIG. The data set contains macrophyte sample information, information on physical and chemical parameters of water, as well as information on land use and hydromorphological degradation for each site in the analysis. It can be concluded that the dataset encompasses sampling sites covering almost the entire gradient of the pressure to be intercalibrated, and hence the complete ecological quality gradient (Wilby et al. 2014).

Table 9. Number of macrophyte samples in the Croatian MedGIG intercalibration dataset broken by RI-HR classification into the five ecological status classes.

	total	H	G	M	P	B
R-M1	**17**	**7**	**2**	**6**	**2**	**-**
R-M2	**25**	**12**	**5**	**5**	**3**	**-**
R-M5	15	4	7	-	4	-

5.3. Description of intercalibration procedure

Step 1: *Calculate the common metric (CM) on the national dataset.*

The method used for the Option 1 intercalibration within the MedGIG was the French IBMR (Haury et al. 2006). The details on how this method was applied as a common metric for the MedGIG intercalibration are given in the MedGIG IC report (Aguiar et al. 2013) and in Aguiar et al. (2014).

The common MedGIG method IBMR (common metric or ICM) was calculated for the national dataset. IBMR was calculated using *Formularie saisie liste floristique calcul IBMR v.3.3*. (https://hydrobio-dce.irstea.fr/telecharger/macrophytes-rivieres-2/)
To calculate ICM-EQR values, IBMRs of the national dataset were divided by the median value of IBMR for benchmark sites, which is 12.09.

Step 2: *Use the associated pressure data to identify sites in the national dataset that meet the criteria established by the GIG for the selection of benchmark or reference sites.*

The procedure followed the approach of MedGIG and the developed common benchmark conditions. Thresholds established in the MedGIG for the final selection of benchmarks (Table 8) were applied to the national dataset. Comparison with the established thresholds for benchmarks (Feio et al. 2014) showed that eight sites (Table 10) fulfilled them.

Table 10. Benchmark sites of Croatian MedGIG selected according to thresholds established by Feio et al. (2014).

Site code	Name of the site	IC-type	HR-EQR	IBMR
40106	Potok Rumin (pritok Cetine)	R-M1	0.688	11.850
40443	Izvor Krke (pritok Une), granični prijelaz	R-M1	0.947	9.500
14006	Una, kod izvorišta Loskun	R-M2	0.797	12.050
14007	Una, nizvodno od D. Kraja	R-M2	0.750	8.730
40102	Cetina, Vinalić	R-M2	0.823	12.130
40104	Cetina, Barišići	R-M2	0.943	14.820
40205	Zrmanja, Palanka	R-M2	0.946	14.710
40199	Zrmanja, most na cesti Kostići-Vukmirice	R-M2	0.950	15.250

Step 3: *Standardise the common metric (CM_bm) against the benchmark according to the approach used in the completed exercise. If benchmark standardization was concluded not to be required in the completed exercise the mean CM value of the joining method's benchmark sites must lie inside the range of mean values of the benchmark sites of the methods already intercalibrated for this conclusion to remain applicable. If the joining method's benchmark sites lie outside of this range the joining method must benchmark standardise its sites relative to the global mean CM value of the benchmark sites included in the completed exercise.*

An overview of how benchmark standardization was carried out in the MedGIG is given in Table 11.

Table 11. Benchmarking approach in the MedGIG and benchmarking of the Joining HR method

	MS	No of BM sites	Mean ICM	Conclusion	
Completed exercise	Spain	21	1.04	0.05	MSs benchmark standardised the ICM by substracting the offset of their benchmark sites
	Portugal	13	0.98	-0.01	
	Italy	15	1.02	0.03	
	France	16	0.97	-0.02	
	Greece	10	0.91	-0.08	
	Cyprus	3	1.03	0.04	
	Slovenia	0			
	Global mean		**0.99**		
	Range		**0.91-1.04**		
Joining MS	Croatia	8	1.02	0.03	The joining HR method benchmark-standardises the ICM by subtracting the offset from the global mean in accordance with the completed exercise.

Step 4: *Use OLS regression to establish the relationship between CM_bm (y) and the EQR of the joining method (x). A specialist case is that when a joining method relies exclusively on the common metric developed in the completed exercise for its classification rather than devising an original method (then being more like Option 1). In such cases, a regression would be meaningless as y is directly dependent on x. The goal for an MS choosing to the use the CM as the basis for their method is simple – after any benchmarking their boundaries must simply lie within one quarter of class of the global mean view.*

The regression fulfils the minimum criteria defined by the EC, 2011 ($r^2 > 0.25$; $p>0.01$; $r > 0.5$). Figure 3 shows regression between CM and RI-EQR.

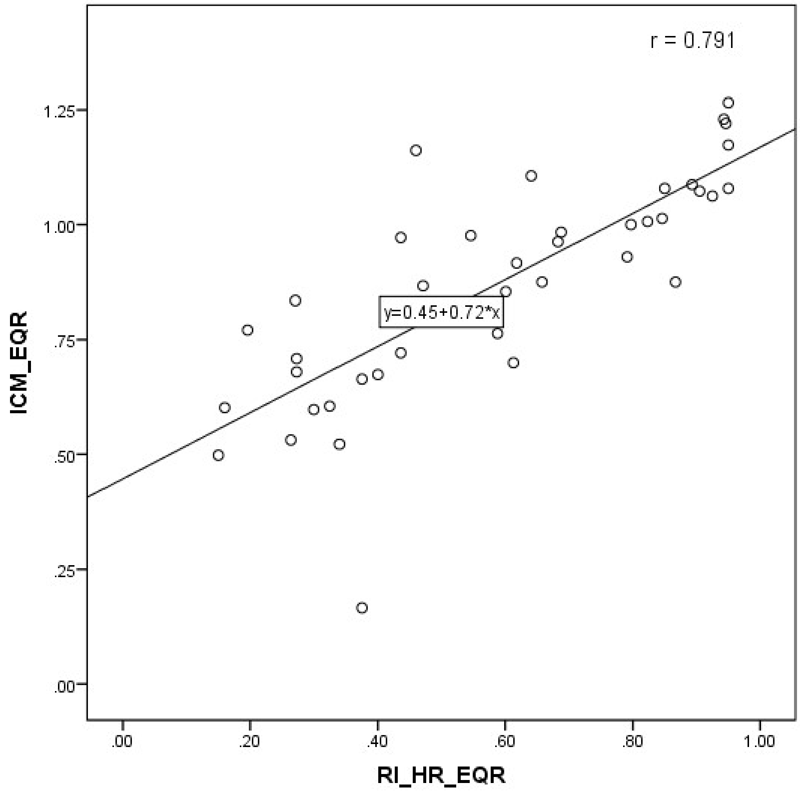

Figure 3. Regression between ICM (IBMR) and national RI-HR EQR ($r^2 = 0.625$, $p = 0.000$; $r = 0.791$ – Pearson correlation coefficient).

Step 5: *Predict the position of the national class boundaries (MP, GM, HG and reference) on the CM_bm scale.*

Using the formula given in Figure 3 the national boundaries can be converted to ICM-boundaries as follows in Table 12.

Table 12. Conversion of the national boundaries into ICM-boundaries

Boundary / reference	National types	RI-HR-EQR	ICM-EQR predicted	ICM-EQR MedGIG	Bias
Max	PF	1	1.17		
	BN	1	1.17		
H/G	PF	0.7	0.954	0.913	0.041
	BN	0.65	0.918	0.913	0.041
G/M	PF	0.49	0.803	0.754	0.049
	BN	0.49	0.803	0.754	0.049

Boundary / reference	National types	RI-HR-EQR	ICM-EQR predicted	ICM-EQR MedGIG	Bias
M/P	PF BN	0.29 0.24	0.659 0.623		

Step 6: *Apply the comparability criteria as summarised in Chapter 6.*

6.1. *Determine the direction of deviation of the national HG and GM boundaries of the joining method on the common metric scale relative to the global mean view defined in the completed exercise.*

The national H/G and G/M boundaries fall above the global view (Table 13).

6.2. *If the national GM boundary on the common metric scale falls below the global view…*

Not relevant.

6.3. *If the national GM boundary on the common metric scale falls above the global view, calculate the amount of this deviation and express it as a proportion of the width of the moderate status class on the common metric scale. If this value is ≤0.25, the boundary meets the comparability criteria. If >0.25 the GM boundary can be lowered until the deviation between the national GM boundary on the common metric scale and the global view on the same scale is ≤0.25 class widths. However, there is no obligation to make this adjustment. If the deviation is equivalent to >0.5 of the moderate class width, an adjustment is strongly recommended since this implies that the global view of the GM boundary of the countries that completed the exercise is closer to the MP boundary of the joining Member State.*

Table 13. Calculation of the amount of G/M bias expressed as a proportion of class width before and after harmonization of the G/M boundary.

	National types	Class width	Bias abs	Bias rel
G/M original	PF BN	0.151 0.115	0.049 0.049	0.321 0.421
G/M adjusted	PF BN	0.166 0.137	0.034 0.027	0.206 0.197

The G/M boundary for national type PF had to be adjusted from 0.49 to 0.47.

The G/M boundary for national type BN hat to be adjusted from 0.49 to 0.46.

6.4. *These steps should then be repeated for the HG boundary. Thus, if the national HG boundary on the common metric scale falls below the global view, calculate the amount of this deviation and express it as a proportion of the width of the high status class on the common metric scale. If this value is ≤0.25, the boundary meets the comparability criteria. If >0.25 the HG boundary must be raised until the deviation between the national HG boundary on the common metric scale and the global view on the same scale is ≤0.25 class widths. If the national HG boundary on the common metric scale falls above the global view, calculate the amount of this deviation and express it as a proportion of the width of the good status class on the common metric scale. If this value is ≤0.25, the boundary meets the comparability criteria. If >0.25, the HG boundary can be lowered until the deviation between the national HG boundary on the common metric scale and the global view on the same scale is ≤0.25 class widths. However, there is no obligation to make this adjustment. If the deviation is equivalent to >0.5 of the good class width, an adjustment is strongly recommended since this implies that the global view of the HG boundary of the countries that completed the exercise is closer to the GM boundary of the joining Member State.*

Table 14. Calculation of the amount of H7G bias expressed as proportion of class width.

	National types	Class width	Bias abs	Bias rel
H/G	PF	0.216	0.041	0.191
	BN	0.252	0.005	0.021

The amount of the deviation expressed as a proportion of class width is ≤ 0.25 and therefore meets the comparability criteria.

Piecewise linear transformation of class boundaries

Since in R-M1 and R-M2 intercalibration types in Croatia two site specific communities were recognized (PF and BN) the class boundaries were accorded using piecewise linear transformation.

Table 15. Classification of EQR values to ecological status classes and associated transformation equations in order to obtain uniform and common class ranges for macrophyte communities

Ecological status class	Community	EQR range	Uniform EQR range	Equation
Very good	PF	>0.65	>0.80	0.80+0.20*(OEK-0.65)/0.35
	BN	>0.60	>0.80	0.80+0.20*(OEK-0.60)/0.40
Good	PF	0.42-0.64	0.60-0.79	0.60+0.20*(OEK-0.42)/0.23
	BN	0.41-0.59	0.60-0.79	0.60+0.20*(OEK-0.41)/0.19
Moderate	PF	0.24-0.41	0.40-0.59	0.40+0.20*(OEK-0.24)/0.18
	BN	0.19-0.40	0.40-0.59	0.40+0.20*(OEK-0.19)/0.22
Bad	PF	0-0.23	0.20-0.39	0.20+0.20*(OEK)/0.24
	BN	0-0.18	0.20-0.39	0.20+0.20*(OEK)/0.19
Poor	PF	-	<0.20	-
	BN	-	<0.20	-

Conclusions

The national assessment method was compared with the finalized IC exercise of the MedGIG following the fit-in procedure of Willby et al. (2014). The analysis revealed a good agreement of the national method with the methods from other member states of the MedGIG. Following the criteria defined in the fit-in-procedure of Wilby et al. (2014), the national assessment method for Croatia with a slight adjusting of the G/M boundary is considered as comparable with the existing macrophyte-based method (IBMR).

6. DESCRIPTION OF THE BIOLOGICAL COMMUNITIES

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT HIGH STATUS

Type-specific reference species are dominant, pressure indictors are rare.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT GOOD STATUS

In good status, reference species are abundant, degradation indicators occur.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT MODERATE STATUS

In moderate status, degradation indicators dominate over reference species.

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Annex A. List of Croatian type specific indicator species for PF (bryophyte community) and BN (herbid community with other morphological types) in R-M1 and R-M2 rivers (A – reference indicators, B – indifferent taxa, C – degradation indicators).

Species	PF	BN
Acorus calamus	C	C
Agrostis stolonifera	B	B
Alisma lanceolatum	C	B
Alysma plantago-aquatica	C	B
Amblystegium serpens	B	B
Amblystegium varium	B	B
Apium repens	A	A
Batrachospermum sp.	A	A
Berula erecta	B	A
Bolboschoenus maritimus	C	C
Brachythecium rivulare	A	A
Bryum pseudotriquetrum	A	A
Butomus umbellatus	C	C
Caliergonella cuspidata	B	B
Callitriche cophocarpa	B	B
Callitriche hamulata	A	A
Callitriche obtusangula	B	B
Callitriche palustris	B	B
Callitriche platycarpa	B	B
Caltha palustris	B	B
Cardamine amara	B	B
Carex acuta	B	B
Carex acutiformis	B	B
Carex elata	B	B
Ceratophyllum demersum	C	C
Ceratophyllum submersum	C	C
Chara aspera	A	A
Chara contraria	A	A
Chara globularis	A	A
Chara hispida	A	A
Chara intermedia	A	A
Chara tomentosa	A	A
Chara vulgaris	A	A
Chara sp.	A	A
Cinclidotus riparius	A	A
Cinclodotus aquaticus	A	A
Cinclodotus danubicus	A	A
Cinclodotus fontinaloides	A	A
Cladophora sp.	C	C
Conocephalum conicum	A	A
Cratoneuron filicinum	A	A
Cyperus longus	B	B
Didymodon tophaceus	A	A
Drepanocladus aduncus	A	A
Egeria densa	C	C
Eleocharis palustris	C	C
Elodea canadensis	C	C
Equisetum arvense	C	C
Equisetum palustre	C	C

Species	PF	BN
Eurhynchium praelongum	B	B
Fontinalis antipyretica	A	A
Fissidens crassipes	B	B
Fissidens rufulus	A	A
Galium palustre	B	B
Glyceria fluitans	B	B
Glyceria maxima	C	C
Glyceria sp.	C	C
Heribaudiella fluviatilis	A	A
Hippuris vulgaris	B	A
Holoschoenus vulgaris	C	B
Hottonia palustris	C	B
Hydrocharis morsus-ranae	C	C
Hygroamblystegium tenax	B	B
Hygrohypnum luridum	A	A
Hydrocharis morsus-ranae	C	C
Hymenostylium recurvirostrum	A	A
Hyophila involuta	B	B
Iris pseudacorus	C	C
Juncus articulatus	B	B
Juncus bulbosus	B	A
Juncus compressus	B	B
Juncus inflexus	B	B
Jungermannia atrovirens	A	A
Lemanea sp.	A	A
Lemna gibba	C	C
Lemna minor	C	C
Lemna trisulca	C	B
Leptodyctium riparium	C	C
Lophocolea bidentata	B	B
Lunularia cruciata	B	B
Lycopus europaeus	C	C
Lysimachia nummularia	C	C
Lysimachia vulgaris	C	C
Lythrum salicaria	C	C
Marchantia polymorpha	B	B
Mentha aquatica	B	B
Myosotis scorpioides	B	B
Myriophyllum spicatum	B	B
Myriophyllum verticillatum	C	B
Najas marina	C	C
Najas minor	C	C
Nasturtium officinale	B	B
Nitella spp.	A	A
Nitellopsis obtusa	A	A
Nuphar lutea	C	C
Nymphaea alba	C	C
Nymphoides peltata	C	B
Oenanthe aquatica	C	C
Oenanthe cf. *fistulosa*	B	B
Palustriella commutata	A	A
Pellia endiviaefolia	A	A

Species	PF	BN
Phalaris arundinacea	C	B
Phragmites australis	C	B
Plagiomnium undulatum	B	B
Platyhypnidium riparioides	A	A
Pohlia ludwigii	B	B
Polygonum amphibium	B	B
Polygonum hydropiper	B	B
Polygonum lapathyfolium	C	C
Potamogeton berchtoldii	C	C
Potamogeton crispus	C	C
Potamogeton gramineus	C	B
Potamogeton lucens	B	B
Potamogeton natans	C	B
Potamogeton nodosus	C	C
Potamogeton pectinatus	C	C
Potamogeton perfoliatus	C	A
Potamogeton pusillus	C	C
Potamogeton trichoides	C	C
Pulicaria dysenterica	C	C
Ranunculus aquatilis	B	B
Ranunculus circinatus	B	B
Ranunculus flammula	B	A
Ranunculus fluitans	B	A
Ranunculus peltatus	B	A
Ranunculus repens	C	C
Ranunculus sceleratus	C	C
Ranunculus trichophyllus	B	A
Riccia fluitans	B	A
Rorippa amphibia	C	B
Rorippa sylvestris	C	C
Rumex hydrolapathum	C	C
Sagittaria sagittifolia	C	B
Scirpus lacustris	C	B
Sparganium emersum	C	B
Sparganium erectum	C	B
Spirodella polyrhiza	C	B
Spirogyra sp.	C	C
Thamnobryum alopecurum	A	A
Tolypela spp.	A	A
Trapa natans	C	C
Typha angustifolia	C	B
Typha latifolia	C	B
Ulva sp.	C	C
Urtica dioica	C	C
Utricularia australis	C	A
Utricularia vulgaris	C	A
Veronica anagalis-aquatica	B	B
Veronica anagalloides	B	B
Veronica beccabunga	B	B
Veronica catenata	B	B
Zanichellia palustris	C	C

Report on fitting the Croatian classification method for benthic macroinvertebrates in rivers to the results of the completed intercalibration of the Eastern-Continental GIG (R-E2 and R-E3)

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Report on fitting a macroinvertebrate classification method with the results of the completed intercalibration of the EC GIG (R-E2 and R-E3)

1. INTRODUCTION

- Croatia;
- Benthic macroinvertebrates;
- R-E2 and R-E3 river types.

2. DESCRIPTION OF NATIONAL ASSESSMENT METHODS

The Water Framework Directive requires comprehensive assessment methods for the evaluation of river ecological statuses according to the benthic macroinvertebrate fauna, which includes taxonomic composition, abundance, the ratio of disturbance of sensitive taxa to tolerant taxa and diversity. It is also required to harmonize national assessment methods under the intercalibration exercise with other Eastern – Continental (EC) Geographic Intercalibration Group (GIG) country methods. The official intercalibration of invertebrate-based methods of ecological status assessment in Eastern Continental rivers was finalized within the EC-GIG intercalibration in 2011 (Opatrilova, 2011).

A new assessment method has been developed for ecological status assessment of rivers belonging to the IC types R-E2 (= HR-R_3C and HR-R_4A) and R-E3 (= HR-R_3D, HR-R_4B and HR-R_4C) based on invertebrates and presented in this report. Both IC types are treated together due to the relatively small data sets (R-E2 type: n=19; R-E3 type: n=13). Because of the similarities between the two types (medium and large lowland rivers) their data set can be considered as somewhat complementary, with type specific development of lower and upper anchors for metric normalization. The method is compliant with the WFD normative definitions and its class boundaries are in line with the results of the completed intercalibration exercise.

The Croatian assessment method based on benthic invertebrates is a modular type with two modules: saprobity and general degradation. The modular system uses the "one-out all-out" principle. Croatian Large Rivers benthic invertebrate assessment method is based on the same approach and it has been successfully intercalibrated (Birk et al., 2016). The system consists of metrics with proven relationships to stressors.

The classification method is verified for WFD compliance and IC feasibility and the class boundaries were compared with agreed boundaries from the EC-GIG intercalibration exercise following the instructions of the CIS Guidance Document 30: "Procedure to fit new or updated classification methods to the results of a completed intercalibration exercise" (Willby et al., 2014). Intercalibration "Option 2" - indirect comparison of assessment methods using a common metric and "continuous benchmarking" approach was used for the intercalibration of methods in EC GIG River Benthic Macroinvertebrate group and the Croatian methodology was compared with finalized results.

2.1. METHODS AND REQUIRED BQE PARAMETERS

The Saprobity module represents normalized values of the Croatian saprobity index (SI_{HR}), which is based on the Pantle Buck index, but with adapted indicator values. The General Degradation module is normalized multimetric index (General $Degradation_{MI}$) that consists of 4 metrics: EPTCBO (Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Bivalvia, Odonata), ASPT (Average score per taxon), Diversity (Margalef Index) and the River fauna index (RFI) that is based on indicator responses to hydromorphological degradation.

The Croatian national method is in accordance with the WFD compliance, as it takes into consideration all the indicative parameters that are mentioned in CIS Guidance document No 14 (2011): taxonomic composition, abundance, disturbance sensitive taxa to insensitive taxa, diversity and absence of major taxonomic groups (Table 1).

Table 1. Overview of the metric groups included in the Croatian national method for the assessment of IC types R-E2 and R-E3

MS	Taxonomic composition	Abundance	Sensitive / tolerant taxa	Diversity	Absence of major taxonomic groups
HR	x	x	x	x	x

Combination rule used in the method:
The Saprobity module is based solely on EQR of the SI_{HR} index. The General $Degradation_{MI}$ equals the average EQR of all four metrics. The final assessment result equals the lower EQR value of the two modules.

Conclusion on the WFD compliance: all the indicative parameters included.

2.2. SAMPLING AND DATA PROCESSING

Description of sampling and data processing:

- Sampling time and frequency;
  The most favorable sampling time is spring (March-April), i.e. before mass swarms of adult insects come out which takes place in May and June. The period of stable and low water levels should last long enough before sampling so that the macrozoobenthic community can be well developed. Sampling shall not be undertaken: during high water levels and up to 3 weeks after high water levels, during all other disturbances caused by natural processes.
- Sampling method;
  All available microhabitats are sampled („multi-habitat sampling ") and 20 sub-samples are collected which are distributed according to the proportion of microhabitat types, with microhabitats that are less than 5% present are not sampled, but are recorded in the protocol. Microhabitat type represents a combination of inorganic and organic substrate. Sub-samples are sampled by raising the substrate that consists of a substrate with accompanying animals from surface size 25 x 25 cm (0.0625 m^2). The channel substrate of each sampling site was classified according to AQEM Consortium (2002).
- Data processing
  EPTCBO (Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Bivalvia, Odonata), ASPT (Average score per taxon) and Diversity (Margalef Index) are calculated using ASTERICS 4.04 software, while the Croatian saprobity index and River fauna index are calculated separately. SI_{HR} is an adapted saprobity index according to Pantle-Buck (1955):

$$SI_{HR} = \frac{\sum SIu_i}{\sum u_i}$$

where:

SI_{HR} = Croatian saprobity index
SI = individual species/taxa indicator value
u_i = number of individuals calculated per 1 m^2

Indicator values of macrozoobenthic taxa (SI) are specific to Croatia.
The River fauna index was calculated according to the following equation:

$$\mathrm{RFI}_{VR_j} = \frac{\sum_{\mathrm{i=1}}^{\mathrm{n}} \mathrm{ac_i} \times \mathrm{Rf_i} \times \mathrm{HW_i}}{\sum_{\mathrm{i=1}}^{\mathrm{n}} \mathrm{ac_i} \times \mathrm{HW_i}}$$

where:
ac_i is the log5 abundance class of the i^{th} taxon,
Rf_i is the river fauna value of the i^{th} taxon,
HW_i is the hydromorphological indicative weight of the i^{th} taxon
n is the number of indicative taxa

Indicator values of macrozoobenthic taxa (RFi and HWi) are specific to IC types R-E2 and R-E3 and are calculated by canonical correspondence analysis of the taxa found in these types with regard to hydromorphological pressure (Urbanič, 2014).

- Identification level;

  It is recommended that identification is conducted as detailed as possible, up to the level of species if possible (Table 2). Required level of macrozoobenthos identification:

Table 2. Level of identification required for the Croatian national assessment (Mihaljević et al., 2011)

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		

2.3. NATIONAL REFERENCE CONDITIONS

The settings for the national reference conditions of some chemical thresholds are given in the legal document Regulation on water quality standards (Uredba o standardu kakvoće voda, NN 96/2019), but this document is currently in the process of revision. Because of this, reference thresholds for this incercalibration fit in procedure follow those of the EC-GIG defined for IC types R-E2 and R-E3 (Opartilova, 2011):

Hydromophological alternation NO or LOW (scoring set at $\leq$2)

NO or LOW:

impoundment, hydropeaking, water abstraction, upstream dam influence, water temperature modification, channelization, alteration of riparian vegetation, local habitat alteration, dykes, toxic risk, water acidification, navigation, recreational use

Land-use in the catchment
<0.8% Urban land cover
< 50 Land Use Index

and

Chemical thresholds:
mean BOD_5 <2.4 mg/l
mean P-PO_4 <0.04 mg/l
mean N-NO_3 <6 mg/l
mean N-NH_4 <0.1 mg/l

It is also important that no point-source or non-point source pollutants are present near the reference site.

2.4. NATIONAL BOUNDARY SETTING

Within the dataset (with complete pressure and biology data) for E2 and E3 IC types, no true reference sites were present, meaning that when calculating reference values for indices (modules) alternative approaches had to be taken. Reference metric values were calculated by adding 20% of the total metric range to the High/Good boundary, whereas the H/G boundaries were determined as the 75th percentile of the benchmark sites.

Croatian saprobity index
The lower anchor of the SI_{HR} represents the worst theoretical value of the metric (on the basis of operational taxa list) and equals 3.6 (for all IC types). The value of the SI_{HR} in type R-E2 ranged from 1.81 to 2.98 and in type R-E3 ranged from 1.54 to 3.48. After selecting benchmark sites from both types, the HIGH/GOOD was calculated as the 75th percentile of the SI_{HR} benchmark values (Table 3). The high/good boundary for the SI_{HR} in R-E2 equaled 1.96 and in R-E3 2.30. Other boundaries were distributed equidistantly to 3.6 separately for each IC type. 20% of the total range (maximum value of SI_{HR} being 3.6) of each type was subtracted from the high/good boundary in order to calculate reference values. For the IC type R-E2 reference values of the SI_{HR} equaled 1.60 and for R-E3 reference values equaled 1.89.

Table 3. Determined benchmark sites of IC type R-E2 and E3 following the criteria of Opartilova (2011)

*"*At least four out of seven parameters used for the screening (chemical parameters + land-use index + ASPT) had to fit within the given range;*

Code	IC Type	Name	LUI	P-PO4 (mgP/l)	N-NO3 (mgN/l)	BOD5 (mgO_2/l)	N-NH4 (mgN/l)	ASPT	Conductivity	SI_{HR}
21085	E2	Bednja, Mali Bukovec	73.73	0.05	1.07	2.33	0.13	6.22	549.97	2.17
15591	E2	Zelina, Božjakovina	109.00	0.12	1.49	2.36	0.15	6.19	610.01	1.96
15355	E2	Česma, Pavlovac	84.71	0.08	0.71	3.78	0.04	5.08	542.83	2.52
16220	E2	Odra, Sisak	124.44	0.051	0.98	1.99	0.25	5.25	523.56	2.01
18005	E2	Sutla, Luke Poljanske	101.73	0.087	1.15	1.99	0.19	5.588	615.78	1.81
15226	E3	Ilova, Maslenjača	50.71	0.041	0.62	2.98	0.1	4.99	360.17	2.59
14001	E3	Una, most na utoku	55.01	0.038	0.62	1.02	0.02	4.96	432.25	2.64
15483	E3	Oteretni kanal Lonja - Strug (Trebež), ustava Trebež	116.5	0.134	0.7	4.14	0.22	5.33	480.11	2.45
16229	E3	Glina, Skela	79.77	0.246	0.78	2.08	0.25	6.35	394.22	1.90

three parameters could be below (or above in case of ASPT) than the given range." For the hydromorphological screening parameters it was required that each site has the appropriate parameter values equal to „no" or „low" status while also allowing for three „medium" statuses at most and just one „high" status as well.

BOD5	*conductivity*	*land-use index (4*artificial + 2*int.agriculture + non-int.agriculture)*	*P-PO4*	*N_NO3*	*N_NH4*	*ASPT*
2.4 - 4.1	*250 - 620*	*50 - 170*	*0.04 - 0.25*	*2.0 - 6.0*	*0.1-0.25*	*5.0 - 6.4*

General deradation

The General Degradation$_{MI}$ equals the average EQRs of four metrics: Diversity (Margalef Index), River fauna index (RFI), ASPT (Average score per taxon) and EPTCBO (number of Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Bivalvia and Odonata taxa). Reference values for each of the metrics were calculated by adding 20% of the metric range to the high/good boundary (Table 4 and 5). The high/good boundaries of all the metrics were calculated as the 75th percentile value of all the benchmark sites. Lower anchors for both R-E2 and R-E3 were set as the worst metric value in both IC-types.

Table 4. Class boundaries and ranges of the metrics used in calculating the General Degradation$_{MI}$ in IC type R-E2

Metric group:	Diversity	Sensitive / tolerant taxa Abundance	Sensitive / tolerant taxa	Absence of major taxonomic groups Taxonomic composition
R-E2 metric boundaries	**Diversity (Margalef Index)**	**RFI**	**ASPT**	**EPTCBO**
Upper anchor	6.13	0.52	6.87	25.2
Lower anchor	2.37	-0.11	1.82	4
High/good boundary	4.83	0.40	6.19	17
Range:				
max	9.46	0.52	6.3	46
min	2.98	-0.11	3.4	5

Table 5. Class boundaries and ranges of the metrics used in calculating the General Degradation$_{MI}$ in IC type R-E3

Metric group:	Diversity	Sensitive / tolerant taxa Abundance	Sensitive / tolerant taxa	Absence of major taxonomic groups Taxonomic composition
R-E3 metric boundaries	**Diversity (Margalef Index)**	**RFI**	**ASPT**	**EPTCBO**
Upper anchor	7.34	0.79	6.35	21.8
Lower anchor	2.37	-0.11	1.82	4
High/good boundary	6.25	0.64	5.59	16
Range:				
max	7.82	0.78	6.71	29
min	5.45	0.02	2.9	4

The transformation of the boundary values of five ecological status classes was defined based on the changes in the portion of sensitive and tolerant taxa (Figure 1). Sensitive and tolerant taxa were determined in calculating the River fauna index with regard to hydromorphological stressors. The ratio of tolerant taxa begin to increase (high/good boundary) at EQR = 0.81, whereas at EQR = 0.62 the portion of tolerant taxa reach the portion of sensitive taxa (good/moderate boundary). The intersection of regression curves representing a portion of tolerant and portion of sensitive taxa occurred approximately at the EQR = 0.56 and at the EQR = 0.45 the portion of tolerant taxa exceed the portion of sensitive taxa (moderate/poor boundary). The portion of tolerant taxa start to dominate at EQR = 0.22 (poor/bad boundary).

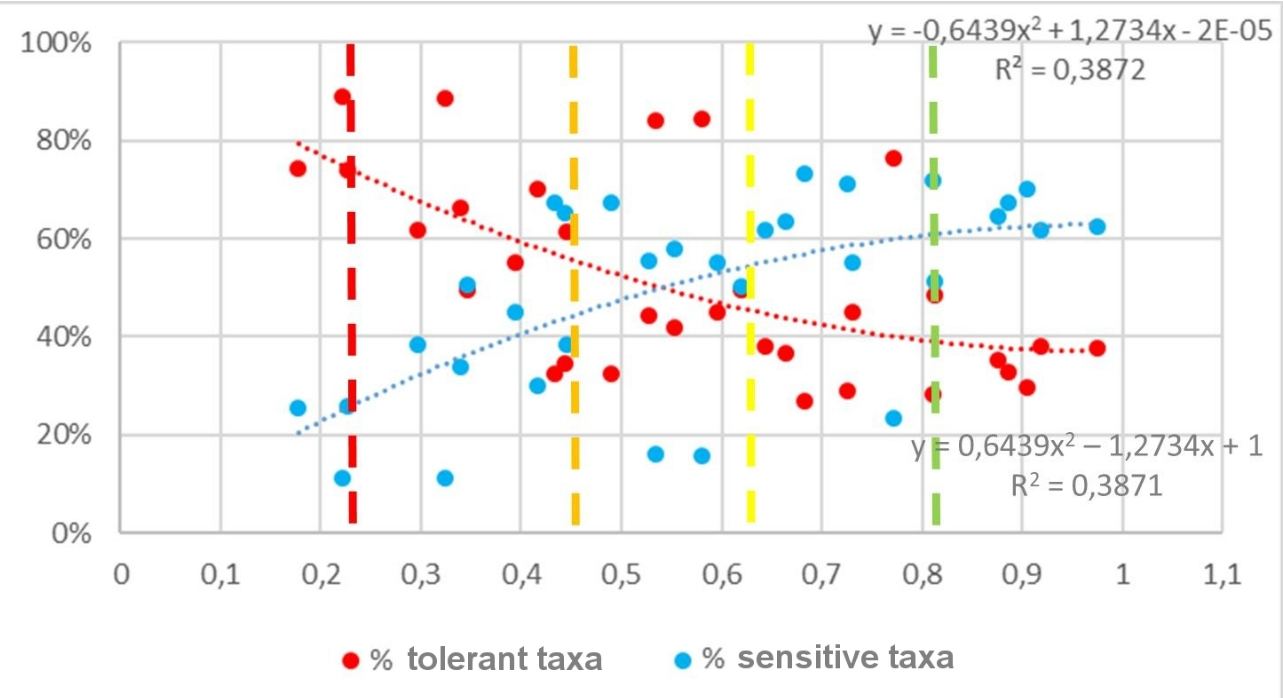

Figure 1. Boundary setting between ecological status classes using changes in a portion of sensitive and tolerant taxa along with the ecological quality ratio of the General Degradation$_{MI}$.

Values of the General Degradation$_{MI}$ for both R-E2 and R-E3 were transformed using following equations:

R-E2 and R-E3

EQR GEN DEG R-E2 and R-E3	**EQR**transform
≥0,81	0,8 + 0,2*(EQR GEN DEG R-E2 and R-E3 - 0,81) / 0,19
0,62 - 0,81	0,6 + 0,2*(EQR GEN DEG R-E2 and R-E3 - 0,62) / 0,19
0,45- 0,62	0,4 + 0,2*(EQR GEN DEG R-E2 and R-E3 - 0,45) / 0,17
0,22 - 0,45	0,2 + 0,2*(EQR GEN DEG R-E2 and R-E3 - 0,22) / 0,23
≤0,22	0,2*(EQR GEN DEG R-E2 and R-E3) / 0,22

2.4. NATIONAL BOUNDARY SETTING

In the intercalibration of types R-E2 and R-E3 the boundaries of the General Degradation$_{MI}$ were transformed. This means that the final EQR represents the “classical” boundaries (0.8; 0.6; etc.), seeing as the final value represents the lower value of the already transformed EQR-s of the two modules.

2.5. PRESSURES ADDRESSED

Various pressures were addressed by the different methods in the finalized IC exercise. Most countries indicated as detected general degradation, hydromorphological degradation and pollution by organic matter.
The Croatian method addresses catchment land use, pollution by organic matter, eutrophication and habitat destruction. The Saprobity module addresses organic pollution, whereas other stressor responses are integrated in the General Degradation module. The lower value of the two modules is

the final score of the site and it gives a direct suggestion on which stressor should be addressed primarily if the score would be less favorable. This method is therefore comparable to the methods which are already successfully intercalibrated.

The following pressure-response relationships have been derived:

1) Saprobity module

R-E2

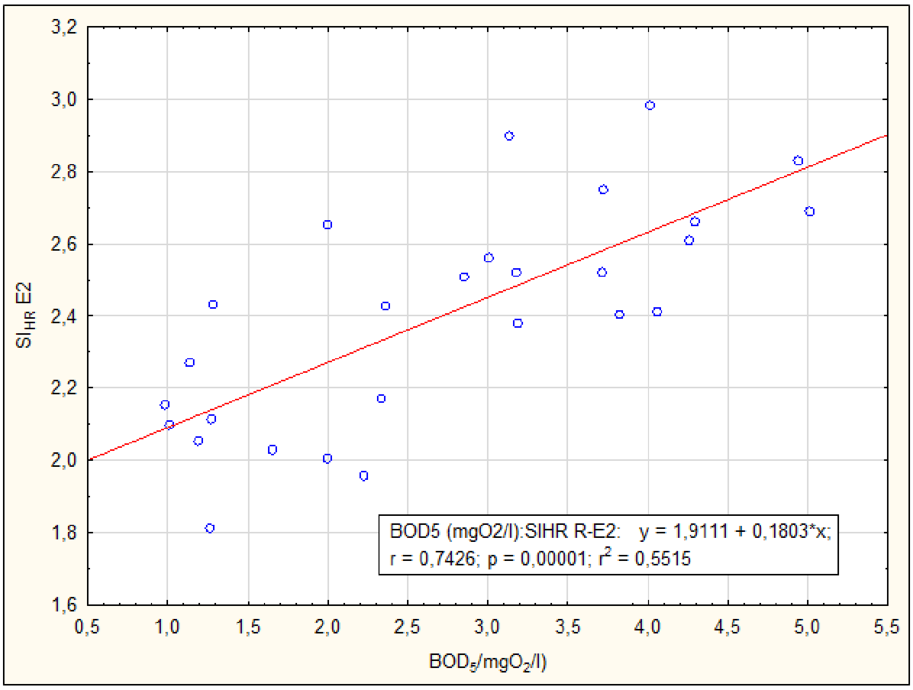

Figure 2. Pressure-Response relationship between biological oxygen demand (BOD_5) and the normalized SI_{HR} values in river type R-E2.

R-E3

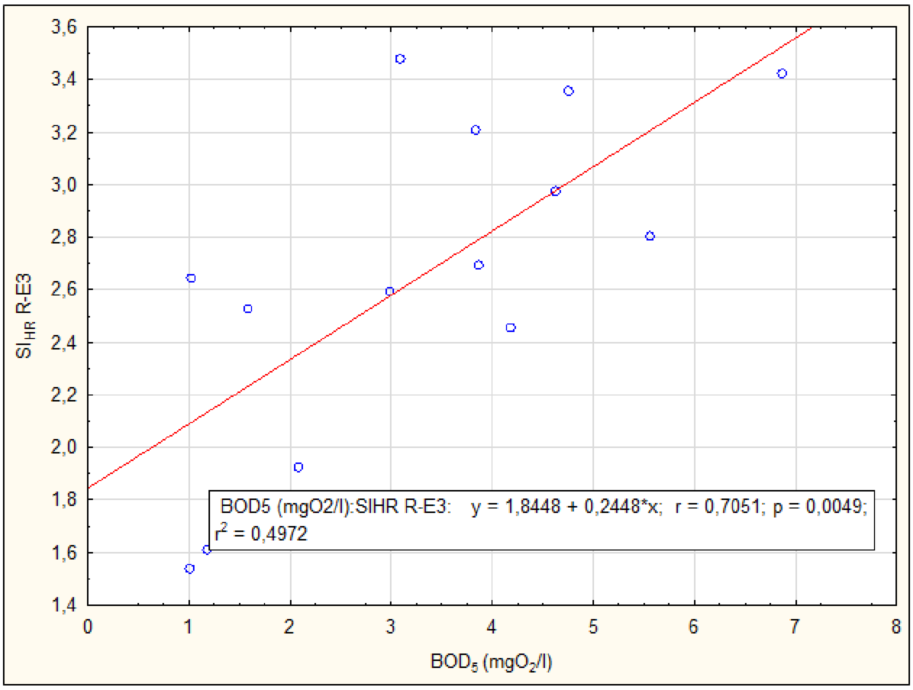

Figure 3. Pressure-Response relationship between biological oxygen demand (BOD_5) and the normalized SI_{HR} values in river type R-E3.

2) General degradation module

A. Land use

R-E2

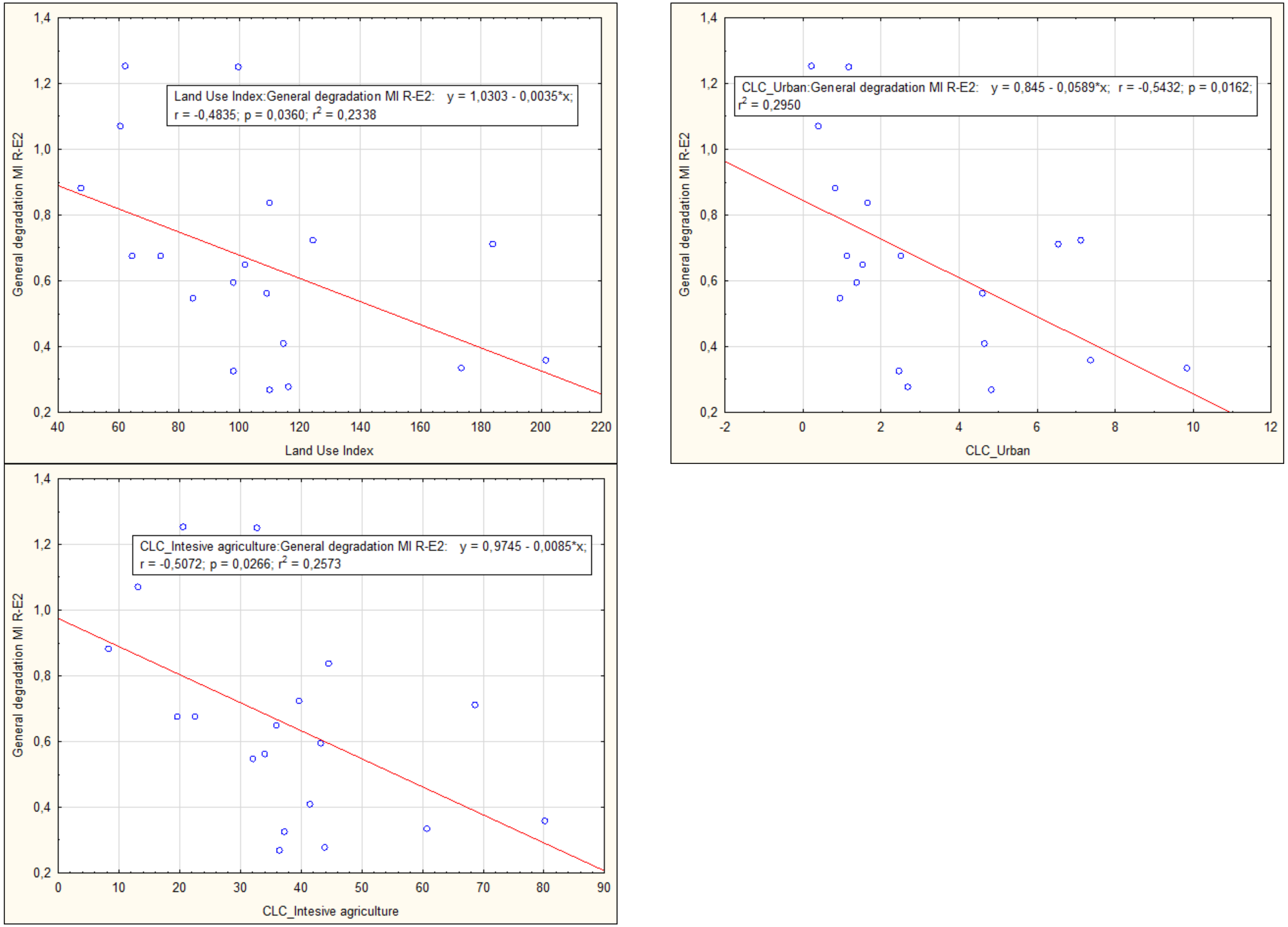

Figure 4. Pressure-Response relationship between the Land Use Index LUI and Corine Land Cover (categories urban and intensive agriculture) against the General Degradation$_{MI}$ for sites of river type R-E2. **No significant regressions were found for land cover in R-E3.**

B. Hydro-chemistry
R-E2

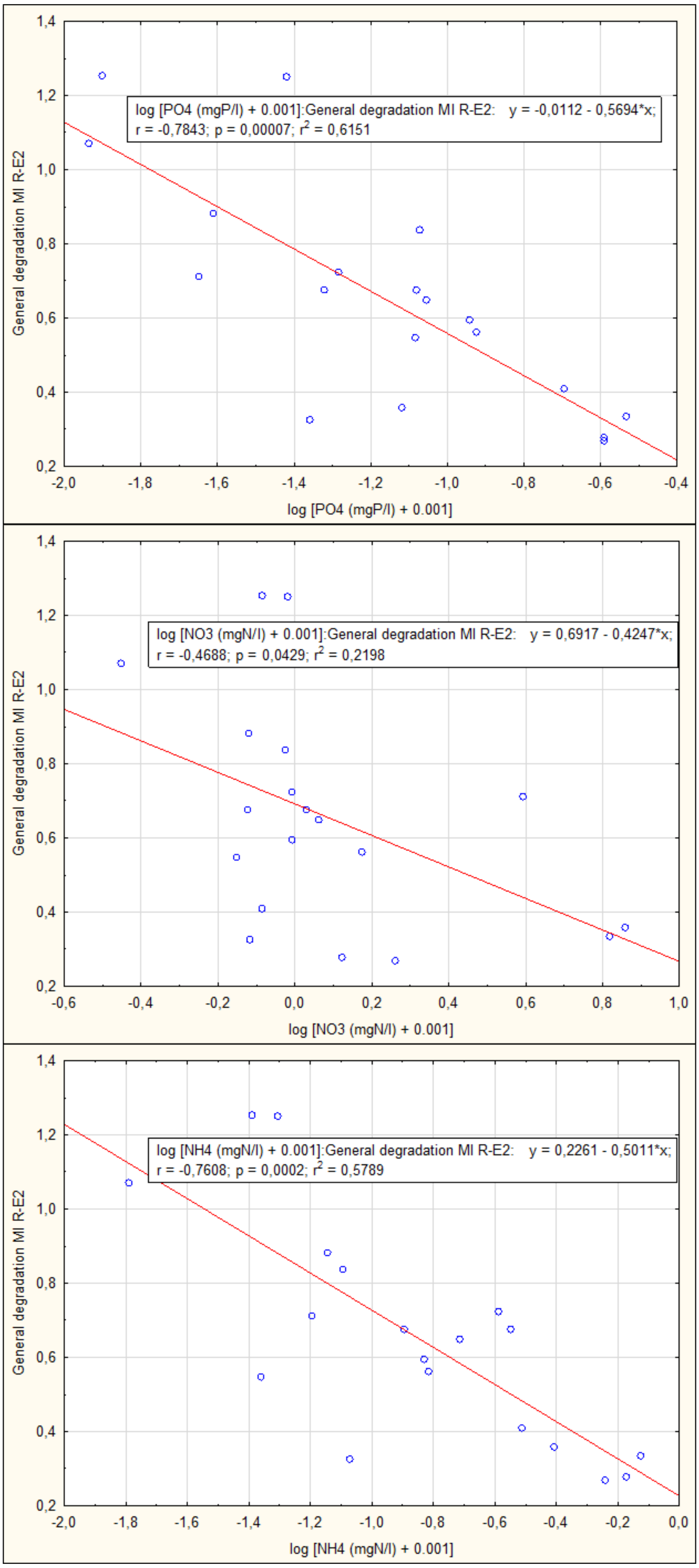

Figure 5. Pressure-Response relationship between chemical water properties against the General Degradation$_{MI}$ for sites of river type R-E2

R-E3

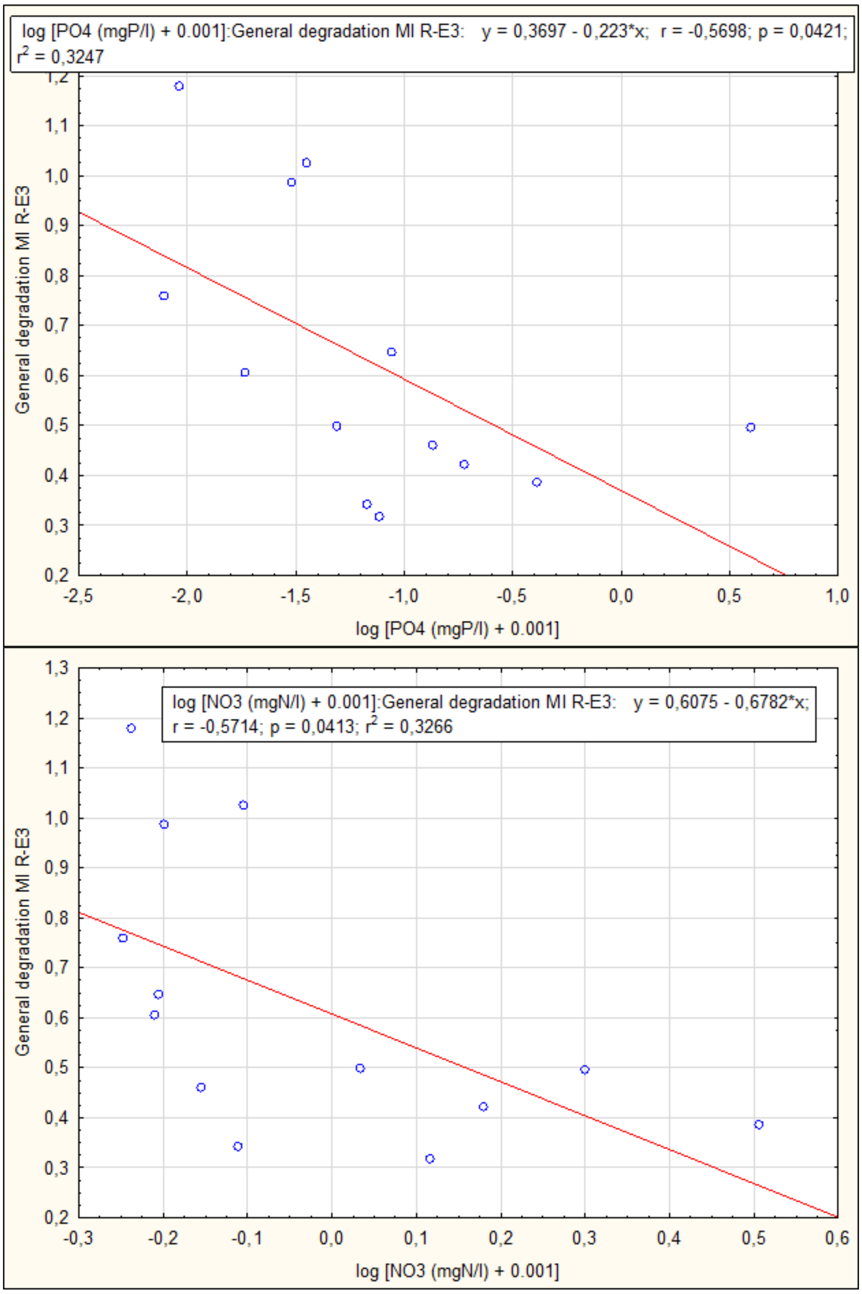

Figure 6. Pressure-Response relationship between chemical water properties against the General Degradation$_{MI}$ for sites of river type R-E3.

C. Hydromorphology

R-E2 and R-E3

Pressure impact relationships between hyromorphology scores and the Geeral degradation$_{MI}$ are jointly displayed for IC typec R-E2 and R-E3 as they were commonly analyzed in the construction of the River fauna index (RFI).

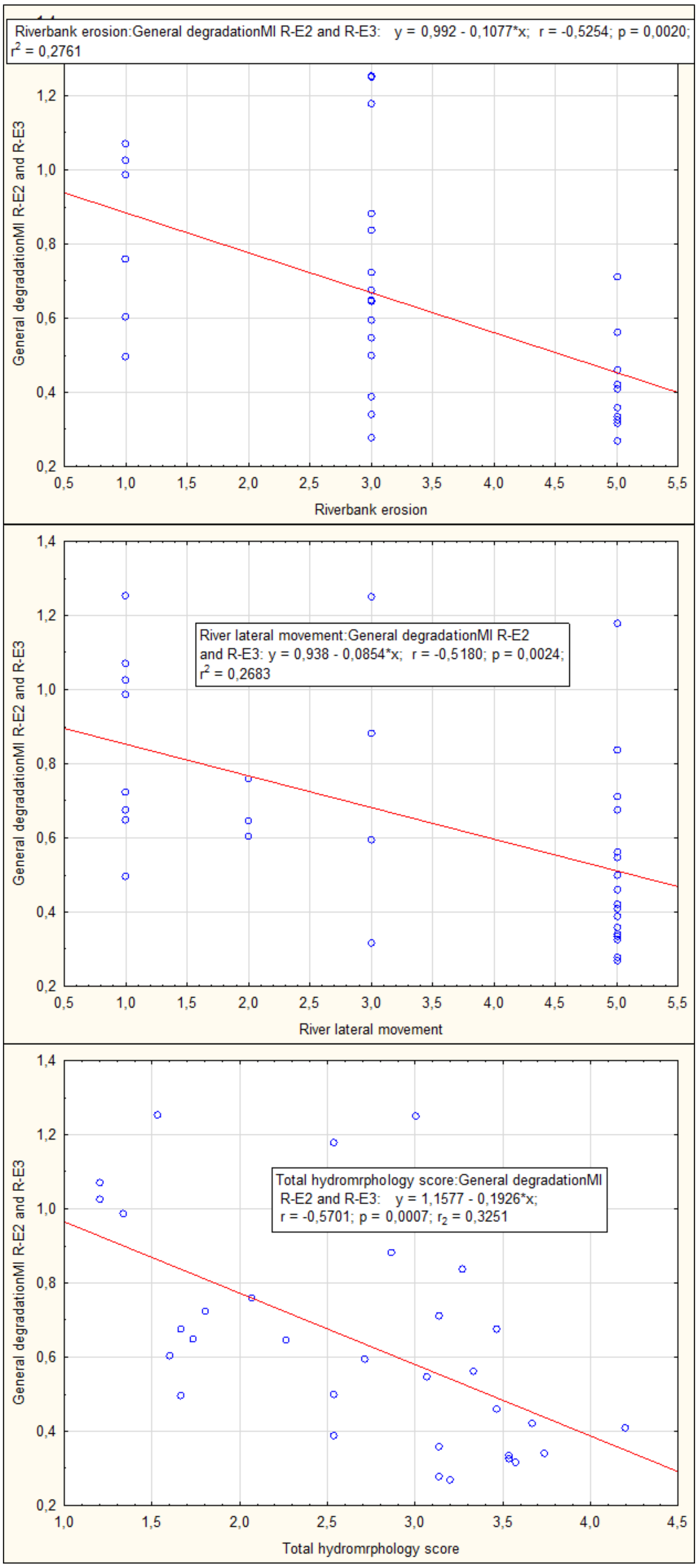

Figure 7. Pressure-Response relationship between hydromorphological river features against the General Degradation$_{MI}$ for sites of river types R-E2 and R-E3

A. Resume

For all four groups of pressures (organic pollution, land use, chemistry, hydromorphological degradation), significant regressions could be found (for land use only for type R-E2). The General Degradation$_{MI}$ showed no significant correlations (regressions) with land use for sites of IC type R-E3 as there are no sites that were not impacted by intensive agriculture (See in chapter 4.2.- all sites have high agricultural land use). It is concluded that both the SI$_{HR}$ and the General Degradation$_{MI}$ clearly respond to anthropogenic impacts and can be used for the assessment of the ecological status.

3. WFD COMPLIANCE CHECKING

The first step in the Intercalibration process requires the checking of national methods considering the WFD compliance criteria. The compliance check showed that the Croatian method fulfils the requirements of the WFD (Table 6).

Table 6. List of the WFD compliance criteria and the WFD compliance checking process and results

Compliance criteria	Compliance checking
Ecological status is classified by one of **five classes** (high, good, moderate, poor and bad).	yes
High, good and moderate ecological status are set in line with the WFD's **normative definitions** (**Boundary setting procedure**)	yes
All relevant parameters indicative of the biological quality element are covered (see Table 1 in the IC Guidance). A **combination rule** to combine parameter assessment into BQE assessment has to be defined. If parameters are missing, Member States need to demonstrate that the method is sufficiently indicative of the status of the QE as a whole	yes
Assessment is adapted to **intercalibration common types** that are defined in line with the typological requirements of the Annex II WFD and approved by WG ECOSTAT	yes
The water body is assessed against **type-specific near-natural reference conditions**	yes
Assessment results are expressed as **EQRs**	yes
Sampling procedure allows for **representative** information about water body quality/ecological status **in space and time**	yes
All data relevant for assessing the biological **parameters** specified in the WFD's normative definitions are covered by the **sampling procedure**	yes
Selected taxonomic level achieves adequate **confidence and precision** in classification	yes

4. IC FEASIBILITY CHECKING

The intercalibration process ideally covers all national assessment methods within a GIG. However, the comparison of dissimilar methods ("apples and pears") has to clearly be avoided. Intercalibration exercise is focused on specific type / biological quality element / pressure combinations. The second step of the process introduces an "IC feasibility check" to restrict the actual intercalibration analysis to methods that address the same common type(s) and anthropogenic pressure(s) and follow a similar assessment concept.

4.1. TYPOLOGY

The EC-GIG includes ten types (not including very large rivers), six of which are relevant for Croatia (Table 7). Two types, R-E2 and R-E3, are treated in this report.
The biological typology of running waters in Croatia was initially established in 2011 (Mihaljević et al., 2011), mainly based on expert opinion, due to general lack of all data types: both biological and pressure data. Today, biological data in most types are sufficient, as well as data on pressures such as water chemistry and land use. The data sets are still lacking hydromorphological scoring from many sites as the hydromorphological evaluation of running waters in Croatia began only recently, in 2017. The current assessment method has equal reference and "worst" metric values for several Croatian types, but in the future, with more data on hydromorphology we wish to fine-tune these values for every type. Hence, the typology will remain as initially determined.

Table 7. IC types of the EC-GIG

Type	Common intercalibration type	Ecoregion (Illies, 1967)	Catchment area [km²]	Altitude [m]	Geology	Channel substrate	Croatian type
R-E1a	Carpathians: small to medium, mid-altitude	10	10 - 1,000	500 - 800	mixed		/
R-E1b	Carpathians: small to medium, mid altitude	10	10 -1,000	200 - 500	mixed		/
R-E2	**Plains: medium-sized, lowland**	**11,12**	**100 - 1,000**	**< 200**	**mixed**	**sand and silt**	**HR-R_3C HR-R_4A**
R-E3	**Plains: large, lowland**	**11,12**	**> 1,000**	**< 200**	**mixed**	**sand, silt and gravel**	**HR-R_3D HR-R_4B HR-R_4C**
R-E4	Plains: medium-sized, mid-altitude	11,12	100 - 1,000	200-500	mixed	sand and gravel	/
R-EX4	Large, mid-altitude	10, 11, 12	> 1,000	200 - 500	mixed	gravel and boulder	/
R-EX5	Plain: small lowland	11, 12	10 -100	< 200	mixed	sand and silt	HR-R_2A HR-R_2B HR-R_3A HR-R_3B
R-EX6	Plain: small, mid-altitude	11, 12	10-100	200-500	mixed	gravel	HR-R_1
R-EX7	Balkan: mid-altitude, small-sized, calcareous, karst spring	5	10 – 100	200 - 500	calcareous	gravel	HR-R_6
R-EX8	Balkan: small to medium-sized, calcareous, karst spring	5	10-1000		calcareous	gravel, sand and silt	HR-R_7 HR-R_8A HR-R_9

Name of HR type		HR TYPE	IC TYPE
Lowland alluvial running waters	Medium lowland alluvial running waters with clay and sand substrate	HR-R_3C	R-E2
	Large lowland alluvial running waters with clay and sand substrate	HR-R_3D	R-E3
Medium lowland running waters		HR-R_4A	R-E2
Large lowland running waters		HR-R_4B	R-E3
Large lowland running waters with spring located in Dinaric eco-region		HR-R_4C	R-E3

4.2. PRESSURES ADDRESSED

The pressure gradient has been assessed for the Corine Land Cover (CLC) as well as the land use index, which is derived from CLC and defined as:

LUI = 4 * CLC urban+2*CLC intensive agriculture + CLC extensive agriculture

The ranges of the CLC and LUI in the two river types are:

CLC/LUI	range R-E2	range R-E3
CLC urban	0.21 – 9.84	0.41 – 2.93
CLC agr.intens.	8.28 – 80.20	10.64 – 47.02
CLC agr. extens.	6.04 – 32.69	13.79 – 28.19
LUI	47.59 – 201.52	40.63 – 119.42

The hydromorphological alteration scale ranges from 1 (no) to 5 (high) and consists of multiple smaller indices. The three main indices: hydrology regime, morphology and flow continuity ranged from 1 to 5 in both river types, whereas the mean hydromorphological score ranged from 1.2 to 4.2 in R-E2 and 1.2 to 3.73 in R-E3.

The ranges for the chemical variables tested are:

Chemical variable	range R-E2	range R-E3
BOD_5 [mg/l]	0.98 – 4.94	1.01 – 6.86
COD [mg/l]	1.20 – 10.45	1.68 – 10.82
PO4-P [mg/l]	0.01 – 0.29	0.01 – 3.94
NO3-N [mg/l]	0.35 – 7.22	0.56 – 3.20
NH4-N [mg/l]	0.02 – 0.75	0.01 – 7.41
Conductivity (µS/cm)	218.04 – 792.82	360.17 – 622.0

The different pressure gradients covered by the national data set are considered to be sufficient.

4.3. ASSESSMENT CONCEPT

Does the national method follow the same assessment concept as other methods in the Intercalibration group? Provide evaluation of IC feasibility regarding assessment concept of the intercalibrated methods.

The pressure gradient covered by the national data set is considered sufficient. The data acceptance criteria as defined in the GIG report from 2011 was used.

The data quality is considered as good, since:

1) the sampling and analytical methodology is comparable among all countries (multi-habitat sampling, at least 500 µm).

2) the identification level used for R-E2 and R-E3 is sufficient

3) the number of sites used for the 2 types is considered sufficiently high.

4.4. CONCLUSION ON THE INTERCALIBRATION FEASIBILITY

The number of sites fully complying in terms of the type criteria is high enough for carrying out the IC exercise. It is concluded that the intercalibration is feasible for the types R-E2 and R-E3.

5. DEMONSTRATING THE COMPLIANCE WITH THE COMPLETED INTERCALIBRATION EXERCISE

5.1. BACKGROUND

- Description of the IC option and benchmark standardization used in the completed IC exercise;
- Selection of the correct procedure to use for intercalibrating new classification method.

5.2. DESCRIPTION OF IC DATASET

Following Figure 1 in the CIS Guidance No. 30 (Willby et al. 2014), case A1 will be applied for the assessment method using invertebrates in the EC GIG river type R-E2 and R-E3. The requirements for fulfilling case A1 are:

i. Full details of the common metric (e.g. species scores and metric weights)

ii. A suitable site x biology dataset covering a range of environmental quality from which the national EQR and common metric can be calculated

iii. Accompanying pressure data in the same format as that used in the completed exercise

iv. Information on the specific thresholds already used in the completed exercise to define reference or alternative benchmark sites (e.g. human population density, extent of agricultural land in the catchment, nutrient concentrations, etc.)

v. Details of exactly how the benchmarking was undertaken in the completed exercise (e.g. creation of a common metric EQR by dividing the observed value by the median common metric value of a set of national reference or benchmark sites). If the completed exercise concluded that benchmarking was not necessary the mean value of the benchmark sites from each country must be provided so that the joining Member State can also judge the need to benchmark its own method

vi. Values of the global mean view of the HG and GM boundaries on the common metric scale for the Member States who participated in the completed exercise.

5.3. DESCRIPTION OF INTERCALIBRATION PROCEDURE

The IC Manual of Willby *et al.* (2014) lists the following steps for Case A1:

1. Calculate the common metric (CM) on the national dataset.

2. Use the associated pressure data to identify sites in the national dataset that meet the criteria established by the GIG for the selection of benchmark or reference sites.

3. Standardize the common metric (CM_bm) against the benchmark according to the approach used in the completed exercise. If benchmark standardization was concluded not to be required in the completed exercise the mean CM value of the joining method's benchmark sites must lie *inside* the range of mean values of the benchmark sites of the methods already intercalibrated for this conclusion to remain applicable. If the joining method's benchmark sites lay *outside* of this range the joining method must benchmark standardize its sites relative to the global mean CM value of the benchmark sites included in the completed exercise. These scenarios are illustrated in Table 1 and 2 of the IC Manual.

4. Use OLS regression to establish the relationship between CM_bm (y) and the EQR of the joining method (x). A specialist case is that when a joining method relies exclusively on the common metric developed in the completed exercise for its classification rather than devising an original method (then being more like Option 1). In such cases, a regression would be meaningless as y is directly dependent on x. The goal for an MS choosing to use the CM as the basis for their method is simple – after any benchmarking their boundaries must simply lie within one-quarter of class of the global mean view.

5. Predict the position of the national class boundaries (MP, GM, HG, and reference) on the CM_bm scale.

6. Apply the comparability criteria as summarized in Chapter 6 of the IC Manual.

- Benchmark standardization;

  Sites identified as alternative benchmark sites based on criteria defined in the EC GIG report (Opatrilova 2011) – see above.

- Calculation of Intercalibration Common metrics (ICM) or Best-Related Intercalibrated National Classification (BRINC);

  The ICM is calculated according to Table 5 in the EC GIG Report (Opatrilova 2011). It includes four metrics: % abundance of EPT taxa (based on individuals), number of EPTCBO taxa (Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Bivalvia and Odonata), ASPT (Average Score per Taxon, based on families), Index of biocoenotic region (functional metric, based on species/genus).

  The correlation of the four metrics with the Croatian EQR was significant in all cases (rEPT = 0.49, rEPTCBO = 0.82, rASPT = 0.75, rIndBiocReg = - 0.68).

  In the GIG Report, the benchmark standardization is described as follows: The raw common metric values were standardized with independently selected benchmark values, separately for each common IC type and a given member state. The benchmark value used for the standardization was obtained as a median value of the metric from selected benchmark samples.

  Given this fact, the common EQR metric derived from alternative benchmark sites is not a true EQR (in the sense of the WFD) because the expected values are not reflecting near-natural conditions (but good status) and therefore, many values are higher than 1.

  The lower anchor (the worse value in the whole IC common dataset) was used for the calculation of the EQR values of one metrics because it showed a constraint gradient (for Index of biocenotic region = 9.0 and ASPT = 1.5).

The EQR value for each of four common metrics was then calculated according to a formula: (measured value – lower anchor)/ (benchmark value – lower anchor). For the two remaining metrics (EPTCBO and % abundance of EPT taxa) the lower anchor was the worst value in the Croatian dataset for E2 and E3 (EPTCBO = 4; EPT% = 0).

The final ICM was calculated as an average of the EQR values of four selected metrics. Following this procedure, the mean iCM values were calculated for the alternative benchmark sites from Croatia.

- Translation of national boundaries to ICM

The common intercalibration metric (normalized benchmark values) and the Croatian national EQRs have been compared in OLS regression (Figure 8).

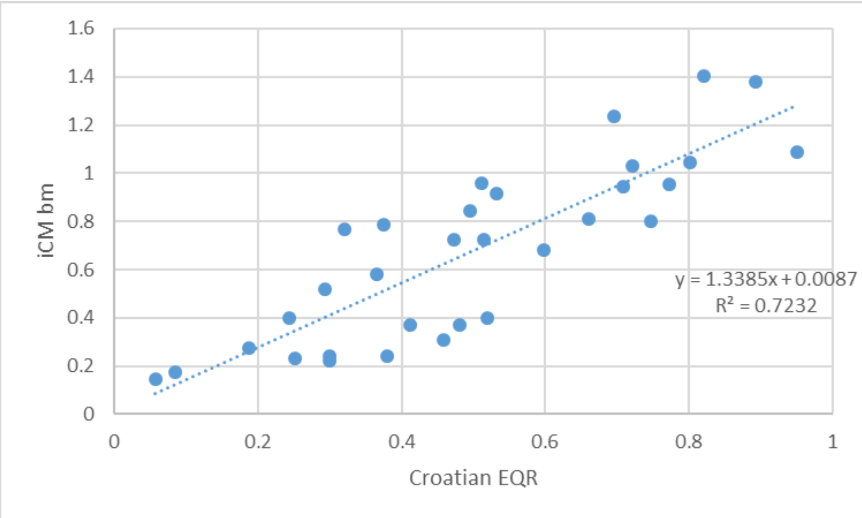

Figure 8. OLS regression to establish the relationship between iCM_bm (y) and the EQR of the joining method (Croatian EQR) for the two IC river types R-E2 and R-E3. The two types are combined.

The Pearson correlation coefficients between the iCM and the national EQR is r = 0.85, p<0.001, n=32 (19 R-E2 + 13 R-E3).

- Calculating boundary bias;

The predicted position of the national class boundaries (MP, GM, HG, and reference) on the CM_bm scale for the two IC river types are listed in table 8.

Table 8. Reference values and class boundaries for the National EQR of the Croatian assessment method in the IC river types R-E2 (= HR-R_3C and HR-R_4A) and R-E3 (= HR-R_3D, HR-R_4B and HR-R_4C) and corresponding iCM values derived from the OLS regression

	National EQR	iCM
Reference values	**1**	**1.3472**
High / Good Boundary	**0.8**	**1.0795**
Good / Moderate Boundary	**0.6**	**0.8118**
Moderate / Poor Boundary	**0.4**	**0.5441**
Poor / Bad Boundary	**0.2**	**0.2764**

5.4. FINAL BOUNDARIES

The global mean views of the H/G and G/M boundaries on the iCM scale in the EC GIG (all types) are:

H/G boundary global mean view of iCM is 1.0291

G/M boundary global mean view of iCM is 0.8309

The adjustment of the boundaries follows chapter 6 in the fit-in guidance of Willby et al. (2014), starting with the G/M boundary.

As the national G/M boundary on the common metric scale falls below the global view, the

amount of this deviation must be calculated:

0.8309 – 0.8118 = 0.0191

and expressed as a proportion of the width of the (national) good status class on the common

metric scale. The width of this class is:

1.0795 – 0.8118 = 0.2677

This gives a proportion of the deviation of:

0.0191 / 0.2677 = 0.07135 (or -0.07135) as it is below the global mean for G / M boundary)

As these values meet the criteria (it must not be >0.25), the G/M boundary does not need changing. There is no obligation to make an adjustment.

In the second step, the H/G boundary is compared. The national view on the common metric scale is 1.0795 and thus slightly above the global view of the finalized IC exercise. The deviation is:

1.0795 – 1.0291 = 0.0504

The deviation expressed as a proportion of the good status class on the stand. iCM scale is:

0.0504/ 0.2677 = 0.18827

As this value is clearly <0.25, the boundary meets the comparability criteria. There is no obligation to make an adjustment. The national boundaries are as listed above in table 8.

6. DESCRIPTION OF THE BIOLOGICAL COMMUNITIES

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT HIGH STATUS

In high status sites of the R-E2 type the EPTCBO (Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Bivalvia, Odonata) groups are represent with around 20 taxa (or more). High local diversity is present at these sites (Margalef index around 5 or more). Taxa sensitive to hydromorphological degradation like taxa from the Plecoptera family Perlodidae can be found. Taxa very sensitive to organic pollution are also present in high abundances.

In high status sites of the R-E3 type the EPTCBO (Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Bivalvia, Odonata) groups are represent with around 20 taxa (or more). High local diversity is present at these sites (Margalef index around 6.5 or more). Taxa sensitive to hydromorphological degradation like taxa from the Trychoptera family Polycentropodidae can be found. Taxa very sensitive to organic pollution are also present in high abundances.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT GOOD STATUS

In good status sites of the R-E2 type the EPTCBO (Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Bivalvia, Odonata) groups are represent with around 15 taxa. Relatively high local diversity is present at these sites (Margalef index around 4.5). Taxa sensitive to hydromorphological degradation like taxa from the Ephemeroptera family Heptageniidae and taxa sensitive to organic pollution are also present.

In good status sites of the R-E3 type the EPTCBO (Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Bivalvia, Odonata) groups are represent with around 15 taxa. Relatively high local diversity is present at these sites (Margalef index around 6). Taxa sensitive to hydromorphological degradation like taxa from the Ephemeroptera family Leptophlebiidae and taxa sensitive to organic pollution are also present.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT MODERATE STATUS

In moderate status sites of the R-E2 type the EPTCBO (Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Bivalvia, Odonata) groups are represent with around 10 taxa. Local diversity is moderate (Margalef index around 4.5). Taxa sensitive to hydromorphological degradation, and organic pollution are also present but in less abundance then tolerant taxa.

In moderate status sites of the R-E3 type the EPTCBO (Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Bivalvia, Odonata) groups are represent with around 10 taxa. Local diversity is moderate (Margalef index around 5.5). Taxa sensitive to hydromorphological degradation, and organic pollution are also present but in less abundance then tolerant taxa.

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Report on fitting the Croatian classification method for benthic macroinvertebrates in rivers to the results of the completed intercalibration of the Eastern-Continental GIG (R-EX5 and R-EX6)

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1. INTRODUCTION

- Croatia;
- Benthic macroinvertebrates;
- R-EX5 and R-EX6 river types.

2. DESCRIPTION OF NATIONAL ASSESSMENT METHODS

The Water Framework Directive requires comprehensive assessment methods for the evaluation of river ecological statuses according to the benthic macroinvertebrate fauna, which includes taxonomic composition, abundance, the ratio of disturbance of sensitive taxa to tolerant taxa and diversity. It is also required to harmonize national assessment methods under the intercalibration exercise with other Eastern – Continental (EC) Geographic Intercalibration Group (GIG) country methods. The official intercalibration of invertebrate-based methods of ecological status assessment in Eastern Continental rivers was finalized within the EC-GIG intercalibration in 2011 (Opatrilova 2011).

A new assessment method has been developed for ecological status assessment of rivers belonging to the IC types R-EX5 (= HR-R_2A; HR-R_2B; HR-R_3A and HR-R_3B) and R-EX6 (= HR-R_1) based on benthic invertebrates and presented in this report. Both IC types are treated together due to the relatively small data set in R-EX6 type (n=24), and absence of pristine sites in R-EX5 as well as bad sites in R-EX6. Because of the similarities between the two types, the (missing) degraded sites in R-EX6 can be considered as complementary to degraded sites in R-EX5 type. The multimetric index uses the same metrics for both river types but with different reference values for each type. The method is compliant with the WFD normative definitions and its class boundaries are in line with the results of the completed intercalibration exercise.

The Croatian assessment method based on benthic invertebrates is a modular type with two modules: saprobity and general degradation. The modular system uses the "one-out all-out" principle. Croatian Large Rivers benthic invertebrate assessment method is based on the same approach and it has been successfully intercalibrated (Birk et al., 2016). The system consists of metrics with proven relationships to stressors.

The classification method is verified for WFD compliance and IC feasibility and the class boundaries were compared with agreed boundaries from the EC-GIG intercalibration exercise following the instructions of the CIS Guidance Document 30: "Procedure to fit new or updated classification methods to the results of a completed intercalibration exercise" (Willby et al. 2014). Intercalibration "Option 2" - indirect comparison of assessment methods using a common metric and "continuous benchmarking" approach was used for the intercalibration of methods in EC GIG River Benthic Macroinvertebrate group and the Croatian methodology was compared with finalized results.

2.1. METHODS AND REQUIRED BQE PARAMETERS

The Saprobity module represents normalized values of the Croatian saprobity index (SI_{HR}), which is based on the Pantle Buck index, but with adapted indicator values. The General Degradation module is normalized multimetric index (General $Degradation_{MI}$) that consists of 4 metrics: Rhithron Type Index, EPT [%] (abundance classes), Diversity (Margalef Index) and the River fauna index (RFI) that is based on indicator responses to hydromorphological degradation.
Combination rule used in the method:
The Saprobity module is based solely on EQR of the SI_{HR} index. The General $Degradation_{MI}$ equals the EQRs of four metrics: 0,2 * Rhithron Type Index + 0,2* EPT [%] (abundance classes) +0,2 * Diversity (Margalef Index) + 0,4 * River fauna index (RFI). The final assessment result equals the lower EQR value of the two modules.

The Croatian national method is in accordance with the WFD compliance, as it takes into consideration all the indicative parameters which are mentioned in CIS Guidance document No 14 (2011): taxonomic composition, abundance, disturbance sensitive taxa to insensitive taxa, diversity and absence of major taxonomic groups (Table 1).

Table 1. Overview of the metric groups included in the Croatian national method for the assessment of IC types R-EX5 and R-EX6

MS	Taxonomic composition	Abundance	Sensitive / tolerant taxa	Diversity	Major taxonomic groups
HR	x	x	x	x	x

2.2. SAMPLING AND DATA PROCESSING

Description of sampling and data processing:

- Sampling time and frequency;
  The most favorable sampling time is spring (March-April), i.e. before mass swarms of adult insects come out which takes place in May and June. The period of stable and low water levels should last long enough before sampling so that the macrozoobenthic community can be well-developed. Sampling shall not be undertaken: during high water levels and up to 3 weeks after high water levels, during all other disturbances caused by natural processes.

- Sampling method;
  All available microhabitats are sampled („multi-habitat sampling ") and 20 sub-samples are collected which are distributed according to the proportion of microhabitat types, with microhabitats that are less than 5% present are not sampled, but are recorded in the protocol. Microhabitat type represents a combination of inorganic and organic substrate. Sub-sample is sampled by raising the substrate that consists of a substrate with accompanying animals from surface size 25 x 25 cm (0.0625 m^2). The channel substrate of each sampling site was classified according to AQEM Consortium (2002).

- Data processing
  Rhithron Type Index, EPT [%] (abundance classes) and Diversity (Margalef Index) are calculated using ASTERICS 4.04 software, while the Croatian saprobity index and River fauna index are calculated separately. SI_{HR} is an adapted saprobity index according to Pantle-Buck (1955):

$$SI_{HR} = \frac{\sum SIu_i}{\sum u_i}$$

where:

SI_{HR} = Croatian saprobity index
SI = individual species/taxa indicator value
u_i = number of individuals calculated per 1 m^2

Indicator values of macrozoobenthic taxa (SI) are specific to Croatia.
The River fauna index was calculated according to the following equation:

$$\mathrm{RFI}_{VR_j} = \frac{\sum_{i=1}^{n} \mathrm{ac_i} \times \mathrm{Rf_i} \times \mathrm{HW_i}}{\sum_{i=1}^{n} \mathrm{ac_i} \times \mathrm{HW_i}}$$

where:
ac_i is the log5 abundance class of the i^{th} taxon,
Rf_i is the river fauna value of the i^{th} taxon,
HW_i is the hydromorphological indicative weight of the i^{th} taxon
n is the number of indicative taxa

Indicator values of macrozoobenthic taxa (RFi and HWi) are specific to IC types R-EX5 and R-EX6 and are calculated by canonical correspondence analysis of the taxa found in these types with regard to hydromorphological pressure (Urbanič, 2014).

- Identification level;

It is recommended that identification is conducted as detailed as possible, up to the level of species if possible. Required level of macrozoobenthos identification:

Table 2. Level of identification required for the Croatian national assessment

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		

2.3. NATIONAL REFERENCE CONDITIONS

The settings for the national reference conditions of some chemical thresholds are given in the legal document Regulation on water quality standards (Uredba o standardu kakvoće voda, NN 96/2019), but this document is currently in the process of revision. Because of this, reference thresholds for this incercalibration fit in procedure follow those of the EC-GIG defined for IC types R-E2 and R-E3 (Opartilova, 2011):

Hydromophological alternation NO or LOW (scoring set at ≤ 2)
NO or LOW:
impoundment, hydropeaking, water abstraction, upstream dam influence, water temperature modification, channelization, alteration of riparian vegetation, local habitat alteration, dykes, toxic risk, water acidification, navigation, recreational use

Land-use in the catchment
<0.8% Urban land cover
< 50 Land Use Index

and

Chemical thresholds:
mean BOD_5 <2.4 mg/l
mean $P\text{-}PO_4$ <0.04 mg/l
mean $N\text{-}NO_3$ <6 mg/l
mean $N\text{-}NH_4$ <0.1 mg/l

It is also important that no point-source or non-point source pollutant are present near the reference site.

2.4. NATIONAL BOUNDARY SETTING

In the intercalibration types R-EX5 and R-EX6, both boundaries of the General Degradation_{MI} were transformed, as well as boundaries for the saprobic index of R-EX6. This means that the final EQR represents the "classical" boundaries (0.8; 0.6; etc.), seeing as the final value represents the lower value of the already transformed EQR-s of the two modules. Although the National classification recognizes four types (H-R-2A, H-R-2B, H-R-3A and H-R-3B) within the intercalibration type R-EX5, the reference values are set equally for all four types. We acknowledge that the lack of type-specific reference values in the method is not substantially reasoned. It is possible that an extended analysis may result in different pressure-impact relationships in different types of rivers. This may result in differentiation of metrics reference values (upper and lower anchors), additional differentiated normalization of the National classification system or possibly weighing the metrics before combination in the future. As the monitoring efforts are ongoing in this region, a greater data set may give a more accurate setting of the reference values for each national biotic river type, as well as the pressure response relationships.

Croatian saprobity index
R-EX5
The majority of rivers belonging to IC type R-EX5 in Croatia are anthropogenically impacted (some even heavily) due to a relatively high ratio of urban areas and even more agricultural areas present in their catchment. The majority of the rivers have been channelized for agricultural land use purposes, or have limited lateral movement because of dykes protecting urban areas and settlements. In R-EX5, no true reference sites were present, meaning that when calculating reference values for indices (modules) alternative approaches had to be taken. Reference metrics values were calculated by adding 20% of the total metric range to the High/Good boundary, whereas the H/G boundaries were determined as the median of the benchmark sites (Table 3).
The lower anchor of the SI_{HR} represents the worst theoretical value of the metric (based on the operational taxa list) and equals 3.6 (for all IC types). The value of the SI_{HR} in type R-EX5 ranged from 1.80 to 3.54. 20% of the total range (maximum value of SI_{HR} being 3.6) was subtracted from the high/good boundary in order to calculate reference values. The reference value of R-EX5 equals 1.90 ($SI_{HR\text{-}ref}$ = 1.90). The high/good boundary for the SI_{HR} equaled 2.26 and other boundaries were distributed equidistantly to 3.6

Table 3. Determined benchmark sites of IC type R-EX5 following the criteria of Opartilova (2011)

Code	Name	LUI	P-PO_4 (mgP/l)	N-NO_3 (mgN/l)	BOD_5 (mgO_2/l)	N-NH_4 (mgN/l)	ASPT	Conductivity	Hidromorp hologicall score	SI_{HR}
15383	Kamešnica, Gregorevac	100.62	0.069	0.752	1.10	0.120	5.75	549	1	2.33
16101	Golinja, Slatina Pokupska	83.20	0.018	0.147	2.20	0.163	6.34	421	1.69	2.00
16107	Veliki Potok, Bukovci	34.69	0.019	0.262	2.27	0.196	6.24	438	1.62	1.95
16234	Svinica, Svinica	76.87	0.025	0.269	1.66	0.226	5.07	435	1.85	2.41
16239	Brijebovina, prije utoka u Sunju, Umetić	49.16	0.021	0.235	2.09	0.218	6.32	486	1.23	2.15
16746	Utinja, Vratečko (prije utoka u Kupu)	91.03	0.026	0.300	1.83	0.225	6.10	466	1	2.79
17606	Presečno, Drašković	104.46	0.029	0.620	3.10	0.200	5.24	607	2.23	2.20
21205	Iskrica, Šaptinovci	89.63	0.084	0.771	3.36	0.113	5.36	475	2.92	2.55

*"*At least four out of seven parameters used for the screening (chemical parameters + land-use index + ASPT) had to fit within the given range; three parameters could be below (or above in case of ASPT) than the given range."*

BOD5	*conductivity*	*land-use index (4*artificial + 2*int.agriculture + non-int.agriculture)*	*P-PO4*	*N_NO3*	*N_NH4*	*ASPT*
2.4 - 4.1	*250 - 620*	*50 - 170*	*0.04 - 0.25*	*2.0 - 6.0*	*0.1-0.25*	*5.0 - 6.4*

R-EX6

The reference value of the SI_{HR} in R-RX6 was determined as the median of all reference sites (n=7, Table 4). The reference value for the SI_{HR} in type R-EX6 equals= 1.68 (Figure 1).

Table 4. Determined reference sites of IC type R-EX6

Name	LUI	P-PO_4 (mgP/l)	N-NO_3 (mgN/l)	BOD_5 (mgO_2/l)	N-NH_4 (mgN/l)	ASPT	Conductivity	Total HYMO score	SI_{HR}
Sivornica, izvorište Psunj	0	0.020	0.540	1.40	0.020	7.00	80	1	1.63
Stipnica kod mjesta G. Stupnica (H.Kostajnica)	13.8	0.010	0.150	0.70	0.030	7.00	413	1.13	1.71
Izvor Duboke rijeke	0.5	0.021	0.220	1.20	0.004	6.56	108	1	1.68
Izvor potoka Dubočanka	0	0.029	0.310	0.70	0.017	6.10	311	1.07	1.66
Sutla, Lupinjak	1	0.012	0.440	0.90	0.039	7.00	281	1.2	1.85
Kamešnica, Kamešnica	0.02	0.022	0.558	1.75	0.036	6.92	458	1	1.83
Bistra, Krainje, Kraljev vrh	9.5	0.019	1.007	1.20	0.040	6.85	224	1.42	1.60

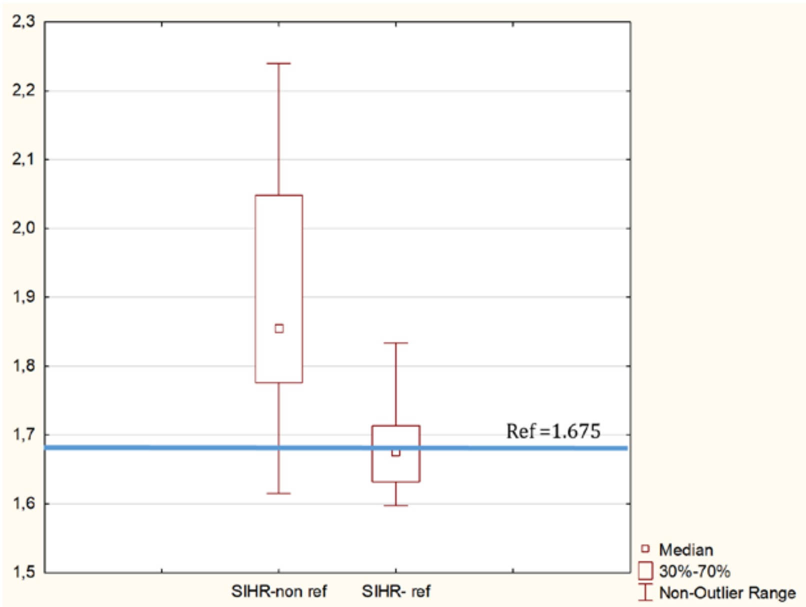


Figure 1. A comparison of the SI_{HR} metric values of reference and non-reference sites of R-EX6. The vertical line is the reference value, for SI_{HR} = 1.68.

The HIGH/GOOD boundary of the SI_{HR} was determined as the median of the benchmark sites of R-EX6 (Table 5). The high/good boundary for the SI_{HR} equaled 1.85 and other boundaries were distributed equidistantly to 3.6.

Table 5. Determined benchmark sites of IC type R-EX6 following the criteria of Opartilova (2011)

Code	Name	LUI	$P\text{-}PO_4$ (mgP/l)	$N\text{-}NO_3$ (mgN/l)	BOD_5 (mgO_2/l)	$N\text{-}NH_4$ (mgN/l)	ASPT	Conductivity	SI_{HR}
17553	Sutla. Prišlin	79.73	0.05	1.07	2.8	0.18	6.13	657.25	1.82
21120	Žarovnica (Sutinska). Žarovnica	70.1	0.03	1.29	1.63	0.1	6.4	566	1.85
21128	Kašina. Kašina	29.25	0.12	0.39	3.32	0.18	6.4	558.67	2.24

*"*At least four out of seven parameters used for the screening (chemical parameters + land-use index + ASPT) had to fit within the given range; three parameters could be below (or above in case of ASPT) than the given range.*

BOD5	conductivity	land-use index (4*artificial + 2*int.agriculture + non-int.agriculture)	P-PO4	N_NO3	N_NH4	ASPT
2.4 - 4.1	250 - 620	50 - 170	0.04 - 0.25	2.0 - 6.0	0.1-0.25	5.0 - 6.4

After determining all the boundaries, a transformation of the EQRs for the saprobity module was conducted:

R-EX6	Ref = 1.68	Lower anchor=3.6
$OEK_{SIHR\ R\text{-}EX6}$	**$OEK_{transform}$**	
≥0.91	$0.8 + 0.2*(OEK_{SIREX6} - 0.91)/0.09$	
0.68 - 0.91	$0.6 + 0.2*(OEK_{SIREX}6 - 0.68)/0.24$	
0.45 - 0.68	$0.4 + 0.2*(OEK_{SIREX6} - 0.45)/0.23$	
0.23 - 0.45	$0.2 + 0.2*(OEK_{SIREX6} - 0.23)/0.23$	
≤0.23	$0.2 + 0.2*(OEK_{SIREX6})/0.23$	

General degradation

R-EX5

No true reference sites that meet all the thresholds were present in R-EX5. The General Degradation$_{MI}$ equals the EQRs of four metrics: 0.2 * Rhithron Type Index + 0.2* EPT [%] (abundance classes) +0.2 * Diversity (Margalef Index) + 0.4 * River fauna index (RFI). For each of the four metrics, the reference value was calculated by adding 20% of the metric range to the high/good boundary (Table 6). The high/good boundary was calculated as the median value of all the benchmark sites. Lower anchors for both R-EX5 and R-EX6 were the same and were set as the worst metric value in both IC-types.

Table 6. Class boundaries and ranges of the metrics used in calculating the General Degradation$_{MI}$ in IC type R-EX5

Metric group:	Functional	Composition/abundance	Richness/diversity	Sensitivity /tolerance
R-EX5 metric boundaries	**Rhithron Type Index**	**EPT [%] (abundance classes)**	**Diversity (Margalef Index)**	**RFI**
Upper anchor	7.99	32.75	9.55	0.178
Lower anchor	1.22	0	0.99	-0.511
High/good boundary	5.97	23.61	7.51	0.105
Range:				
max	11.32	45.69	11.23	0.368
min	1.22	0	0.99	-0.511

R-EX6

Reference values for each of the metrics used in the calculation of the General Degradation$_{MI}$ for IC type R-EX6 were determined as the median metric value within the reference sites (Table 7; Figure 2).

Table 7. Class boundaries and ranges of the metrics used in calculating the General Degradation$_{MI}$ in IC type R-EX6

Metric group:	Functional	Composition/abundance	Richness/diversity	Sensitivity /tolerance
R-EX6 metric boundaries	**Rhithron Type Index**	**EPT [%] (abundance classes)**	**Diversity (Margalef Index)**	**RFI**
Upper anchor	10.9	49.01	5.59	0.054
Lower anchor	1.22	0	0.99	-0.511
max	13.2	55.81	9.32	0.177
min	4.5	17.09	2.21	-0.147

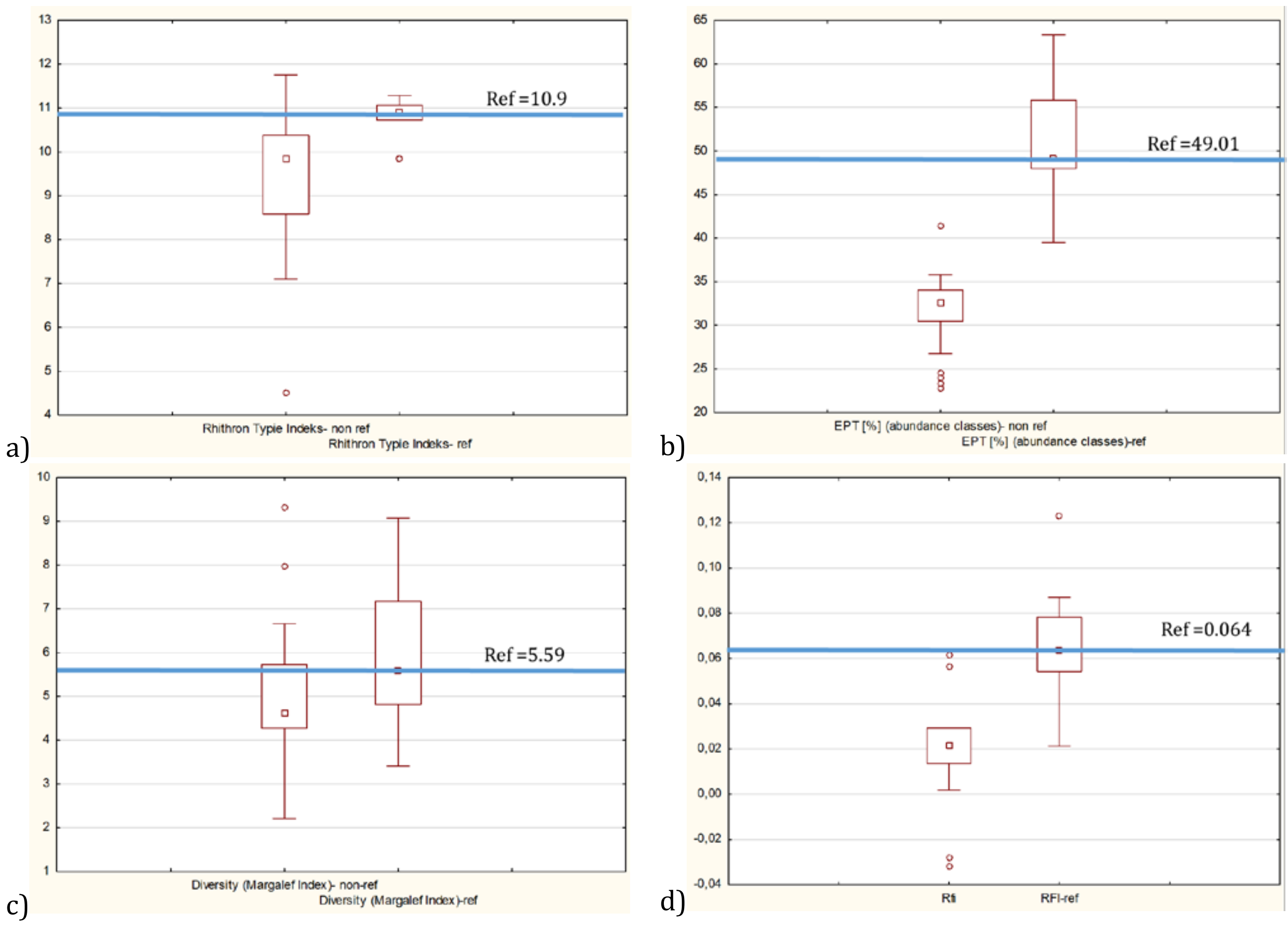


Figure 2. A comparison of four metric values: a) Rhithron type index; b) EPT % (abundance classes); c) Maraglef index and d) River fauna index (RFI) of reference and non-reference sites of R-EX6. The vertical lines represent the reference value for each metric.

Boundary values of five ecological status classes were defined based on the changes in the portion of sensitive and tolerant taxa (Figure 3). Sensitive and tolerant taxa were determined in calculating the River fauna index with regard to hidromorphological stressors. The ratio of tolerant taxa begins to increase (high/good boundary) at EQR = 0.82, whereas at EQR = 0.68 the portion of tolerant taxa reach the portion of sensitive taxa (good/moderate boundary). The intersection of regression curves representing a portion of tolerant and portion of sensitive taxa occurred approximately at the EQR = 0.62 and at the EQR = 0.55 the portion of tolerant taxa exceeds the portion of sensitive taxa (moderate/poor boundary). The portion of tolerant taxa start to dominate at EQR = 0.30 (poor/bad boundary).

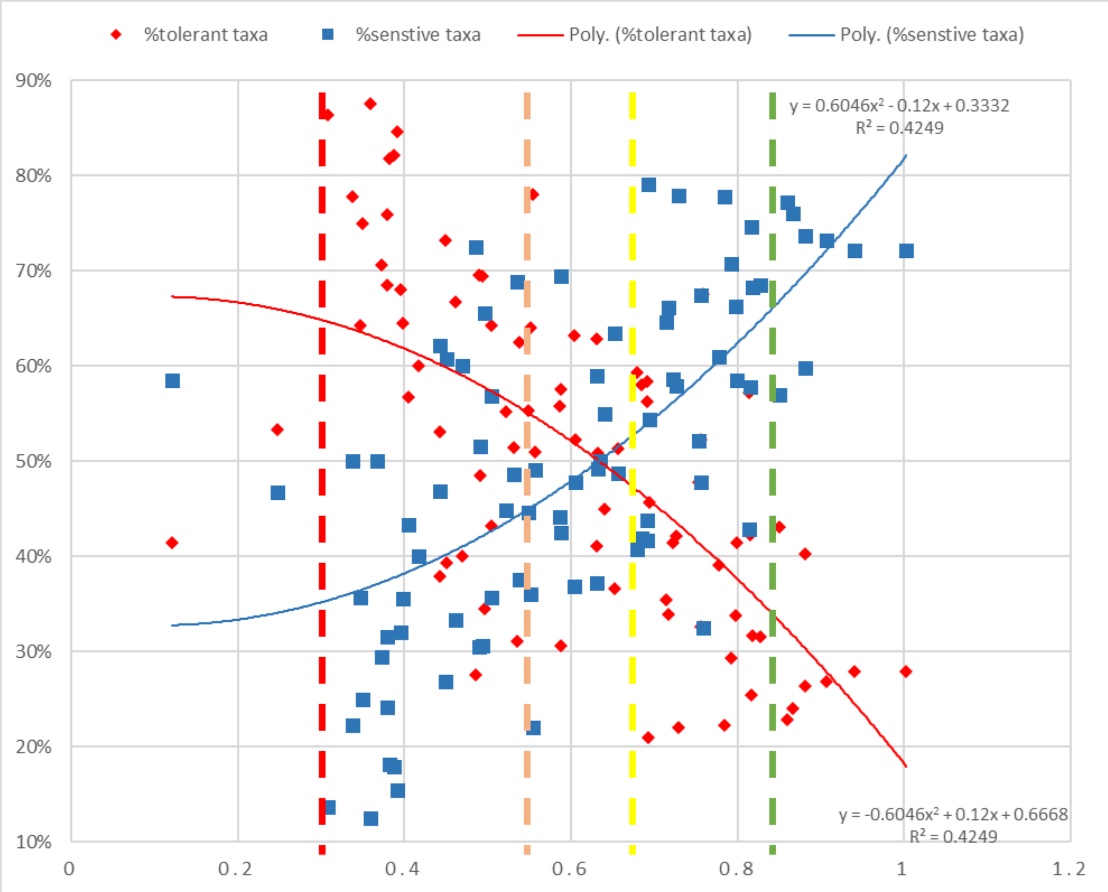


Figure 3. Boundary setting between ecological status classes using changes in a portion of sensitive and tolerant taxa along with the ecological quality ratio of the General Degradation$_{MI}$.

Values of the General Degradation$_{MI}$ for both R-EX5 and R-EX6 were transformed using the following equations:

R-EX5 and R-EX 6

EQR$_{GEN\ DEG\ R\text{-}EX5\ and\ R\text{-}EX6}$	**EQR$_{transform}$**
≥0.82	0.8 + 0.2*(EQR$_{GEN\ DEG\ R\text{-}EX5\ and\ R\text{-}EX6}$ – 0.82) / 0.18
0.68 – 0.82	0.6 + 0.2*(EQR$_{GEN\ DEG\ R\text{-}EX5\ and\ R\text{-}EX6}$ – 0.68) / 0.14
0.55- 0.68	0.4 + 0.2*(EQR$_{GEN\ DEG\ R\text{-}EX5\ and\ R\text{-}EX6}$ – 0.55) / 0.13
0.30 – 0.55	0.2 + 0.2*(EQR$_{GEN\ DEG\ R\text{-}EX5\ and\ R\text{-}EX6}$ – 0.30) / 0.25
≤0.30	0.2*(EQR$_{GEN\ DEG\ R\text{-}EX5\ and\ R\text{-}EX6}$) / 0.30

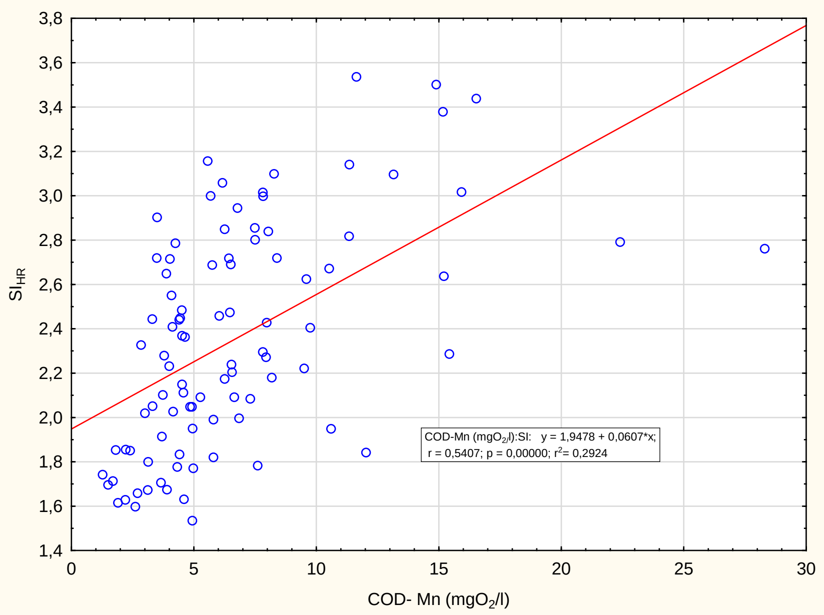
2.5. PRESSURES ADDRESSED

Various pressures were addressed by the different methods in the finalized IC exercise. Most countries indicated as detected general degradation, hydromorphological degradation and pollution by organic matter.

The Croatian method addresses catchment land use, pollution by organic matter, eutrophication and habitat destruction. The Saprobity module addresses organic pollution, whereas other stressor responses are integrated into the General degradation module. **The lower value of the two modules is the final score of the site and it gives a direct suggestion on which stressor should be addressed primarily if the score would be less favorable.** This method is therefore comparable to the methods which are already successfully intercalibrated.

The following pressure-response relationships have been derived:

1) Saprobity module



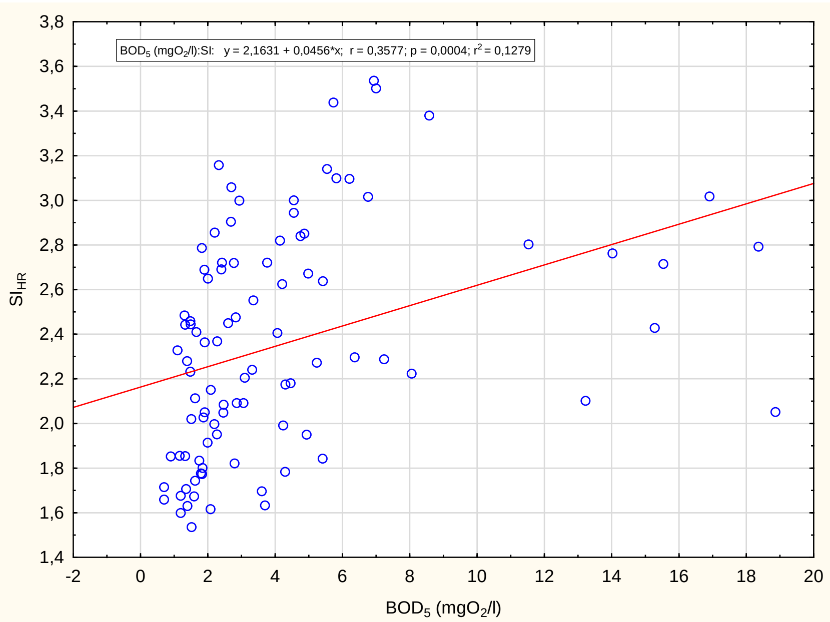


Figure 4. Pressure-Response relationship between chemical oxygen demand (COD) and biological oxygen demand (BOD) against the SI_{HR} values in river types R-EX5 and R-EX6.

2) General degradation module

Land Use

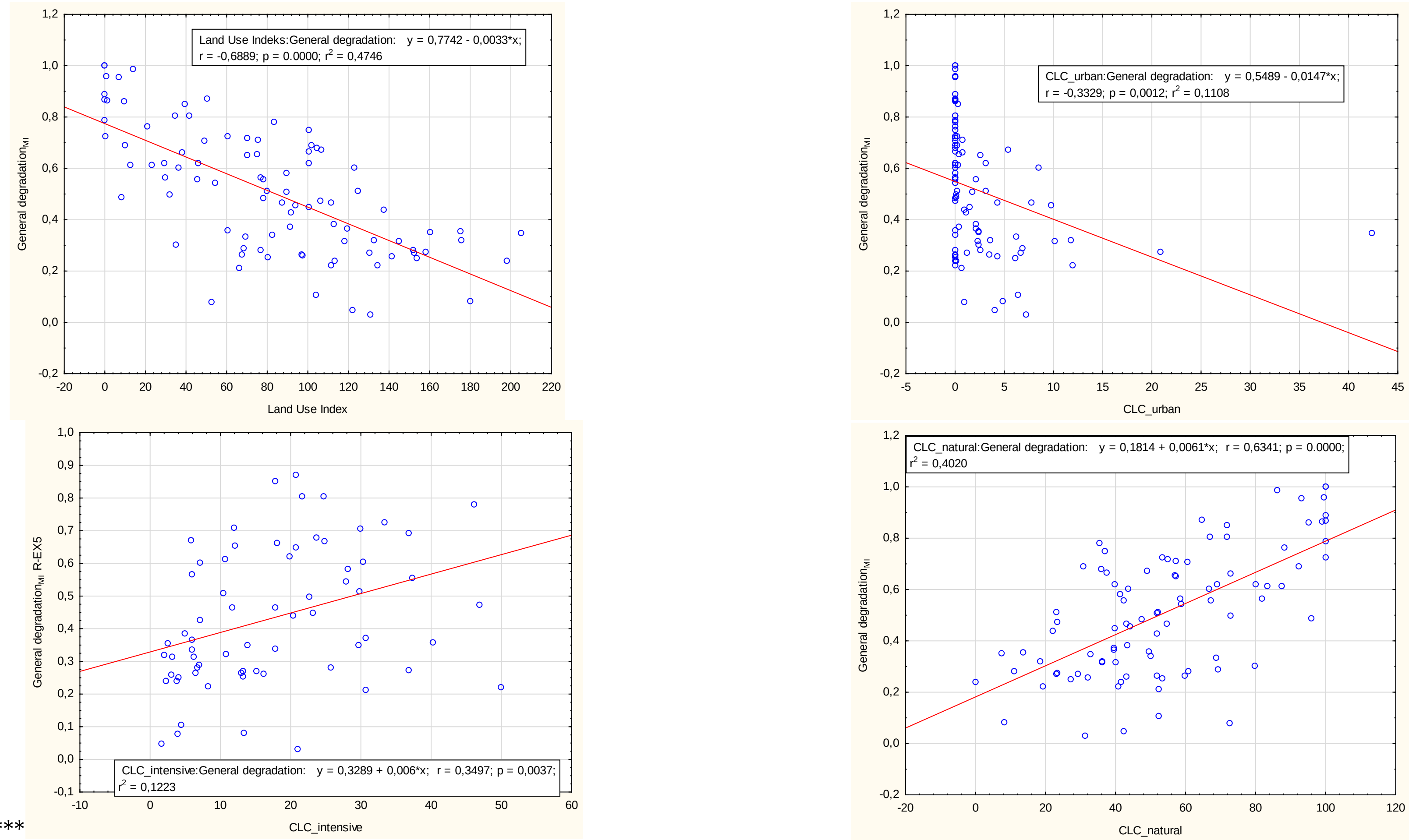


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Figure 5. Pressure-Response relationship between the Land Use Index LUI and Corine Land Cover (categories urban, intensive agriculture and natural) against the General Degradation$_{MI}$ for sites of river type R-EX5 and R-EX6. ***The CLC_intensive category is plotted against the General Degradation$_{MI}$ values of R-EX5 only because there were little sites with intensive agriculture present in the mid altitude types of R-EX6

A. Hydro-chemistry

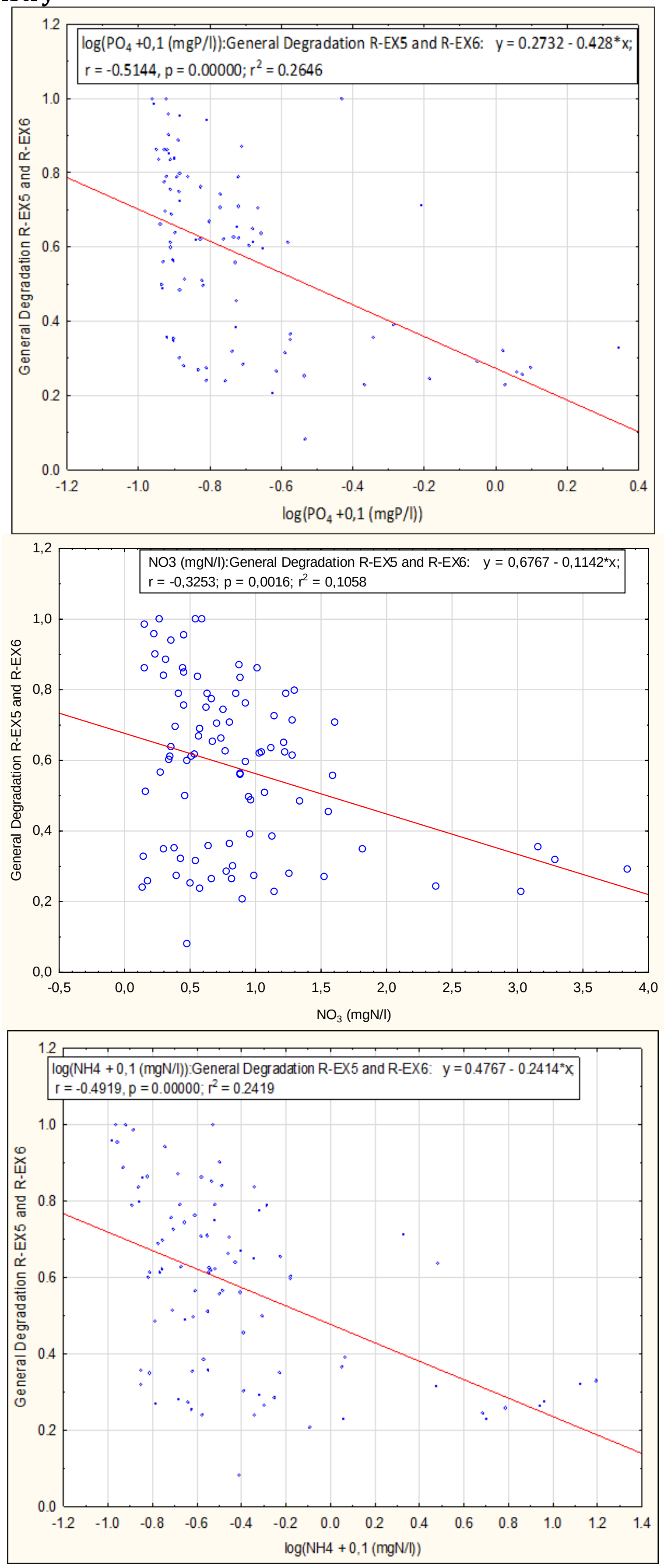

Figure 6. Pressure-Response relationship between chemical water properties against the General Degradation$_{MI}$ for sites of river type R-EX5 and R-EX6.

B. Hydromorphology

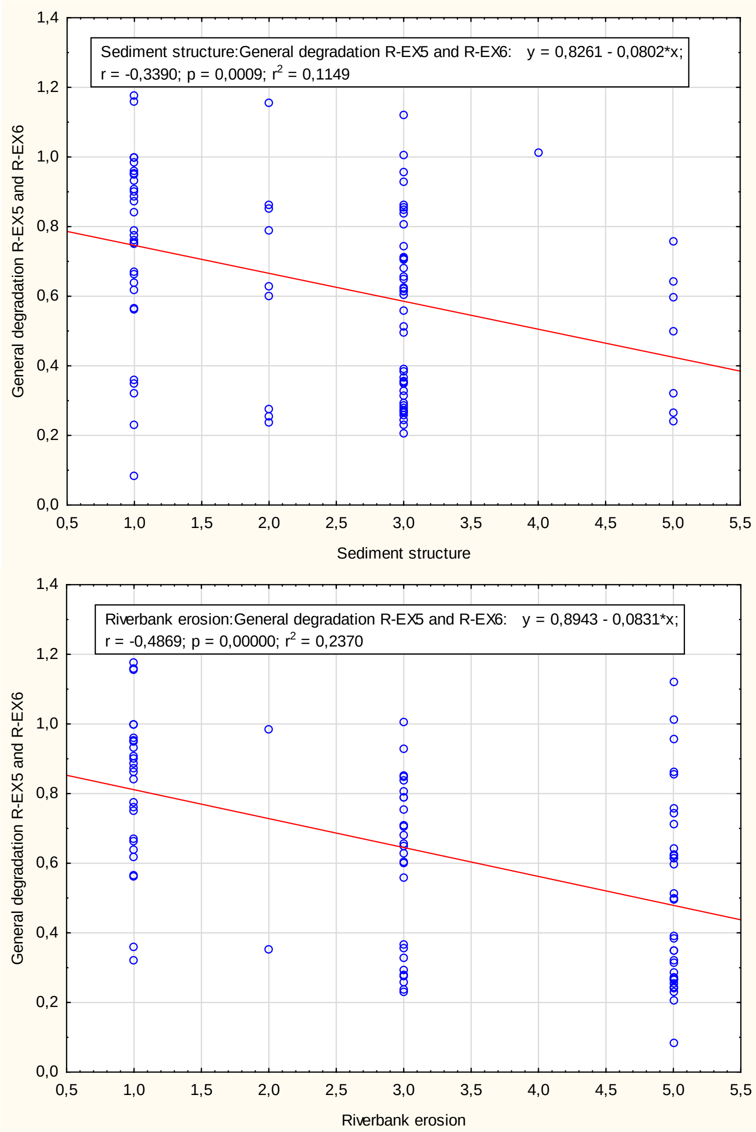

Figure 7. Pressure-Response relationship between hydromorphological river features against the General Degradation$_{MI}$ for sites of river type R-EX5 and R-EX6.

C. Resume

For all four groups of pressures (organic pollution, land use, chemistry, hydromorphological destruction), significant regressions could be found. It is concluded that both the SI_{HR} and the General $Degradation_{MI}$ clearly respond to anthropogenic impacts and can be used for the assessment of the ecological status.

3. WFD COMPLIANCE CHECKING

The first step in the Intercalibration process requires the checking of national methods considering the following WFD compliance criteria. The compliance check showed that the Croatian method fulfils the requirements of the WFD (Table 8).

Table 8. List of the WFD compliance criteria and the WFD compliance checking process and results

Compliance criteria	Compliance checking
Ecological status is classified by one of **five classes** (high. good. moderate. poor and bad).	yes
High. good and moderate ecological status are set in line with the WFD's **normative definitions** (**Boundary setting procedure**)	yes
All relevant parameters indicative of the biological quality element are covered (see Table 1 in the IC Guidance). A **combination rule** to combine parameter assessment into BQE assessment has to be defined. If parameters are missing. Member States need to demonstrate that the method is sufficiently indicative of the status of the QE as a whole	yes
Assessment is adapted to **intercalibration common types** that are defined in line with the typological requirements of the Annex II WFD and approved by WG ECOSTAT	yes
The water body is assessed against **type-specific near-natural reference conditions**	yes
Assessment results are expressed as **EQRs**	yes
Sampling procedure allows for **representative** information about water body quality/ecological status **in space and time**	yes
All data relevant for assessing the biological **parameters** specified in the WFD's normative definitions are covered by the **sampling procedure**	yes
Selected taxonomic level achieves adequate **confidence and precision** in classification	yes

4. IC FEASIBILITY CHECKING

The intercalibration process ideally covers all national assessment methods within a GIG. However, the comparison of dissimilar methods ("apples and pears") has clearly to be avoided. Intercalibration exercise is focused on specific type / biological quality element / pressure combinations. The second step of the process introduces an "IC feasibility check" to restrict the actual intercalibration analysis to methods that address the same common type(s) and anthropogenic pressure(s) and follow a similar assessment concept.

4.1. TYPOLOGY

The EC-GIG includes ten types (not including very large rivers), five of which are relevant for Croatia (Table 9). Two types, R-EX5 and R-EX6, are treated in this report.
The biotic typology of running waters in Croatia was initially established in 2011 (Mihaljević et al., 2011), mainly based on expert opinion, due to a general lack of all data types: both biological and pressure data. Today, biological data in most types are sufficient, as well as data on pressures such as water chemistry and land use. The hydromorphological data sets are still lacking from many sites as the hydromorphological evaluation of running waters in Croatia began only recently, in 2017. The current assessment method has equal reference and "worst" metric values for several Croatian types, but in the future, with more data primarily on hydromorphology we could fine-tune these values for every type. Hence, the typology will remain as initially determined.

Table 9. IC types of the EC-GIG

Type	Common intercalibration type	Ecoregion (Illies. 1967)	Catchment area [km2]	Altitude [m]	Geology	Channel substrate	Croatian type
R-E1a	Carpathians: small to medium. mid-altitude	10	10 - 1.000	500 - 800	mixed		/
R-E1b	Carpathians: small to medium. mid altitude	10	10 -1.000	200 - 500	mixed		/
R-E2	Plains: medium-sized. lowland	11.12	100 - 1.000	< 200	mixed	sand and silt	HR-R_3C HR-R_4A
R-E3	Plains: large. lowland	11.12	> 1.000	< 200	mixed	sand. silt and gravel	HR-R_3D HR-R_4B HR-R_4C
R-E4	Plains: medium-sized. mid-altitude	11.12	100 - 1.000	200-500	mixed	sand and gravel	/
R-EX4	Large. mid-altitude	10. 11. 12	> 1.000	200 - 500	mixed	gravel and boulder	/
R-EX5	**Plain: small lowland**	**11. 12**	**10 -100**	**< 200**	**mixed**	**sand and silt**	**HR-R_2A HR-R_2B HR-R_3A HR-R_3B**
R-EX6	**Plain: small. mid-altitude**	**11. 12**	**10-100**	**200-500**	**mixed**	**gravel**	**HR-R_1**
R-EX7	Balkan: mid-altitude. small-sized. calcareous. karst spring	5	10 – 100	200 - 500	calcareous	gravel	HR-R_6
R-EX8	Balkan: small to medium-sized. calcareous. karst spring	5	10-1000		calcareous	gravel. sand and silt	HR-R_7 HR-R_8A HR-R_9

The number of sites sharing the common types R-EX5 and R-EX6 are:

R-EX5 (= HR-R_2A; HR-R_2B; HR-R_3A; HR-R_3B): 67

R-EX6 (= HR-R_1): 24

Table 10. Croatian National types falling in the category of IC types R-EX-5 and R-EX6 of the EC-GIG

<table>
<tr><th colspan="2">Name of HR type</th><th>HR Type</th><th>IC Type</th></tr>
<tr><td colspan="2">Small mountain and upland running waters</td><td>HR-R_1</td><td>R-EX6</td></tr>
<tr><td rowspan="2">Small lowland running waters</td><td>Small lowland running waters with clay and sand substrate</td><td>HR-R_2A</td><td rowspan="4">R-EX5</td></tr>
<tr><td>Small lowland running waters with gravel and pebble substrate</td><td>HR-R_2B</td></tr>
<tr><td rowspan="2">Lowland alluvial running waters</td><td>Small lowland alluvial running waters with gravel and pebble substrate</td><td>HR-R_3A</td></tr>
<tr><td>Small lowland alluvial running waters with clay and sand substrate</td><td>HR-R_3B</td></tr>
</table>

4.2. PRESSURES ADDRESSED

The pressure gradient has been assessed for the Corine Land Cover (CLC) as well as the land use index, which is derived from CLC and defined as:

LUI = 4 * CLC urban+2*CLC intensive agriculture + CLC extensive agriculture

The ranges of the CLC and LUI in the two river types are:

CLC/LUI	range R-EX5	range R-EX6
CLC urban	0 – 47.62	0 – 9.73
CLC agr.intens.	0.87 – 97.69	0 – 37.61
CLC agr. extens.	1.6 – 49.88	0 – 37.35
LUI	23.21 – 205.15	0 – 100.60

The hydromorphological alteration scale ranges from 1 (no) to 5 (high) and consists of multiple smaller indices. The three main indices: hydrology regime, morphology and flow continuity ranged from 1 to 5 in both river types, whereas the mean hydromorphological score ranged from 1 to 4.74 in R-EX5 and 1 to 4.69 in R-EX6.

The ranges for the chemical variables tested are:

Chemical variable	range R-EX5	range R-EX6
BOD_5 [mg L–1]	1.1 – 55.25	1.17 – 20.88
COD [mg L–1]	2.84 – 40.92	1.27 – 7.61
PO_4-P [mg L–1]	0.01 – 2.10	0.02 – 0.12
NO_3-N [mg L–1]	0.14 – 31.58	0.34 – 1.56
NH_4-N [mg L–1]	0.04 – 15.51	0.03 – 2.94
Conductivity (µS/cm)	77.88 – 1045.36	121.67 – 722.88

The different pressure gradients covered by the national data set are considered to be sufficient.

4.3. ASSESSMENT CONCEPT

Does the national method follow the same assessment concept as other methods in the Intercalibration group? Provide evaluation of IC feasibility regarding assessment concept of the intercalibrated methods

The pressure gradient covered by the national data set is considered sufficient. The data acceptance criteria as defined in the GIG report from 2011 was used.

The data quality is considered as good, since:

1) the sampling and analytical methodology is comparable among all countries (multi-habitat sampling, at least 500 µm).

2) the identification level used for R-EX5 and R-EX6 is sufficient

3) the number of sites used for the 2 types is considered sufficiently high.

4.4. CONCLUSION ON THE INTERCALIBRATION FEASIBILITY

The number of sites fully complying in terms of the type criteria is high enough for carrying out the IC exercise. It is concluded that the intercalibration is feasible for the types R-EX5 and R-EX6.

5. DEMONSTRATING THE COMPLIANCE WITH THE COMPLETED INTERCALIBRATION EXERCISE

5.1. BACKGROUND

- Description of the IC option and benchmark standardization used in the completed IC exercise;
- Selection of the correct procedure to use for intercalibrating new classification method.

5.2. DESCRIPTION OF IC DATASET

Following Figure 1 in the CIS Guidance No. 30 (Willby et al. 2014), case A1 will be applied for the assessment method using invertebrates in the EC GIG river type R-EX5 and R-EX6. The requirements for fulfilling case A1 are:

i. Full details of the common metric (e.g. species scores and metric weights)

ii. A suitable site x biology dataset covering a range of environmental quality from which the national EQR and common metric can be calculated

iii. Accompanying pressure data in the same format as that used in the completed exercise

iv. Information on the specific thresholds already used in the completed exercise to define reference or alternative benchmark sites (e.g. human population density, extent of agricultural land in the catchment. nutrient concentrations, etc.)

v. Details of exactly how the benchmarking was undertaken in the completed exercise (e.g. creation of a common metric EQR by dividing the observed value by the median common metric value of a set of national reference or benchmark sites). If the completed exercise concluded that benchmarking was not necessary the mean value of the benchmark sites from each country must be provided so that the joining Member State can also judge the need to benchmark its own method

vi. Values of the global mean view of the HG and GM boundaries on the common metric scale for the Member States who participated in the completed exercise.

5.3. DESCRIPTION OF INTERCALIBRATION PROCEDURE

The IC Manual of Willby *et al.* (2014) lists the following steps for Case A1:

1. Calculate the common metric (CM) on the national dataset.

2. Use the associated pressure data to identify sites in the national dataset that meet the criteria established by the GIG for the selection of benchmark or reference sites.

3. Standardise the common metric (CM_bm) against the benchmark according to the approach used in the completed exercise. If benchmark standardisation was concluded not to be required in the completed exercise the mean CM value of the joining method's benchmark sites must lie *inside* the range of mean values of the benchmark sites of the methods already intercalibrated for this conclusion to remain applicable. If the joining method's benchmark sites lie *outside* of this range the joining method must benchmark standardise its sites relative to the global mean CM value of the benchmark sites included in the completed exercise. These scenarios are illustrated in Table 1 and 2 of the IC Manual.

4. Use OLS regression to establish the relationship between CM_bm (y) and the EQR of the joining method (x). A specialist case is that when a joining method relies exclusively on the common metric developed in the completed exercise for its classification rather than devising an original method (then being more like Option 1). In such cases, a regression would be meaningless as y is directly dependent on x. The goal for an MS choosing to use the CM as the basis for their method is simple – after any benchmarking their boundaries must simply lie within one-quarter of class of the global mean view.

5. Predict the position of the national class boundaries (MP, GM, HG, and reference) on the CM_bm scale.

6. Apply the comparability criteria as summarised in Chapter 6 of the IC Manual.

- Benchmark standardization;

  Sites identified as alternative benchmark sites based on criteria defined in the EC GIG report (Opatrilova 2011) – see above.

- Calculation of Intercalibration Common metrics (ICM) or Best-Related Intercalibrated National Classification (BRINC);

  The ICM is calculated according to Table 5 in the EC GIG Report (Opatrilova 2011). It includes four metrics: % abundance of EPT taxa (based on individuals), number of EPTCBO taxa (Ephemeroptera, Plecoptera, Trichoptera, Coleoptera, Bivalvia and Odonata), ASPT (Average Score per Taxon, based on families), Index of biocoenotic region (functional metric, based on species/genus).

  The correlation of the four metrics with the Croatian EQR was significant in all cases (rEPT = 0.70, rEPTCBO = 0.81, rASPT = 0.84, rIndBiocReg = -0.71).

  In the GIG Report, the benchmark standardization is described as follows: The raw common metric values were standardized with independently selected benchmark values, separately for each common IC type and a given member state. The benchmark value used for the standardization was obtained as a median value of the metric from selected benchmark samples.

  Given this fact, the common EQR metric derived from alternative benchmark sites is not a true EQR (in the sense of the WFD) because the expected values are not reflecting near-natural conditions (but good status) and therefore, many values are higher than 1.

The lower anchor (the worse value in the whole common dataset) was used for the calculation of the EQR values of two metrics because they showed a constraint gradient (for ASPT it was 1.5 and for Index of biocenotic region 9.0).

The EQR value for each of four common metrics was then calculated according to a formula: (measured value – lower anchor)/ (benchmark value – lower anchor). For two remaining metrics (EPTCBO and % abundance of EPT taxa) lower anchor was equal to zero.

The final ICM was calculated as an average of the EQR values of four selected metrics. Following this procedure, the mean iCM values were calculated for the alternative benchmark sites from Croatia. The mean and median iCM values are:

- Translation of national boundaries to ICM

The common intercalibration metric (normalized benchmark values) and the Croatian national EQRs have been compared in OLS regression (Figure 8).

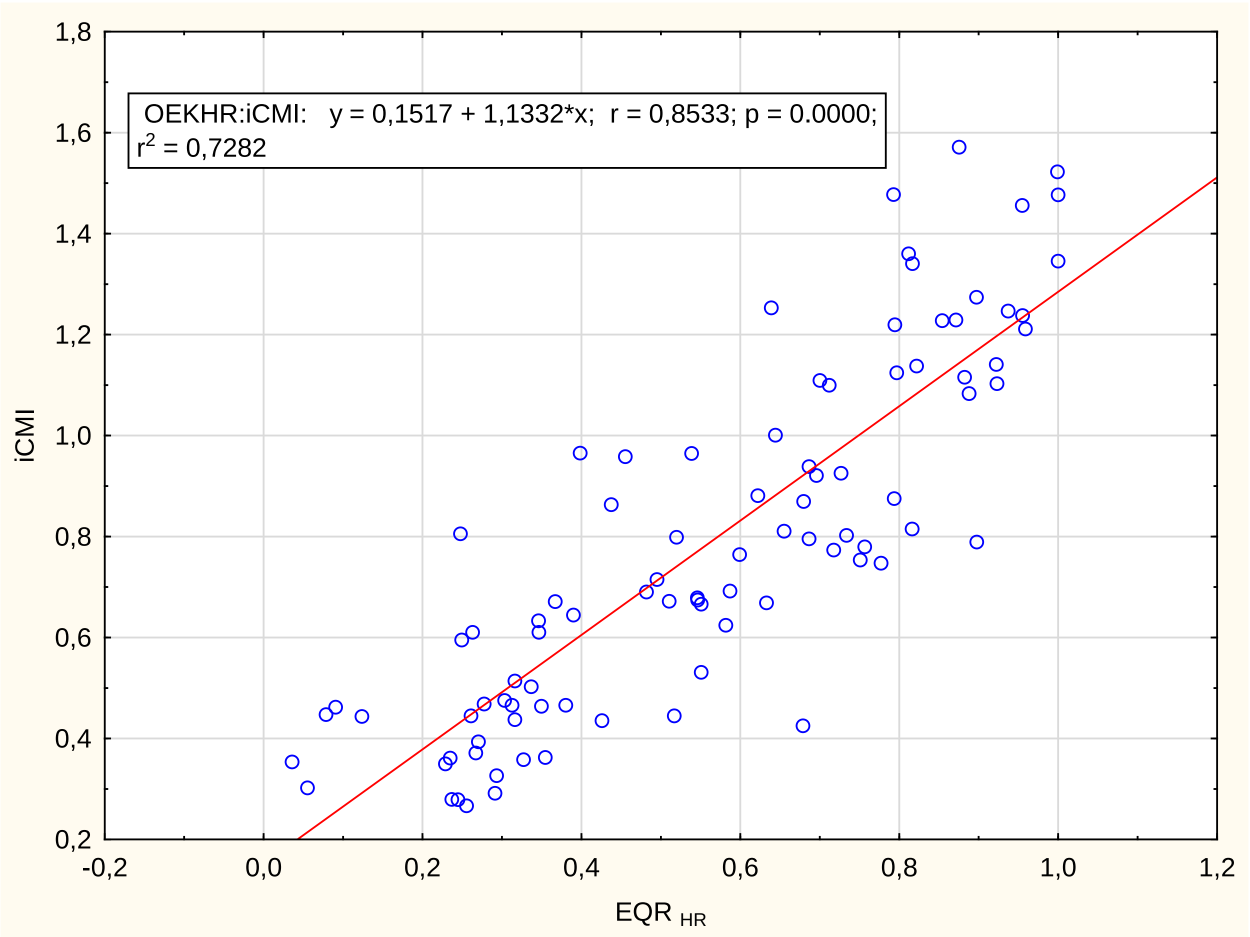

Figure 8. OLS regression to establish the relationship between iCM_bm (y) and the EQR of the joining method (Croatian EQR) for the two IC river types R-EX5 and R-EX6. The two types are combined, as the regression coefficients are not significantly different.

The Pearson correlation coefficients between the iCM and the national EQR is r = 0.856. p<0.001. n=91 (67 R-EX5 + 14 R-EX6).

- Calculating boundary bias;

The predicted position of the national class boundaries (MP, GM, HG and reference) on the CM bm scale for the two IC river types are listed in table 11.

Table 11. Reference values and class boundaries for the National EQR of the Croatian assessment method in the IC river types R-EX5 and R-EX6 and corresponding iCM values derived from the OLS regression

	National EQR	iCM
Reference values	**1**	**1.2849**
High / Good Boundary	**0.8**	**1.0583**
Good / Moderate Boundary	**0.6**	**0.8316**
Moderate / Poor Boundary	0.4	0.605
Poor / Bad Boundary	0.2	0.3783

5.4. FINAL BOUNDARIES

The global mean views of the H/G and G/M boundaries on the iCM scale in the EC GIG (all types) are:

H/G boundary global mean view of iCM is 1.0291

G/M boundary global mean view of iCM is 0.8309

The adjustment of the boundaries follows chapter 6 in the fit-in guidance of Willby et al. (2014), starting with the G/M boundary.

As the national G/M boundary on the common metric scale falls below the global view, the

amount of this deviation must be calculated:

0.8309 – 0.83162 = 0.00072

and expressed as a proportion of the width of the (national) good status class on the common

metric scale. The width of this class is:

1.05826 – 0.8034 = 0.22664

This gives a proportion of the deviation of:

0.00072 / 0.22664 = 0.003177

As these values meet the criteria (it must not be >0.25), the G/M boundary does not need changing. There is no obligation to make an adjustment.

In the second step, the H/G boundary is compared. The national view on the common metric scale is 1.05826 and thus slightly above the global view of the finalized IC exercise. The deviation is:

1.05826– 1.0291= 0.02916

The deviation expressed as a proportion of the good status class on the stand. iCM scale is:

0.02916/ 0.22664 = 0.128662

As this value is clearly <0.25, the boundary meets the comparability criteria. There is no obligation to make an adjustment. The national boundaries are as listed above in Table 11.

6. DESCRIPTION OF THE BIOLOGICAL COMMUNITIES

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT HIGH STATUS

In high status sites of the R-EX5 type the Ephemeroptera, Plecoptera and Trichoptera individuals represent around 25 % (or more) of the total macroinvertebrate abundance. High local diversity is present at these sites. Taxa sensitive to hydromorphological degradation, such as *Electrogena affinis* (Ephemeroptera) can be found. Taxa very sensitive to organic pollution are also present in high abundances.

In high status sites of the R-EX6 type the Ephemeroptera, Plecoptera and Trichoptera individuals represent around 45 % (or more) of the total macroinvertebrate abundance. High local diversity is present at these sites. Taxa sensitive to hydromorphological degradation, such as *Eukiefferiella devonica* (Diptera-Chironomidae) can be found. Taxa very sensitive to organic pollution are also present in high abundances.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT GOOD STATUS

In good status sites of the R-EX5 type the Ephemeroptera, Plecoptera and Trichoptera individuals represent around 20 % of the total macroinvertebrate abundance. Relatively high local diversity is present at these sites. Taxa sensitive to hydromorphological degradation and organic pollution are also present.

In good status sites of the R-EX5 type the Ephemeroptera, Plecoptera and Trichoptera individuals represent around 35 % of the total macroinvertebrate abundance. Relatively high local diversity is present at these sites. Taxa sensitive to hydromorphological degradation and organic pollution are also present.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT MODERATE STATUS

In moderate status sites of the R-EX5 type the Ephemeroptera, Plecoptera and Trichoptera individuals represent around 15 % of the total macroinvertebrate abundance. Local diversity is moderate. Taxa sensitive to hydromorphological degradation and organic pollution are also present but in less abundance then tolerant taxa.

In moderate status sites of the R-EX6 type the Ephemeroptera, Plecoptera and Trichoptera individuals represent around 25 % of the total macroinvertebrate abundance. Local diversity is moderate. Taxa sensitive to hydromorphological degradation and organic pollution are also present but in less abundance then tolerant taxa.

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Report on fitting the Croatian classification method for benthic macroinvertebrates classification method to the results of the completed intercalibration of the Mediterranean GIG (R-M1 and R-M2)

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Report on fitting a macroinvertebrate classification method with the results of the completed intercalibration of the MED GIG (M1 and M2)

1. INTRODUCTION

- Croatia;
- Benthic macroinvertebrates;
- M1 and M2 river types.

2. DESCRIPTION OF NATIONAL ASSESSMENT METHODS

The Water Framework Directive requires comprehensive assessment methods for the evaluation of river ecological statuses according to the benthic macroinvertebrate fauna, which includes taxonomic composition, abundance, ratio of disturbance of sensitive taxa to tolerant taxa and diversity. It is also required to harmonize national assessment methods under the intercalibration exercise with other Mediterranean (MED) Geographic Intercalibration Group (GIG) country methods. The official intercalibration of invertebrate-based methods of ecological status assessment in Mediterranean rivers was finalized within the MED-GIG intercalibration in 2011 (Feio, 2011). Croatia did not join the official IC round because it became a member state of the EU in the second half of 2013.

A new assessment method has been developed for ecological status assessment of rivers belonging to the IC types R-M1 (= HR-R_11A; HR-R_14A; HR-R_15A; HR-R_17) and R-M2 (= HR-R_12; HR-R_13; HR-R_14B; HR-R_15B; HR-R_18) based on macroinvertebrates and presented in this report. Both IC types are treated together due to the relatively small data sets (R-M1 type n=14, and R-M2 type n=18). Because of the similarities between the two types (both Mediterranean highly seasonal rivers with similar geology), their data can be considered as complementary for statistical analysis. The multimetric index uses the same metrics for both river types but with different reference values for each type. The method is compliant with the WFD normative definitions and its class boundaries are in line with the results of the completed intercalibration exercise.

The Croatian assessment method based on benthic macroinvertebrates is a modular type with two modules: saprobity and general degradation. The modular system uses the “one-out all out” principle. Croatian Large Rivers benthic invertebrate assessment method is based on the same approach and it has been successfully intercalibrated (Birk et al., 2016). The system consists of metrics with proven relationships to stressors.

The classification method is verified for WFD compliance and IC feasibility and the class boundaries were compared with agreed boundaries from the MED-GIG intercalibration exercise following the instructions of the CIS Guidance Document 30: “Procedure to fit new or updated classification methods to the results of a completed intercalibration exercise” (Willby et al. 2014).

2.1. METHODS AND REQUIRED BQE PARAMETERS

The Saprobity module represents normalized values of the Croatian saprobity index (SI_{HR}), which is based on the Pantle Buck index, but with adapted indicator values. The General Degradation module is a multimetric index (General Degradation MI) that consists of 5 metrics: Average Score Per Taxon (ASPT), r-dominance, EPT [%] (abundance classes), Diversity (Margalef Index) and the River fauna index (RFI) that is based on indicator responses to hydromorphological degradation.

The Croatian national method is in accordance with the WFD compliance, as it takes into consideration all the indicative parameters, which are mentioned in CIS Guidance document No 14 (2011): taxonomic composition, abundance, disturbance sensitive taxa to insensitive taxa, diversity and absence of major taxonomic groups (Table 1).

Table 1. Overview of the metric groups included in the Croatian national method for the assessment of IC types R-M1 and R-M2

MS	Taxonomic composition	Abundance	Sensitive / tolerant taxa	Diversity	Major taxonomic groups
HR	x	x	x	x	x

Combination rule used in the method:
The Saprobity module is based solely on EQR of the Croatian saprobity index (SI_{HR}). The General Degradation module is a multimetric index (General Degradation $_{MI}$) that equals the average EQR of 5 metrics: Average Score Per Taxon (ASPT), r-dominance, EPT [%] (abundance classes), Diversity (Margalef Index) and the River fauna index (RFI). The final assessment result equals the lower EQR value of the two modules. Two metrics belonging to the Sensitivity/tolerance group have been chosen for the general degradation module and one for the saprobic module, but they all show correlations to different pressure gradients. SI_{HR} responds to parameters linked to organic pollution such as biological oxygen demand, ASPT responds to all types of land use pressure (Corine Land Cover) and RFI responds to hydromorphological alternation. Although the three metrics belong to the same metric group, they are in no means redundant amongst themselves.

Conclusion on the WFD compliance:
all the indicative parameters included.

2.2. SAMPLING AND DATA PROCESSING

Description of sampling and data processing:

- Sampling time and frequency;
  The most favorable sampling time is spring (March-April), i.e. before mass swarms of adult insects emerge which takes place in May and June. The period of stable and low water levels should last long enough before sampling so that the macrozoobenthic community can be well-developed. Sampling shall not be undertaken: during high water levels and up to 3 weeks after high water level events, and during all other disturbances caused by natural processes.
- Sampling method;
  All available microhabitats are sampled („multi-habitat sampling") and 20 sub-samples are collected which are distributed according to the proportion of microhabitat types. Microhabitats that are represented by less than 5% are not sampled, but are recorded in the protocol. Microhabitat type represents a combination of inorganic and organic substrate. Sub-sample is sampled by raising the substrate that consists of substrate with accompanying animals from area of 25 × 25 cm (0.0625 m^2). The channel substrate of each sampling site is classified according to AQEM Consortium (2002).

- Data processing
  Average Score Per Taxon (ASPT), r-dominance, EPT [%] (abundance classes) and Diversity (Margalef Index) are calculated using ASTERICS 4.04 software, while the Croatian saprobity index and River fauna index are calculated separately. Croatian Saprobity Index (SI_{HR}) is an adapted saprobity index according to Pantle-Buck (1955):

$$SI_{HR} = \frac{\sum SIu_i}{\sum u_i}$$

where:

SI_{HR} = saprobity index
SIu_i = individual species/taxa indicator value
u_i = number of individuals calculated per 1 m^2

Indicator values of macrozoobenthic taxa (SI) are specific to Croatia.

The River fauna index was calculated according to the following equation:

$$\mathrm{RFI}_{VR_j} = \frac{\sum_{i=1}^{n} \mathrm{ac_i} \times \mathrm{Rf_i} \times \mathrm{HW_i}}{\sum_{i=1}^{n} \mathrm{ac_i} \times \mathrm{HW_i}}$$

where:
ac_i is the log5 abundance class of the i^{th} taxon,
Rf_i is the river fauna value of the i^{th} taxon,
HW_i is the hydromorphological indicative weight of the i^{th} taxon
n is the number of indicative taxa

Indicator values of macrozoobenthic taxa (RFi and HWi) are specific to IC types R-M1 and 2 and are calculated by canonical correspondence analysis of the taxa found in these types with regard to hydromiphological pressure (Urbanič, 2014).

- Identification level;

  It is recommended that identification is conducted as detailed as possible, up to the level of species if possible. Required level of macrozoobenthos identification:

  Table 2. Level of identification required for the Croatian national assessment

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		

2.3. NATIONAL REFERENCE CONDITIONS

Reference thresholds for land use in catchment follow those of the MED-GIG defined for IC types R-M1 and R-M2 (Feio, 2011)

Pressure variables	RM1+RM2
% Artificial areas (catchm)	≤1
% Intensive agriculture (catchm)	≤11
% Extensive agriculture (catchm)	≤32
% Semi-natural areas (catchm)	≥68

Hydromophological alternation NO or LOW (scoring set at ≤2)

NO or LOW:

impoundment, hydropeaking, water abstraction, upstream dam influence, water temperature modification, channelization, alteration of riparian vegetation, local habitat alteration, dykes, toxic risk, water acidification, navigation, recreational use

The settings for the national reference conditions are given in the legal document "Regulation on water quality standards"(Uredba o standardu kakvoće voda, N.N. 73/2013). The reference values in this document refer to chemical thresholds only. Reference values were calculated from type specific reference sites (reference sites determined by expert judgement) as the median value of a chemical parameter. Although this document is currently under revision (because a great amount of criteria are "expert judgement" based), we used chemical thresholds to distinguish true reference sites from the reference+benchmark combination given by Feio (2011).

Table 3. National reference conditions for chemical parameters

IC type	Parameters							
	Acidity	Oxygen regime		Nutrients				
	pH	BOD_5	COD-Mn	Ammonia	Nitrates	Total N	Orthophosphate	Total P
		mg O_2/l	mg O_2/l	mg N/l	mg N/l	mg N/l	mg P/l	mg P/l
R-M1	7.4-8.5	1.6	2	0.01	0.4	0.6	0.01	0.02
R-M2	7.4-8.5	1.9	2.5	0.01	0.4	0.6	0.01	0.02

2.4. NATIONAL BOUNDARY SETTING

The final EQR represents the "classical" boundaries (0.8; 0.6; etc.) and the final score represents the lower value of the EQR-s of the modules: Saprobity and General Degradation.
Although the National classification recognizes four types (H-R-11A, H-R-14A, H-R-15A and H-R-17) within the intercalibration type R-M1 and six types (H-R-12, H-R-13, H-R-13A, H-R-14B, H-R-15B and H-R-18) within the intercalibration type R-M2, the reference values are set equally for all types within an IC type. We acknowledge that the lack of type-specific reference values in the method is not substantially reasoned. It is possible that an extended analysis will result in different pressure-impact relationships in different types of rivers. This may result in differentiation of reference values (upper and lower anchors) for the metrics, additional differentiated normalization of the National classification system or possibly weighing the metrics before combination in the future. As the monitoring efforts are ongoing in this region of Croatia, a greater data set may possibly give a more accurate setting of the reference values for each national biotic river type, as well as the pressure response relationships.

Saprobity index

R-M1

The lower anchor of the saprobity index represents the worst theoretical value of the metric (based on the operational taxa list) and equals 3.6 (for all IC types). The setting of reference values was done based on alternative benchmark sites, since there are only two true reference sites by the national reference conditions. The procedure followed the approach of the MED GIG. The median of the metric values from the three benchmark sites is defined as H/G boundary. Using the inverse EQR (ref = H/G boundary / 0.8), a (theoretical) reference value for all the metrics was calculated. After calculating the EQRs of the saprobity index, the HIGH/GOOD boundary was determined as the median of the benchmark sites of R-M1 (Table 4). The high/good boundary for the saprobity index equaled 1.52. The upper anchor in R-M1 was calculated by retracting 20% from the median of the high/good boundary and equaled 1.21. Other boundaries were distributed equidistantly to 3.6. The value of the saprobity index in type R-M1 ranged from 1.06 to 2.40 (Table 4).

Table 4. Sites of IC type R-M1 against the criteria for reference sites (green highlighted reference sites according to Feio, 2011 and blue according to the National standards and Feio, 2011) and their values of the saprobity index.

	R-M1	O2 (%)	N-NH4+ (mg/L)	N-NO3- (mg/L)	P-PO43- (mg/L)	P-Total (mg/L)	% Artificial areas (catchm)	% Intensive agriculture (catchm)	% Extensive agriculture (catchm)	% Semi-natural areas (catchm)	Saprobity Index value
Code	Site/ criteria	73.72-127.92	≤0.09	≤1.15	≤0.06	≤0.07	≤1	≤11	≤32	≥68	
40106	Potok Rumin (pritok Cetine)	117.30	0.044	0.479	0.043	0.019	0	1.274	15.68	83.048	1.21
40108	Vojskova (pritok Cetine). Čitluk	109.17	0.036	0.404	0.023	0.012	8.826	10.653	0.02	80.49	1.25
40198	Kobilica (pritok Zrmanje). Kusac	110.56	0.006	0.273	0.007	0.002	0	0	35.41	64.58	1.34
40429	Vrba. kod mjesta Vrba	165.06	0.0472	0.071	0.033	0.020	0.396	6.043	8.37	85.18	1.55
40430	Orašnica. prije utoka u Krku	78.41	0.139	0.287	0.023	0.008	11.955	6.769	7.61	73.65	1.82
40431	Orašnica. Kninsko polje	98.08	0.048	0.348	0.029	0.016	2.430	7.988	6.38	83.20	1.58
40432	Vrba. Ojdanići	104.25	0.067	0.284	0.032	0.011	0.721	6.096	7.56	85.61	1.48
40443	Izvor Krke (pritok Une). granični prijelaz	106.07	0.193	0.376	0.005	0.005	0	7.658	21.30	71.03	1.58
40213	Krupa. Manastir	103.84	0.013	0.236	0.010	0.007	0	0	8.43	91.56	1.97
40218	Krupa. u selu Mandići. 300 m nizvodno od izvorišta	100.24	0.012	0.257	0.010	0.003	0	0	0.91	99.08	2.13
31008	Mufrin. Valenti	90.44	0.039	1.18	0.059	0.019	0	1.082	32.59	66.32	2.40
31031	kanal Botonega. 200 m od utoka u Mirnu	98.84	0.041	0.606	0.075	0.025	0	1.664	28.40	69.93	2.01
31070	Pazinčica Dubravica	93.13	0.015	0.381	0.061	0.043	0.683	4.726	26.38	68.20	2.11
31071	Pazinčica. ponor	77.69	5.968	1.94	0.477	0.368	2.534	4.440	25.28	67.73	2.05
31082	Boljunčica. nizvodno od mjesta Brus	107.41	0.034	0.21	0.058	0.012	0.196	1.105	12.84	85.85	1.10
								median of benchmark values:			**1.52**
								upper anchor:			**1.21**

R-M2

The lower anchor of the saprobity index represents the worst theoretical value of the metric and equals 3.6 (for all IC types). The value of the saprobity index in type R-M2 ranged from 1.17 to 3.32 (Table 5). The upper anchor in R-M2 was calculated as the median of saprobity index values from national reference sites and equaled 1.62.

Table 5. Sites of IC type R-M2 against the criteria for reference sites (according to Feio. 2011) and their values of the saprobity index. Benchmark sites are highlighted.

	R-M2	O2 (%)	N-NH4+ (mg/L)	N-NO3- (mg/L)	P-PO43- (mg/L)	P-Total (mg/L)	% Artificial areas (catchm)	% Intensive agriculture (catchm)	% Extensive agriculture (catchm)	% Semi-natural areas (catchm)	Saprobity Index value
Code	Site/ criteria	73.72-127.92	≤0.09	≤1.15	≤0.06	≤0.07	≤1	≤11	≤32	≥68	
14006	Una. kod izvorišta Loskun	99.56	0.011	0.501	0.011	0.011	0.35	6.57	15.98	77.09	1.642
31010	Mirna. Portonski most	105.04	0.0157	0.609	0.009	0.066	0.74	4.49	24.38	70.37	1.902
31011	Mirna. Kamenita vrata	111.18	0.035	0.627	0.022	0.064	2.53	5.04	21.62	70.80	2.219
31016	Obuhvatni kanal Srednja Mirna	84.52	0.033	0.556	0.016	0.049	0	1.55	34.16	64.28	2.469
31017	Stara Mirna. Gradinje	91.31	0.036	0.746	0.044	0.107	2.10	2.03	29.44	66.41	2.366
31024	Raša. most Mutvica	100.68	0.0152	1.144	0.010	0.040	0.35	12.89	16.58	70.17	2.393
31025	Obuhvatni kanal Krapanj. most u naselju Raša	101.16	3.562	2.876	1.181	1.659	25.70	11.75	16.27	46.26	3.316
40102	Cetina. Vinalić	91.66	0.0155	0.316	0.003	0.012	0.53	6.81	11.30	81.35	1.292
40104	Cetina. Barišići	106.89	0.025	0.372	0.002	0.004	0	0.43	2.26	97.30	1.166
40200	Zrmanja. Butiga	110.52	0.03	0.357	0.002	0.005	0	3.11	21.70	75.18	1.605
40205	Zrmanja. Palanka	100.60	0.015	0.361	0.003	0.014	0	0	26.84	73.16	1.622
40208	Zrmanja. Žegar	101.40	0.015	0.271	0.003	0.012	0	3.42	20.95	75.63	1.874
40416	Krka. nizvodno od Knina	99.17	0.029	0.353	0.003	0.011	1.64	11.65	9.17	77.52	1.829
40441	Krka. Marasovine	91.57	0.030	0.361	0.007	0.015	1.05	7.19	13.10	78.64	1.598
40453	Butižnica. HE Golubić	97.50	0.015	0.181	0.003	0.013	0.19	0.16	10.51	89.13	1.523
40454	Butižnica. Bulin most	99.12	0.021	0.231	0.007	0.022	0.72	2.54	15.87	80.86	1.574
40515	Norin. Vid	76.11	0.019	0.932	0.012	0.019	5.15	11.68	6.34	76.81	1.701
								upper anchor:			**1.62**

General degradation

R-M1

The General Degradation module consists of 5 metrics: Average Score Per Taxon (ASPT), r-dominance, EPT [%] (abundance classes), Diversity (Margalef Index) and the River fauna index (RFI). The setting of reference values was done based on alternative benchmark sites, since there are only two true reference sites by the national reference conditions. The procedure followed the approach of the MED GIG. Using the inverse EQR (ref = H/G boundary / 0.8), a (theoretical) reference value for all the metrics was calculated. The lower anchor was set at the lowest value in the whole dataset of both IC types (M1 and M2)

Table 6. Metrics and their upper and lower anchors used in the calculation of the General Degradation module for IC type R-M1. Benchmark sites are highlighted.

Code	Site/Metric	- EPT [%] (abundance classes)	Diversity (Margalef Index)	- r-Dominance	Average score per Taxon	River fauna index(RFI)
40198	Kobilica (pritok Zrmanje). Kusac	26.027	2.574	2.142	7	0.124
40106	Potok Rumin (pritok Cetine)	26.19	2.591	1.747	5.429	0.109
40108	Vojskova (pritok Cetine). Čitluk	37.5	4.342	3.287	6.45	0.116
40429	Vrba. kod mjesta Vrba	28.767	5.044	16.86	6	0.104
40430	Orašnica. prije utoka u Krku	11.538	5.115	38.614	4.87	0.107
40431	Orašnica. Kninsko polje	15.894	3.764	9.592	4.938	0.094
40432	Vrba. Ojdanići	20.625	4.891	5.884	5.905	0.079
40443	Izvor Krke (pritok Une). granični prijelaz	44.828	5.484	21.488	7.25	0.141
31008	Mufrin. Valenti	10.784	3.129	9.286	5.267	0.081
31031	kanal Botonega. 200 m od utoka u Mirnu	24.706	4.811	20.833	6.333	-0.033
31070	Pazinčica Dubravica	19.149	2.132	14.158	4.75	0.097
31071	Pazinčica. ponor	25.203	2.694	39.24	5.667	0.119
31082	Boljunčica. nizvodno od mjesta Brus	26.389	2.031	25	5.625	0.084
40213	Krupa. Manastir	26.496	3.177	0.219	7.05	0.119
40218	Krupa. u selu Mandići	37.864	2.368	0.511	7.235	0.122
	Upper anchor	33.12	3.97	1.4	7.5	0.137
	Lower anchor	0	1.27	68.18	3.14	-0.857

R-M2

The General Degradation module consists of 5 metrics: Average Score Per Taxon (ASPT), r-dominance, EPT [%] (abundance classes), Diversity (Margalef Index) and the River fauna index (RFI). The upper anchor in R-M2 was calculated as the median of index values from National criteria reference sites (Table 7). The lower anchor was set at the lowest value in the whole dataset of both IC types (M1 and M2).

Table 7. Metrics and their upper and lower anchors used in the calculation of the General Degradation module for IC type R-M2. Benchmark sites are highlighted.

Code	Site/Metric	- EPT [%] (abundance classes)	Diversity (Margalef Index)	- r-Dominance	Average score per Taxon	River fauna index(RFI)
14006	Una. kod izvorišta Loskun	35.849	4.118	12.032	6.333	0.129
31010	Mirna. Portonski most	29.054	3.639	39.264	6.684	-0.164
31011	Mirna. Kamenita vrata	27.811	5.048	21.674	5.632	0.092
31016	Obuhvatni kanal Srednja Mirna	13.861	3.074	5.737	4.625	-0.223
31017	Stara Mirna. Gradinje	24.46	4.561	18.389	5.412	-0.146
31024	Raša. most Mutvica	22.892	4.08	28.723	5.389	-0.215
31025	Obuhvatni kanal Krapanj. most u naselju Raša	0	1.265	68.183	3.143	-0.857
40102	Cetina. Vinalić	33.077	3.821	4.609	6	0.097
40104	Cetina. Barišići	38.938	2.418	4.363	7	0.111
40200	Zrmanja. Butiga	30	5.349	2.951	6.053	0.111
40205	Zrmanja. Palanka	30	5.136	4.507	6.682	0.138
40208	Zrmanja-Žegar	24.823	7.91	9.793	5.821	0.118
40416	Krka. nizvodno od Knina	2.837	4.495	5.813	4.214	0.107
40441	Krka. Marasovine	30.631	3.811	2.521	6.722	0.116
40453	Butižnica. HE Golubić	18.889	2.403	4.98	5.273	0.078
40454	Butižnica. Bulin most	26.667	3.873	2.035	6.368	0.081
40515	Norin. Vid	13.043	7.066	17.163	4.903	0.089
	Upper anchor	30.63	4.12	4.61	6.33	0.119
	Lower anchor	0	1.27	68.18	3.14	-0.857

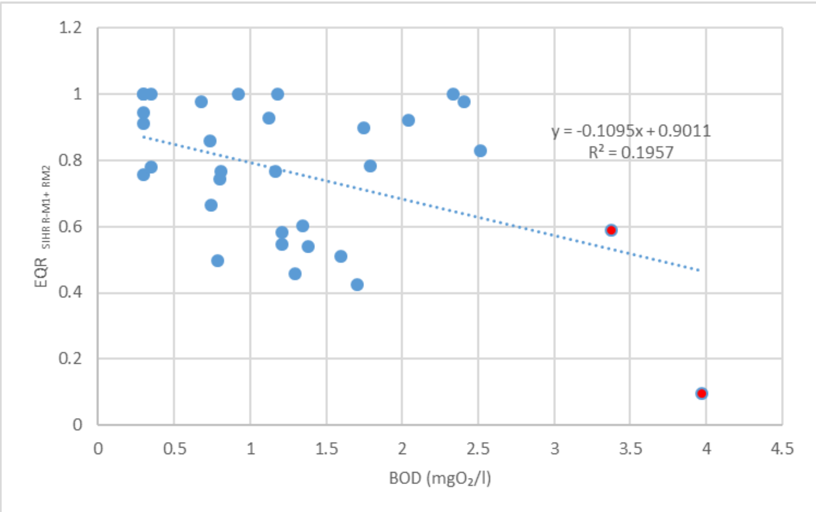
2.5. PRESSURES ADDRESSED

The Croatian method addresses: 1) catchment land use, 2) pollution by organic matter and 3) habitat destruction (hydromorphological alteration). The Saprobity module addresses organic pollution, whereas other stressor responses are integrated in the General degradation module. The pressure / index graphs show responses of the two IC types together. Seeing as the types are similar (only catchment sizes differ) and the number of sites per one type alone is relatively low: R-M1 type n=14 and R-M2 type n=18. It is also important to note that, although a relatively wide gradient of all analyzed pressures is present, only few sites in this region have very high specific pressure values. These sites are marked as extremes or outliers in the scatterplots, but are still valuable for assessing the pressure-impact relationship, as they are nevertheless representative for M1 and M2 stream types, taking into consideration the specificity of the MED-GIG area in question where there is comparably very little pressure. Two sites that are standing out are definitely type-specific. However, they are respectively: a) highly hydromorphologically changed and situated within an urban area with some communal waste inputs and b) specific because the catchment area that includes agriculturally covered floodplain near upstream of the sampling site while sampling site itself is boulder dominated fast flowing canyon site, highly typical for the MED-GIG. Due to discharge regime and porosity of carbonate substrate, the agricultural runoff is common.
The lower value of the two modules is the final score of the site and it gives a direct suggestion on which stressor should be addressed primarily if the score would be less favorable. This method is therefore comparable to the methods that are already successfully intercalibrated.

The following pressure-response relationships for the Croatian assessment method have been derived:

1) **Saprobity:**

The correlation between the National EQR and both the BOD (r=-0.442) and COD (r=-0.622) parameters are significant ($p<0.05$). When excluding the outliers (colored red in Fig 1), the relationship between the National EQR and COD remain significant (r= 0.421; $p<0.05$), whereas the relationship between the National EQR and BOD is not significant (r=0.201, $p>0.05$).



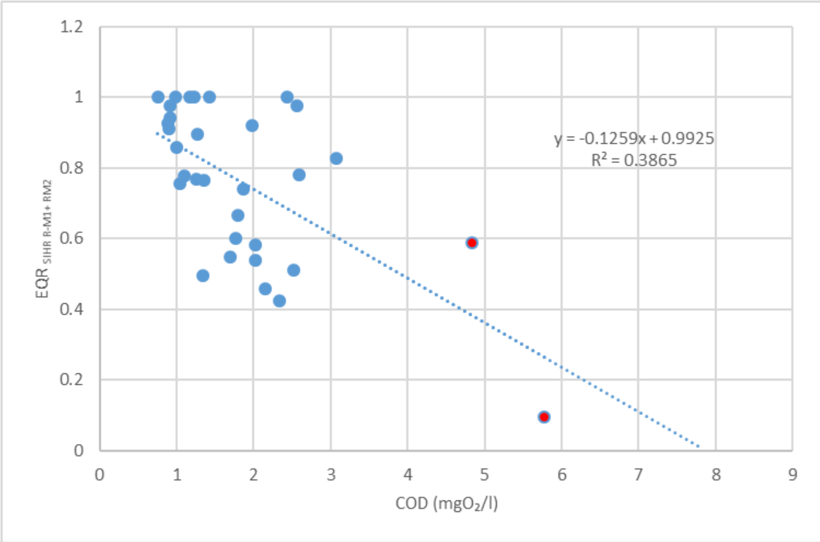
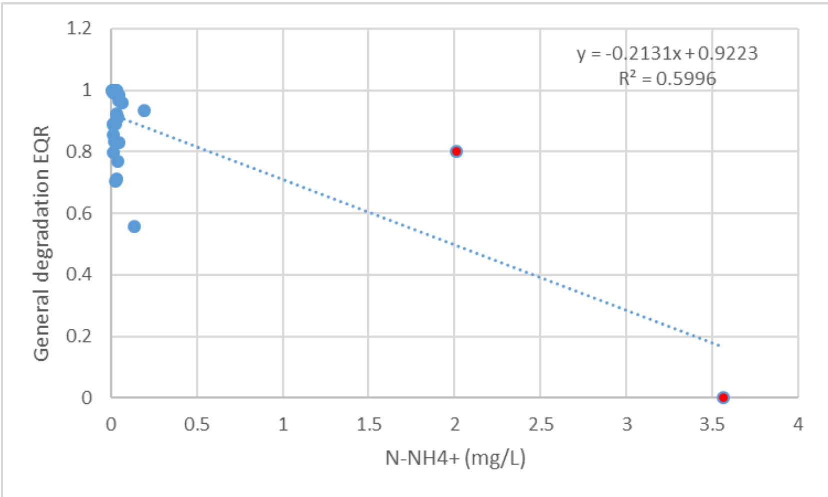


Figure 1. Pressure-Response relationship between biological oxygen demand (BOD) and chemical oxygen demand (COD) against the SI_{HR} values in river types R-M1 and R-M2.

2) General Degradation:

A. Hydro-chemistry

The correlation between the National EQR and the ammonia (r=-0.774), nitrate (r=-0.725) and orthophosphate (r=-0.808) concentrations are significant ($p<0.05$). When excluding the outliers (colored red in Fig 2), the relationship between the National EQR and the ammonia (r=-0.327), nitrate (r=-0.279) and orthophosphate (r=-0.209) concentrations were no longer significant ($p>0.05$), although retained negative trend relationships.



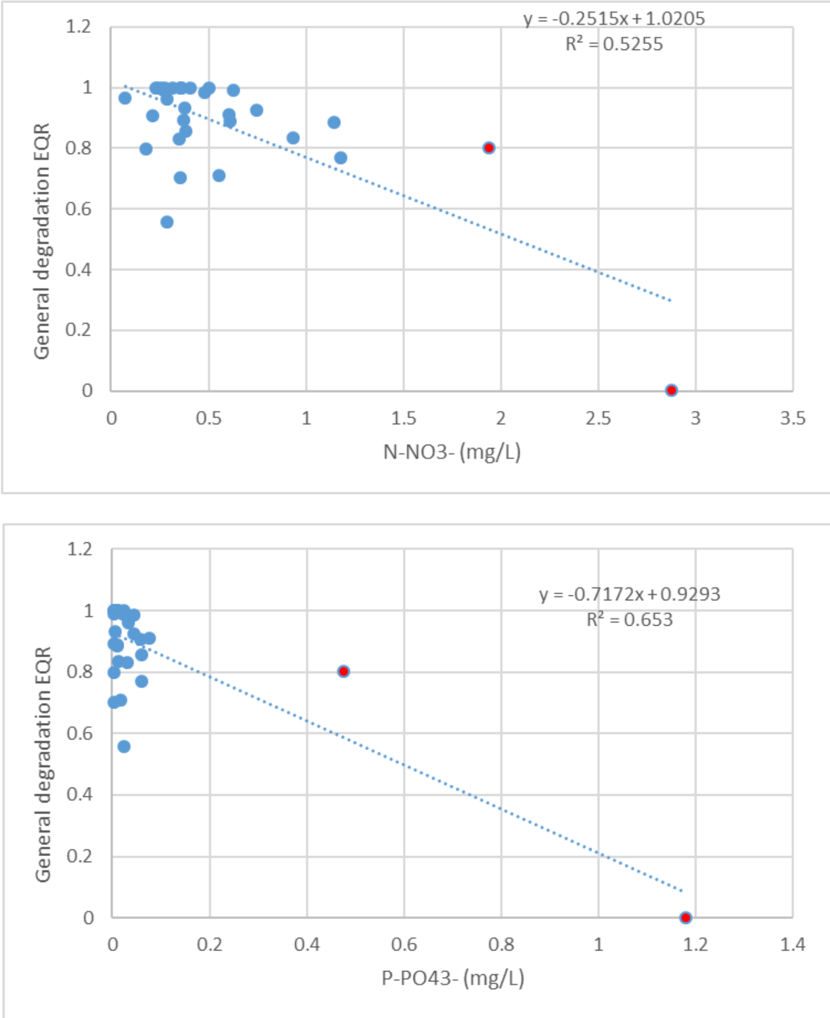


Figure 2. Pressure-Response relationship between chemical water properties against General Degradation module scores for sites of river types R-M1 and R-M2.

B. Land use

The correlation between the National EQR and the artificial areas (r=-0.835), natural areas in the catchment (r=0.554) and the Land Use Index (r=-0.818) are significant (p<0.05). When excluding the outliers (colored red in Fig 3), the relationship between the National EQR and the artificial areas (r=-0.447) and the Land Use Index (r=-0.458) remain significant (p<0.05), whereas the relationship between the National EQR and natural areas in the catchment (r=0.26) did not remain significant (p>0.05).

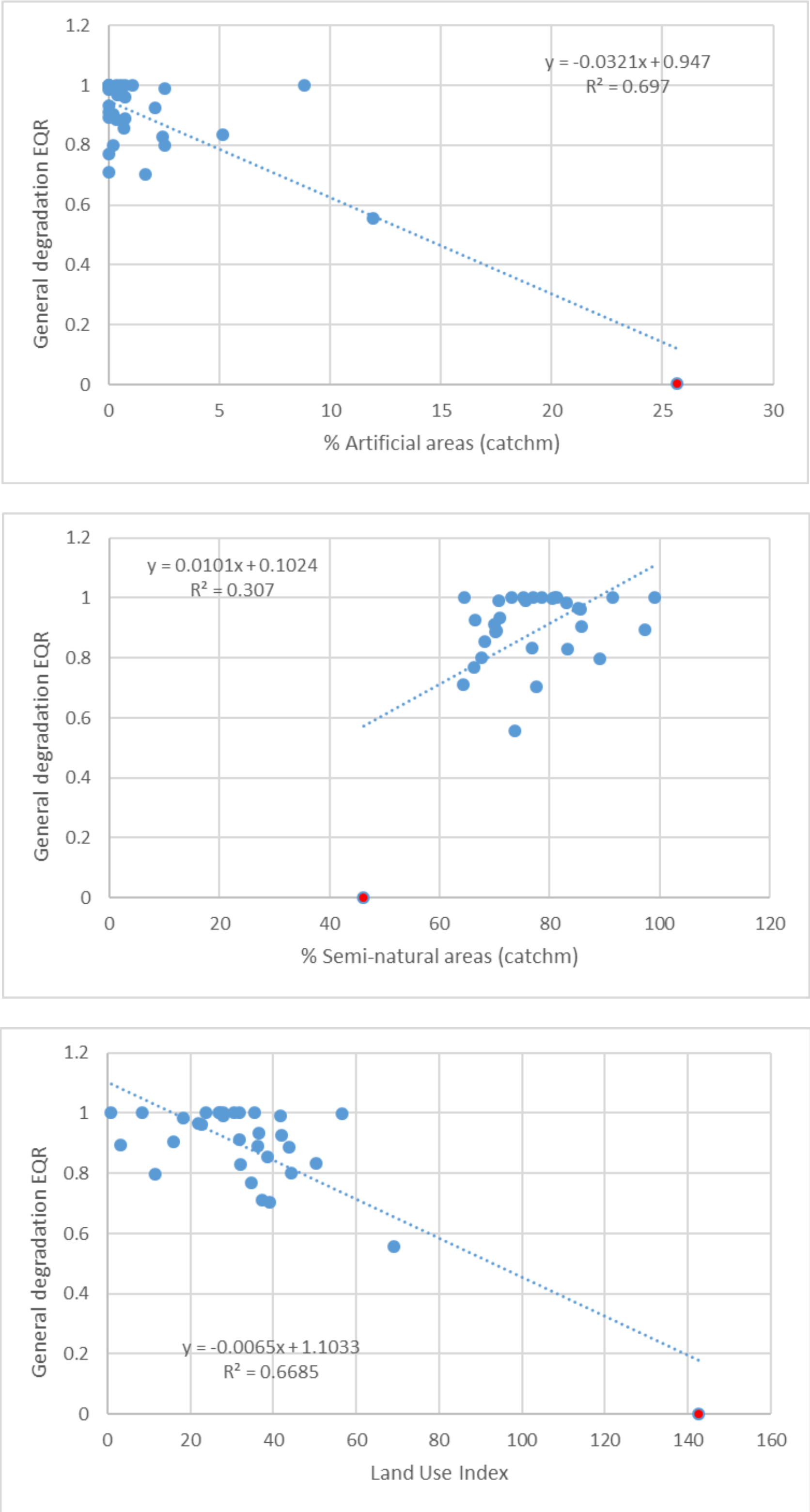


Figure 3. Pressure-Response relationship between Corine Land Cover (categories urban and natural) and the Land Use Index LUI and against General Degradation module scores for sites of river types R-M1 and R-M2.

C. Hydromorphology

The correlation between the National EQR and the hydromorphological scores of sediment structure (r=-0.423), riverbank erosion (r=-0.385) and hydrology score (r=-0.368) are significant ($p<0.05$). When excluding the outliers (colored red in Fig 4), the relationship between the National EQR and the hydromorphological scores of sediment structure (r=-

0.250), riverbank erosion (r=-0.239) and hydrology score (r=-0.205) were no longer significant (p>0.05), although retained negative trend relationships.

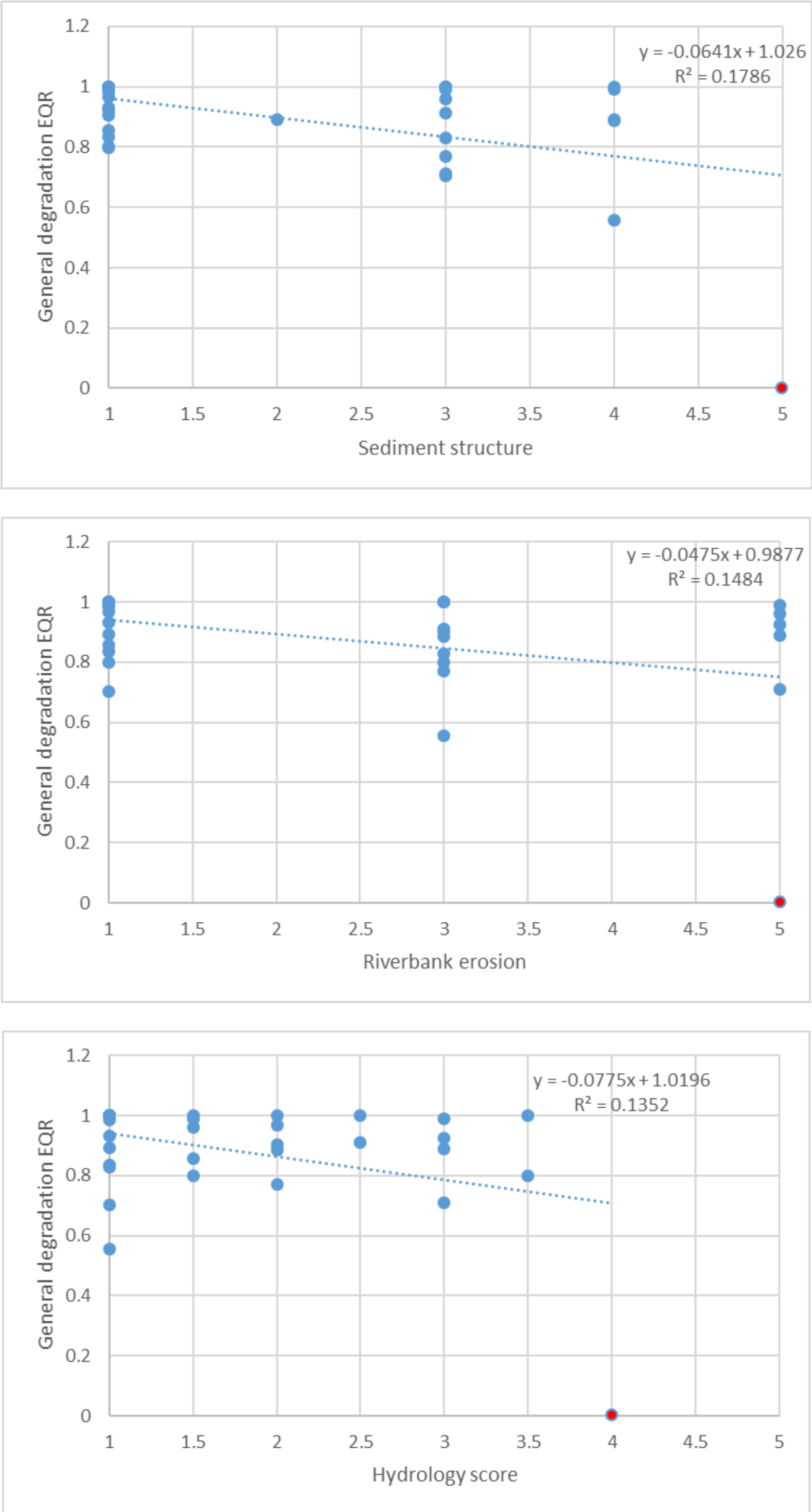


Figure 4. Pressure-Response relationship between hydromorphological river features against General Degradation module scores for sites of river types R-M1 and R-M2.

D. Resume

For all three groups of pressures (land use. chemistry. hydromorphological alteration). significant regressions could be found. Some relationships were found to be relatively weak and mostly driven by outliers, although even after excluding the outliers, the relationships retained the same trend (if not significance). It is concluded that both the Saprobity Index and the General Degradation Multimetric Index clearly respond to anthropogenic impacts and can be used for the assessment of the ecological status.

3. WFD COMPLIANCE CHECKING

The first step in the Intercalibration process requires the checking of national methods considering the following WFD compliance criteria.

Table 8. List of the WFD compliance criteria and the WFD compliance checking process and results.

Compliance criteria	Compliance checking
Ecological status is classified by one of **five classes** (high. good. moderate. poor and bad).	yes
High. good and moderate ecological status are set in line with the WFD's **normative definitions** (**Boundary setting procedure**)	yes
All relevant parameters indicative of the biological quality element are covered (see Table 1 in the IC Guidance). A **combination rule** to combine parameter assessment into BQE assessment has to be defined. If parameters are missing. Member States need to demonstrate that the method is sufficiently indicative of the status of the QE as a whole	yes
Assessment is adapted to **intercalibration common types** that are defined in line with the typological requirements of the Annex II WFD and approved by WG ECOSTAT	yes
The water body is assessed against **type-specific near-natural reference conditions**	yes
Assessment results are expressed as **EQRs**	yes
Sampling procedure allows for **representative** information about water body quality/ecological status **in space and time**	yes
All data relevant for assessing the biological **parameters** specified in the WFD's normative definitions are covered by the **sampling procedure**	yes
Selected taxonomic level achieves adequate **confidence and precision** in classification	yes

4. IC FEASIBILITY CHECKING

The intercalibration process ideally covers all national assessment methods within a GIG. However, the comparison of dissimilar methods ("apples and pears") has to clearly be avoided. The Intercalibration exercise focused on specific type / biological quality element / pressure combinations. The second step of the process introduces an "IC feasibility check" to restrict the actual intercalibration analysis to methods that address the same common type(s) and anthropogenic pressure(s), and follow a similar assessment concept.

4.1. TYPOLOGY

The biological typology of running waters in Croatia was initially established in 2011 (Mihaljević et al. 2011), mainly based on expert opinion, due to general lack of all data types: both biological and pressure data. Today, biological data in almost all types are sufficient, as well data on pressures such as water chemistry and land use. The data sets are still lacking hydromorphological scoring from many sites as the hydromorphological evaluation of running waters in Croatia began only recently, in 2017. The current assessment method has equal reference and "worst" metric values for several Croatian types, but in the future, with more data on hydromorphology we wish to fine-tune these values for every type. Hence, the typology will remain as initially determined.

Table 9. Overview of common intercalibration types in the Mediterranean rivers GIG and MS sharing the types.

Common IC type	Type characteristics
R-M1	**catchment <100 km^2; mixed geology (except siliceous); highly seasonal**
R-M2	**catchment 100-1000 km^2 ; mixed geology (except siliceous); highly seasonal**
R-M3	catchment 1000-10000 km^2 ; mixed geology (except siliceous); highly seasonal
R-M4	non-siliceous streams; highly seasonal
R-M5	temporary rivers

The typology in Croatia is more precise, distinguishing Mediterranean rivers in type R-M1 and R-M2 not only by catchment size, but also by altitude. Rivers of the sub-ecoregion Istria (Istrian peninsula; HR-R-17 and HR-R-18) are assessed separately from the rest of the rivers of the Dinaric coastal sub-ecoregion due to some geological differences.

Table 10. Comparison of the intercalibration types with the Croatian typology.

Common IC type	Croatian typology	Croatian type characteristics
R-M1	HR-R_11A	Small lowland and upland rivers of the Dinaric coastal sub-ecoregion
	HR-R_14A	Small lowland rivers with a channel drop >5 ‰ of the Dinaric coastal sub-ecoregion
	HR-R_15A	Small and medium rivers in karst polje of the Dinaric coastal sub-ecoregion
	HR-R_17	Small lowland and upland rivers of the Dinaric coastal sub-ecoregion Istria
R-M2	HR-R_12	Medium and large upland rivers of the Dinaric coastal sub-ecoregion
	HR-R_13	Medium and large lowland rivers of the Dinaric coastal sub-ecoregion
	HR-R_13A	Large lowland rivers of the Dinaric coastal sub-ecoregion with barrage pools
	HR-R_14B	Medium lowland rivers with a channel drop >5 ‰ rivers of the Dinaric coastal sub-ecoregion
	HR-R_15B	Medium rivers in karst polje of the Dinaric coastal sub-ecoregion
	HR-R_18	Medium lowland rivers of the Dinaric coastal sub-ecoregion Istria

4.2. PRESSURES ADDRESSED

The pressure gradient has been assessed for the Corine Land Cover (CLC) as well as the land use index, which is derived from CLC and defined as:

LUI = 4 * CLC urban+2*CLC intensive agriculture + CLC extensive agriculture

The ranges of the CLC and LUI in the two river types are:

CLC/LUI	range R-M1	range R-M2
CLC urban	0 – 11.96	0 – 25.70
CLC agr. intens.	0 – 10.65	0 – 12.89
CLC agr. extens.	0.02 – 35.42	2.26 – 34.17
CLC natural	63.85 – 99.08	46.26 – 97.30
LUI	0.92 – 68.98	3.14 – 142.60

The hydromorphological alteration scale ranges from 1 (no) to 5 (high) and consists of multiple smaller indices. The three main indices: hydrology regime, morphology and flow continuity ranged from 1 to 5 in both river types, whereas the mean hydromorphological score ranged from 1 to 3.57 in M1 and 1 to 4.29 in M2.

The ranges for the chemical variables tested are:

Chemical variable	range R-M1	range R-M2
BOD_5 [mg L^{-1}]	0.3 – 3.376	0.3 – 3.971
COD [mg L^{-1}]	0.756 – 4.836	0.892 – 5.775
PO_4^-P [mg L^{-1}]	0.003 – 0.368	0.002 – 1.181
NO_3^-N [mg L^{-1}]	0.072 – 1.944	0.181 – 2.877
NH_4^-N [mg L^{-1}]	0.006 – 5.968	0.011 – 3.563
Total P [mg L^{-1}]	0.006 – 0.477	0.005 – 1.659

The different pressure gradients covered by the national data set are considered sufficient.

4.3. ASSESSMENT CONCEPT

Does the national method follow the same assessment concept as other methods in the Intercalibration group? Provide evaluation of IC feasibility regarding assessment concept of the intercalibrated methods

Table 11. Data acceptance criteria used for the data quality control and describing the data acceptance checking process and results (Feio 2011).

Data acceptance criteria	Data acceptance checking Croatia	
Data requirements (obligatory and optional)	Common pressure data. Common environmental data. Correctly checked typologies, geographical location, and biotic data, all properly introduced in harmonized excel files.	+
The sampling and analytical methodology	All MS sampling methods use a multi-habitat approach. All MS have indicated a response of their indices to pressure using statistical tools.	+
Level of taxonomic precision required and taxa lists with codes	Family level is required	+
The minimum number of sites / samples per intercalibration type	Minimum of 15 benchmark sites by IC type are available.	-*
Sufficient covering of all relevant quality classes per type	Yes	-*

*relevant for the previous IC exercise, but not for join-in procedure

4.4. CONCLUSION ON THE INTERCALIBRATION FEASIBILITY

The number of sites fully complying in terms of the type criteria is high enough for carrying out the IC exercise. It is concluded that the intercalibration is feasible for the types R-M1 and R-M2

5. DEMONSTRATING THE COMPLIANCE WITH THE COMPLETED INTERCALIBRATION EXERCISE

5.1. BACKGROUND

Following the CIS Guidance No. 30 (Willby et al. 2014), case A1 will be applied for the assessment method using macroinvertebrates in the MED GIG river types R-M1 and R-M2.

5.2. DESCRIPTION OF IC DATASET

i. Full details of the common metric (e.g. species scores and metric weights)

ii. A suitable site x biology dataset covering a range of environmental quality from which the national EQR and common metric can be calculated

iii. Accompanying pressure data in the same format as that used in the completed exercise *iv. Information on the specific thresholds already used in the completed exercise to define reference or alternative benchmark sites (e.g. human population density. extent of agricultural land in the catchment. nutrient concentrations. etc.)*

v. Details of exactly how the benchmarking was undertaken in the completed exercise (e.g. creation of a common metric EQR by dividing the observed value by the median common metric value of a set of national reference or benchmark sites). If the completed exercise concluded that benchmarking was not necessary the mean value of the benchmark sites from each country must be provided so that the joining Member State can also judge the need to benchmark its own method

vi. Values of the global mean view of the HG and GM boundaries on the common metric scale for the Member States who participated in the completed exercise.

5.3. DESCRIPTION OF INTERCALIBRATION PROCEDURE

1*. Calculate the common metric (CM) on the national dataset.*

2. Use the associated pressure data to identify sites in the national dataset that meet the criteria established by the GIG for the selection of benchmark or reference sites.

3. Standardize the common metric (CM_bm) against the benchmark according to the approach used in the completed exercise. If benchmark standardization was concluded not to be required in the completed exercise the mean CM value of the joining method's benchmark sites must lie inside the range of mean values of the benchmark sites of the methods already intercalibrated for this conclusion to remain applicable. If the joining method's benchmark sites lie outside of this range the joining method must benchmark standardize its sites relative to the global mean CM value of the benchmark sites included in the completed exercise. These scenarios are illustrated in Table 1 and 2 of the IC Manual.

4. Use OLS regression to establish the relationship between CM_bm (y) and the EQR of the joining method (x). A specialist case is that when a joining method relies exclusively on the common metric developed in the completed exercise for its classification rather than devising an original method (then being more like Option 1). In such cases, a regression would be meaningless as y is directly dependent on x. The goal for an MS choosing to use the CM as the basis for their method is simple – after any benchmarking their boundaries must simply lie within one quarter of class of the global mean view.

5. Predict the position of the national class boundaries (MP. GM. HG and reference) on the CM_bm scale.

6. Apply the comparability criteria as summarized in Chapter 6 of the IC Manual.

- Benchmark standardization;

In the MED GIG the reference sites are referred to as "benchmarks" as they represent the best available values determined for the abiotic data for the Mediterranean region. In Feio (2011) these two terms seem to be synonymized, so we also use the two as synonyms in this intercalibration fitting in procedure.

Table 12. Criteria for identifying benchmarking sites for the MED GIG.

Pressure variables	**Benchmarks are accepted if RM1+RM2**
Channelization (classes 1-4)	≤ 2
Bank alteration (classes 1-4)	≤ 2
Connectivity (classes 1-4)	≤ 2
Local habitat alteration (classes 1-4)	≤ 2
Stream Flow (classes 1-4)	≤ 2
Upstream dams influence (classes 1-4)	≤ 2
Hydropeaking (classes 1-4)	≤ 2
Riparian Vegetation (classes 1-4)	≤ 2
DO (mg/L) [1]	6.39-13.70
O_2 (%)	73.72-127.92
N-$NH4^+$ (mg/L)	≤0.09
N-NO_3^- (mg/L)	≤1.15
P-Total (mg/L)	≤0.07
P-PO_4^{3-} (mg/L)	≤0.06
% Artificial areas (catchm)	≤1
% Intensive agriculture (catchm)	≤11
% Extensive agriculture (catchm)	≤32
% Semi-natural areas (catchm)	≥68

- Calculation of Intercalibration Common metrics (ICM) or Best-Related Intercalibrated National Classification (BRINC);

  The ICM includes the following six metrics: average score per taxon, log 10 (sel_EPTD+1), 1-GOLD, total number of taxa Families, number of EPT taxa (Families) and the Shannon-Wiener diversity index. The index is described in Buffagni et al. (2006). The main steps are:

  - Normalization of the single metrics by dividing the original values by the 75th percentile of high status sites within the national data sets

  - Calculation the iCM:

    iCM = a * ASPT + b * log10(sel_EPTD+1) + c * 1-GOLD + d * No. Fam + e * EPT + f * H'

    a – f weights for the six metrics:

    - ASPT a = 0.333
    - log10(sel_EPTD+1) b = 0.266
    - 1-GOLD c = 0.067
    - No. Fam d = 0.167
    - EPT e = 0.083
    - Shannon-Wiener H' f = 0.083

- Normalization of the iCM (_norm. iCM) and of the EQR (_ norm. EQR) by dividing the original values by the 75th percentile of benchmark sites within the national data sets (Table 13)

Table 13. Metric normalization and intercalibration common metric iCM calculation for the comparison with the national assessment methodology. Benchmark sites are highlighted.

IC Type	Code	Site	Average score per Taxon	Norm. ASPT	log(sel EPTD+1)	Norm log(sel EPTD+1)	(1-GOLD)	Norm (1-GOLD)	Number of Families	Norm. Num. Of Fam.	- EPT-Taxa	Norm. EPT	Diversity (Shannon-Wiener-Index)	Norm H'	National EQR	iCM	norm ICM
M1	40198	Kobilica (pritok Zrmanje), Kusac	7.00	1.03	0.33	0.60	0.96	1.08	17.00	0.64	8.00	0.67	0.39	0.16	0.93	0.75	0.78
M1	40106	Potok Rumin (pritok Cetine)	5.43	0.80	0.40	0.73	0.91	1.02	17.00	0.64	6.00	0.50	1.20	0.51	0.86	0.72	0.75
M1	40108	Vojskova (pritok Cetine), Čitluk	6.45	0.95	0.51	0.94	0.93	1.04	29.00	1.08	18.00	1.50	1.59	0.68	0.94	1.00	1.04
M1	40429	Vrba, kod mjesta Vrba	6.00	0.88	0.54	0.99	0.75	0.84	26.00	0.97	11.00	0.92	2.46	1.05	0.85	0.94	0.98
M1	40430	Orašnica, prije utoka u Krku	4.87	0.72	0.53	0.98	0.21	0.23	39.00	1.46	6.00	0.50	2.00	0.86	0.63	0.87	0.91
M1	40431	Orašnica, Kninsko polje	4.94	0.73	0.34	0.64	0.85	0.95	24.00	0.90	5.00	0.42	1.93	0.83	0.75	0.73	0.76
M1	40432	Vrba, Ojdanići	5.91	0.87	0.34	0.64	0.78	0.87	32.00	1.20	9.00	0.75	2.05	0.88	0.83	0.85	0.89
M1	40443	Izvor Krke (pritok Une), granični prijelaz	7.25	1.07	0.62	1.14	0.67	0.75	34.00	1.27	21.00	1.75	2.87	1.23	0.84	1.17	1.22
M1	31008	Mufrin, Valenti	5.27	0.78	0.42	0.78	0.86	0.97	23.00	0.86	4.00	0.33	1.12	0.48	0.68	0.74	0.77
M1	31031	kanal Botonega, 200 m od utoka u Mirnu	6.33	0.93	0.41	0.75	0.55	0.61	30.00	1.12	11.00	0.92	2.83	1.21	0.62	0.91	0.96
M1	31070	Pazinčica Dubravica	4.75	0.70	0.00	0.00	0.05	0.06	14.00	0.52	5.00	0.42	1.82	0.78	0.50	0.42	0.44
M1	31071	Pazinčica, ponor	5.67	0.83	0.51	0.95	0.08	0.09	19.00	0.71	8.00	0.67	2.10	0.90	0.66	0.78	0.82
M1	31082	Boljunčica, nizvodno od mjesta Brus	5.63	0.83	0.00	0.00	0.08	0.09	13.00	0.49	6.00	0.50	1.76	0.75	0.62	0.47	0.49
M1	40213	Krupa, Manastir	7.05	1.04	0.49	0.91	0.71	0.80	23.00	0.86	12.00	1.00	1.54	0.66	0.94	0.92	0.96
M1	40218	Krupa, u selu Mandići	7.24	1.07	0.63	1.17	0.99	1.10	19.00	0.71	10.00	0.83	1.51	0.65	1.02	0.98	1.03
M2	40205	Zrmanja, Palanka	6.68	0.98	0.55	1.02	0.76	0.85	29.00	1.08	14.00	1.17	2.78	1.19	0.86	1.03	1.08
M2	40200	Zrmanja, Butiga	6.05	0.89	0.20	0.38	0.42	0.47	24.00	0.90	11.00	0.92	2.23	0.95	0.87	0.73	0.77
M2	14006	Una, kod izvorišta Loskun	6.33	0.93	0.56	1.03	0.84	0.94	26.00	0.97	15.00	1.25	2.10	0.90	0.85	0.99	1.03
M2	31010	Mirna, Portonski most	6.68	0.98	0.45	0.84	0.58	0.65	25.00	0.93	11.00	0.92	2.01	0.86	0.74	0.90	0.94
M2	31011	Mirna, Kamenita vrata	5.63	0.83	0.51	0.94	0.57	0.64	30.00	1.12	14.00	1.17	2.55	1.09	0.60	0.94	0.99
M2	31016	Obuhvatni kanal Srednja Mirna	4.63	0.68	0.31	0.57	0.82	0.91	25.00	0.93	3.00	0.25	1.46	0.62	0.49	0.67	0.70
M2	31017	Stara Mirna, Gradinje	5.41	0.80	0.40	0.74	0.73	0.82	24.00	0.90	8.00	0.67	2.65	1.13	0.54	0.82	0.85
M2	31024	Raša, most Mutvica	5.39	0.79	0.36	0.67	0.62	0.69	23.00	0.86	10.00	0.83	2.29	0.98	0.52	0.78	0.82
M2	31025	Obuhvatni kanal Krapanj, most u naselju Raša	3.14	0.46	0.00	0.00	0.00	0.00	10.00	0.37	0.00	0.00	1.10	0.47	0.00	0.26	0.27
M2	40515	Norin, Vid	4.90	0.72	0.48	0.88	0.59	0.66	45.00	1.68	8.00	0.67	2.63	1.12	0.75	0.95	0.99
M2	40453	Butižnica, HE Golubić	5.27	0.78	0.00	0.00	0.94	1.05	14.00	0.52	5.00	0.42	1.34	0.57	0.73	0.50	0.52
M2	40454	Butižnica, Bulin most	6.37	0.94	0.47	0.86	0.96	1.07	24.00	0.90	10.00	0.83	1.77	0.76	0.88	0.90	0.94
M2	40441	Krka, Marasovine	6.72	0.99	0.47	0.88	0.89	0.99	21.00	0.79	11.00	0.92	2.30	0.98	0.87	0.92	0.96
M2	40416	Krka, nizvodno od Knina	4.21	0.62	0.38	0.70	0.71	0.79	28.00	1.05	2.00	0.17	2.43	1.04	0.68	0.72	0.75
M2	40102	Cetina, Vinalić	6.00	0.88	0.45	0.84	0.94	1.05	29.00	1.08	13.00	1.08	1.10	0.47	0.95	0.90	0.94
M2	40104	Cetina, Barišići	7.00	1.03	0.50	0.93	0.85	0.95	18.00	0.67	12.00	1.00	1.69	0.72	0.88	0.91	0.95
M2	40208	Zrmanja-Žegar	5.82	0.86	0.38	0.70	0.57	0.63	37.00	1.38	18.00	1.50	3.02	1.29	0.75	0.98	0.99
	75th percentile of the high status M1 and M2 sites		6.79		0.54		0.89		26.75		12.00		2.34			0.96	

- Translation of national boundaries to ICM or BRINC:

The regression between the normalized EQR values from the national assessment method and the normalized iCM is highly significant (Pearson corr. R=0.6738; $p<0.001$). The OLS regression of the two variables is shown in figure 4.

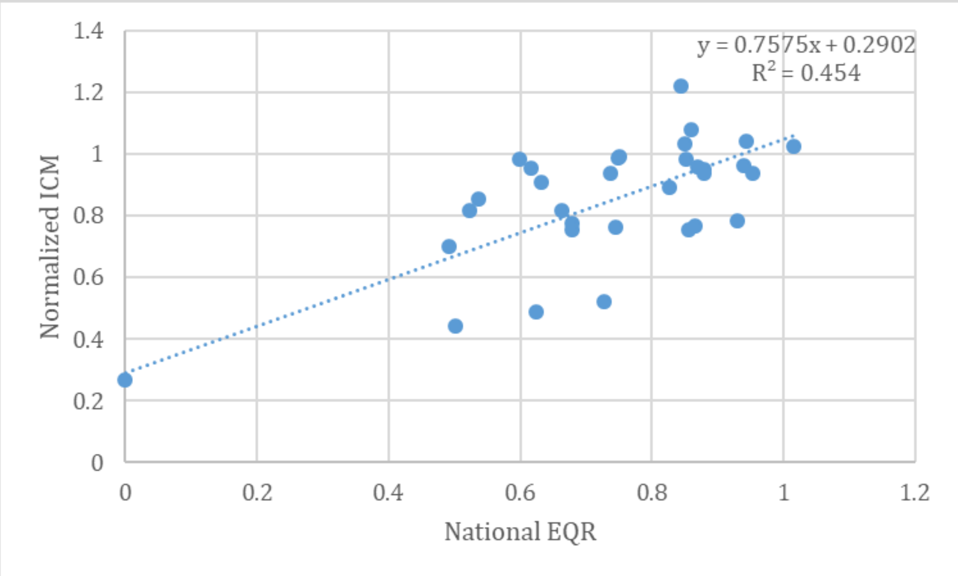


Figure 4. OLS regression to establish the relationship between normalized iCM (y) and the normalized nEQR of the joining method (x) for the IC river type R-M1 and R-M2.

- Calculating boundary bias;

The global mean views of the H/G and G/M boundaries for IC river types R-M1 and R-M2 in the MED GIG can be derived from the final table of harmonized class boundaries (Feio. 2011). The average weighted by MS is:

H/G boundary global mean view of iCM is 0.87928

± 0.25 class width: 0.8962 – 1.0477

G/M boundary global mean view of iCM is 0.70831

± 0.25 class width: 0.8962 – 0.7447

Calculating boundary bias for R-M1 and R-M2:

National EQR	Norm. ICM R-M1 and R-M2	Global mean view	Deviation from global mean view	Class width	deviation expressed as proportion
1	1.0477				
0.8	0.8962	0.87928	0.01692	0.1515	11%
0.6	0.7447	0.70831	0.03639	0.1515	24%
0.4	0.5932				
0.2	0.4417				

Harmonizing of boundaries

The national H/G and G/M boundary on the common metric scale falls above the global mean view for M1 and M2. The amount of the deviation expressed as a proportion of the width of the (national) good and high status classes on the common metric scale is <0.25 for all the boundaries. Therefore, there is no need to adjust the boundaries.

5.4. FINAL BOUNDARIES

Final boundaries for R-M1and R-M2:

National EQR	Norm. ICM R-M1 and R-M2
1	1.0477
0.8	0.8962
0.6	0.7447
0.4	0.5932
0.2	0.4417

6. DESCRIPTION OF THE BIOLOGICAL COMMUNITIES

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT HIGH STATUS

In high status sites of the R-M1 type the Ephemeroptera. Plecoptera and Trichoptera individuals represent around 30 % (or more) of the total macroinvertebrate abundance. High local diversity is present at these sites (Margalef index around 4 or more). The proportion of r- strategist taxa is relatively low (around 5 % or less). Taxa sensitive to hydromorphological degradation and taxa very sensitive to organic pollution are present in high abundances.

In high status sites of the R-M2 type the Ephemeroptera. Plecoptera and Trichoptera individuals represent around 35 % (or more) of the total macroinvertebrate abundance. High local diversity is present at these sites (Margalef index around 5 or more). The proportion of r- strategist taxa is relatively low (around 5 % or less). Taxa sensitive to hydromorphological degradation and taxa very sensitive to organic pollution are present in high abundances.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT GOOD STATUS

In good status sites of the R-M1 type the Ephemeroptera. Plecoptera and Trichoptera individuals represent around 25 % (or more) of the total macroinvertebrate abundance. High local diversity is present at these sites (Margalef index around 4). The proportion of r- strategist taxa is relatively low (around 15 %). Taxa sensitive to hydromorphological degradation and taxa very sensitive to organic pollution are present.

In good status sites of the R-M2 type the Ephemeroptera. Plecoptera and Trichoptera individuals represent around 25 % of the total macroinvertebrate abundance. Relatively high local diversity is present at these sites (Margalef index around 4). The proportion of r-strategist taxa is relatively low (around 10 %). Taxa sensitive to hydromorphological degradation and taxa very sensitive to organic pollution are present.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT MODERATE STATUS

In moderate status sites of the R-M1 type the Ephemeroptera. Plecoptera and Trichoptera individuals represent around 20 % of the total macroinvertebrate abundance. High local diversity is present at these sites (Margalef index around 3). The proportion of r-strategist taxa is relatively low (around 20 %). Taxa sensitive to hydromorphological degradation and taxa very sensitive to organic pollution are also present but in less abundance then tolerant taxa.

In moderate status sites of the R-M2 type the Ephemeroptera. Plecoptera and Trichoptera individuals represent around 20 % of the total macroinvertebrate abundance. High local diversity is present at these sites (Margalef index around 3). The proportion of r- strategist taxa is relatively low (around 20 %). Taxa sensitive to hydromorphological degradation and taxa very sensitive to organic pollution are also present but in less abundance then tolerant taxa.

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Report on fitting the Croatian classification method for benthic macroinvertebrates classification method to the results of the completed intercalibration of the Mediterranean GIG (R-M5)

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Report on fitting the Croatian classification method for benthic macroinvertebrates classification method to the results of the completed intercalibration of the Mediterranean GIG (R-M5)

1. INTRODUCTION

- Croatia;
- Benthic macroinvertebrates;
- M5 river type.

2. DESCRIPTION OF NATIONAL ASSESSMENT METHODS

The Water Framework Directive requires comprehensive assessment methods for the evaluation of river ecological statuses according to the benthic macroinvertebrate fauna, which includes taxonomic composition, abundance, ratio of disturbance of sensitive taxa to tolerant taxa and diversity. It is also required to harmonize national assessment methods under the intercalibration exercise with other Mediterranean (MED) Geographic Intercalibration Group (GIG) country methods. The official intercalibration of invertebrate-based methods of ecological status assessment in Mediterranean rivers was finalized within the MED-GIG intercalibration in 2011 (Feio, 2011). Croatia did not join the official IC round because it became a member state of the EU in the second half of 2013.

A new assessment method has been developed for ecological status assessment of rivers belonging to the IC type R-M5 (= HR-R_16A; HR-R_16B; HR-R_19) based on invertebrates and presented in this report. The IC type has a relatively small, but sufficient data set (R-M5 type n=19) consisting of biological data and all pressures needed for intercalibration fit in procedures. The newly proposed multimetric index is compliant with the WFD normative definitions and its class boundaries are in line with the results of the completed intercalibration exercise.

The Croatian assessment method based on benthic invertebrates is a modular type with two modules: saprobity and general degradation. The modular system uses the "one-out all out" principle. Croatian Large Rivers benthic invertebrate assessment method is based on the same approach and it has been successfully intercalibrated (Birk et al., 2016). The system consists of metrics with proven relationships to stressors.

The classification method is verified for WFD compliance and IC feasibility and the class boundaries were compared with agreed boundaries from the MED-GIG intercalibration exercise following the instructions of the CIS Guidance Document 30: "Procedure to fit new or updated classification methods to the results of a completed intercalibration exercise" (Willby et al. 2014).

2.1. METHODS AND REQUIRED BQE PARAMETERS

The Saprobity module represents normalized values of the Croatian saprobity index (SI_{HR}), which is based on the Pantle Buck index, but with adapted indicator values. The General Degradation module is a multimetric index (General Degradation MI) that consists of 4 metrics: Index of Biocoenotic Region, EPT taxa, Number of families and Diversity (Shannon-Wiener-Index).

The Croatian national method is in accordance with the WFD compliance, as it takes into consideration all the indicative parameters which are mentioned in CIS Guidance document No 14 (2011): taxonomic composition, abundance, disturbance sensitive taxa to insensitive taxa, diversity and absence of major taxonomic groups (Table 1).

Table 1. Overview of the metric groups included in the Croatian national method for the assessment of IC type R-M5

MS	Taxonomic composition	Abundance	Sensitive / tolerant taxa	Diversity	Absence of major taxonomic groups
HR	x	x	x	x	x

Combination rule used in the method:
The Saprobity module is based solely on the EQR of the Croatian saprobity index (SI_{HR}). The General Degradation module is a multimetric index (General Degradation$_{MI}$) that equals the EQR values of 4 metrics as: 0.4*Index of Biocoenotic Region + 0.2*EPT taxa + 0.2*Number of families + 0.2*Diversity (Shannon-Wiener-Index). The final assessment result equals the lower EQR value of the two modules. SI_{HR} responds to parameters linked to organic pollution such as biological oxygen demand, whereas the General Degradation module responds to land use pressure (Corine Land Cover), hydromorphological alternation and hydro-chemical pressures

Conclusion on the WFD compliance:
Most indicative parameters included.

2.2. SAMPLING AND DATA PROCESSING

Description of sampling and data processing:

- Sampling time and frequency;
  The most favorable sampling time is spring (March-April), i.e. before mass swarms of adult insects emerge which takes place in May and June. If no flow-phases occur in this period, surveys should be timed before the drying events. The period of stable and low water levels should last long enough before sampling so that the macrozoobenthic community can be well-developed. Sampling shall not be undertaken: during high water levels and up to 3 weeks after high water level events, and during all other disturbances caused by natural processes.
- Sampling method;
  All available microhabitats are sampled („multi-habitat sampling“) and 20 sub-samples are collected which are distributed according to the proportion of microhabitat types. Microhabitats that are represented by less than 5% are not sampled, but are recorded in the protocol. Microhabitat type represents a combination of inorganic and organic substrate. Sub-sample is sampled by raising the substrate that consists of substrate with accompanying animals from area of 25 × 25 cm (0.0625 m^2). The channel substrate of each sampling site is classified according to AQEM Consortium (2002).
- Data processing
  Index of Biocoenotic Region, EPT taxa, Number of families and Diversity (Shannon-Wiener-

Index) are calculated using ASTERICS 4.04 software, whereas the Croatian saprobity index is calculated separately. Croatian Saprobity Index (SI_{HR}) is an adapted saprobity index according to Pantle-Buck (1955):

$$SI_{HR} = \frac{\sum SIu_i}{\sum u_i}$$

where:

SI_{HR} = saprobity index
SIu_i = individual species/taxa indicator value
u_i = number of individuals calculated per 1 m^2

Indicator values of macrozoobenthic taxa (SI) are specific to Croatia.

- Identification level;

  It is recommended that identification is conducted as detailed as possible, up to the level of species if possible. Required level of macrozoobenthos identification:

Table 2. Level of identification required for the Croatian national assessment

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		

2.3. NATIONAL REFERENCE CONDITIONS

The settings for the national reference conditions of some chemical thresholds are given in the legal document Regulation on water quality standards (Uredba o standardu kakvoće voda, NN 96/2019), but this document is currently in the process of revision. Because of this, reference thresholds for this incercalibration fit in procedure follow those of the MED-GIG defined for IC type R-M5 (Feio, 2011):
In the MED GIG the reference sites are referred to as "benchmarks" as they represent the best available values determined for the abiotic data for the Mediterranean region. In Feio (2011) these two terms seem to be synonymized, so we also use the two as synonyms in this intercalibration fitting in procedure. Setting of the national reference conditions for general degradation follow those of the MED-GIG defined for IC types R-M5 (Table 3).

Table 3 – Criteria for identifying benchmarking sites for the MED GIG.

Pressure variables	Benchmarks are accepted if RM5
Channelization (classes 1-4)	≤ 2
Bank alteration (classes 1-4)	≤ 2
Connectivity (classes 1-4)	≤ 2
Local habitat alteration (classes 1-4)	≤ 2
Stream Flow (classes 1-4)	≤ 2
Upstream dams influence (classes 1-4)	≤ 2
Hydropeaking (classes 1-4)	≤ 2
Riparian Vegetation (classes 1-4)	≤ 2
DO (mg/L) [1]	6.39-13.70
O_2 (%)	60.34-127.92
N-$NH4^+$ (mg/L)	≤0.09
N-NO_3^- (mg/L)	≤1.15
P-Total (mg/L)	≤0.07
P-PO_4^{3-} (mg/L)	≤0.06
% Artificial areas (catchm)	≤1
% Intensive agriculture (catchm)	≤11
% Extensive agriculture (catchm)	≤32
% Semi-natural areas (catchm)	≥68

2.4. NATIONAL BOUNDARY SETTING

The final EQR represents the "classical" boundaries (0.8; 0.6; etc.), seeing as the final score represents the lower value of the already transformed EQR-s of the modules: Saprobity and General Degradation. The setting of reference values was done based on alternative benchmark sites, since true reference sites are lacking (as seen in the Bulgarian example by Wolfram et al., 2016). The procedure followed the approach of the Med GIG. The median of the metric values from the four benchmark sites is defined as H/G boundary. Using the inverse EQR (ref = H/G boundary / 0.8), a (theoretical) reference value of all the metrics was calculated.

Although the National classification recognizes three types (= HR-R_16A; HR-R_16B; HR-R_19) within the intercalibration type R-M5, the reference values are set equally for all types within an IC type. We acknowledge that the lack of type-specific reference values in the method is not substantially reasoned. It is possible that an extended analysis will result in different pressure-impact relationships in different types of rivers. This may result in differentiation of reference values (upper and lower anchors) for the metrics, additional differentiated normalization of the National classification system or possibly weighing the metrics before combination in the future. As the monitoring efforts are ongoing in this region of Croatia, a greater data set may possibly give a more accurate setting of the reference values for each national biotic river type, as well as the pressure response relationships.

Saprobity index

The lower anchor of the saprobity index represents the worst theoretical value of the metric (based on the operational taxa list) and equals 3.6. The median of the metric values from the four benchmark sites is defined as H/G boundary. Using the inverse EQR (ref = H/G boundary / 0.8), a (theoretical) reference value for all the metrics was calculated. The high/good boundary for the saprobity index equaled 1.66. The upper anchor in R-M5 was calculated by retracting 20% from the high/good boundary and equaled 1.19. The value of the saprobity index in type R-M5 ranged from 1.28 to 2.60 (Table 4).

Table 4. Sites of IC type R-M5 against the criteria for reference sites (according to Feio, 2011) and their values of the saprobity index. Benchmark sites are highlighted.

	R-M5	**O_2 (%)**	**N-NH_4^+ (mg/L)**	**N-NO_3^- (mg/L)**	**P-PO_4^{3-} (mg/L)**	**P-Total (mg/L)**	**% Semi-natural areas (catchm)**	**% Artificial areas (catchm)**	Saprobity Index value
Code	Site/ criteria	73.72-127.92	≤0.09	≤1.15	≤0.06	≤0.07	≥68	≤1	
30081	Dubračina. Crikvenica (igralište)	106.69	0.02	0.43	0.01	0.02	89.77	3.51	1.29
30082	Suha Novljanska Ričina. 1 km uzvodno od ušća	123.95	0.02	0.35	0.01	0.04	88.86	7.05	1.45
30084	Suha Ričina Bašćanska. poslije Jurandvora	88.30	0.01	0.40	0.00	0.01	78.30	2.76	2.37
31009	Krvar. most na cesti Motovun-Pazin	98.00	0.01	0.43	0.00	0.07	63.85	0.00	1.94
31013	Bračana. uzvodno od ceste Buzet-Motovun	106.51	0.04	0.53	0.04	0.07	75.97	0.00	2.05
31014	Mala Huba. most na cesti Buzet-Motovun	100.61	0.05	0.83	0.02	0.05	52.52	4.91	2.35
31018	Draga Baredine. most Štuparija	94.64	0.02	0.35	0.01	0.07	68.67	0.00	1.30
31021	Raša. most Potpićan	97.77	0.01	0.44	0.01	0.05	66.85	0.54	2.60
31040	Dragonja. ušće. kod Kaštela	99.78	0.02	0.14	0.07	0.11	65.43	0.10	2.25
40140	Pritok Cetine uzvodno od Vinalića	88.67	0.02	0.39	0.01	0.01	68.05	1.30	1.37
40141	Zduški potok. prije utoka u Cetinu	107.88	0.03	0.48	0.02	0.03	29.90	4.18	1.28
40143	Donji kanal. pritok Cetine kod Trilja	58.14	1.19	0.77	0.21	0.36	77.22	0.82	1.83
40211	Jaruga. Ražanac	100.45	0.93	0.20	0.00	0.00	37.60	5.87	2.07
40220	Jaruga/Mijanovac. Zvjerinac	100.81	0.03	0.17	0.02	0.03	70.77	0.62	1.54
40221	Vodotok Bokanjac. prije ulaska u tunel	90.36	0.30	0.79	0.00	0.01	66.09	6.27	2.17
40315	Jaruga. Benkovac	82.63	0.18	1.69	0.06	0.07	51.79	9.22	1.50
40318	Bašćica. uzvodno od Posedarja	93.34	0.01	0.62	0.00	0.01	21.34	5.90	1.92
40507	Šipovača. Jelavića most	78.22	0.06	0.55	0.00	0.02	52.95	10.41	2.23
40702	Taranta. uzvodno od Srebrenog	117.40	0.03	0.65	0.03	0.04	53.03	17.69	1.88

General degradation

The General Degradation module consists of 4 metrics: Index of Biocoenotic Region, EPT taxa, Number of families and Diversity (Shannon-Wiener-Index). The median of the metric values from the four benchmark sites is defined as H/G boundary. Using the inverse EQR (ref = H/G boundary / 0.8), a reference value of all the metrics was calculated. (Table 5). The lower anchor for all metrics was set at the lowest value in the whole dataset.

Table 5. Metrics and their upper and lower anchors used in the calculation of the General Degradation module for IC type R-M5. Benchmark sites are highlighted.

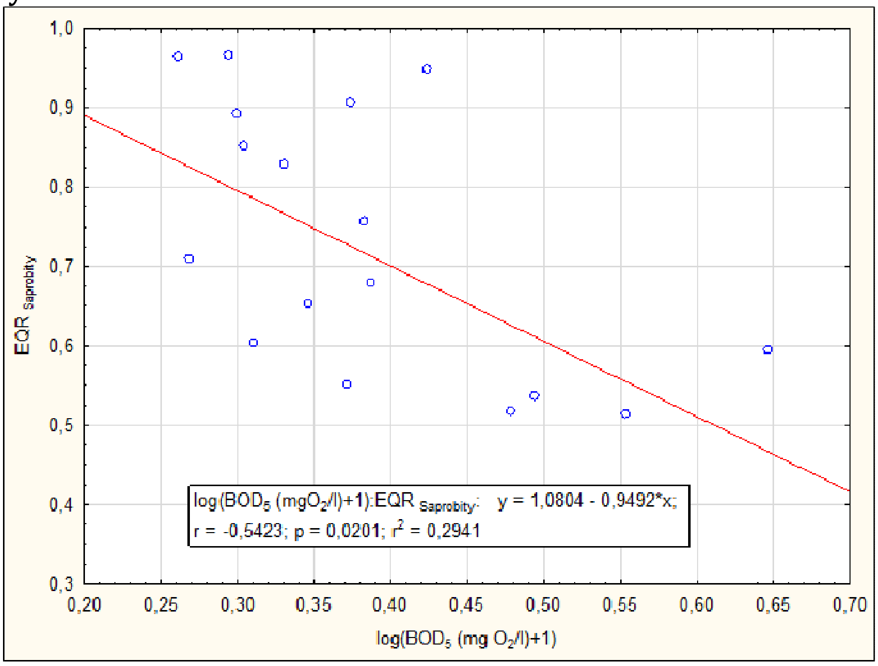
Name	Index of Biocoenotic Region	EPT taxa	Number of families	Diversity (Shannon-Wiener-Index)
Zduški potok, prije utoka u Cetinu	5.85	2	17	1.31
Potok Kotluša - pritok Cetine uzvodno od Vinalića	4.70	17	33	1.87
Šipovača, Jelavića most	5.59	9	25	2.83
Donji kanal, pritok Cetine kod Trilja	6.80	5	20	2.59
Dubračina, Crikvenica (igralište)	6.00	21	26	2.62
Suha Ričina Bašćanska, poslije Jurandvora	6.36	9	19	1.86
Suha Novljanska Ričina, 1 km uzvodno od ušća	6.60	5	19	1.80
Taranta, uzvodno od Srebrenog	5.71	3	14	1.46
Bašćica, uzvodno od Posedarja	7.60	6	20	1.84
Jaruga/Mijanovac, Zvjerinac	4.70	5	33	2.12
Jaruga (Krivac), Benkovac	4.70	2	16	1.36
Vodotok Bokanjac, prije ulaska u tunel	9.00	2	11	1.31
Jaruga, Ražanac	7.14	11	16	3.22
Mala Huba, most na cesti Buzet-Motovun	6.83	5	20	2.27
Bračana, uzvodno od ceste Buzet-Motovun	5.78	21	18	2.62
Dragonja, ušće, kod Kaštela	6.72	14	31	2.93
Draga Baredine, most Štuparija	6.45	15	31	2.34
Raša, most Potpićan	5.52	6	22	1.76
Krvar, most na cesti Motovun-Pazin	3.93	14	21	2.37
Upper anchor	6.25	20	32.5	2.93
Lower anchor	9	2	11	1.31
Min	9	2	11	1.31
Max	3.93	21	33	3.22
Median of benchmark sites	5.24	16	26	2.35

2.5. PRESSURES ADDRESSED

The Croatian method addresses: 1) pollution by organic matter; 2) hydrochemical pressures; 3) catchment land use, and 4) habitat destruction (hydromorphological alteration). The Saprobity module addresses organic pollution, whereas other stressor responses are integrated in the General degradation module. The lower value of the two modules is the final score of the site and it gives a direct suggestion on which stressor should be addressed primarily if the score would be less favorable. This method is therefore comparable to the methods which are already successfully intercalibrated.

The following pressure-response relationships for the Croatian assessment method have been derived:

1) **Saprobity:**



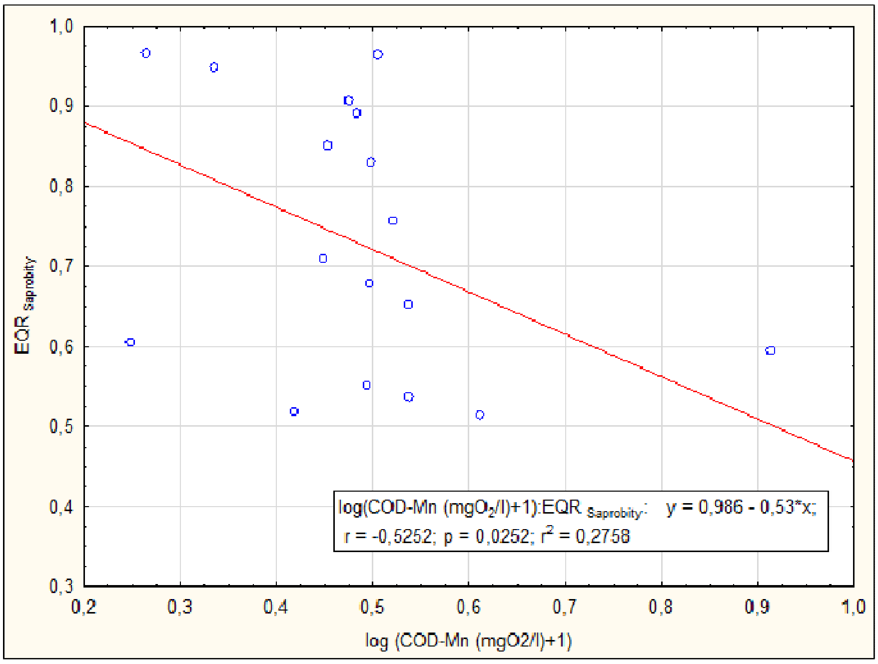
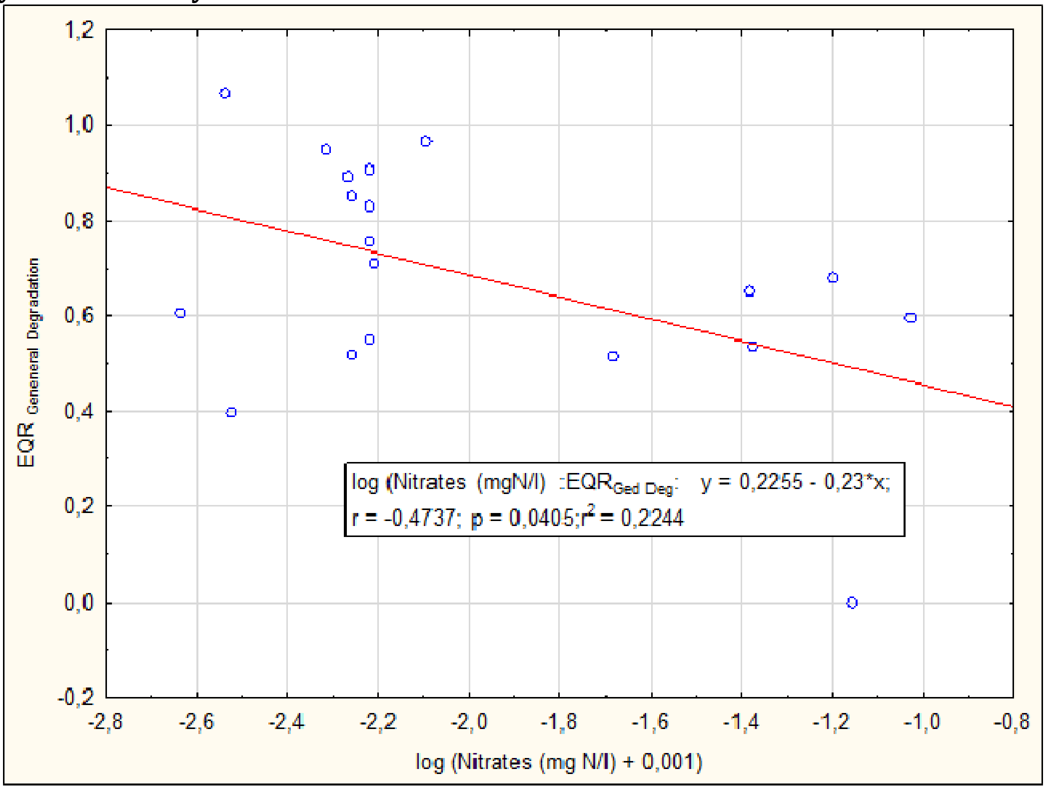


Figure 1. Pressure-Response relationship between biological oxygen demand (BOD) and chemical oxygen demand (COD) against the EQR values of the saprobity module in river type R-M5.

2) General Degradation:

A. Hydro-chemistry



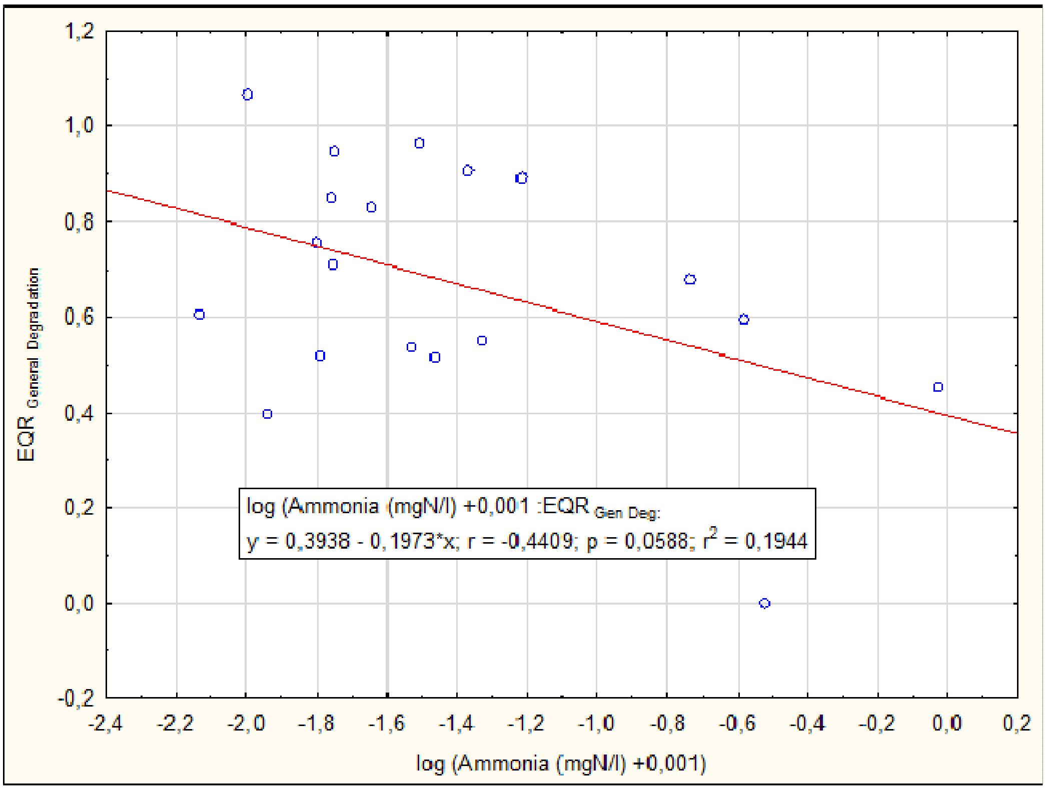
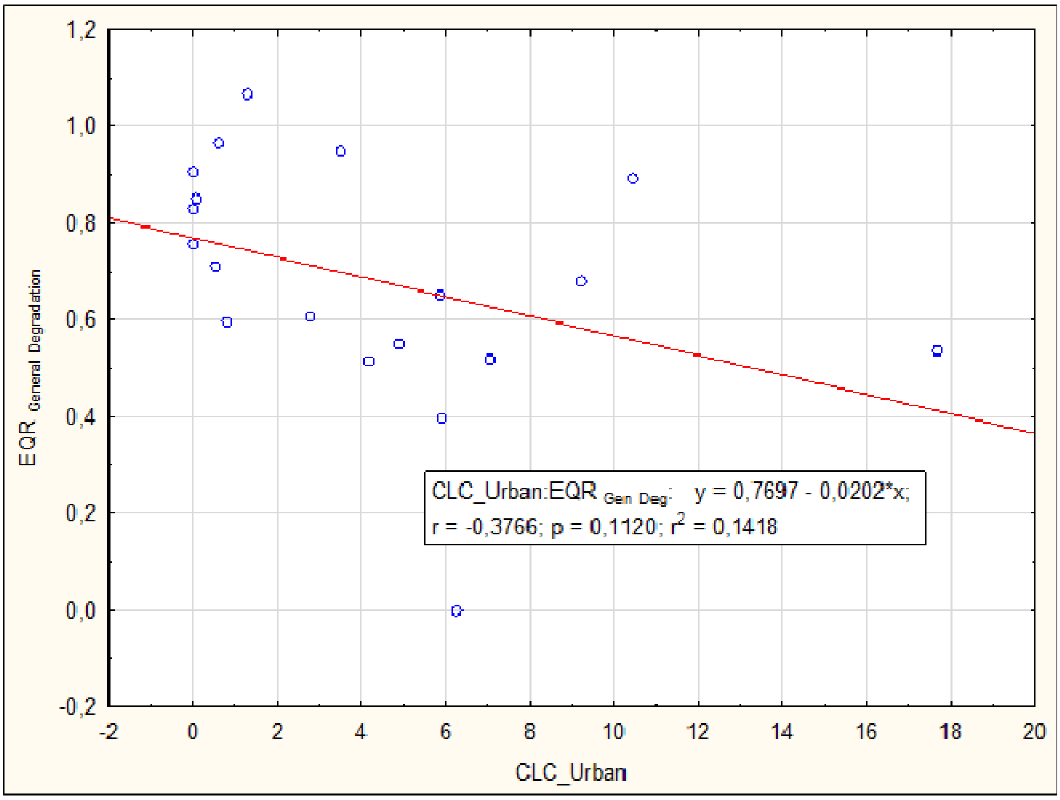


Figure 2. Pressure-Response relationship between chemical water properties against EQR of General Degradation module scores for sites of river types R-M5.

B. Land use



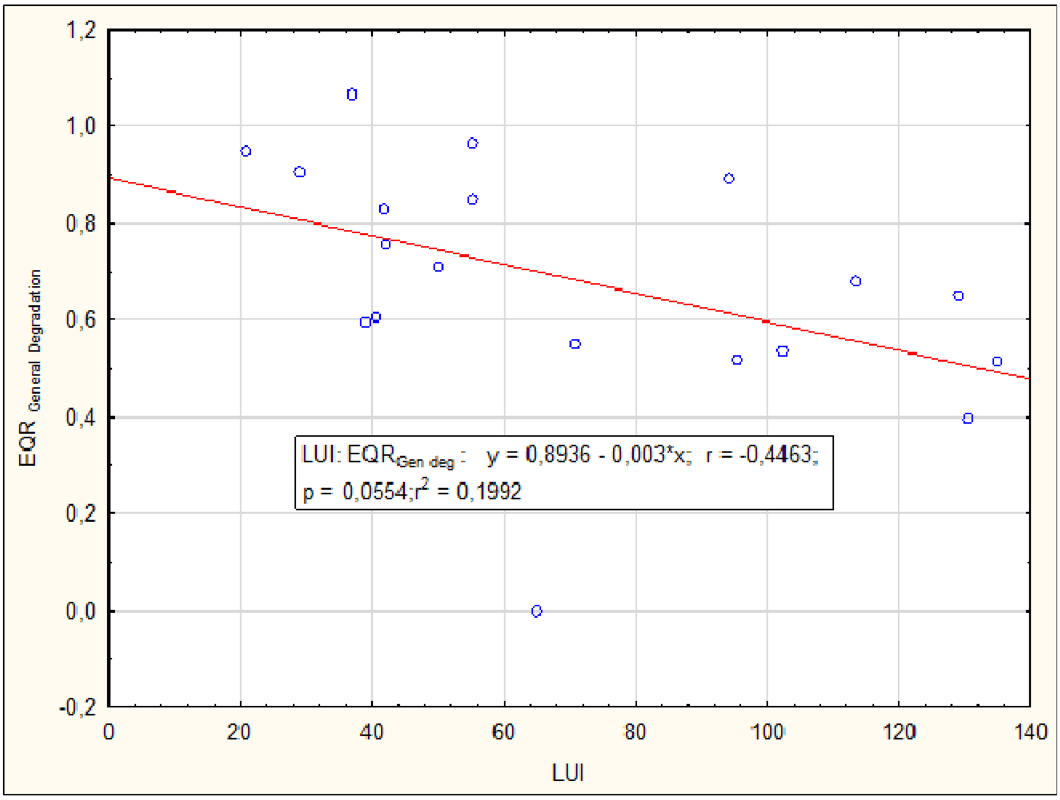
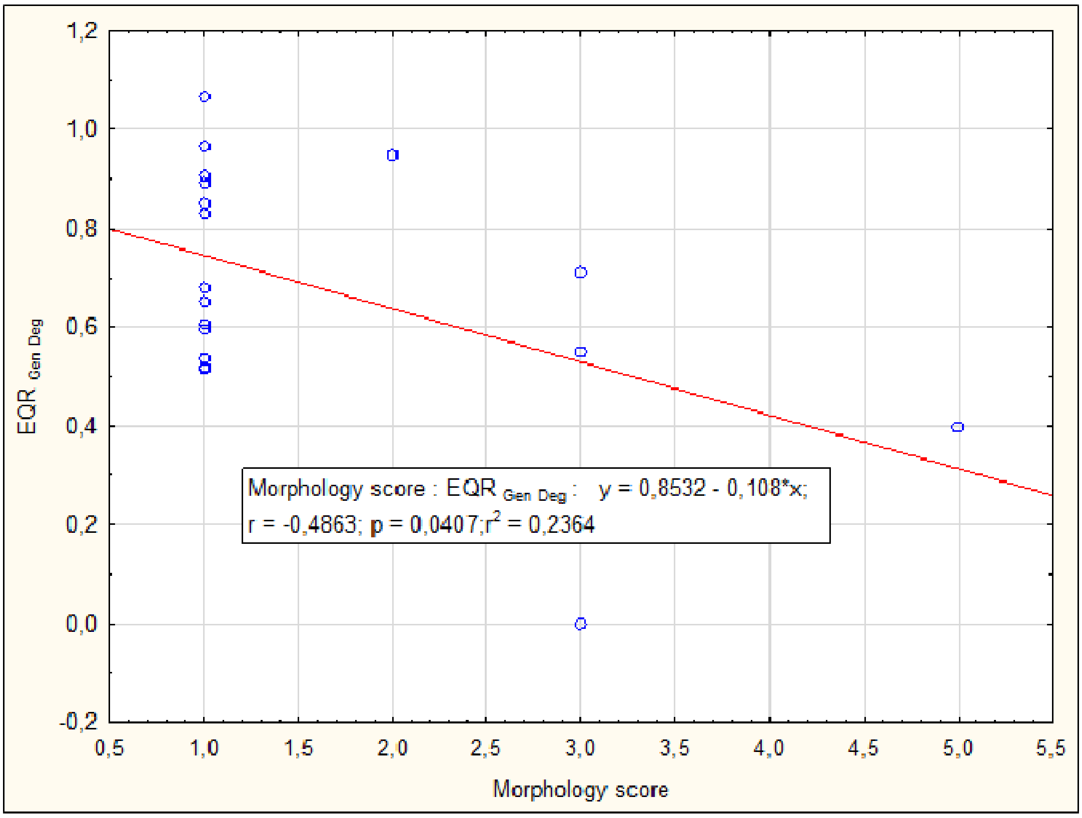


Figure 3. Pressure-Response relationship between Corine Land Cover (categorie urban) and the Land Use Index LUI and against EQR of General Degradation module scores for sites of river types R-M5.

C. Hydromorphology



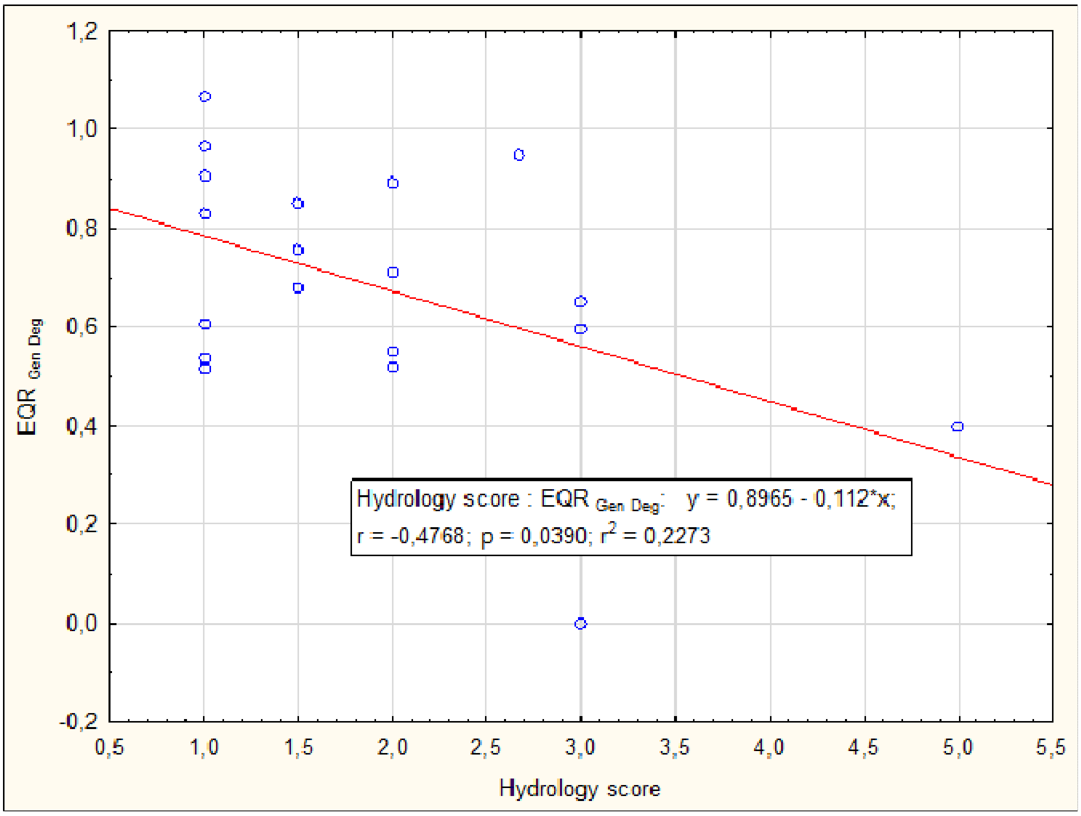


Figure 4. Pressure-Response relationship between morphological and hydrological river features against General Degradation module scores for sites of river types R-M5.

D. Resume

For variables associated with organich enrichment, chemistry pressures and hydromorphology, significant regressions could be found. In the case of land use pressures, an evident trend is visible, although it is not statistically significant. It is concluded that both the Saprobity Index and the General Degradation Multimetric Index clearly respond to anthropogenic impacts and can be used for the assessment of the ecological status.

3. WFD COMPLIANCE CHECKING

The first step in the Intercalibration process requires the checking of national methods considering the following WFD compliance criteria.

Table 6. List of the WFD compliance criteria and the WFD compliance checking process and results.

Compliance criteria	Compliance checking
Ecological status is classified by one of **five classes** (high, good, moderate, poor and bad).	yes
High, good and moderate ecological status are set in line with the WFD's **normative definitions** (**Boundary setting procedure**)	yes
All relevant parameters indicative of the biological quality element are covered (see Table 1 in the IC Guidance). A **combination rule** to combine parameter assessment into BQE assessment has to be defined. If parameters are missing, Member States need to demonstrate that the method is sufficiently indicative of the status of the QE as a whole	yes
Assessment is adapted to **intercalibration common types** that are defined in line with the typological requirements of the Annex II WFD and approved by WG ECOSTAT	yes
The water body is assessed against **type-specific near-natural reference conditions**	yes
Assessment results are expressed as **EQRs**	yes
Sampling procedure allows for **representative** information about water body quality/ecological status **in space and time**	yes
All data relevant for assessing the biological **parameters** specified in the WFD's normative definitions are covered by the **sampling procedure**	yes
Selected taxonomic level achieves adequate **confidence and precision** in classification	yes

4. IC FEASIBILITY CHECKING

The intercalibration process ideally covers all national assessment methods within a GIG. However, the comparison of dissimilar methods (“apples and pears”) has clearly to be avoided. Intercalibration exercise is focused on specific type / biological quality element / pressure combinations. The second step of the process introduces an “IC feasibility check” to restrict the actual intercalibration analysis to methods that address the same common type(s) and anthropogenic pressure(s), and follow a similar assessment concept.

4.1. TYPOLOGY

The biological typology of running waters in Croatia was initially established in 2011 (Mihaljević et al.. 2011), mainly based on expert opinion, due to general lack of all data types: both biological and pressure data. Today, biological data in almost all types are sufficient, as well data on pressures such as water chemistry and land use. The data sets are still lacking hydromorphological scoring from many sites as the hydromorphological evaluation of running waters in Croatia began only recently, in 2017. The current assessment method has equal reference and “worst” metric values for several Croatian types, but in the future, with more data on hydromorphology we wish to fine-tune these values for every type. Hence, the typology will remain as initially determined.

Table 7. Overview of common intercalibration types in the Mediterranean rivers GIG and MS sharing the types.

Common IC type	Type characteristics
R-M1	catchment <100 km^2; mixed geology (except siliceous); highly seasonal
R-M2	catchment 100-1000 km^2 ; mixed geology (except siliceous); highly seasonal
R-M3	catchment 1000-10000 km^2 ; mixed geology (except siliceous); highly seasonal
R-M4	non-siliceous streams; highly seasonal
R-M5	**temporary rivers**

The typology in Croatia is more precise, distinguishing temporary Mediterranean rivers by not only catchment size, but also geographical subecoregions. Rivers of the sub-ecoregion Istra (Istrian peninsula; HR-R-19) are assessed separately from the rest of the rivers of the Dinaric coastal subecoregion (HR-R_16A and HR-R_16B) due to some geological differences.

Table 8. Comparison of the intercallibartion types with the Croatian typology.

Common IC type	Croatian typology	Croatian type characteristics
R-M5	HR-R_16A	Small and medium temporary foothill rivers of the Dinaric coastal subecoregion
	HR-R_16B	Small lowland temporary rivers of the Dinaric coastal subecoregion
	HR-R_19	Small temporary rivers of the Istria subecoregion

4.2. PRESSURES ADDRESSED

The pressure gradient has been assessed for the Corine Land Cover (CLC) as well as the land use index, which is derived from CLC and defined as:

LUI = 4 * CLC urban+2*CLC intensive agriculture + CLC extensive agriculture

The ranges of the CLC and LUI in the two river types are:

CLC/LUI	range R-M5
CLC urban	0 – 17.69
CLC agr. intens.	0 – 52.17
CLC agr. extens.	1.11 – 38.61
CLC natural	21.34 – 89.77
LUI	20.75 – 134.81

The morphological and hydrological alteration scale ranges from 1 (no) to 5 (high; Fig 4).

The ranges for the chemical variables tested are:

Chemical variable	range R-M5
BOD_5 [mg/l]	0.825 – 3.431
COD [mg/l]	0.768 – 7.816
PO_4-P [mg/l]	0.00 – 0.368
NO_3-N [mg/l]	0.171 – 1.689
NH_4-N [mg/l]	0.006 – 0.931
Total P [mg/l]	0.365 – 18.516

The different pressure gradients covered by the national data set are considered to be sufficient.

4.3. ASSESSMENT CONCEPT

Does the national method follow the same assessment concept as other methods in the Intercalibration group? Provide evaluation of IC feasibility regarding assessment concept of the intercalibrated methods

Table 9. Data acceptance criteria used for the data quality control and describing the data acceptance checking process and results (Feio 2011).

Data acceptance criteria	Data acceptance checking Croatia	
Data requirements (obligatory and optional)	Common pressure data, common environmental data, correctly checked typologies and geographical location and biotic data, all properly introduced in harmonized excel files.	+
The sampling and analytical methodology	All MS sampling methods use a multi-habitat approach. All MS have indicated a response of their indices to pressure using statistical tools.	+
Level of taxonomic precision required and taxa lists with codes	Family level is required	+
The minimum number of sites / samples per intercalibration type	minimum of 15 benchmark sites by IC type are available.	-*
Sufficient covering of all relevant quality classes per type	Yes	-*

*relevant for the previous IC exercise, but not for join-in procedure

4.4. CONCLUSION ON THE INTERCALIBRATION FEASIBILITY

The number of sites fully complying in terms of the type criteria is high enough for carrying out the IC exercise. It is concluded that the intercalibration is feasible for the type R-M5

5. DEMONSTRATING THE COMPLIANCE WITH THE COMPLETED INTERCALIBRATION EXERCISE

5.1. BACKGROUND

- Following the CIS Guidance No. 30 (Willby et al. 2014), case A1 will be applied for the assessment method using invertebrates in the Med GIG river types R-M1 and R-M2.

5.2. DESCRIPTION OF IC DATASET

i. Full details of the common metric (e.g. species scores and metric weights)

ii. A suitable site x biology dataset covering a range of environmental quality from which the national EQR and common metric can be calculated

iii. Accompanying pressure data in the same format as that used in the completed exercise

iv. Information on the specific thresholds already used in the completed exercise to define reference or alternative benchmark sites (e.g. human population density, extent of agricultural land in the catchment, nutrient concentrations, etc.)

v. Details of exactly how the benchmarking was undertaken in the completed exercise (e.g. creation of a common metric EQR by dividing the observed value by the median common metric value of a set of national reference or benchmark sites). If the completed exercise concluded that benchmarking was not necessary the mean value of the benchmark sites from each country must be provided so that the joining Member State can also judge the need to benchmark its own method

vi. Values of the global mean view of the HG and GM boundaries on the common metric scale for the Member States who participated in the completed exercise.

5.3. DESCRIPTION OF INTERCALIBRATION PROCEDURE

1. *Calculate the common metric (CM) on the national dataset.*

2. Use the associated pressure data to identify sites in the national dataset that meet the criteria established by the GIG for the selection of benchmark or reference sites.

3. Standardise the common metric (CM_bm) against the benchmark according to the approach used in the completed exercise. If benchmark standardisation was concluded not to be required in the completed exercise the mean CM value of the joining method's benchmark sites must lie inside the range of mean values of the benchmark sites of the methods already intercalibrated for this conclusion to remain applicable. If the joining method's benchmark sites lie outside of this range the joining method must benchmark standardise its sites relative to the global mean CM value of the benchmark sites included in the completed exercise. These scenarios are illustrated in Table 1 and 2 of the IC Manual.

4. Use OLS regression to establish the relationship between CM_bm (y) and the EQR of the joining method (x). A specialist case is that when a joining method relies exclusively on the common metric developed in the completed exercise for its classification rather than devising an original method (then being more like Option 1). In such cases a regression would be meaningless as y is directly dependent on x. The goal for an MS choosing to use the CM as the basis for their method is simple – after any benchmarking their boundaries must simply lie within one quarter of class of the global mean view.

5. Predict the position of the national class boundaries (MP, GM, HG and reference) on the CM_bm scale.

6. Apply the comparability criteria as summarised in Chapter 6 of the IC Manual.

- Benchmark standardization;

  Benchmark sites are listed in chapter 2.4 as the reference criteria for the national assessment methodology was the same as for types R-M5 of the MED GIG provided by Feio (2011).

- Calculation of Intercalibration Common metrics (ICM) or Best-Related Intercalibrated National Classification (BRINC);

  The ICM includes the following 6 metrics are used: average score per taxon, log 10 (sel_EPTD+1), 1-GOLD, total number of taxa Families, number of EPT taxa (Families) and the Shannon-Wiener diversity index. The index is described in Buffagni et al. (2006). The main steps are:

  - Normalization of the single metrics by dividing the original values by the 75th percentile of high status sites within the national data sets
  - Calculation the iCM:

    iCM = a * ASPT + b * log10(sel_EPTD+1) + c * 1-GOLD + d * No.Fam + e * EPT + f * H‘

    a – f weights for the six metrics:

    - ASPT a = 0.333
    - log10(sel_EPTD+1) b = 0.266
    - 1-GOLD c = 0.067
    - No.Fam d = 0.167
    - EPT e = 0.083
    - Shannon-Wiener H‘ f = 0.083

- Normalization of the iCM (_norm. iCM) and of the EQR (_ norm. EQR) by dividing the original values by the 75th percentile of benchmark sites within the national data sets (Table 10)

Table 10. Metric normalization and intercalibration common metric iCM calculation for the comparison with the national assessment methodology.

Code	Site	Average score per Taxon	Norm. ASPT	log(sel EPTD+1)	Norm log(sel EPTD+1)	(1-GOLD)	Norm (1-GOLD)	Number of Families	Norm. Num. Of Fam.	- EPT-Taxa	Norm. EPT	Diversity (Shannon-Wiener-Index)	Norm H'	National EQR	iCM	norm ICM
M5																
40141	Zduški potok, prije utoka u Cetinu	4.23	0.67	0.51	0.97	0.08	0.12	17.00	0.65	2.00	0.13	1.31	0.56	0.51	0.65	0.64
40140	Potok Kotluša - pritok Cetine uzvodno od Vinalića	6.70	1.06	0.56	1.05	0.61	0.96	33.00	1.27	17.00	1.06	1.87	0.80	0.93	1.06	1.04
40507	Šipovača, Jelavića most	6.17	0.98	0.51	0.95	0.71	1.11	25.00	0.96	9.00	0.56	2.83	1.20	0.57	0.96	0.94
40143	Donji kanal, pritok Cetine kod Trilja	5.39	0.85	0.56	1.05	0.21	0.33	20.00	0.77	5.00	0.31	2.59	1.10	0.60	0.83	0.82
30081	Dubračina, Crikvenica (igralište)	5.78	0.92	0.57	1.08	0.89	1.40	26.00	1.00	21.00	1.31	2.62	1.11	0.95	1.05	1.03
30084	Suha Ričina Bašćanska, poslije Jurandvora	5.42	0.86	0.42	0.80	0.71	1.12	19.00	0.73	9.00	0.56	1.86	0.79	0.51	0.81	0.79
30082	Suha Novljanska Ričina, 1 km uzvodno od ušća	5.30	0.84	0.41	0.78	0.89	1.40	19.00	0.73	5.00	0.31	1.80	0.76	0.52	0.79	0.78
40702	Taranta, uzvodno od Srebrenog	4.31	0.68	0.29	0.55	0.34	0.54	14.00	0.54	3.00	0.19	1.46	0.62	0.54	0.57	0.56
40318	Bašćica, uzvodno od Posedarja	5.67	0.90	0.54	1.01	0.29	0.46	20.00	0.77	6.00	0.38	1.84	0.78	0.40	0.82	0.81
40220	Jaruga/Mijanovac, Zvjerinac	4.91	0.78	0.50	0.94	0.54	0.85	33.00	1.27	5.00	0.31	2.12	0.90	0.85	0.88	0.86
40315	Jaruga (Krivac), Benkovac	4.25	0.67	0.43	0.80	0.26	0.41	16.00	0.62	2.00	0.13	1.36	0.58	0.68	0.63	0.61
40221	Vodotok Bokanjac, prije ulaska u tunel	3.03	0.48	0.00	0.00	0.08	0.12	11.00	0.42	2.00	0.13	1.31	0.56	0.00	0.30	0.29
40211	Jaruga, Ražanac	5.48	0.87	0.46	0.86	0.58	0.91	16.00	0.62	11.00	0.69	3.22	1.37	0.64	0.85	0.84
31014	Mala Huba, most na cesti Buzet-Motovun	4.82	0.76	0.00	0.00	0.68	1.06	20.00	0.77	5.00	0.31	2.27	0.96	0.52	0.56	0.55
31013	Bračana, uzvodno od ceste Buzet-Motovun	6.42	1.02	0.51	0.95	0.63	0.99	18.00	0.69	21.00	1.31	2.62	1.11	0.64	0.98	0.96
31040	Dragonja, ušće, kod Kaštela	5.81	0.92	0.42	0.79	0.34	0.54	31.00	1.19	14.00	0.88	2.93	1.24	0.56	0.93	0.91
31018	Draga Baredine, most Štuparija	6.20	0.98	0.57	1.08	0.89	1.40	31.00	1.19	15.00	0.94	2.34	1.00	0.83	1.07	1.05
31021	Raša, most Potpićan	5.17	0.82	0.36	0.68	0.52	0.81	22.00	0.85	6.00	0.38	1.76	0.75	0.42	0.74	0.73
31009	Krvar, most na cesti Motovun-Pazin	4.67	0.74	0.29	0.55	0.64	1.01	21.00	0.81	14.00	0.88	2.37	1.00	0.69	0.75	0.74
Metric median of benchmark sites		**6.31**		**0.53**		**0.64**		**26.00**		**16.00**		**2.35**			**1.02**	

- Translation of national boundaries to ICM or BRINC:

The correlation between the EQR values from the national assessment method and the normalized iCM is statistically significant (Spearman corr. R=0,688; p<0,05), meeting the requirement set during the intercalibration exercise (R≥0,5). The OLS regression of the two variables is shown in figure 5.

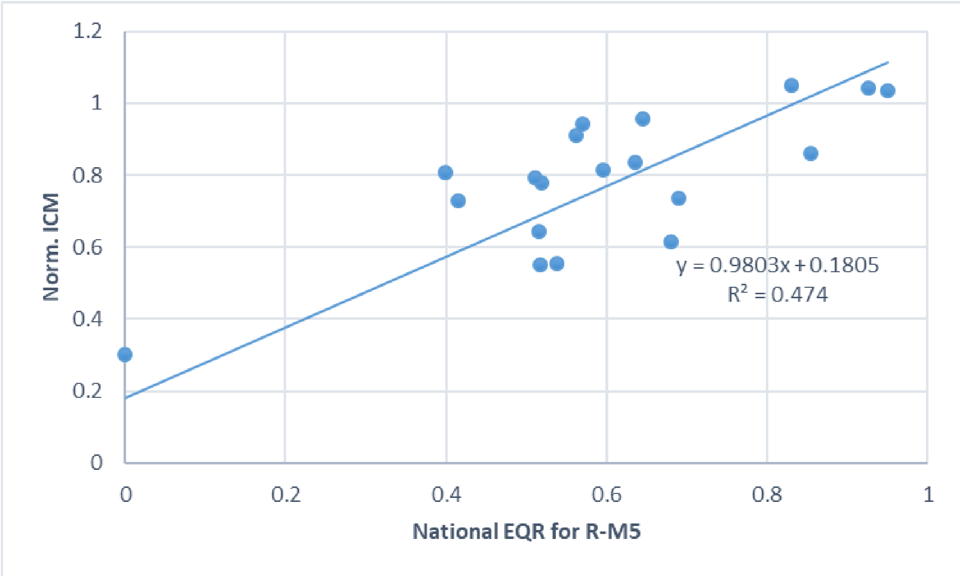


Figure 5. OLS regression to establish the relationship between normalized iCM (y) and the EQR of the joining method (x) for the IC river type R-M5.

- Calculating boundary bias;

  The global mean views of the H/G and G/M boundaries for IC river types R-M5 in the MedGIG can be derived from the final table of harmonized class boundaries (Feio, 2011).

  H/G boundary global mean view of iCM is 0.975

  G/M boundary global mean view of iCM is 0.722

Calculating boundary bias for R-M5:

National EQR	Norm. ICM R-M5	Global mean view	Deviation from global mean view	Class width	deviation expressed as proportion
1	1.1608				
0,8	0.96474	0.975	-0.01026	0.19606	-5%
0,6	0.76868	0.722	0.04668	0.19606	24%
0,4	0.57262				
0,2	0.37656				

Harmonizing of boundaries

The national H/G boundary on the common metric scale falls below the global mean view the and G/M boundary is above the global mean view for M5. The amount of the deviation expressed as a proportion of the width of the (national) good and high status classes on the common metric scale is ≤0.25 for all the boundaries. Therefore, there is no need to adjust the boundaries.

5.4. FINAL BOUNDARIES

Final boundaries for R-M5:

National EQR	Norm. ICM R-M5
1	1.1608
0.8	0.96474
0.6	0.76868
0.4	0.57262
0.2	0.37656

6. DESCRIPTION OF THE BIOLOGICAL COMMUNITIES

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT HIGH STATUS

In high status sites of the R-M5 are dominated by rheophile invertebrate taxa, showing values of the Rhithron Type Index of 8 and more. Taxa sensitive to hydromorphological degradation and taxa very sensitive to organic pollution are present in high abundances.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT GOOD STATUS

In good status sites of the R-M5 rheophile invertebrate taxa are present in high numbers, showing values of the Rhithron Type Index of around 6. Taxa sensitive to hydromorphological degradation and taxa very sensitive to organic pollution are present.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT MODERATE STATUS

In moderate status sites of the R-M5 rheophile invertebrate taxa are abundant, but generalist also present in relatively high abundaces, values of the Rhithron Type Index of around 5. Taxa sensitive to hydromorphological degradation and taxa very sensitive to organic pollution are also present but in less abundance then tolerant taxa.

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Report on fitting the Croatian classification method for fish in rivers to the results of the completed intercalibration of the Fish Cross GIG (Danubian Group)

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Report on fitting the Croatian classification method for fish in rivers to the results of the completed intercalibration of the Fish Cross GIG (Danubian Group)

1. INTRODUCTION

- Member State: Croatia;
- BQE: Fish;
- Water body category (type): Rivers.

Croatia did not participate in the Fish Cross GIG intercalibration exercise, since the Croatian fish-based method of ecological status assessment in the river types was under development during the intercalibration exercise (see Intercalibration Technical Report Cross-GIG rivers - Fish fauna, 2012). The objective of this report is to present Croatian fish index for rivers (CFIR) which is national method for ecological status assessment in the rivers based on fish, and to prove that it is compliant with the WFD normative definitions and its class boundaries are in line with the results of the completed intercalibration exercise of the Fish Cross GIG (Danubian Group).

Croatian rivers and streams belong to two different watersheds – Black Sea (Danube) watershed comprises waters from northern and central part of Croatia, whereas southern river basins belong to the Adriatic watershed. Croatian national typology of natural rivers divides Croatian rivers into three ecoregions: rivers of the Black Sea watershed mostly belong to the Pannonian ecoregion (national types HR-R_1, HR-R_2A, HR-R_2B, HR-R_3A, HR-R_3B, HR-R_3C, HR-R_3D, HR-R_4A, HR-R_4B, HR-R_4C, HR-R_5B, HR-R_5C and HR-R_5D), whereas rivers of the Adriatic watershed mostly belong to the Dinaric coastal ecoregion (national types HR-R_11A, HR-R_11B, HR-R_12, HR-R_13, HR-R_13A, HR-R_14A, HR-R_14B, HR-R_14C, HR-R_15A, HR-R_15B, HR-R_16A, HR-R_16B, HR-R_17, HR-R_18 and HR-R_19). Designation of rivers belonging to these two ecoregion to CROSS GIG fish intercalibration groups is obvious: rivers of the Pannonian ecoregion fall under the Danube IC group, whereas rivers of the Dinaric coastal ecoregion belong to the Mediterranean group. The third ecoregion based on the national typology, Dinaric continental ecoregion (national types HR-R_6, HR-R_7, HR-R_8A, HR-R_8B, HR-R_9, H-R_10A and HR-R_10B), comprises rivers from the central, mostly mountainous part of Croatia that belong to both watersheds (Black Sea and Adriatic). Based on geographic location and ecological characters of those river types, it is agreed that rivers belonging to this ecoregion will be presented together with the Pannonian ecoregion and included into the Danube group for intercalibration. Thereafter, in this report we present methodology for the ecological status assessment based on fish in national river types that belong to the Pannonian and Dinaric continental ecoregions, as well as comparison with the CROSS GIG Danubian group intercalibration exercise. Noteworthy, national types HR-R_5B, HR-R_5C and HR-5_5D concern very large rivers and, thereafter, are not included into classification method described here.

2. DESCRIPTION OF NATIONAL ASSESSMENT METHODS

2.1. METHODS AND REQUIRED BQE PARAMETERS

For the estimation of the ecological status of natural rivers and streams in Croatia, a fish based index was developed, following requirements of the Water Framework Directive (WF) 2000/60/EC. The development of the **Croatian fish index for rivers (CFIR)** included procedures and methods previously identified as the best practices (Hering et al. 2006, Argillier et al. 2013), but taking into account also specific characters of Croatian watersheds, considering both, river communities and anthropogenic pressures. In the development of CFIR, following procedures were implemented:

- Field sampling of fish
- Obtaining of relevant environmental parameters
- Calculating fish fauna metrics
- Selection of relevant environmental parameters and pressure proxies, as well as fish fauna metrics that respond to at least one pressure proxy
- Ecological Quality Ratios calculations
- Multimetrix index generation
- Ecological quality class boundaries implementation

Following the mentioned procedure we have designed the Croatian fish index for rivers that documents well the relationships between fish and pressures occurring in their habitats, as requested by the WFD.

The classification method is verified for WFD compliance (Table 1) and IC feasibility and the class boundaries were compared with agreed boundaries from the CROSS GIG Danube Group intercalibration exercise following the instructions of the CIS Guidance Document 30: "Procedure to fit new or updated classification methods to the results of a completed intercalibration exercise" (Willby et al. 2014).

Table 1. Overview of the metrics included in the national method

HR	Taxonomic composition	Abundance	Disturbance of sensitive taxa	Age structure
CFIR	yes	yes	yes	no*

**Age structuring estimations were considered at first steps, but they did not confirm to requirements of statistical analyses and no pressure-responses was established, so age structure metrics are not included in the final index.*

2.2. FISH FAUNA SAMPLING

Description of sampling and data processing

Fish sampling must be in accordance with HRN EN 14962:2007, Water quality – Guidance on the scope and selection of fish sampling methods, and HRN EN 14011:2005 Water quality -- Sampling of fish with electricity (EN 14011:2003).

- Sampling time and frequency:
  Sampling is conducted in late summer or early autumn in the continental part of Croatia (Danube river basin). For the purpose of comparing obtained results, repeated sampling in individual monitoring localities should be conducted at the same time of the year.

Sampling site (locality) is identified by ensuring that the sampled section covers the diversity of all types of natural microhabitats and man-induced microhabitats. Sampling site has to be large enough to include living area of dominant species and to include all characteristic river habitats (faster and slower parts, sidearms….), i.e. it has to be representative of fish community in order to be able to evaluate density and age structure of each species in ichthyopopulation. Simultaneously with covering as many habitats as possible during selection of sampling localities, it should also be taken into consideration easy access to the sampling site itself and previous knowledge on a certain locality.

Selected localities have to be representative of the status on a section of running waters whose length is (according to FAME, 2004):

- 1 km, for small running waters (catchment area size < 100 km2); 500 m upstream and 500 m downstream from the initial sampling site),
- 5 km, for medium running waters (catchment area size 100 – 1 000 km2) and
- 10 km, for large running waters (catchment area size > 1 000 km2).

- Sampling method:
  Electrofishing represents a universal standard method for river sampling. This sampling method enables the best estimation of population density, species abundance, number of organisms and fish biomass, age structure and mutual relationships of fish species samples, and it also represents the least harmful fishing method when compared to other methods.

Electric fishing generator is used to catch fish in three ways:

- Wading in the river,
- From river bank or
- On board a boat.

Wadable running waters, shallow watercourses up to 15 m width, are sampled in their entire width using a backpack generator. Prior to sampling, the sampling section is delimited with nets to prevent fish from escaping. In the delimited area fishing is conducted twice with the same fishing effort. If the probability of catching type-specific species in the first two catches is less than 50%, sampling has to be repeated once more. If sampling is not done using a backpack electric generator, the electric generator should be placed on the river bank and an anode with a long electric cable on fiberglass handle used.

In larger running waters where the depth (> 0,7 m) and habitat diversity prevent efficient sampling from the bank or by wading in the riverbed, a special electric fishing boat is used. Electric generators with different powers are used for fishing in running waters of different size and depth:

- minimum 2,5 kW – small running waters and fishing by wading in the river and from the bank,
- minimum 5 kW – medium running waters and fishing on board a boat,
- minimum 7,5 kW (recommended ≥ 10 kW) – large and very large running waters and fishing on board a boat.

Electric generator enabling fishing with pulsating current, and direct current (with or without pulsating option) was used because it is least harmful to fish, and provides the best results whereas alternating current shall not be used. Depending on the size of running waters, fishing was done using:

- one anode with known ring diameter (for example 50 cm) and with a net on a fiberglass handle 2.5 m long,
- four or more anodes placed at a distance of 50 cm between each other, placed on a construction mounted on board a boat adapted for electric fishing (fishing efficiency can be increased by expanding the electric field in most cases by increasing the number of anodes used for fishing).

Fishing is done downstream with the boat moving along the bank, covering as many existing habitats as possible especially places where fish might be hiding.

Fishing alongside both banks in periods longer than 20 minutes or 250 m in length, depending on the size of running water was conducted, and sampling the length in equivalent to 10 widths of the watercourse, trying to cover all available microhabitats, and in large and very large river sampling covered even up to 1000 m of the river length in order to include a representative sample of fish community.

During each sampling time during which electric generator was used for fishing was recorded, and GPS used to determine the distance that was crossed. Based on these data it was possible to calculate catch per unit effort (CPUE) and surface covered by the sampling. During each repeated electrical fishing, it is of vital importance to use electrical generator with the same power and with it sweep the same surface in equal time as during the first sampling.

- Species determination, measuring and handling:

All caught fish were determined based on morphological features using determination keys (Kottelat and Freyhof, 2007; Vuković and Ivanović, 1971; Povž and Sket, 1990; Miller and Loates 1997). In the case of doubt (hybrids, closely related species, young individuals), those individuals were put in 4 % - formaldehyde solution and taken to the laboratory for precise determination.
During determination, total body length (TL) was measured using ichthyometer from the beginning of the head to the tip of the tail fin, expressed in mm. Based on that data compared with literature, quality of obtained samples was assessed, because domination of smaller or larger individuals than expected indicates stress in the population. Total body length (TL) was measured by the person that caught it in order to return fish back to water as soon as possible. During measuring body length, noticed anomalies (visible external skin, subcutaneous or fin damage, parasites, deformations, tumors, lesions) were noted. If the number of individuals with outside anomalies is higher than usual, than stress is present in that population and it doesn't represent natural state of population. Mortality of sampled individuals by electrofishing method was less than 1%.

Environmental data describing each sampling site are collected both in the field and using literature or Internet sources. Site position is recorded with GPS, site length (m), river width (m) and description of sampling site are recorded at the field protocol which should be overwritten into the database at home. Photo of sampling site was taken and file number of a photo recorded at the field protocol too.

Description of fish fauna metrics used to describe fish communities in Croatian rivers

All sampled fish species were classified in groups according to their preferences for reproductive substrate (litophilic, LITH; phytophilic, PHYT; phyto-litophilic, PHLI; pelagophilic, PEL; psamophilic, PSAM; ostracophilic, OSTR; species that spawn in the sea, SEA), feeding preferences (herbivores, HERB; invertivores, INV; omnivores, OMNI; piscivores, PISC; and detritivores, DETR) and habitat preferences (benthopelagic, WCOL and benthic, BENT) (Table 2).

Table 2. Ecological characters of fish species from Croatian rivers.

Species	Habitat preferences	Spawning substrate	Feeding strategy	Ecological requirements
Abramis brama	BENT	PHLI	OMNI	Euritopic
Alburnoides bipunctatus	WCOL	LITH	INV	Reophilic
Alburnus alburnus	WCOL	PHLI	OMNI	Euritopic
Ameiurus melas	BENT	PHLI	OMNI	Limnophilic
Babka gymnotrachelus	BENT	PHLI	OMNI	Euritopic
Barbatula barbatula	BENT	PSAM	INV	Reophilic
Barbus balcanicus	BENT	LITH	INV	Reophilic
Barbus barbus	BENT	LITH	INV	Reophilic
Blicca bjoerkna	BENT	PHYT	OMNI	Euritopic
Carassius carassius	BENT	PHYT	OMNI	Euritopic
Carassius gibelio	BENT	PHYT	OMNI	Euritopic
Chondrostoma nasus	BENT	LITH	HERB	Reophilic
Cobitis elongata	BENT	LITH	INV	Reophilic
Cobitis elongatoides	BENT	PHYT	INV	Reophilic
Ctenopharyngodon idella	WCOL	PEL	HERB	Euritopic
Cyprinus carpio	BENT	PHYT	OMNI	Euritopic
Esox lucius	WCOL	PHYT	PISC	Euritopic
Eudontomyzon vladykovi	BENT	LITH	DETR	Reophilic
Gobio obtusirostris	BENT	PSAM	INV	Reophilic
Gymnocephalus baloni	BENT	PHLI	INV	Euritopic

Gymnocephalus cernua	BENT	PHLI	INV	Euritopic
Lepomis gibbosus	WCOL	LITH	INV	Limnophilic
Leuciscus aspius	WCOL	LITH	PISC	Reophilic
Leuciscus idus	WCOL	PHLI	OMNI	Reophilic
Leuciscus leuciscus	WCOL	LITO	OMNI	Reophilic
Misgurnus fossilis	BENT	PHYT	INV	Reophilic
Neogobius fluviatilis	BENT	LITO	INV	Euritopic
Neogobius melanostomus	BENT	LITO	INV	Euritopic
Oncorhynchus mykiss	WCOL	LITH	INV/PISC	Reophilic
Perca fluviatilis	WCOL	PHLI	INV/PISC	Euritopic
Phoxinus phoxinus	WCOL	LITH	INV	Reophilic
Ponticola kessleri	BENT	LITH	INV	Euritopic
Proterorhinus semilunaris	BENT	LITH	INV/PISC	Euritopic
Pseudorasbora parva	WCOL	PHLI	OMNI	Euritopic
Rhodeus amarus	WCOL	OSTR	OMNI	Euritopic
Romanogobio kesslerii	BENT	PSAM	INV	Reophilic
Romanogobio vladykovi	BENT	PSAM	INV	Reophilic
Rutilus rutilus	WCOL	PHLI	OMNI	Euritopic
Rutilus virgo	BENT	PHYT	INV	Reophilic
Sabanejewia balcanica	BENT	PHYT	INV	Reophilic
Salmo trutta	WCOL	LITH	INV/PISC	Reophilic
Sander lucioperca	WCOL	PHYT	PISC	Euritopic
Scardinius erythrophthalmus	WCOL	PHYT	OMNI	Limnophilic
Silurus glanis	BENT	PHYT	PISC	Euritopic
Squalius cephalus	WCOL	LITH	OMNI	Reophilic
Tinca tinca	BENT	PHYT	OMNI	Limnophilic
Umbra krameri	BENT	PHLI	INV	Limnophilic
Vimba vimba	BENT	LITO	INV	Reophilic

After field investigation, determination and measurement of all individuals, we have prepared a total of 103 metrics that describe fish communities (Table 3). Metrics belonging to four metric types have been prepared (following Furse et al. 2006), but also several additional metrics, similarly as conducted in previous fish-based indices assessments (for example Petriki et al. 2017). Noteworthy, collocation of certain fish metrics under metric types (as defined by Furse et al. 2006) is sometimes arbitrary, because the same metric can sometimes be collocated under more than one metric type. For example, proportion of individuals and biomass of species belonging to certain feeding or habitat preferences type can be addressed as functional metric, because they correspond with ecological functions of taxa, but also as sensitivity/tolerance metric, since they will be changed as a response to certain stressors. Nevertheless, all metric types are well represented in the metrics that describe fish community of Croatian flowing waters.

Table 3. Overview of the metrics included in the analyses with their abbreviations in brackets.

Composition/ abundance metrics	Richness/ diversity metrics	Sensitivity/ tolerance metrics	Functional metrics	Other metrics
Proportion of native species (pSn) Proportion of non-native species (pSa) Proportion of litophilic species (pLITH) Proportion of phytophilic species (pPHYT) Proportion of phyto-lithophilic species (pPHLI) Proportion of pelagophilic species (pPEL) Proportion of psammophilic species (pPSAM) Proportion of ostracophilic species (pOSTR) Proportion of species spawning in the sea (pSEA) Proportion of herbivorous species (pHERB) Proportion of invertivorous species (pINV) Proportion of omnivourous species p(OMNI) Proportion of piscivourous species (pPISC)	Total number of species (S) Number of native species (Sn) Number of non-native species (Sa) Proportion of Salmoniform species (pSALM) Proportion of Cypriniform species (pCYPR) pSALM/pCYPR pPERC (proportion of Perciform species)/pCYPR Shannon index (H) Reciprocal Simpson index (1/S) Margalef index (Ml) Alpha index (A) Berger-Parker index (d) Shannon index based on native species (Hnat) Reciprocal Simpson index for native species (1/S) Margalef index for native species (Mlnat) Alpha index for native species (Anat)	Proportion of native individuals (uSn) Proportion of non-native individuals (uSa) Proportion of litophilic individuals (uLITH) Proportion of phytophilic individuals (uPHYT) Proportion of phyto-lithophilic individuals (uPHLI) Proportion of pelagophilic individuals (uPEL) Proportion of psamophilic individuals (uPSAM) Proportion of ostracophilic individuals (pOSTR) Proportion of individuals spawning in the sea (uSEA) Proportion of herbivorous individuals (uHERB) Proportion of invertivorous individuals (uINV)	Number of lithophilic species (LITH) Number of phytophilic species, (PHYT) Number of phyto-lithophilic species (PHLI) Number of pelagophilic species (PEL) Number of psammophilic species (PSAM) Number of ostracophilic species (OSTR) Number of species spawning in the sea (SEA) Number of herbivorous species (HERB) Number of invertivorous species (INV) Number of omnivourous species (OMNI) Number of piscivourous species (PISC) Number of detritivorous species (DETR) Number of benthopelagic species (WCOL) Number of benthic species (BENT) Proportion of phytophilic species biomass (bPHYT) Proportion of phyto-lithophilic species biomass (bPHLI) Proportion of pelagophilic species biomass (bPEL) Proportion of psammophilic species biomass (bPSAM) Proportion of ostracophilic species biomass (bOSTR)	Total biomass (B) Biomass of native individuals (Bnat) Biomass of non-native individuals (Balo) Total length of the most abundant species based on the number of individuals (TLmaxn) Total length of the most abundant species based on the biomass (TLmaxb)

Composition/ abundance metrics	Richness/ diversity metrics	Sensitivity/ tolerance metrics	Functional metrics	Other metrics
Proportion of detritivorous species (pDETR) pPISC/pINV Proportion of benthopelagic species (pWCOL) Proportion of benthic species (pBENT)	Berger-Parker index for native species (dnat) Hnat-H (Hdif) 1/Snat-1/S (1/Sdif) Mlnat-Ml (Mldif) Anat-A (Adif) dnat-d (ddif) Hnat/H (Hrat) 1/Snat/1/S (1/Srat) Mlnat/Ml (Mlrat) Anat/A (Arat) dnat/d (drat)	Proportion of omnivourous individuals (uOMNI) Proportion of piscivourous individuals (uPISC) Proportion of detritivorous individuals (uDETR) uPISC/uINV Proportion of benthopelagic individuals (uWCOL) Proportion of benthic individuals (uBENT) Proportion of Salmoniform individuals (uSALM) Proportion of Cypriniform individuals (uCYPR) uSALM/uCYPR uPERC (proportion of Perciform individuals)/uCYPR Proportion of native individuals' biomass (bnat) Proportion of non-native individuals' biomass (balo)	Proportion of biomass of species spawning in the sea (bSEA) Proportion of herbivorous species biomass (bHERB) Proportion of invertivorous species biomass (bINV) Proportion of omnivourous species biomass (bOMNI) Proportion of piscivourous species biomass (bPISC) Proportion of detritivorous species biomass (bDETR) bPISC/bINV Proportion of benthopelagic species biomass (bWCOL) Proportion of benthic species biomass (bBENT) Proportion of Salmoniform species biomass (bSALM) Proportion of Cypriniform species biomass (bCYPR) bSALM/bCYPR	

2.4. ENVIRONMENTAL PARAMETERS AND PRESSURE PROXIES

Description of environmental parameters and indicators of anthropogenic pressures investigated in Croatian rivers

Altogether 21 parameters describing habitat conditions and anthropogenic pressures were assessed, including the hydrological, morphological and physico-chemical components (alkalinity, conductivity, pH, transparency, temperature, concentrations of ammonia, concentrations of organic carbon, molecular ammonium, nitrates, nitrites, nitrogen, phosphorous, total organic carbon, dissolved organic carbon, dissolved orthophosphates, dissolved oxygen, oxygen saturation, biological oxygen consumption and chemical oxygen consumption; representation of unnatural, modified shores (NNLC assessed according to ArcGIS 10); hydrological regime; longitudinal continuity; morphological conditions. Average values of all physico-chemical parameters considering warmer part of the year (from April to September) were included into further analyses.

2.5. STATISTIC ANALYSES FOR METRIC SELECTION

Detailed description of statistical analyses employed for metric selection and pressure-response relationships

Two sets of parameters were prepared (one describing fish communities and the second one concerning environmental parameters and pressure proxies), metrics in both of them were subjected to similar procedures in order to choose the ones that are not intercorrelated, that have normal distribution and for which a clear pressure-response relationship can be confirmed.

Parameters were first standardized. Log-transformation was used for count measures and logistic model for proportions, whereas diversity indices and measures derived from them were considered as already standardized measures.

After standardization Pearson's correlation coefficient was calculated among all the metrics inside each data set and in cases where coefficient was higher than 0,7; one or more metrics were excluded and the one with better ecological interpretation was retained. In cases where ecological interpretation was not clear, both variables were included in the next step and the one with no or lower pressure-response relationship was excluded later.

Responses of fish fauna metrics on all environmental parameters and pressure proxies were analysed by stepwise linear regression. Metrics that were significantly correlated with at least one pressure ($R^2>0.4$ and significance level, $p<0.05$) were checked for complying with linear regression assumptions (normal distribution, linearity and absence of multicollinearity). In rare cases where no significant response had $R^2>0.4$, responses with $R^2>0.2$ and statistical significance were taken into consideration. If both conditions were met (significant correlation with at least one pressure and linear assumptions), those metrics were considered for the index development. Again, correlation coefficients were calculated among metrics of both data sets and, finally, in cases of significant correlations, metrics for which better pressure-response relationships were obtained, were included in the index calculation.

2.6. PRESSURE-RESPONSE RELATIONSHIPS AND SELECTED METRICS

Description of the pressure-response relationships

Clear pressure-response relationships have been established for all investigated river types or combinations of river types. In Table 4 metrics for fish communities showing clear response to particular pressure are listed, which also show normal distribution and satisfy presumptions of linearity. In the table 4 pressure-responses are presented by ecoregions and national river types for all river types included in the Danube IC group (Pannonian and Dinaric continental ecoregions based on the national typology).

Table 4. Fish fauna metrics that showed significant response to certain pressure. River types and names are based on the Croatian national typology.

ECOREGION	RIVER TYPE	DESCRIPTION OF THE RIVER TYPE	PRESSURE RESPONSE	R^2	p
PANNONIAN	HR-R_1, HR-R_2A & HR-R_2B	Small mountain, mid-altitude and lowland rivers	Number of native species (Sn) shows response to phosphorus concentration (P)	0,25	0,00024
			Proportion of psammophilic species (pPSAM) shows response to dissolved oxygen concentration (O_2)	0,264	0,0001
			Difference between Shannon indices based on native species and the whole community (Hdif) shows response to dissolved nitrogen concentration (N)	0,22	0,00051
	HR-R_3A, HR-R_3B, HR-R_3C, HR-R_3D, HR-R_4A, HR-R_4B & HR-R_4C	Medium, large and alluvial lowland rivers	Number of non-native species (Sa) shows response to water temperature (temp)	0,545	0,00000
			Ration between Shannon indices based the native species and the whole community (Hrat) shows response to the dissolved oxygen concentration (O_2)	0,256	0,00098
DINARIC CONTINENTAL	HR-R_6, HR-R_7, HR-R_8A & HR-R_8B	Small, medium and large mountain and mid-altitude rivers, as well as medium and large lowland rivers	Proportion of invertivorous individuals (uINV) shows response to dissolved ammonia	0,53	0,00011
			Proportion of omnivorous individuals (uOMNI) shows response to dissolved ammonia	0,503	0,00019
			Proportion of benthopelagic species	0,361	0,00237

			(pWCOL) shows response to suspended particles concentration		
	HR-R_9, HR-R_10A & HR-R_10B	Rivers in karstic fields and temporary rivers	Proportion of non-native species (pSa) shows response to water temperature	0,584	0,04737
			Proportion of native species (uSn) shows response to nitrates concentration in water	0,6136	0,04033
			Proportion of piscivorous individuals (uPISC) shows response to the dissolved oxygen concentration (O_2)	0,593	0,04515

Descriptions of responses of fish community metrics to pressures in small (mountain, mid-altitude and lowland) rivers in Pannonian ecoregion (types HR-R_1, HR-R_2A and HR-R_2B)

- Number of native species (Sn) shows statistically significant response to phosphorus concentration (R^2=0,25, p=0,00024; Figure 1). Phosphorus in watercourses appears as a result of pollution and eutrophication and is considered one of the best indicators, but also the strongest causes of eutrophication (Correll 1998, Yang et al. 2008). That is especially applicable to small watercourses, where water is very clean and concentration of dissolved phosphorus is low. Since fish communities of small watercourses are adapted to these conditions and are mostly composed of stenovalent, sensitive species, a slight change of conditions leads to change in community and lowering of native species number.
- Proportion of psammophilic species (pPSAM) shows response to concentration of dissolved oxygen (R^2=0,264, p=0.0001; Figure 2). Lowering of oxygen concentration, especially in the small watercourses is an indicator of pollution and eutrophication, in correlation with enhancing microbiological degradation. Psammophilic species, which spawn on sandy substrates represent sensitive component of fish community and first react to oxygen lowering in watercourses.
- The difference between Shannon's index based on native species and on all species (Hdif) shows response to concentration of dissolved nitrogen (R^2=0,22, p=0,00051; Figure 3). Similar as phosphorus, nitrogen is considered as an important cause and indicator of eutrophication, it reaches watercourses by rinsing of agricultural fields. For fertilization of agricultural fields nitrogen-rich fertilizers are often used, by rinsing they end up in water enhancing eutrophication and deteriorating water quality. Concentrations of nitrogen in watercourses can be increased also form other pollutants. Fish community metrics show significant response to concentration of nitrogen increase in the water (Hdif), pointing to higher tolerance of non-native species to this element. In watercourses with nitrogen increase there is higher probability that non-native species will form stabile population.

Thereafter, Croatian fish index for rivers (CFIR) for river types HR-R_1, HR-R_2A and HR-R_2B (small mountain, mid-altitude and lowland rivers in the Pannonian ecoregion) shall be based on the following fish fauna metrics: Sn, pPSAM and Hdif, that incorporate response of fish communities to phosphorous, dissolved oxygen concentration and nitrogen concentrations.

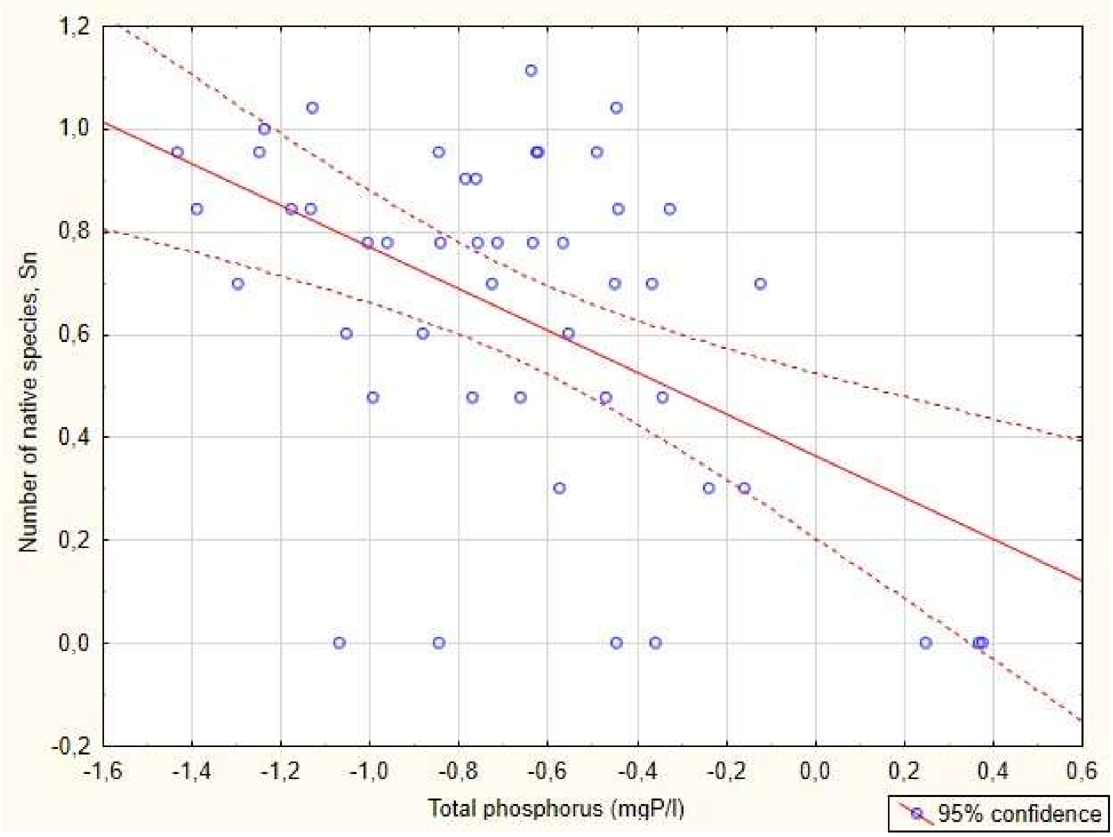

Figure 1. Scatterplot of the linear regression between the number of native species (Sn) and the phosphorus concentration, based on the standardized values of metrics.

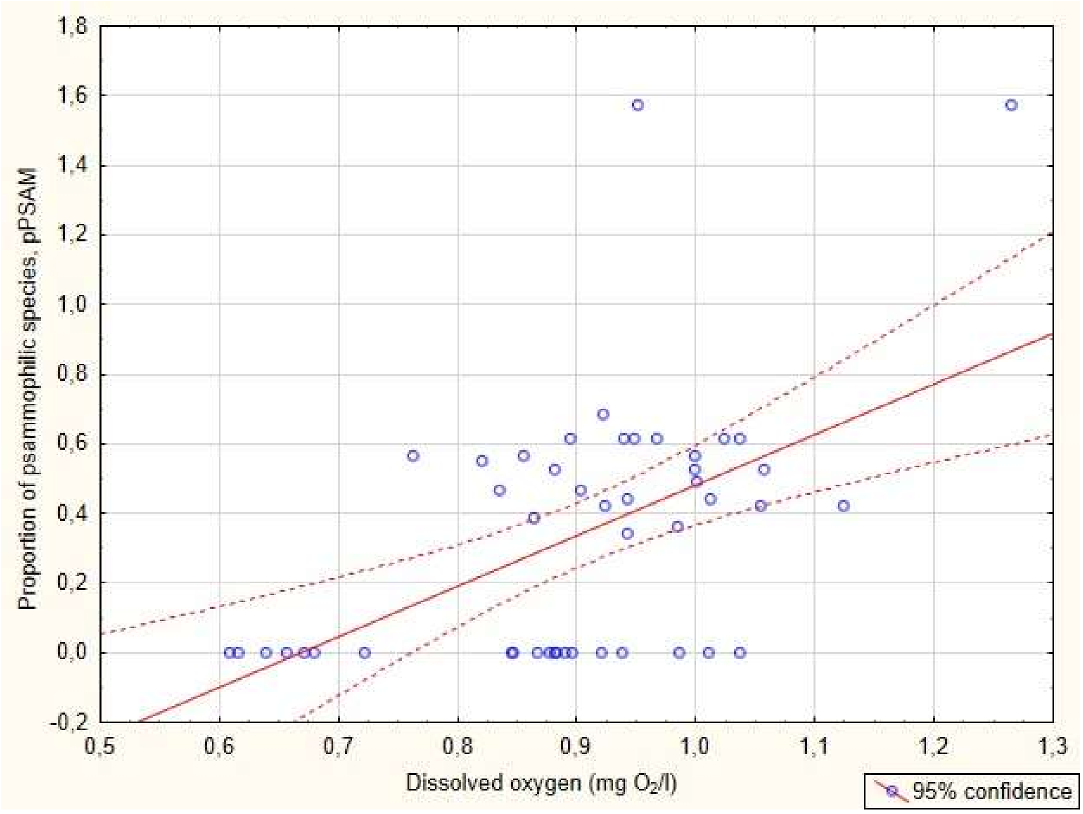

Figure 2. Scatterplot of the linear regression between the proportion of psammophilic species (pPSAM) and the dissolved oxygen concentration, based on the standardized values of metrics.

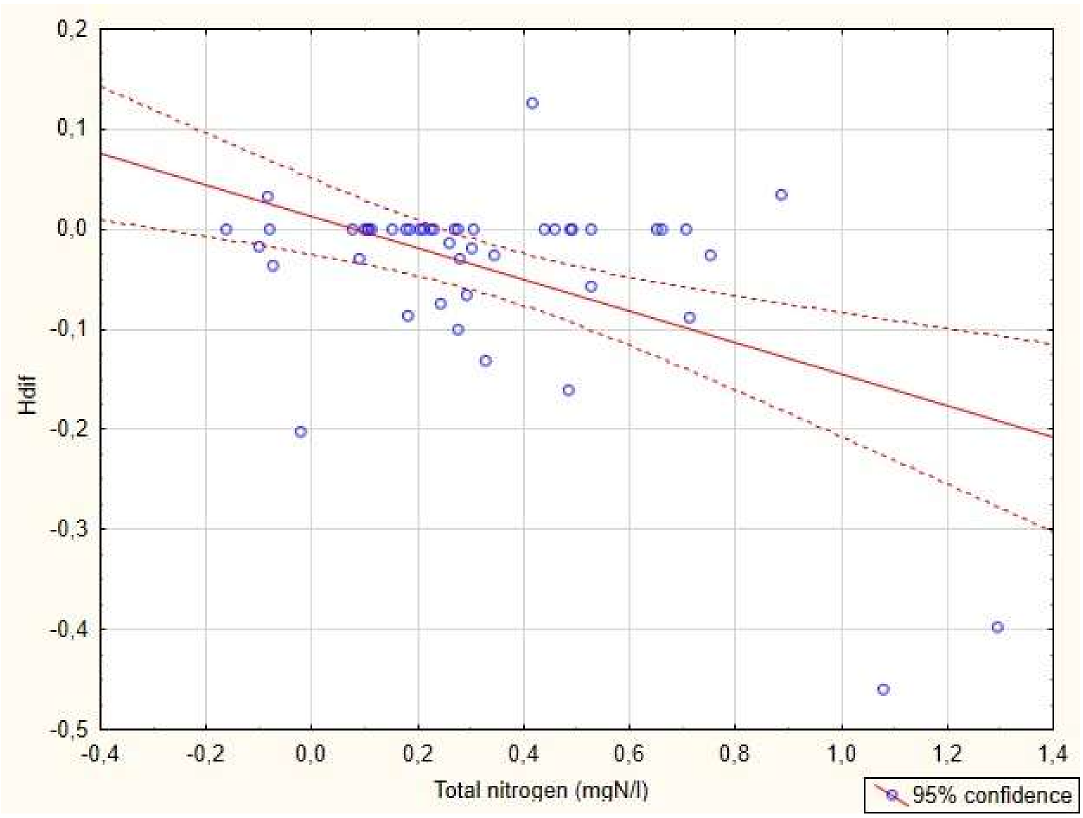

Figure 3. Scatterplot of the linear regression between difference between Shannon index based on the native species and the same index based on the whole community (Hdif) and the nitrogen concentration in water, based on the standardized values of metrics.

Descriptions of metrics of fish community to pressures in lowland alluvial, medium and large watercourses of the Pannonian ecoregion (types HR-R_3A, HR-R_3B, HR-R_3C, HR-R_3D, HR-R_4A, HR-R_4B and HR-R_4C)

- The number of non-native (Sa) species shows response to temperature (R^2=0,545, p=0,000; Figure 4). The increase in water temperature is usually a consequence of hydrological and morphological changes in watercourses, e.g. slowing down the flow with dams and barriers which leads to increase in temperature. Temperature changes can be caused by other environment modifications and often occur in combination with eutrophication. Climatic changes are also important factor influencing water temperature. Increased temperature is often suboptimal or completely unsuitable for native species, especially sensitive ones, while invasive species have wider ecological valency and tolerate temperature fluctuations.
- Relation between Shannon's index based on native species and the same index based on all recorded species (Hrat) shows response to concentration of dissolved oxygen (R^2=0,256, p=0,00098; Figure 5). As for the previous type, decrease in oxygen concertation, which is a common consequence of pollution, eutrophication and microbial degradation, can better be tolerated by non-native invasive species and they easily establish stable populations in such conditions.

Thereafter, Croatian fish index for rivers (CFIR) for river types HR-R_3A, HR-R_3B, HR-R_3C, HR-R_3D, HR-R_4A, HR-R_4B and HR-R_4C (lowland alluvial, medium and large rivers in the Pannonian ecoregion) shall be based on the following fish fauna metrics: Sa and Hrat, that incorporate response of fish communities to water temperature and dissolved oxygen concentration.

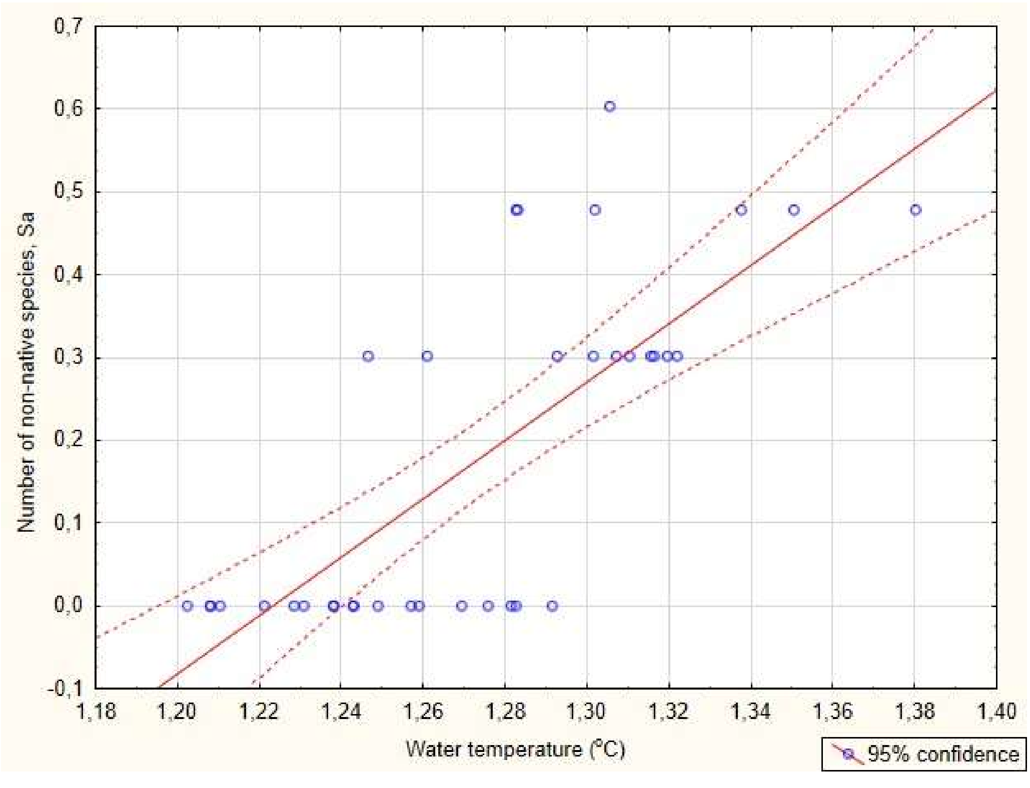

Figure 4. Scatterplot of the linear regression between the number of non-native species (Sa) and the water temperature, based on the standardized values of metrics.

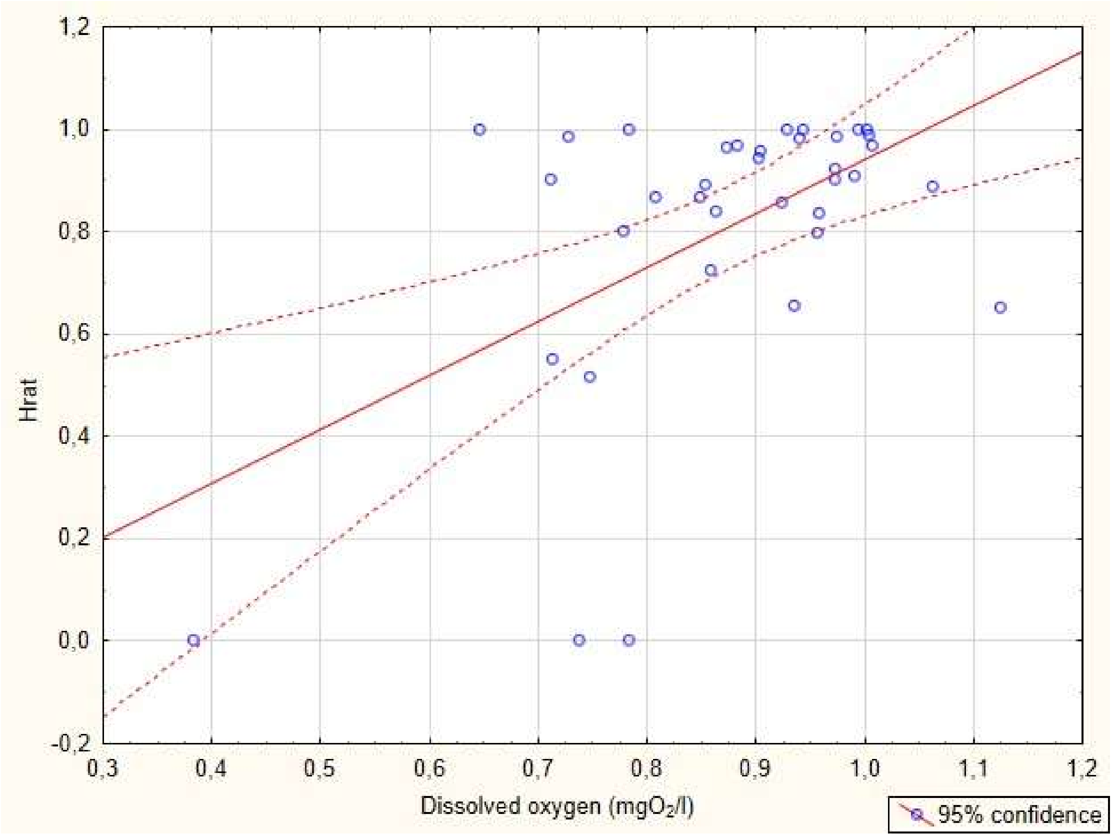

Figure 5. Scatterplot of the linear regression between the ratio between Shannon indices based on native species and the whole community and the dissolved oxygen concentrations, based on the standardized values of metrics.

Descriptions of metrics of fish community to pressures in small, medium and large mountain and mid-altitude rivers, as well as and lowland medium and large lowland rivers of the Dinaric continental ecoregion (types HR-R_6, HR-R_7, HR-R_8A and HR-R_8B)

- Proportion of individuals of invertivorous species (uINV) shows response to concentration of ammonia in water (R^2=0,53, p=0,00011; Figure 6), and similar response was obtained for proportion of omnivores (uOMNI) (R^2=0,503, p=0,00019; Figure 7). Increased concentrations of ammonium and ammonium ions in watercourses are usually a consequence of intensive use of artificial fertilizers and leaching from agricultural land, animal waste, industrial and urban wastewater and bacterial activity (Bouwman 1990, Jana 1994). Also, high concentration of foreign species in watercourse can lead to increase of ammonium and ammonium ions from excretion and microbial decomposition of dead fish. On the other side, ammonium is toxic for many fish species and salmonid species are particularly sensitive. Ammonium ions dissolved in the water can lead to ionic imbalance in fish blood (Eddy 1999), and molecular form which can easily entrance fish body can shift to ionic form which is significantly more toxic within the body and causes cell damage (Levit 2010). The two fish metrics which display response to concentration of ammonium naturally interchange in the water column, since number of invertivores is higher in the upper parts of rivers and streams while in downstream river stretches there is higher portion of omnivores. Since this water types encompass upper and lower parts of watercourses both of those metrics were included in calculations. The ecological quality ratio for the proportion of individuals of omnivorous species in localities in the upper parts of watercourses is sometimes higher than one (which is then counted as 1), in order to enable its correspondence to habitats in different parts of watercourses.

- Number of species that feed in the water column, so called benthopelagic species (pWCOL) shows response to concentration of suspended particles (R^2=0,361, p=0,00237; Figure 8). It is clear that concentration of suspended particles in water column can affect species that feed there, e.g. reduced visibility is leading to reduced feeding, abrasions on the skin can lead to infections and even clogging of the gills and blood vessels of the fish. Increase in concentration of suspended particles in water is connected to different kinds of water pollution.

Thereafter, Croatian fish index for rivers (CFIR) for river types HR-R_6, HR-R_7, HR-R_8A and HR-R_8B (small, medium and large mountain and mid-altitude rivers, as well as medium and large lowland rivers in the Dinaric continental ecoregion) shall be based on the following fish fauna metrics: uINV, uOMNI and pWCOL, that incorporate response of fish communities to ammonia and suspended particles concentrations.

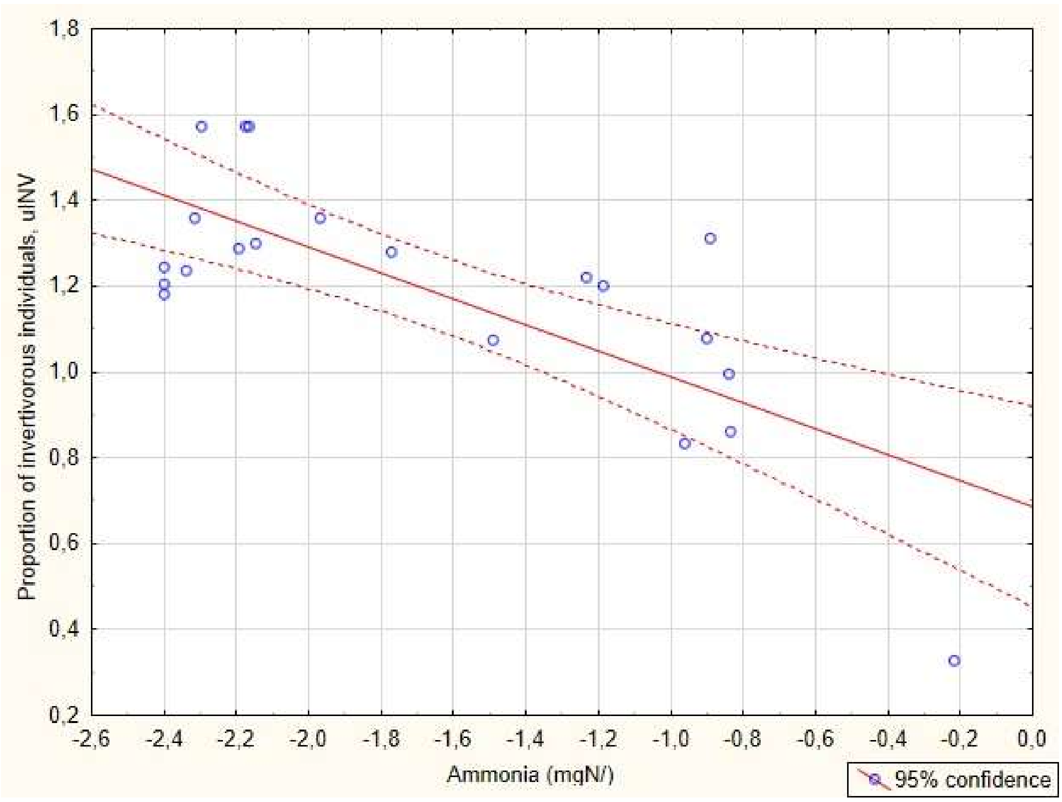

Figure 6. Scatterplot of the linear regression between the proportion of individuals belonging to invertivorous species and ammonia concentrations, based on the standardized values of metrics.

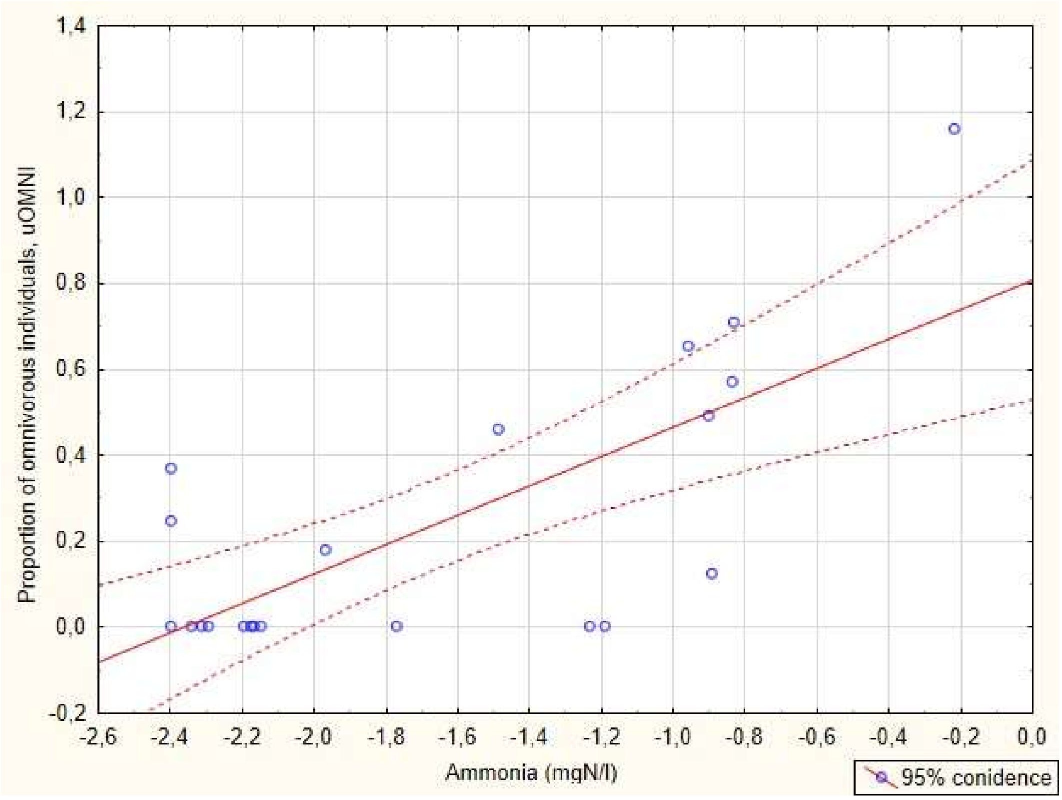

Figure 7. Scatterplot of the linear regression between the proportion of individuals belonging to omnivorous species and ammonia concentrations, based on the standardized values of metrics.

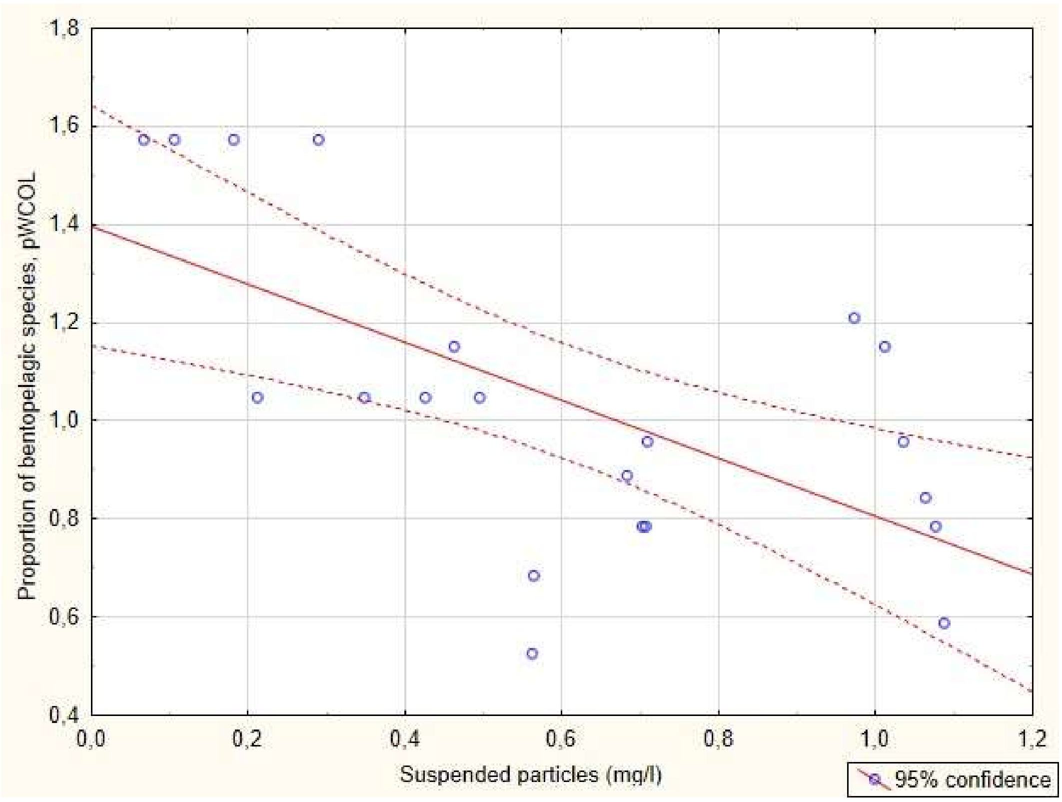

Figure 8. Scatterplot of the linear regression between the proportion of benthopelagic species and suspended particles, based on the standardized values of metrics.

Descriptions of metrics of fish community to pressures in rivers in karst fields and temporary rivers of the Dinaric continental ecoregion (types HR-R_9, HR-R_10A and HR-R_10B)

- Proportion of non-native species (pSa) shows response to water temperature (R^2=0,584, p=0,04737; Figure 9). As it was previously mentioned, native species adapted to specific conditions (especially in karst field streams and temporary streams) often cannot tolerate significant changes in temperature. On the other hand, invasive species (which are generalists and opportunists by nature) take advantage of conditions unfavourable to the indigenous community and create stable populations.
- Proportion of individuals of native species (uSn) shows response to nitrates (R^2=0,614, p=0,04033; Figure 10). Nitrates are, beside phosphorus, one of the major indicators and drivers of eutrophication, even though they cause by indirect chemical mechanism. Increase of nitrates in water is a consequence of intensive agricultural production and other forms of pollution.
- Proportion of individuals of piscivore species (uPISC) show response to concentration of dissolved oxygen in water (R^2=0,593, p=0,04515; Figure 11). Decrease in oxygen concentration usually follows enhanced microbial degradation, which is a common consequence of eutrophication and pollution. Howver, it can also be connected to increase of water temperature since solubility of oxygen in water is lower at higher temperatures.

Thereafter, Croatian fish index for rivers (CFIR) for river types HR-R_9, HR-R_10A and HR-R_10B (rivers in karstic fields and temporary rivers in the Dinaric continental ecoregion) shall be based on the following fish fauna metrics: pSa, uSn and uPISC, that incorporate response of fish communities to water temperature, nitrates and dissolved oxygen concentrations.

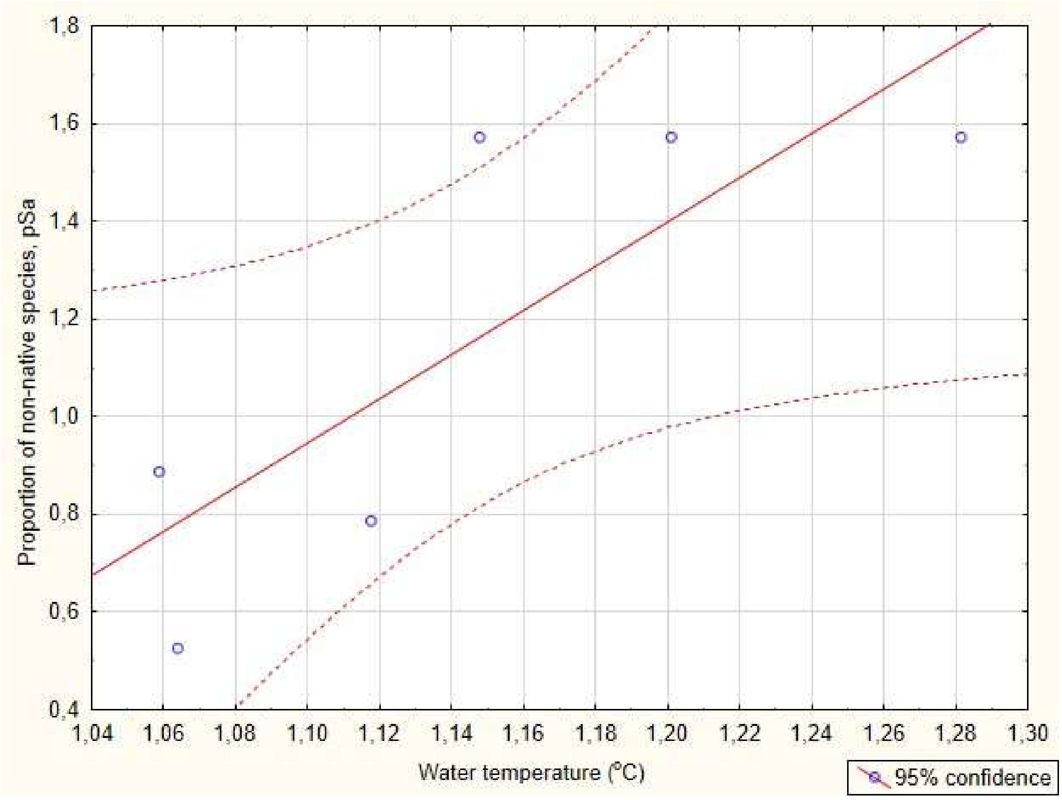

Figure 9. Scatterplot of the linear regression between the proportion of non-native species (pSa) and water temperature, based on the standardized values of metrics.

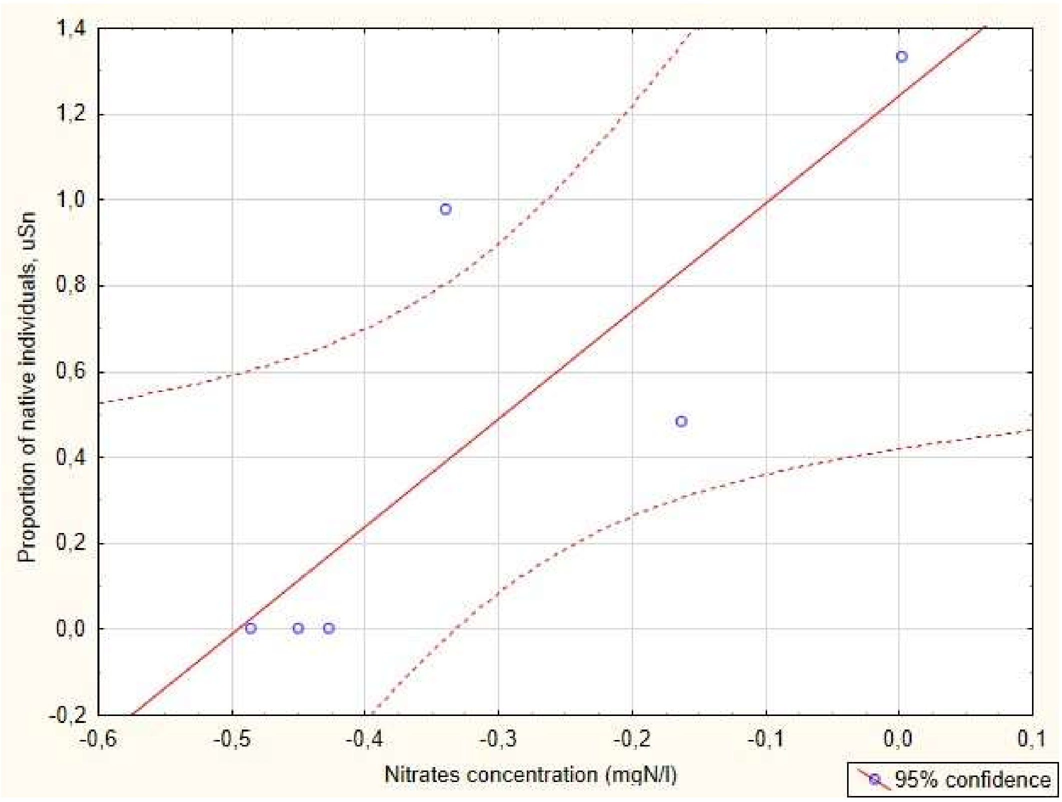

Figure 10. Scatterplot of the linear regression between the proportion of individuals belonging to native species (uSn) and nitrates concentration, based on the standardized values of metrics.

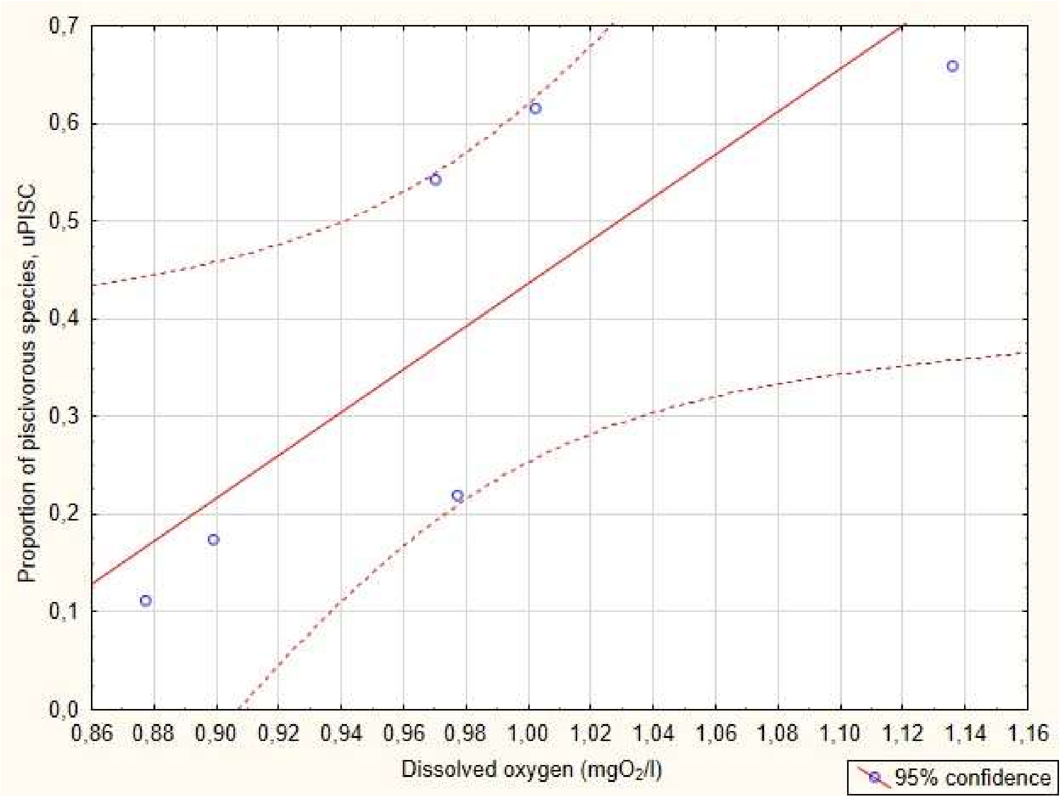

Figure 11. Scatterplot of the linear regression between the proportion of piscivorous species (uPISC) and dissolved oxygen concentration, based on the standardized values of metrics.

2.7. UPPER AND LOWER ANCHORS FOR ECOLOGICAL QUALITY RATIOS

Description of estimation of upper and lower anchors for Ecological Quality Ratios (EQRs)

Reference conditions were determined for each river type/group of types and responses to certain pressures were calculated. Since some river types comprised only a small number of locations that are influenced by various anthropogenic pressures, it was impossible to identify a satisfactory number of watercourses in which the fish community is in optimal condition, in order to determine the reference sites for all metrics of the fish community for all national river types. Moreover, there is a lack of estimated reference conditions for some of the physico-chemical parameters, disabling extrapolation of reference conditions of fish metrics in cases where they show significant response to certain environmental parameter. Therefore, for determining The Ecological Quality Ratios we applied the approach proposed by Furse et al. (2006) for cases where it is not possible to determine reference locations – we set the upper and lower anchors for individual metrics of fish communities (which had shown pressure response). The upper anchor was defined as value observed or proposed for natural, stabile community (reference condition for certain river type) and the lower anchor as the worst possible value obtained for severely altered communities or expert assessment of the worst possible conditions (e.g. for the metrics tackling the proportion of non-native species, the worst possible situation is that only non-native species have been recorded on a locality).

Inside some national river types there are localities with undisturbed, or almost undisturbed fish communities and they present national reference conditions for certain river types (Table 5). In those communities chosen fish metrics have the best possible or almost the best possible values, yielding Ecological quality ratios (as described in the next chapter) of 1 or nearly 1.

Noteworthy, reference communities, in cases when they could be identified, are referent considering their structure. Particularly, they exhibit reference values of those fish community metrics that were, following the described procedure, chosen for inclusion in the Croatian fish index for rivers. Understandably, this does not mean that all fish communities belonging to a certain national river type should have, under natural type-specific conditions, the same composition or even comprise the same number of species. Namely, fish community belonging to a certain river type are very diverse concerning species composition and richness which is a natural consequence of ecological and evolutionary differences.

Table 5. National reference localities under river types belonging to Pannonian and Dinaric continental ecoregion where they could be identified, and the explanation for river types under which no reference localities could be identified, but reference values of fish community metrics were estimated.

NATIONAL RIVER TYPE	LOCALITY CODE	LOCALITY NAME	COMPOSITION OF FISH COMMUNITY
HR-R_1	21114	Ivanečka Železnica, influence	Fish community is natural and very rich, comprising 9 native fish species.
HR-R_2A & HR-R_2B	On neither locality there is an undisturbed or nearly undisturbed fish community (all communities are more severely modified). Thereafter, identification of reference localities is not possible, yet reference conditions were estimated based on expert judgement and literature data.		
HR-R_3A, HR-R_3B, HR-R_3C & HR-R_3D	21052	Boščak II	Natural fish community comprised of 6 native typical species.
	21049	Bistrec – Rakovnica I	Natural fish community comprised of 5 native typical species.
	21050	Bistrec – Rakovnica II	Natural, rich fish community comprised of 9 native typical species.
HR-R_4A, HR-R_4B & HR-R_4C	18005	Sutla, Luke Poljanske	Natural fish community comprised of 7 native typical species.
HR-R_6	30018	Curak, before influence to Kupicu	Natural fish community comprised of 7 native typical species.
HR-R_7	Neither locality can be considered reference, even though several communities express very high or even maximal Ecological quality ratios for chosen fish community metrics. Namely, those are specific localities with communities comprising very small number of species (which is their natural state evinced by Ecological quality ratios, but do not resemble type-specific structure of fish communities), or non-native species are present,		

	but they ecologically resemble native species inducing lesser lowering of the Ecological quality ratios, but we cannot consider them undisturbed.
HR-R_8A & HR-R_8B	On neither locality there is an undisturbed or nearly undisturbed fish community (all communities are more severely modified). Thereafter, identification of reference localities is not possible, yet reference conditions were estimated based on expert judgement and literature data. These types comprise very small number of localities that could be included in analyses (only 2).
HR-R_9	On neither locality there is an undisturbed or nearly undisturbed fish community (all communities are more severely modified). Thereafter, identification of reference localities is not possible, yet reference conditions were estimated based on expert judgement and literature data. These types comprise very small number of localities that could be included in analyses (only 2).
HR-R_10A & HR-R_10B	On neither locality there is an undisturbed or nearly undisturbed fish community (all communities are more severely modified). Thereafter, identification of reference localities is not possible, yet reference conditions were estimated based on expert judgement and literature data.

2.8. ECOLOGICAL QUALITY RATIOS CALCULATION

Description of Ecological Quality Ratios calculation

Ecological quality ratios (EQRs) were calculated separately for all chosen metrics of fish community (metrics that had shown significant response to a pressure, that are not intercorelated and meet the conditions of normality and linearity and are statistically significantly correlated with pressure) inside each type/group of types. For that purpose, formula form Furse et al. (2006) was used:

$EQR_{metrics}$ = (Metrics value – Lower limit) / (Upper limit – Lower limit), for metrics decreasing with increasing pressure

or

$EQR_{metrics}$ = 1 – (Metrics value – Lower limit) / (Upper limit – Lower limit), for metrics increasing with increasing pressure.

As already explained, upper and lower anchors for each metrics were determined as the best or the worst possible conditions, either observed based on reference localities, or estimated as reference values.

2.9. GENERATION OF CROATIAN FISH INDEX FOR RIVERS

Description of the methodology used to calculate Croatian fish index for rivers (CFIR)

As it was already described, in the previous steps we identified fish metrics that proved to be suitable for inclusion in the multimetric index, because they show significant responses to certain pressures, are significantly correlated with pressures, but are not intercorrelated. Finally, Croatian fish index for rivers (CFIR) was calculated separately for each river type/group of types as the sum of the ecological quality ratios determined for a certain river type/group of types divided by the number of ecological quality ratios included.

$$CFIR = \frac{EQR1 + EQR2 + \cdots + EQRn}{n}$$

CFIR is multimetric index which integrates multiple metrics and simplifies decision making because one value can be used to assess and monitor the quality of streams (Furse et al. 2006). Since the index combines the effects of different fish metrics, it combines responses to multiple pressures.

2.10. NATIONAL BOUNDARY SETTING

Ecological status classes were defined by dividing the EQR values to a five-class equidistant scale according to the prescriptions of the WFD (Table 6). Namely, CFIR expresses the relationship of the observed metrics in certain fish community with the same metrics under reference conditions (in natural, stabile, undisturbed fish community of certain river type) and ecological status of certain locality can be designated as belonging to one out of five classes, in accordance with the requirements of the WFD. CFIR values close to 1 imply undisturbed or only slightly disturbed fish communities, whereas values closer to 0 represent communities that are significantly modified as a consequence of anthropogenic pressures. The EQRs, and thereafter also CFIR, were developed in comparison with reference conditions (conditions in natural, undisturbed fish communities), so class boundaries were set following the suggestion of Furse et al. (2006) – equidistal arrangement of class boundaries from 1 (reference conditions) to 0 for five ordinal rating categories for assessment of impairment in accordance with WFD requirements.

Table 6. CFIR classification – class boundaries setting for biological element fish.

EQR value intervals	Ecological Quality State (EQS)
0,80 – 1,0	high / very good
0,60 – 0,79	good
0,40 – 0,59	moderate
0,21 – 0,39	poor
0,0 – 0,20	bad

3. WFD COMPLIANCE CHECKING

The first step in the Intercalibration process requires checking whether national methods are in accordance with the WFD compliance criteria (Table 7).

Table 7. List of the WFD compliance criteria and the WFD compliance checking process and results.

Compliance criteria	Compliance checking
Ecological status is classified by one of **five classes** (high, good, moderate, poor and bad).	yes
High, good and moderate ecological status are set in line with the WFD's **normative definitions** (**Boundary setting procedure**)	yes
All relevant parameters indicative of the biological quality element are covered (see Table 1 in the IC Guidance). A **combination rule** to combine parameter assessment into BQE assessment has to be defined. If parameters are missing, Member States need to demonstrate that the method is sufficiently indicative of the status of the QE as a whole	yes, with the exception of age structure because it did not provide pressure-response answers
Assessment is adapted to **intercalibration common types** that are defined in line with the typological requirements of the Annex II WFD and approved by WG ECOSTAT	yes
The water body is assessed against **type-specific near-natural reference conditions**	yes
Assessment results are expressed as **EQRs**	yes
Sampling procedure allows for **representative** information about water body quality/ecological status **in space and time**	yes
All data relevant for assessing the biological **parameters** specified in the WFD's normative definitions are covered by the **sampling procedure**	yes
Selected taxonomic level achieves adequate **confidence and precision** in classification	yes (species level)

4. IC FEASIBILITY CHECKING

The intercalibration process ideally covers all national assessment methods within a GIG. However, the comparison of dissimilar methods (“apples and oranges”) has clearly to be avoided. Intercalibration exercise is focused on specific type / biological quality element / pressure combinations. The second step of the process introduces an “IC feasibility check” to restrict the actual intercalibration analysis to methods that address the same common type(s) and anthropogenic pressure(s), and follow a similar assessment concept.

4.1. TYPOLOGY

The Fish Cross GIG did not use the common intercalibration typology in its IC work (Mediterranean GIG, Eastern Continental GIG *etc.*). Instead, following regional groups were used:

Nordic Group
Lowland-Midland Group
Alpine-type Mountains Group
Mediterranean South-Atlantic
Danubian Group

Croatian rivers belong to the Mediterranean South-Atlantic Group (Adriatic River Basin) and to the Danubian Group (Danube River Basin). Although located in the Danube River Basin, Danube, Drava and Sava rivers are not considered here, since they are included in the intercalibration exercise for very large rivers.

Croatia fits national fish classification method to the results of the completed intercalibration exercise of the Fish Cross GIG Danubian Group, along with the Czech Republic, Slovakia, Hungary, Bulgaria and Romania. Intercalibration common types are not used, and all the river sites were considered together for the IC exercise. In connection with the GIG agreed common data base included in the IC process, Croatian data are not divided according to their typological characteristics.
Fourteen (14) Croatian national river types are included in the common database of the Cross GIG Danubian Group. The national types correspond with the Eastern Continental IC common river types, according to their abiotic characteristics, catchment area, altitude, geology and substrate (Table 8).

Table 8. Croatian river types, with the respective common Eastern Continental IC river types, included in the Fish Cross GIG Danube Group

<table>
<tr><th>ECOREGION</th><th>SUBREGION</th><th colspan="2">NATIONAL TYPE NAME</th><th>NATIONAL TYPE</th><th>IC TYPE</th></tr>
<tr><td rowspan="5" colspan="2">PANNONIAN ECOREGION (11 HUNGARIAN LOWLANDS)</td><td colspan="2">Small mountain and mid-altitude rivers</td><td>HR-R_1</td><td>EX6</td></tr>
<tr><td rowspan="2">Small lowland rivers</td><td>Small lowland rivers with clay and sand substrate</td><td>HR-R_2A</td><td rowspan="2">EX5</td></tr>
<tr><td>Small lowland rivers with gravel and pebble substrate</td><td>HR-R_2B</td></tr>
<tr><td rowspan="2">Lowland alluvial rivers</td><td>Small lowland alluvial rivers with gravel and pebble substrate</td><td>HR-R_3A</td><td rowspan="2">EX5</td></tr>
<tr><td>Small lowland alluvial rivers with clay and sand substrate</td><td>HR-R_3B</td></tr>
</table>

ECOREGION	SUBREGION	NATIONAL TYPE NAME		NATIONAL TYPE	IC TYPE
			Medium lowland alluvial rivers with clay and sand substrate	HR-R_3C	E2
			Large lowland alluvial rivers with clay and sand substrate	HR-R_3D	E3
		Medium and large lowland rivers	Medium lowland rivers	HR-R_4A	E2
			Large lowland rivers	HR-R_4B	E3
			Large lowland rivers with spring in Dinaric Western Balkan	HR-R_4C	
DINARIC ECOREGION (5 DINARIC WESTERN BALKAN)	DINARIC CONTINENTAL SUB-ECOREGION	Small mountain and mid-altitude rivers		HR-R_6	EX7
		Medium and large mountain and mid-altitude rivers		HR-R_7	EX8
		Medium and large lowland rivers		HR-R_8A	EX8
		Medium mountain and mid-altitude rivers in karst field (krško polje)		HR-R_9	EX8

4.2. PRESSURES ADDRESSED

The most important common pressures for IC are considered: water quality alteration, hydro-morphological modifications, connectivity disruption. The hydromorphological alteration scale ranges from 1 (no) to 5 (high) and consists of multiple smaller indices. The three main indices: hydrology regime, morphology and flow continuity ranged from 1 to 5, whereas the mean hydromorphological score ranged from 1 to 4,53.

The ranges for the chemical variables tested are:

Chemical variable	range
BOD_5 [mg L^{-1}]	0,55 – 7,244
COD [mg L^{-1}]	0,731 – 15,42
PO_4^-P [mg L^{-1}]	0,0 – 0,959
NO_3^-N [mg L^{-1}]	0,0004 – 4,494
NH_4^-N [mg L^{-1}]	0,0 – 4,917
Total P [mg L^{-1}]	0,002 – 1,614

The following pressure-response relationships have been derived:

1. ORGANIC ENRICHMENT

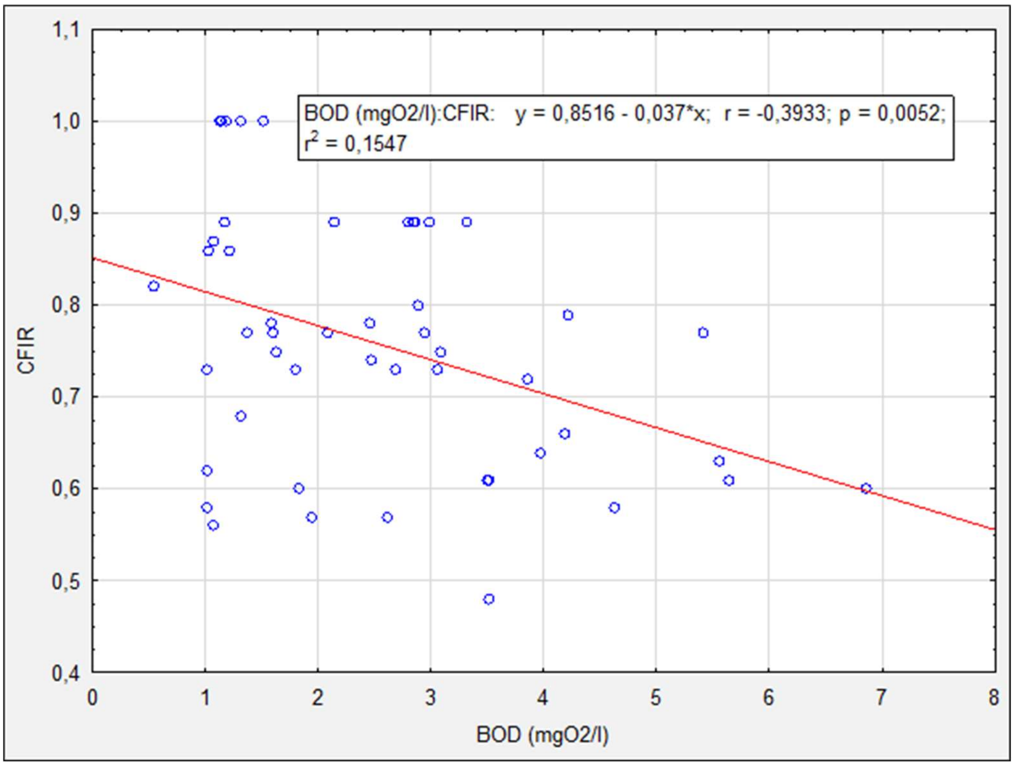

Figure 12. Pressure-response relationship between biological oxygen demand (BOD) and the EQR_{CFIR} values in the Danubian region of Croatia

2. HYDRO-CHEMISTRY

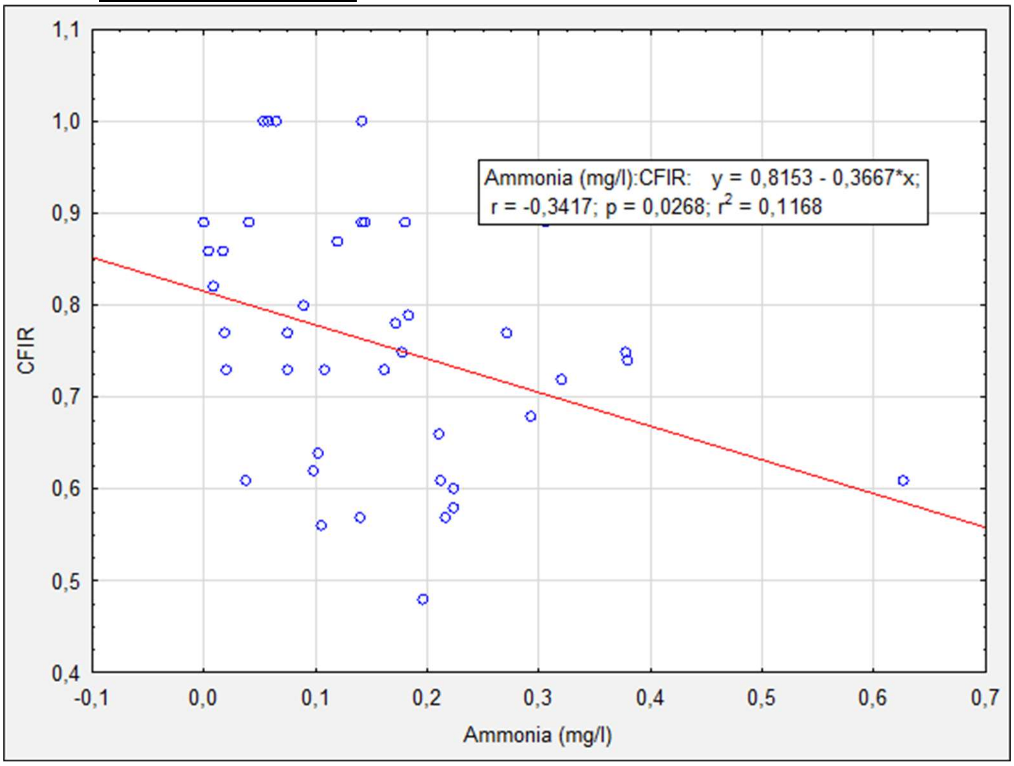

Figure 13. Pressure-response relationship between ammonia and the EQR_{CFIR} values in the Danubian region of Croatia

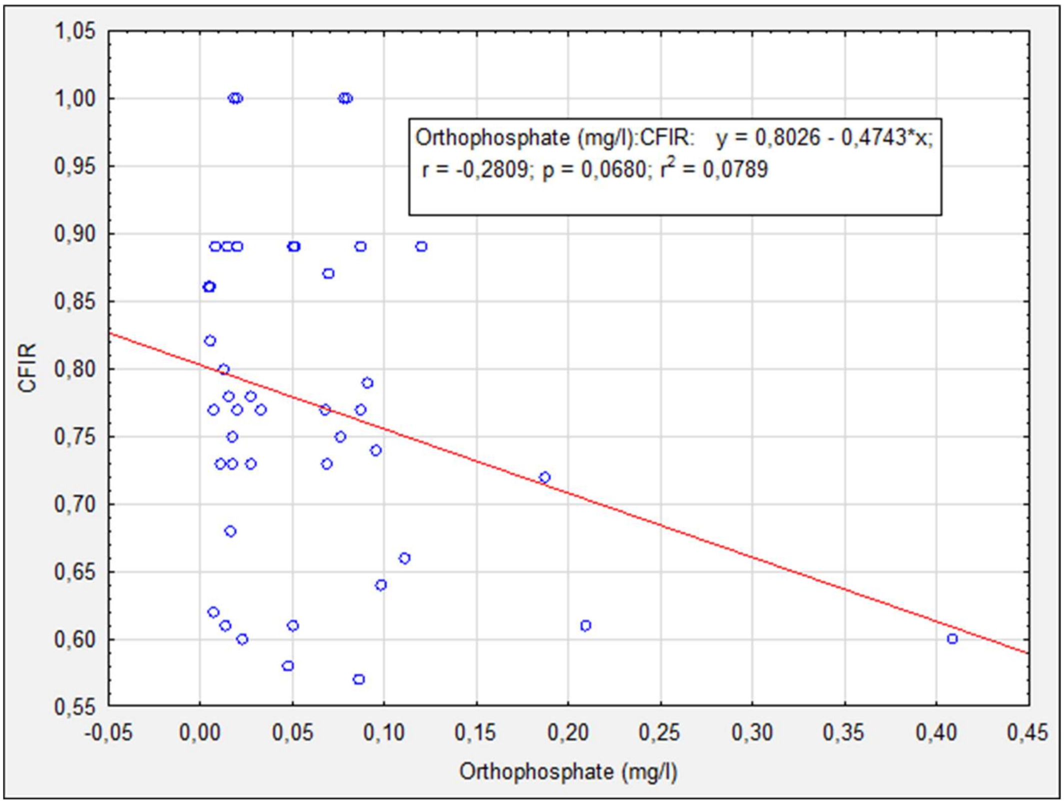

Figure 14. Pressure-response relationship between orthophosphates and the EQR_{CFIR} values in the Danubian region of Croatia

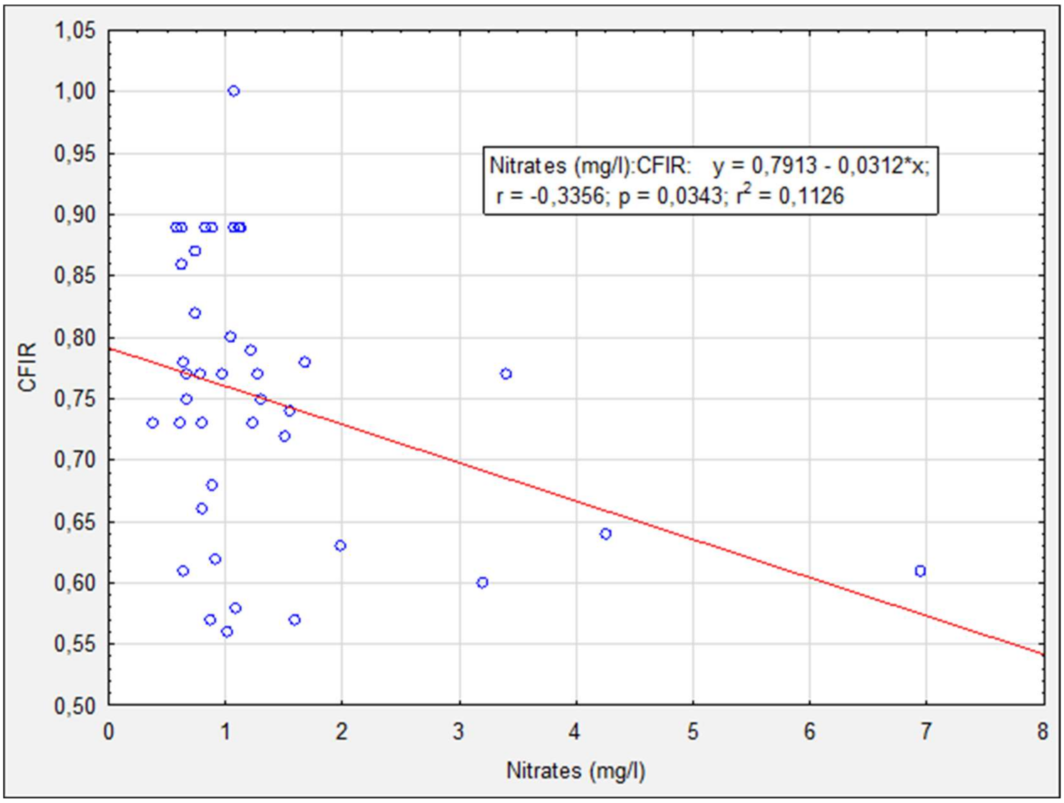

Figure 15. Pressure-response relationship between nitrates and the EQR_{CFIR} values in the Danubian region of Croatia

3. HYDROMORPHOLOGY

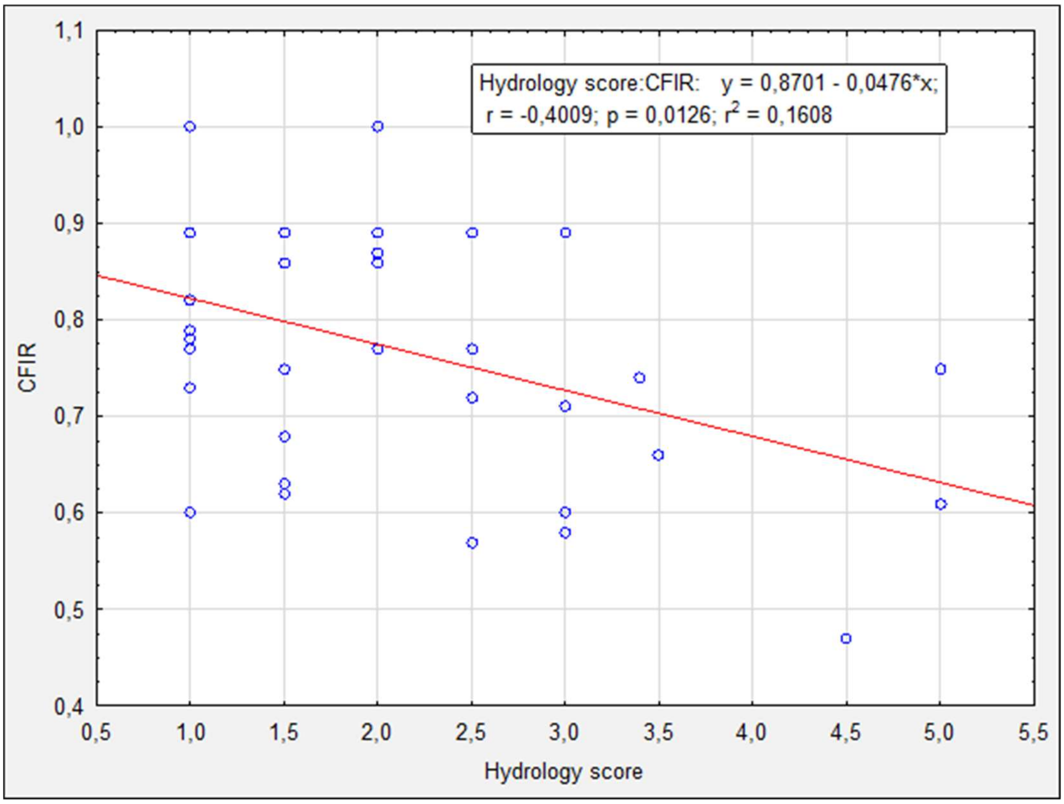

Figure 16. Pressure-response relationship between the river mean hydromorphological score and the EQR_{CFIR} values in the Danubian region of Croatia

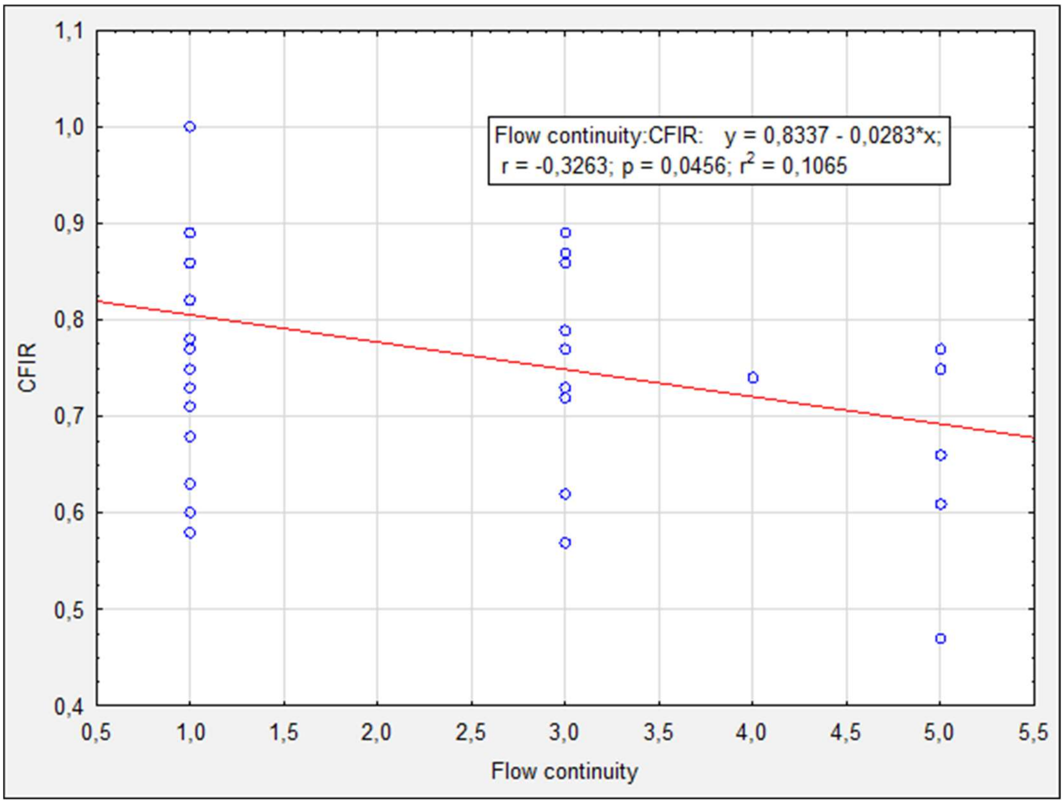

Figure 17. Pressure-response relationship between flow continuity and the EQR_{CFIR} values in the Danubian region of Croatia

4. RESUME

For three groups of pressures (organic enrichment, chemistry and hydromorphology), significant regressions could be found. Although the national fish based index (CFIR) does show evident trends when compared to land use, no significant regressions were calculated. It is concluded that the national fish based index (CFIR) clearly responds to anthropogenic impacts and can be used for the assessment of the ecological status.

4.3. ASSESSMENT CONCEPT

The Croatian national method under IC process is the Croatian fish index for rivers (CFIR). This index is a multimetric index that compares fish communities metrics to reference conditions, as described above. The Croatian national method follows the same assessment concept as other methods in the intercalibration group.

4.4. CONCLUSION ON THE INTERCALIBRATION FEASIBILITY

The number of sites fully complying in terms of the type criteria is high enough for carrying out the IC exercise.

5. DEMONSTRATING THE COMPLIANCE WITH THE COMPLETED INTERCALIBRATION EXERCISE

5.1. BACKGROUND

- **Description of the IC option and benchmark standardization used in the completed IC exercise;**

The comparison of reference conditions between countries is completed. Reference sites defined using common criteria at the European scale were used.

The common metrics (Table 9) are based on ecological and biological traits and are standardized in terms of natural environmental conditions, which means that they are independent of biogeographical conditions and environmental gradients.

Table 9. The metrics used to calculate the Salmonid and the Cyprinid Fish Index (EFI+ Manual 2009, WFD Intercalibration Phase 2: Milestone report, 2011)

Zone / Index	Metric name	Detailed name - guild
Salmonid	Ni.O2.Intol	Density (number of individuals per 100 m^2 in the 1 run of a sample site) of species **intolerant to oxygen depletion**, always more than 6 mg/l O_2 in water.
	Ni.Hab.Intol.150	Density (number of individuals per 100 m^2 in the 1. run of a sample site) ≤ 150 mm (total length) of species **intolerant to habitat degradation**.
Cyprinid	Ric.RHt.Par	Richness (number of species in the 1. run of a sample site) of species requiring a **rheophilic reproduction habitat**, i.e. preference to spawn in running waters.
	Ni.LITHO	Density (number of individuals per 100m^2 in the 1. run of a sample site) of species requiring **lithophilic** reproduction habitat, species which spawn exclusively on gravel, rocks, stones, cobble or pebbles. Their hatchlings are photophobic.

For each zone, the index value is the mean of the two metric values:

Salm.Fish.Index = (Ni.Hab.150 + Ni.O2.Intol) / 2

Cypr.Fish.Index = (Ric.RH.Par + Ni.LITHO) / 2

A second version of common metrics has been developed in 2010 (WFD Intercalibration Phase 2: Milestone report – October 2011).

This second version does not consider anymore the two river zones. Only two metrics are used to compute the fish index for all sites (see Table 4).

$$Fish.Index = (Ni.O2.Intol + Ric.RHt.Par) / 2$$

This second version is only applicable in the Lowland, Nordic and Danubian Group, but not in the Mediterranean Group because of the rarity of oxygen-intolerant species in this area. Croatia is located in the Danubian Group and the intercalibration process was done according to the scheme adopted in this group.

- **Selection of the correct procedure to use for intercalibrating new classification method.**

IC Option 2 without piecewise transformation was chosen for intercalibration of the Croatian national method. Total number of reference sites for Croatia is 6. It is sufficient for benchmarking process according to Guidance Document No. 30 (European Union 2015).

5.2. DESCRIPTION OF IC DATASET

For intercalibration of the Croatian method the dataset consisted of 55 sites sampled within the standard monitoring program in 2015, 2016 and 2017. These sites represented all of the abiotic river types that are assessed with the national assessment method and have a wide pressure gradient. We took over 80 sites located in Danubian Group in consideration, but only 55 of them had all the necessary pressure impact data to proceed with analysis.

5.3. DESCRIPTION OF INTERCALIBRATION PROCEDURE

- Benchmark standardization:

  Benchmark standardization was conducted according to the scheme used in completed IC exercise (WFD Intercalibration Phase 2: Milestone report, 2011) and in other IC guiding documents. The Intercalibration Common Metrics (ICM) calculated for all sites in the Croatian national dataset were divided by a median value of ICM for 6 benchmark sites.

- Calculation of Intercalibration Common metrics (ICM) or Best-Related Intercalibrated National Classification (BRINC):

  Intercalibration Common metrics (ICM) were calculated for all sites in the Croatian national dataset with the original EFI+ software (EFI+ Manual 2009). According to the second version of CM adopted in the completed IC exercise for Danubian Group (WFD Intercalibration Phase 2: Milestone report, 2011), the final value of ICM was calculated using the formula described in chapter 5.1. ICM values were than benchmark standardized, as described above, into ICM_{bm}. These ICM_{bm} values were than related to the national EQR-s using OLS regression. The OLS regressions were analyzed separately for the four index groups (types).

- Translation of national boundaries to ICM or BRINC:

  The results of OLS regression between ICM_{bm} and national EQRs for a total of 106 sites from the Croatian national dataset are presented in Figures 18-21. The correlation was significant (R >

0,5; p < 0,05) for all four indices types. The R value (correlation coefficient) of the OLS regression and was > 0,5 for all four types (0,504, 0,534, 0,503 and 0,951). The OLS regression for all four types fitted the boundaries within the range of the values for other three countries in the Danubian Group od the completed IC exercise (Table 10). For the Croatian national method, classes are as follows H/G=0,8; G/M=0,6; M/B=0,4 and B/P=0,2. The regression formula was used to translate the national boundaries to ICM_{bm} scale. These data were compared with values obtained for Danubian Group of completed IC exercise (Table 10).

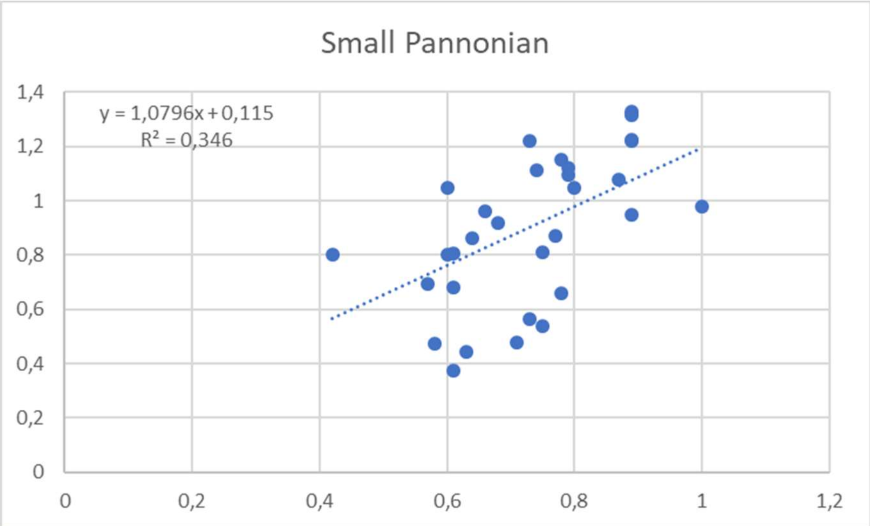

Figure 18. OLS regression between ICMbm and Croatian national index for river types HR-R_1 and HR-R_2 (small Pannonian rivers).

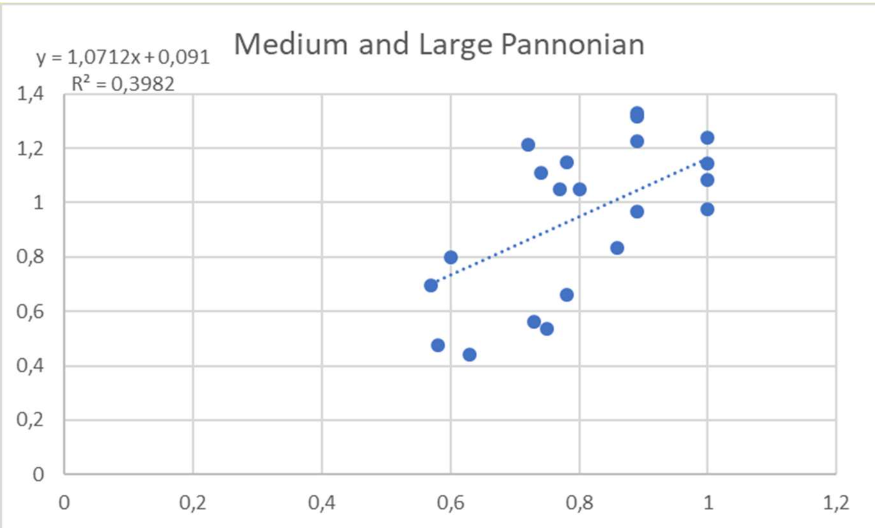

Figure 19. OLS regression between ICMbm and Croatian national index for river types HR-R_3 and HR-R_4 (medium, large and alluvial Pannonian rivers).

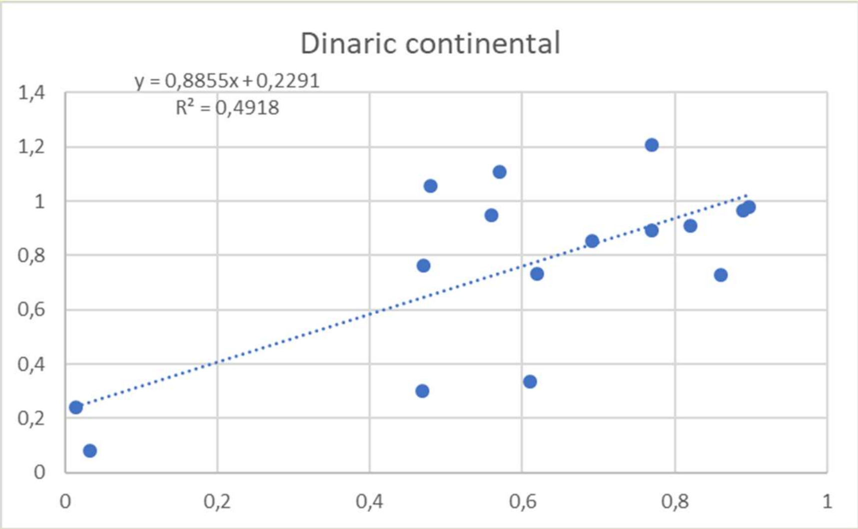

Figure 20. OLS regression between ICMbm and Croatian national index for river types HR-R_6, HR-R_7 and HR-R_8 (small, medium and large, mountain, mid-altitude and lowland rivers in the Dinaric continental ecoregion).

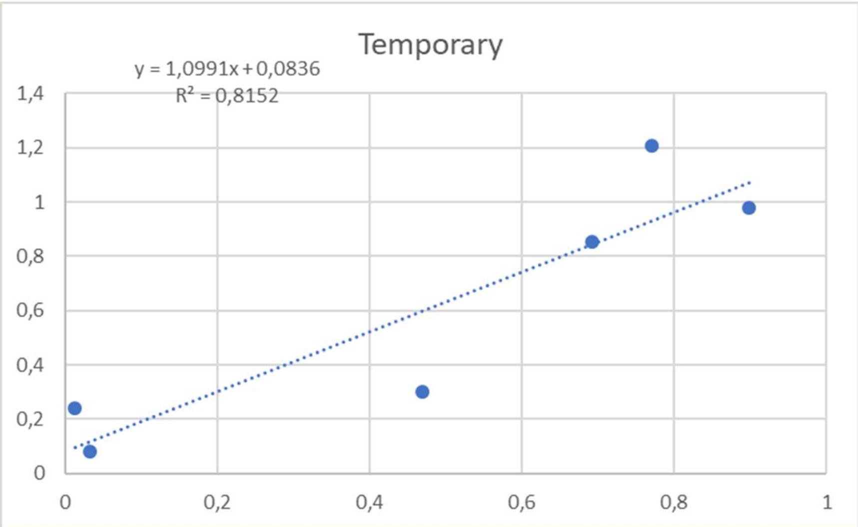

Figure 21. OLS regression between ICMbm and Croatian national index for river types HR-R_9 and HR-R_10 (rivers in karstic fields and temporary rivers in the Dinaric continental ecoregion).

- Calculating boundary bias:

  The Croatian National index boundaries for HRIR in the Danubian region of Croatia are as follows:

High	0,8-1
Good	0,6-0,79
Moderate	0,4-0,59
Bad	0,2-0,39
Poor	<0,2

According to Guidance Document No. 30 (European Union 2015) the boundary bias was calculated by subtracting the mean value of G/M or H/G boundary on ICM_{bm} scale from Croatian index boundaries transposed to this scale with OLS regression. After EQR and piecewise transformation, the boundaries (+/- a fourth of a class) are compared to the boundaries of the national methods already intercalibrated in the Danubian catchment. After checking the overlap between the boundary intervals of the Croatian indices and the already intercalibrated in the Danube catchment. After checking the overlap between the boundary intervals of the four Croatian indices and the already intercalibrated national boundaries from CZ, RO and SK, we can conclude that all the boundaries (both G/M and H/G) overlap (Table 10 and Figures 22 & 23).

Table 10. Raw values (raw) and predicted values (fit) of the High-Good (H/G) and the Good-Moderate (G/M) boundaries and the associated intervals: lower(lwr) and upper (upr) values, which correspond to one fourth of a class; expressed in common metrics for three countries in the Danubian Group of completed IC exercise and for four indices types in Pannonian and Dinaric continental Croatia.

Method_country Type	H/G raw	G/M raw	H/G fit	H/G lwr	H/G upr	G/M fit	G/M flwr	G/M upr
CZ_index	0,78	0,585	0,928	0,894	0,967	0,791	0,757	0,826
RO_EFI_salmonid	0,911	0,755	0,895	0,861	0,914	0,759	0,704	0,793
SK_FIS_index	0,71	0,57	0,943	0,91	1,012	0,811	0,778	0,844
HR_index 1 small Pann	0,8	0,6	0,979	0,925	1,033	0,763	0,709	0,817
HR_index 2 Medium and Large Pann	0,8	0,6	0,948	0,894	1,002	0,734	0,680	0,787
HR_index 3	0,8	0,6	0,938	0,893	0,982	0,760	0,716	0,805
HR_index 4	0,8	0,6	0,963	0,908	1,018	0,743	0,688	0,798

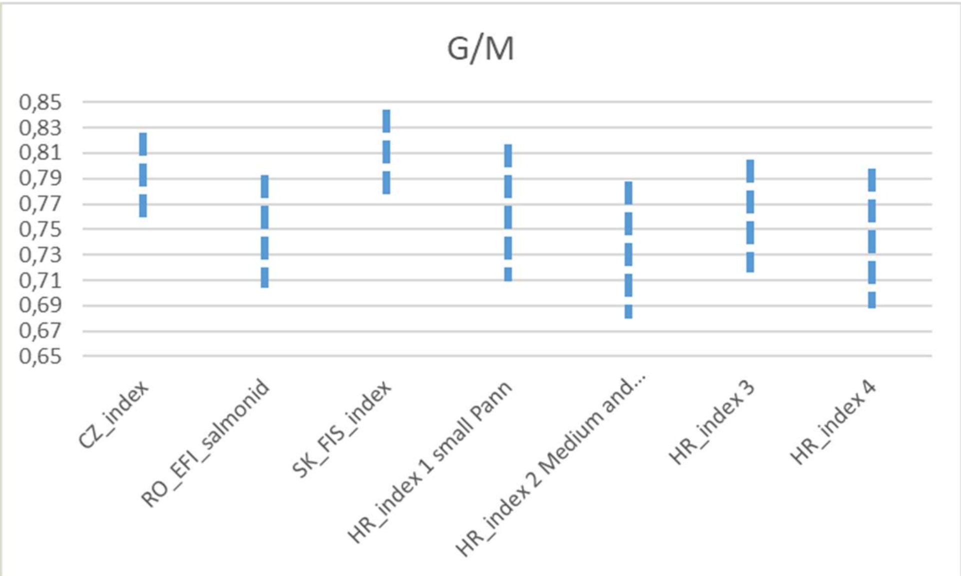

Figure 22. Class boundaries G/M ±0.25 class width of the intercalibrated national methods on the common metric scale compared with the global mean of the finalized method (Croatia with four methods for Pannonian and Dinaric rivers).

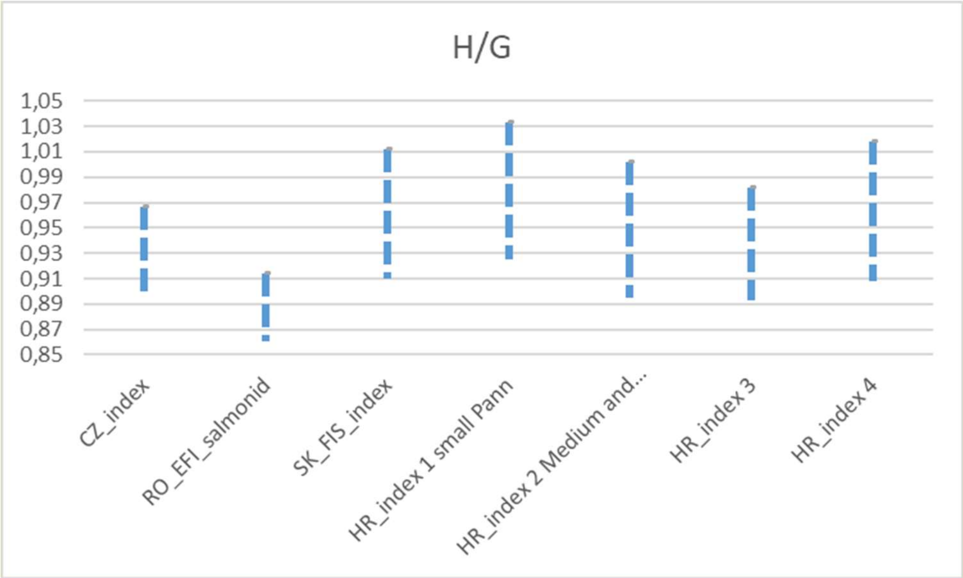

Figure 23. Class boundaries H/G ±0.25 class width of the intercalibrated national methods on the common metric scale compared with the global mean of the finalized method (Croatia with four methods for Pannonian and Dinaric rivers).

5.4. FINAL BOUNDARIES

According to Guidance Document no. 30 (European Union 2015) the boundary bias of the newly formed boundaries for the Croatian method was assessed as not significant. It amounted to up to 0,17 of a moderate class width for G/M boundary and up to 0,25 of a high class width for H/G boundary, so in both cases for all four indices types it was less or equal to the acceptable level of 0,25 class width (Table 10,).

The Croatian National EQR newly harmonized boundaries for CFIR in the Danubian region of Croatia are as follows:

High	0,8-1
Good	0,6-0,79
Moderate	0,4-0,59
Bad	0,21-0,39
Poor	0-0,20

6. DESCRIPTION OF THE BIOLOGICAL COMMUNITIES

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT HIGH STATUS

In general, fish assemblages in high status contain most type specific species, characteristic for natural community of certain national river type. Proportion of non-native species and individuals belonging to non-native species is very low or they are not present. Ecological Quality Ratios important for certain river type are mostly above 0,8, indicating small or absent anthropogenic modifications.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT GOOD STATUS

Assemblages indicating good ecological status of rivers comprise high proportion of native species characteristic for natural communities of the certain river type. Nevertheless, non-native species are often present with low or moderate proportion of species and/or individuals. Ecological Quality Ratios are slightly to moderately (0,2-0,4) reduced in comparison to high ecological status. Small to moderate negative consequences of anthropogenic pressures can be noticed.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT MODERATE STATUS

Moderate ecological status is estimated based on the communities with a significantly reduced proportion of native species, higher proportion and sometime even dominance of non-native species and disrupted composition of fish communities. Ecological Quality Ratios are about half (0,4-0,6) of EQRs in natural communities characteristic for certain river type. Negative consequences of anthropogenic pressures led to significant disturbances in structures and compositions of fish communities.

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Report on fitting the Croatian classification method for fish in rivers to the results of the completed intercalibration of the Fish Cross GIG (Mediterranean Group)

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Report on fitting the Croatian classification method for fish in rivers to the results of the completed intercalibration of the Fish Cross GIG (Mediterranean Group)

1. INTRODUCTION

- Member State: Croatia;
- BQE: Fish;
- Water body category (type): Rivers.

Croatia did not participate in the Fish Cross GIG intercalibration exercise, since the Croatian fish-based method of ecological status assessment in the river types was under development during the intercalibration exercise (see Intercalibration Technical Report Cross-GIG rivers - Fish fauna, 2012). The objective of this report is to present Croatian fish index for rivers (CFIR) which is national method for ecological status assessment in the rivers based on fish, and to prove that it is compliant with the WFD normative definitions and its class boundaries are in line with the results of the completed intercalibration exercise of the Fish Cross GIG (Mediterranean Group).

Croatian rivers and streams belong to two different watersheds – Black Sea (Danube) watershed comprises waters from northern and central part of Croatia, whereas southern river basins belong to the Adriatic watershed. Croatian national typology of natural rivers divides Croatian rivers into three ecoregions: rivers of the Black Sea watershed mostly belong to the Pannonian ecoregion (national types HR-R_1, HR-R_2A, HR-R_2B, HR-R_3A, HR-R_3B, HR-R_3C, HR-R_3D, HR-R_4A, HR-R_4B, HR-R_4C, HR-R_5B, HR-R_5C and HR-R_5D), whereas rivers of the Adriatic watershed mostly belong to the Dinaric coastal ecoregion (national types HR-R_11A, HR-R_11B, HR-R_12, HR-R_13, HR-R_13A, HR-R_14A, HR-R_14B, HR-R_14C, HR-R_15A, HR-R_15B, HR-R_16A, HR-R_16B, HR-R_17, HR-R_18 and HR-R_19). Designation of rivers belonging to these two ecoregions to CROSS GIG fish intercalibration groups is obvious: rivers of the Pannonian ecoregion fall under the Danube IC group, whereas rivers of the Dinaric coastal ecoregion belong to the Mediterranean group. The third ecoregion based on the national typology, Dinaric continental ecoregion (national types HR-R_6, HR-R_7, HR-R_8A, HR-R_8B, HR-R_9, H-R_10A and HR-R_10B), comprises rivers from the central, mostly mountainous part of Croatia that belong to both watersheds (Black Sea and Adriatic). Based on geographic location and ecological characters of those river types, it is agreed that rivers belonging to this ecoregion will be presented together with the Pannonian ecoregion and included into the Danube group for intercalibration. In this report we present methodology for the ecological status assessment based on fish in national river types that belong to the Dinaric coastal ecoregion, as well as comparison with the CROSS GIG Mediterranean group intercalibration exercise.

2. DESCRIPTION OF NATIONAL ASSESSMENT METHODS

2.1. METHODS AND REQUIRED BQE PARAMETERS

For the estimation of the ecological status of natural rivers and streams in Croatia, a fish based index was developed, following requirements of the Water Framework Directive (WF) 2000/60/EC. The development of the **Croatian fish index for rivers (CFIR)** included procedures and methods previously identified as the best practices (Hering et al. 2006, Argillier et al. 2013), but taking into account also specific characters of Croatian watersheds, considering both, river communities and anthropogenic pressures. In the development of CFIR, following procedures were implemented:

- Field sampling of fish
- Obtaining of relevant environmental parameters
- Calculating fish fauna metrics
- Selection of relevant environmental parameters and pressure proxies, as well as fish fauna metrics that respond to at least one pressure proxy
- Ecological Quality Ratios calculations
- Multimetrix index generation
- Ecological quality class boundaries implementation

Following the mentioned procedure, we have designed the Croatian fish index for rivers that documents well the relationships between fish and pressures occurring in their habitats, as requested by the WFD.

The classification method is verified for WFD compliance (Table 1) and IC feasibility and the class boundaries were compared with agreed boundaries from the CROSS GIG Mediterranean Group intercalibration exercise following the instructions of the CIS Guidance Document 30: “Procedure to fit new or updated classification methods to the results of a completed intercalibration exercise” (Willby et al. 2014).

Table 1. Overview of the metrics included in the national method

HR	Taxonomic composition	Abundance	Disturbance of sensitive taxa	Age structure
CFIR	yes	yes	yes	no*

**Age structuring estimations were considered at first steps, but they did not confirm to requirements of statistical analyses and no pressure-responses was established, so age structure metrics are not included in the final index.*

2.2. FISH FAUNA SAMPLING

Description of sampling and data processing

Fish sampling must be in accordance with HRN EN 14962:2007, Water quality – Guidance on the scope and selection of fish sampling methods, and HRN EN 14011:2005 Water quality -- Sampling of fish with electricity (EN 14011:2003).

- Sampling time and frequency:
  Sampling is conducted in late summer or early autumn in the continental part of Croatia (Danube river basin). For the purpose of comparing obtained results, repeated sampling in individual monitoring localities should be conducted at the same time of the year.

Sampling site (locality) is identified by ensuring that the sampled section covers the diversity of all types of natural microhabitats and man-induced microhabitats. Sampling site has to be large enough to include living area of dominant species and to include all characteristic river habitats (faster and slower parts, sidearms….), i.e. it has to be representative of fish community in order to be able to evaluate density and age structure of each species in ichthyopopulation. Simultaneously with covering as many habitats as possible during selection of sampling localities, it should also be taken into consideration easy access to the sampling site itself and previous knowledge on a certain locality.

Selected localities have to be representative of the status on a section of running waters whose length is (according to FAME, 2004):

- 1 km, for small running waters (catchment area size < 100 km2); 500 m upstream and 500 m downstream from the initial sampling site),
- 5 km, for medium running waters (catchment area size 100 – 1 000 km2) and
- 10 km, for large running waters (catchment area size > 1 000 km2).

- Sampling method:
  Electrofishing represents a universal standard method for river sampling. This sampling method enables the best estimation of population density, species abundance, number of organisms and fish biomass, age structure and mutual relationships of fish species samples, and it also represents the least harmful fishing method when compared to other methods.

Electric fishing generator is used to catch fish in three ways:

- Wading in the river,
- From river bank or
- On board a boat.

Wadable running waters, shallow watercourses up to 15 m width, are sampled in their entire width using a backpack generator. Prior to sampling, the sampling section is delimited with nets to prevent fish from escaping. In the delimited area fishing is conducted twice with the same fishing effort. If the probability of catching type-specific species in the first two catches is less than 50%, sampling has to be repeated once more. If sampling is not done using a backpack electric generator, the electric generator should be placed on the river bank and an anode with a long electric cable on fiberglass handle used.

In larger running waters where the depth (> 0,7 m) and habitat diversity prevent efficient sampling from the bank or by wading in the riverbed, a special electric fishing boat is used. Electric generators with different powers are used for fishing in running waters of different size and depth:

- minimum 2,5 kW – small running waters and fishing by wading in the river and from the bank,
- minimum 5 kW – medium running waters and fishing on board a boat,
- minimum 7,5 kW (recommended ≥ 10 kW) – large and very large running waters and fishing on board a boat.

Electric generator enabling fishing with pulsating current, and direct current (with or without pulsating option) was used because it is least harmful to fish, and provides the best results whereas alternating current shall not be used. Depending on the size of running waters, fishing was done using:

- one anode with known ring diameter (for example 50 cm) and with a net on a fiberglass handle 2.5 m long,
- four or more anodes placed at a distance of 50 cm between each other, placed on a construction mounted on board a boat adapted for electric fishing (fishing efficiency can be increased by expanding the electric field in most cases by increasing the number of anodes used for fishing).

Fishing is done downstream with the boat moving along the bank, covering as many existing habitats as possible especially places where fish might be hiding.

Fishing alongside both banks in periods longer than 20 minutes or 250 m in length, depending on the size of running water was conducted, and sampling the length in equivalent to 10 widths of the watercourse, trying to cover all available microhabitats, and in large and very large river sampling covered even up to 1000 m of the river length in order to include a representative sample of fish community.

During each sampling time during which electric generator was used for fishing was recorded, and GPS used to determine the distance that was crossed. Based on these data it was possible to calculate catch per unit effort (CPUE) and surface covered by the sampling. During each repeated electrical fishing, it is of vital importance to use electrical generator with the same power and with it sweep the same surface in equal time as during the first sampling.

- Species determination, measuring and handling:

All caught fish were determined based on morphological features using determination keys (Kottelat and Freyhof, 2007; Vuković and Ivanović, 1971; Povž and Sket, 1990; Miller and Loates 1997). In the case of doubt (hybrids, closely related species, young individuals), those individuals were put in 4 % - formaldehyde solution and taken to the laboratory for precise determination.
During determination, total body length (TL) was measured using ichthyometer from the beginning of the head to the tip of the tail fin, expressed in mm. Based on that data compared with literature, quality of obtained samples was assessed, because domination of smaller or larger individuals than expected indicates stress in the population. Total body length (TL) was measured by the person that caught it in order to return fish back to water as soon as possible. During measuring body length, noticed anomalies (visible external skin, subcutaneous or fin damage, parasites, deformations, tumors, lesions) were noted. If the number of individuals with outside anomalies is higher than usual, than stress is present in that population and it doesn't represent natural state of population. Mortality of sampled individuals by electrofishing method was less than 1%.

Environmental data describing each sampling site are collected both in the field and using literature or Internet sources. Site position is recorded with GPS, site length (m), river width (m) and description of sampling site are recorded at the field protocol which should be overwritten into the database at home. Photo of sampling site was taken and file number of a photo recorded at the field protocol too.

2.3. FISH FAUNA METRICS

Description of fish fauna metrics used to describe fish communities in Croatian rivers

All sampled fish species were classified in groups according to their preferences for reproductive substrate (litophilic, LITH; phytophilic, PHYT; phyto-litophilic, PHLI; pelagophilic, PEL; psamophilic, PSAM; ostracophilic, OSTR; species that spawn in the sea, SEA), feeding preferences (herbivores, HERB; invertivores, INV; omnivores, OMNI; piscivores, PISC; and detritivores, DETR) and habitat preferences (benthopelagic, WCOL and benthic, BENT) (Table 2).

Table 2. Ecological characters of fish species from Croatian rivers.

Species	Habitat preferences	Spawning substrate	Feeding strategy	Ecological requirements
Abramis brama	BENT	PHLI	OMNI	Euritopic
Alburnoides bipunctatus	WCOL	LITH	INV	Reophilic
Alburnus alburnus	WCOL	PHLI	OMNI	Euritopic
Ameiurus melas	BENT	PHLI	OMNI	Limnophilic
Babka gymnotrachelus	BENT	PHLI	OMNI	Euritopic
Barbatula barbatula	BENT	PSAM	INV	Reophilic
Barbus balcanicus	BENT	LITH	INV	Reophilic
Barbus barbus	BENT	LITH	INV	Reophilic
Blicca bjoerkna	BENT	PHYT	OMNI	Euritopic
Carassius carassius	BENT	PHYT	OMNI	Euritopic
Carassius gibelio	BENT	PHYT	OMNI	Euritopic
Chondrostoma nasus	BENT	LITH	HERB	Reophilic
Cobitis elongata	BENT	LITH	INV	Reophilic
Cobitis elongatoides	BENT	PHYT	INV	Reophilic
Ctenopharyngodon idella	WCOL	PEL	HERB	Euritopic
Cyprinus carpio	BENT	PHYT	OMNI	Euritopic
Esox lucius	WCOL	PHYT	PISC	Euritopic
Eudontomyzon vladykovi	BENT	LITH	DETR	Reophilic
Gobio obtusirostris	BENT	PSAM	INV	Reophilic
Gymnocephalus baloni	BENT	PHLI	INV	Euritopic

Gymnocephalus cernua	BENT	PHLI	INV	Euritopic
Lepomis gibbosus	WCOL	LITH	INV	Limnophilic
Leuciscus aspius	WCOL	LITH	PISC	Reophilic
Leuciscus idus	WCOL	PHLI	OMNI	Reophilic
Leuciscus leuciscus	WCOL	LITO	OMNI	Reophilic
Misgurnus fossilis	BENT	PHYT	INV	Reophilic
Neogobius fluviatilis	BENT	LITO	INV	Euritopic
Neogobius melanostomus	BENT	LITO	INV	Euritopic
Oncorhynchus mykiss	WCOL	LITH	INV/PISC	Reophilic
Perca fluviatilis	WCOL	PHLI	INV/PISC	Euritopic
Phoxinus phoxinus	WCOL	LITH	INV	Reophilic
Ponticola kessleri	BENT	LITH	INV	Euritopic
Proterorhinus semilunaris	BENT	LITH	INV/PISC	Euritopic
Pseudorasbora parva	WCOL	PHLI	OMNI	Euritopic
Rhodeus amarus	WCOL	OSTR	OMNI	Euritopic
Romanogobio kesslerii	BENT	PSAM	INV	Reophilic
Romanogobio vladykovi	BENT	PSAM	INV	Reophilic
Rutilus rutilus	WCOL	PHLI	OMNI	Euritopic
Rutilus virgo	BENT	PHYT	INV	Reophilic
Sabanejewia balcanica	BENT	PHYT	INV	Reophilic
Salmo trutta	WCOL	LITH	INV/PISC	Reophilic
Sander lucioperca	WCOL	PHYT	PISC	Euritopic
Scardinius erythrophthalmus	WCOL	PHYT	OMNI	Limnophilic
Silurus glanis	BENT	PHYT	PISC	Euritopic
Squalius cephalus	WCOL	LITH	OMNI	Reophilic
Tinca tinca	BENT	PHYT	OMNI	Limnophilic
Umbra krameri	BENT	PHLI	INV	Limnophilic
Vimba vimba	BENT	LITO	INV	Reophilic

After field investigation, determination and measurement of all individuals, we have prepared a total of 103 metrics that describe fish communities (Table 3). Metrics belonging to four metric types have been prepared (following Furse et al. 2006), but also several additional metrics, similarly as conducted in previous fish-based indices assessments (for example Petriki et al. 2017). Noteworthy, collocation of certain fish metrics under metric types (as defined by Furse et al. 2006) is sometimes arbitrary, because the same metric can sometimes be collocated under more than one metric type. For example, proportion of individuals and biomass of species belonging to certain feeding or habitat preferences type can be addressed as functional metric, because they correspond with ecological functions of taxa, but also as sensitivity/tolerance metric, since they will be changed as a response to certain stressors. Nevertheless, all metric types are well represented in the metrics that describe fish community of Croatian flowing waters.

Table 3. Overview of the metrics included in the analyses with their abbreviations in brackets.

Composition/ abundance metrics	Richness/ diversity metrics	Sensitivity/ tolerance metrics	Functional metrics	Other metrics
Proportion of native species (pSn) Proportion of non-native species (pSa) Proportion of litophilic species (pLITH) Proportion of phytophilic species (pPHYT) Proportion of phyto-lithophilic species (pPHLI) Proportion of pelagophilic species (pPEL) Proportion of psammophilic species (pPSAM) Proportion of ostracophilic species (pOSTR) Proportion of species spawning in the sea (pSEA) Proportion of herbivorous species (pHERB) Proportion of invertivorous species (pINV) Proportion of omnivourous species p(OMNI) Proportion of piscivourous species (pPISC)	Total number of species (S) Number of native species (Sn) Number of non-native species (Sa) Proportion of Salmoniform species (pSALM) Proportion of Cypriniform species (pCYPR) pSALM/pCYPR pPERC (proportion of Perciform species)/pCYPR Shannon index (H) Reciprocal Simpson index (1/S) Margalef index (Ml) Alpha index (A) Berger-Parker index (d) Shannon index based on native species (Hnat) Reciprocal Simpson index for native species (1/S) Margalef index for native species (Mlnat) Alpha index for native species (Anat)	Proportion of native individuals (uSn) Proportion of non-native individuals (uSa) Proportion of litophilic individuals (uLITH) Proportion of phytophilic individuals (uPHYT) Proportion of phyto-lithophilic individuals (uPHLI) Proportion of pelagophilic individuals (uPEL) Proportion of psamophilic individuals (uPSAM) Proportion of ostracophilic individuals (pOSTR) Proportion of individuals spawning in the sea (uSEA) Proportion of herbivorous individuals (uHERB) Proportion of invertivorous individuals (uINV)	Number of lithophilic species (LITH) Number of phytophilic species, (PHYT) Number of phyto-lithophilic species (PHLI) Number of pelagophilic species (PEL) Number of psammophilic species (PSAM) Number of ostracophilic species (OSTR) Number of species spawning in the sea (SEA) Number of herbivorous species (HERB) Number of invertivorous species (INV) Number of omnivourous species (OMNI) Number of piscivourous species (PISC) Number of detritivorous species (DETR) Number of benthopelagic species (WCOL) Number of benthic species (BENT) Proportion of phytophilic species biomass (bPHYT) Proportion of phyto-lithophilic species biomass (bPHLI) Proportion of pelagophilic species biomass (bPEL) Proportion of psammophilic species biomass (bPSAM) Proportion of ostracophilic species biomass (bOSTR)	Total biomass (B) Biomass of native individuals (Bnat) Biomass of non-native individuals (Balo) Total length of the most abundant species based on the number of individuals (TLmaxn) Total length of the most abundant species based on the biomass (TLmaxb)

Composition/ abundance metrics	Richness/ diversity metrics	Sensitivity/ tolerance metrics	Functional metrics	Other metrics
Proportion of detritivorous species (pDETR) pPISC/pINV Proportion of benthopelagic species (pWCOL) Proportion of benthic species (pBENT)	Berger-Parker index for native species (dnat) Hnat-H (Hdif) 1/Snat-1/S (1/Sdif) Mlnat-Ml (Mldif) Anat-A (Adif) dnat-d (ddif) Hnat/H (Hrat) 1/Snat/1/S (1/Srat) Mlnat/Ml (Mlrat) Anat/A (Arat) dnat/d (drat)	Proportion of omnivourous individuals (uOMNI) Proportion of piscivourous individuals (uPISC) Proportion of detritivorous individuals (uDETR) uPISC/uINV Proportion of benthopelagic individuals (uWCOL) Proportion of benthic individuals (uBENT) Proportion of Salmoniform individuals (uSALM) Proportion of Cypriniform individuals (uCYPR) uSALM/uCYPR uPERC (proportion of Perciform individuals)/uCYPR Proportion of native individuals' biomass (bnat) Proportion of non-native individuals' biomass (balo)	Proportion of biomass of species spawning in the sea (bSEA) Proportion of herbivorous species biomass (bHERB) Proportion of invertivorous species biomass (bINV) Proportion of omnivourous species biomass (bOMNI) Proportion of piscivourous species biomass (bPISC) Proportion of detritivorous species biomass (bDETR) bPISC/bINV Proportion of benthopelagic species biomass (bWCOL) Proportion of benthic species biomass (bBENT) Proportion of Salmoniform species biomass (bSALM) Proportion of Cypriniform species biomass (bCYPR) bSALM/bCYPR	

2.4. ENVIRONMENTAL PARAMETERS AND PRESSURE PROXIES

Description of environmental parameters and indicators of anthropogenic pressures investigated in Croatian rivers

Altogether 21 parameters describing habitat conditions and anthropogenic pressures were assessed, including the hydrological, morphological and physico-chemical components (alkalinity, conductivity, pH, transparency, temperature, concentrations of ammonia, concentrations of organic carbon, molecular ammonium, nitrates, nitrites, nitrogen, phosphorous, total organic carbon, dissolved organic carbon, dissolved orthophosphates, dissolved oxygen, oxygen saturation, biological oxygen consumption and chemical oxygen consumption; representation of unnatural, modified shores (NNLC assessed according to ArcGIS 10); hydrological regime; longitudinal continuity; morphological conditions. Average values of all physico-chemical parameters considering warmer part of the year (from April to September) were included into further analyses.

2.5. STATISTIC ANALYSES FOR METRIC SELECTION

Detailed description of statistical analyses employed for metric selection and pressure-response relationships

Two sets of parameters were prepared (one describing fish communities and the second one concerning environmental parameters and pressure proxies), metrics in both of them were subjected to similar procedures in order to choose the ones that are not intercorrelated, that have normal distribution and for which a clear pressure-response relationship can be confirmed.

Parameters were first standardized. Log-transformation was used for count measures and logistic model for proportions, whereas diversity indices and measures derived from them were considered as already standardized measures.

After standardization Pearson's correlation coefficient was calculated among all the metrics inside each data set and in cases where coefficient was higher than 0,7; one or more metrics were excluded and the one with better ecological interpretation was retained. In cases where ecological interpretation was not clear, both variables were included in the next step and the one with no or lower pressure-response relationship was excluded later.

Responses of fish fauna metrics on all environmental parameters and pressure proxies were analysed by stepwise linear regression. Metrics that were significantly correlated with at least one pressure ($R^2>0.4$ and significance level, $p<0.05$) were checked for complying with linear regression assumptions (normal distribution, linearity and absence of multicollinearity). If both conditions were met (significant correlation with at least one pressure and linear assumptions), those metrics were considered for the index development. Again, correlation coefficients were calculated among metrics of both data sets and, finally, in cases of significant correlations, metrics for which better pressure-response relationships were obtained, were included in the index calculation.

2.6. PRESSURE-RESPONSE RELATIONSHIPS AND SELECTED METRICS

Description of the pressure-response relationships

Clear pressure-response relationships have been established for all investigated river types or combinations of river types. In the Table 4 metrics for fish communities showing clear response to particular pressure are listed, which also show normal distribution and satisfy presumptions of linearity. In the Table 4 pressure-responses are presented by ecoregions and national river types for all river types included in the Mediterranean IC group (Dinaric coastal ecoregion based on the national typology).

Table 4. Fish fauna metrics that show significant response to certain pressure. River types and names are based on the Croatian national typology.

ECOREGION	NATIONAL RIVER TYPES	DESCRIPTION OF RIVER TYPES	PRESSURE RESPONSES	R^2	p
DINARIC COASTAL	HR-R_11A, HR-_11B, HR-R_14A, HR-R_14B & HR-_14C	Lowland and mid-altitude small rivers and lowland short rivers with > 5‰ slope	Proportion of omnivorous species (pOMNI) shows response to dissolved oxygen concentration (O_2)	0,62	0,0122
			Ratio between proportion of piscivorous and invertivore individuals (uPISC/uINV) shows response to chemical oxygen consumption (KPK)	0,506	0,0289
			Proportion of piscivorous individuals (uPISC) shows response to ammonia concentration	0,477	0,0349
			Shannon index based on native species (Hnat) shows response on nitrates concentration	0,606	0,014
	HR-R_12, HR-R_13, HR-R_13A, HR-R_15A & HR-R_15B	Mid-altitude and lowland rivers, and rivers in karstic fields	Proportion of litophilic species (pLITH) shows response to phosphorous concentration (P)	0,472	0,0014
			Proportion of phytophilic species (pFITO) shows response to phosphorous concentration (P)	0,640	0,0000
			Proportion of individuals belonging to native species (uSn) shows response to the proportion of non-native species (pSa)	0,392	0,00425
			Number of invertivore species (INV) shows response to the conductivity	0,493	0,001
	HR-R_16A & HR-R_16B	Temporary rivers	Proportion of individuals belonging to Cypriniformes (uCYPR) shows response to suspended particles concentration	0,429	0,04651
			Proportion of piscivorous individuals (uPISC) shows response to ammonia concentration	0,458	0,039

			Ratio between Shannon indices based on native species and the whole community (Hrat) shows response to nitrates concentration	0,465	0,03737
	HR-R_17, HR-R_18 & HR-R_19	Lowland and mid-altitude small, medium and temporary rivers in Istria	Proportion of phytophilic species (pFITO) shows response to phosphorous concentration (P)	0,489	0,01
			Proportion of invertivorous species (pINV) shows response to alkalinity	0,419	0,0186
			Alpha index based on native species shows response to conductivity	0,678	0,00113
			Ratio between Shannon indices based on native species and the whole community (Hrat) shows response to nitrates concentrations	0,378	0,026

Descriptions of metrics of fish community to pressures in small mid-altitude and lowland rivers and lowland short rivers with more than 5 ‰ slope in the Dinaric coastal ecoregion (types HR-R_11A, HR-R_11B, HR-R_14A, HR-R_14B and H-R_14C)

- Proportion of omnivore species (pOMNI) shows response to concentration of dissolved oxygen (R^2=0,62, p=0,0122; Figure 1). Response of sensitive components of the fish community to changes in oxygen concentration was recorded in watercourses of Pannonian ecoregion as well, so we can confirm that stabile oxygen concentration is crucial for preserving native ichthyofauna of small streams.
- Ratio of proportion of piscivore and invertivore individuals (uPISC/uINV) shows response to chemical oxygen demand (KPK) (R^2=0,506, p=0,0289; Figure 2). Oxygen consumption is indicator of eutrophication, and importance of oxygen for maintaining stable structures of ichthyocenoses of small streams was previously described.
- Proportion of individuals belonging to piscivorous (uPISC) species shows response to ammonia concentration in water (R2=0,477, p=0,0349; Figure 3). Presence of ammonium and ammonium ions in water can be direct consequence of water pollution as a result of unresolved sewage systems and/or industrial wastewater, leaching of agricultural land, as well as metabolism and microbial degradation. Ammonium ions and molecular ammonium are toxic for fish and due to the increase in their concentration, the disappearance of more sensitive species and disturbances in ichthyocenoses first occurs, which was confirmed by the methods used in this study. Furthermore, we can conclude that piscivore species, which are native to these types of

watercourses, are especially sensitive to increase of ammonia and that their proportion in fish community reduces with higher ammonia concentrations.

- Shannon's diversity index based on native species (Hnat) shows response to concentration of dissolved nitrates in water (R^2=0,606, p=0,014; Figure 4). Increase of nitrates in watercourses often occurs due to rinsing of agricultural fields. Nitrates can also cause high eutrophication as a result of indirect chemical mechanism (Smolders et al. 2010).

Thereafter, Croatian fish index for rivers (CFIR) for river types HR-R_11A, HR-R_11B, HR-R_14A, HR-R_14B and HR-R_14C (small mid-altitude and lowland rivers and short rivers with > 5‰ slope in the Dinaric coastal ecoregion) shall be based on the following fish fauna metrics: pOMNI, uPISC/uINV, uPISC and Hnat, that incorporate response of fish communities to dissolved oxygen concentration, chemical oxygen demand, ammonia and nitrates concentrations.

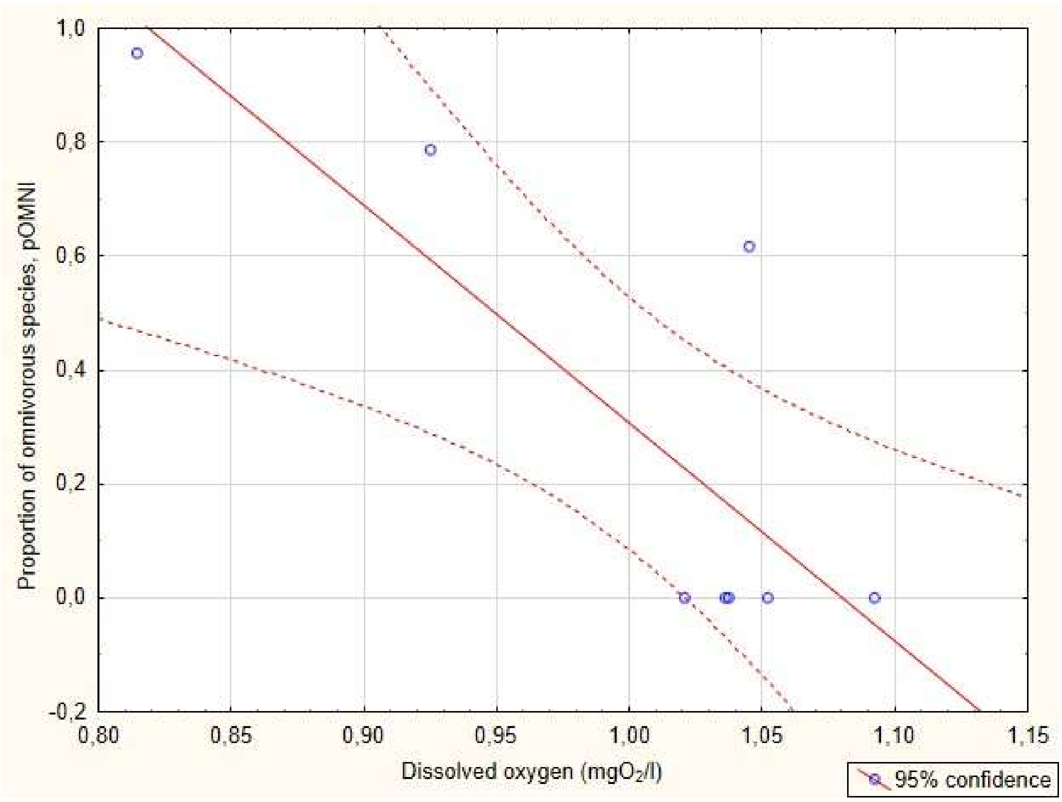

Figure 1. Scatterplot of the linear regression between the proportion of omnivore species (pOMNI) and the concentration of dissolved oxygen, based on the standardized values of metrics.

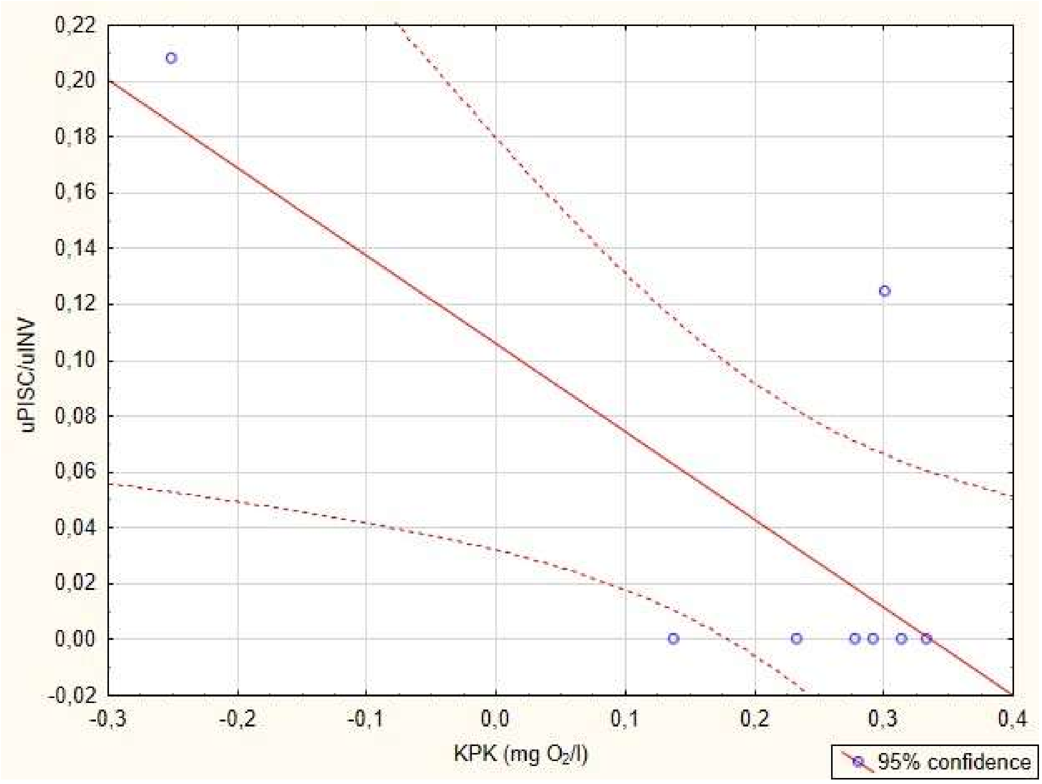

Figure 2. Scatterplot of the linear regression between the ratio of proportion of piscivore an invertivore individuals (uPISC/uINV) and the chemical oxygen demand, based on the standardized values of metrics.

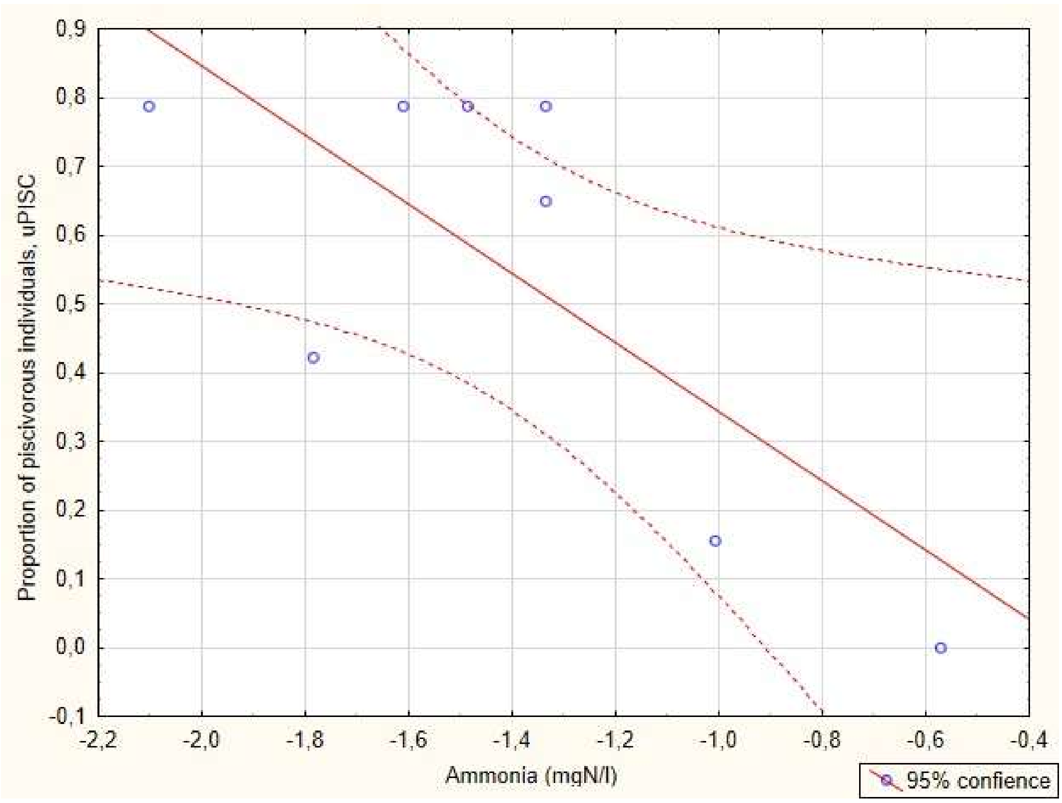

Figure 3. Scatterplot of the linear regression between the proportion of piscivore individuals (uPISC) and ammonia concentration, based on the standardized values of metrics.

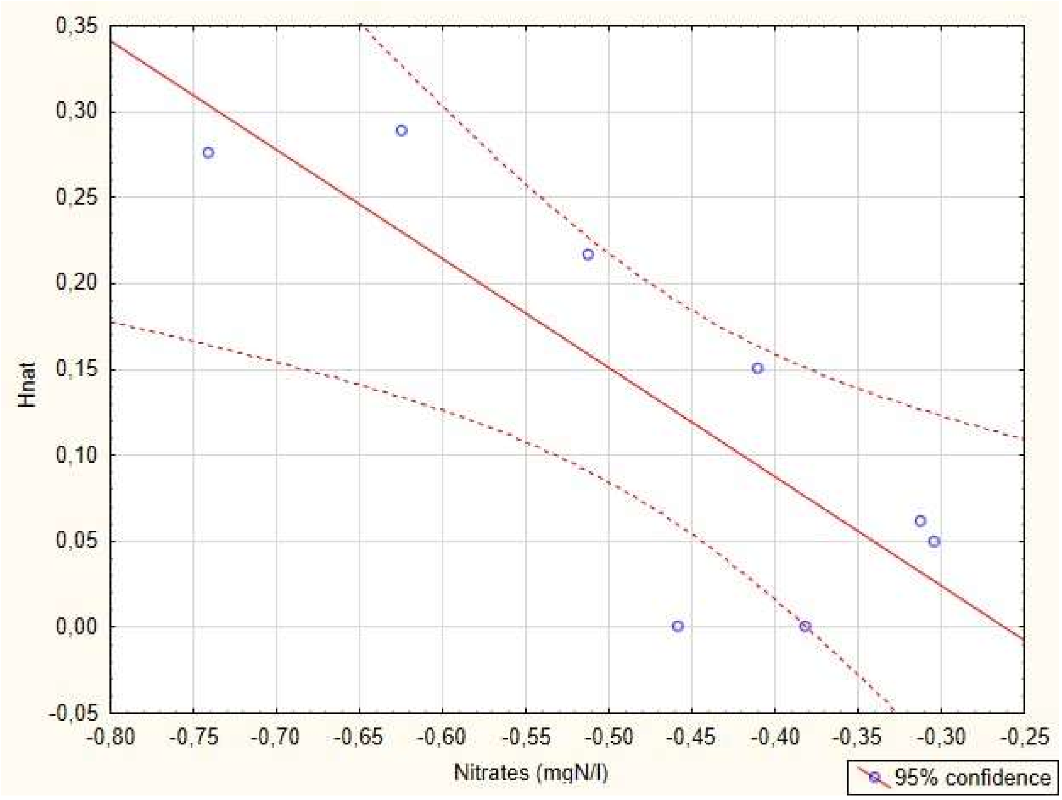

Figure 4. Scatterplot of the linear regression between the Shannon index based on native species (Hnat) and nitrates concentration, based on the standardized values of metrics.

Descriptions of metrics of fish community to pressures in mid-altitude and lowland medium and large rivers and small and medium rivers in karst fields in the Dinaric coastal ecoregion (types HR-R_12, HR-R_13, HR-R_13A, HR-R_15A and HR-R_15B)

- Proportion of lithophilic species (pLITH) shows response to phosphorus concentration (R^2=0,472, p=0,0014; Figure 5). Phosphorus is one of the most significant causes and best indicators of eutrophication in watercourses. Two fish metrics show significant correlation to phosphorus concentration, proportion of lithophilic species is negatively correlated to phosphorus concentration while correlation factor between proportion of phytophilic species and concentration of phosphorus is positive. Apparently, eutrophication leads to changes in fish communities which decreases proportion of lithophilic and increases proportion of phytophilic species.
- Proportion of phytophilic species (pPHYT) shows response to concentration of phosphorus (R^2=0,640, p=0,0000; Figure 6). As it was previously mentioned, proportion of phytophilic species increases with phosphorus concentration, that is, with enhanced eutrophication.
- Proportion of native species individuals (uSn) shows response to proportion of non-native species species (pSa) (R^2=0,392, p=0,00425; Figure 7). Considering that all non-native fish species in watercourses of the Dinaric coastal ecoregion were introduced as a result of anthropogenic activity and not as a consequence of some other pressures, they can be considered as a pressure on native fish community. Their negative influence on native species is more pronounced than physicochemical, morphological or hydrological changes. As expected, proportion of native species individuals shows a significant response to proportion of non-native species in the sample.

- Number of invertivore species (INV) shows response to electrical conductivity (R^2=0,493, p=0,001; Figure 8). Conductivity increases in cases of inflow of untreated wastewater (from industries, but also from cities and villages) into watercourses, given that wastewaters have a high conductivity. Conductivity depends on water temperature and it increases with higher temperatures (Horne and Goldman 1994, Bhateria and Jain 2016). Fish and other freshwater organisms generally do not tolerate well changes of conductivity, which reflect changes of the salt concentration in the water. Increased salinity affects their osmoregulation and they consume significantly more energy for osmoregulation or cannot perform it at all.

Thereafter, Croatian fish index for rivers (CFIR) for river types HR-R_12, HR-R_13, HR-R_13A, HR-R_15A and HR-R_15B (mid-altitude and lowland medium and large rivers and small and medium rivers in karst fields in the Dinaric coastal ecoregion) shall be based on the following fish fauna metrics: pLITH, pPHYT, uSn and INV, that incorporate response of fish communities to phosphorus concentration, proportion of non-native species and conductivity.

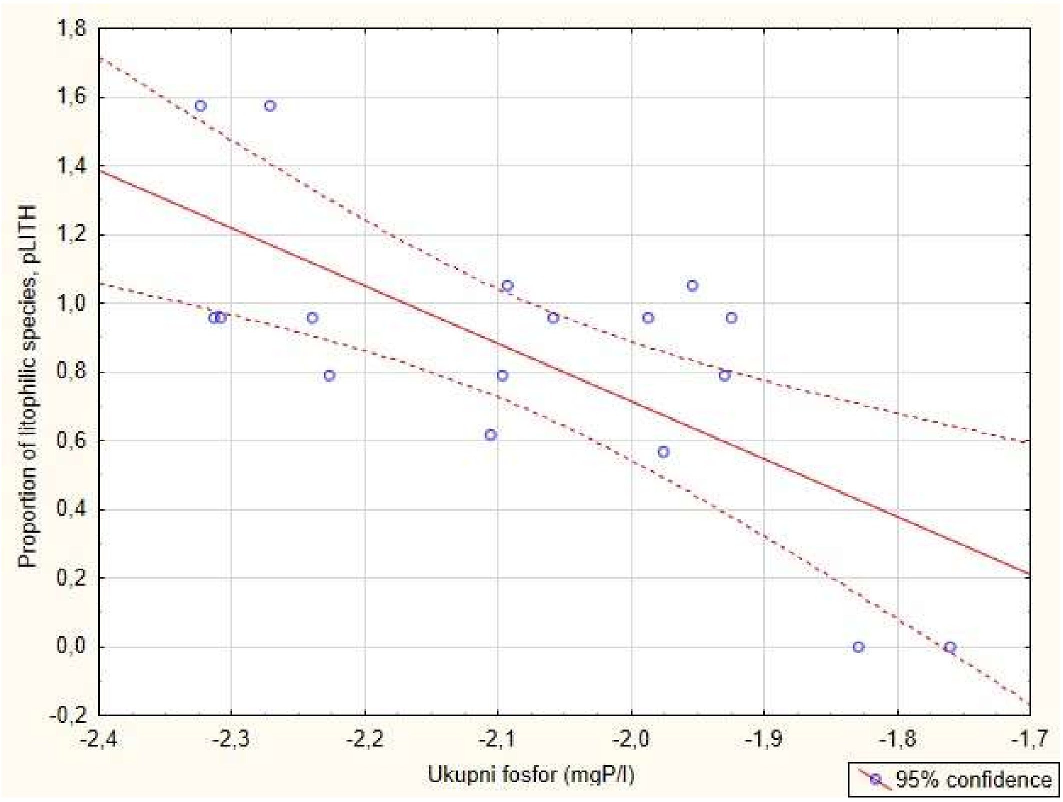

Figure 5. Scatterplot of the linear regression between the proportion of litophilic species (pLITH) and phosphorous concentration, based on the standardized values of metrics.

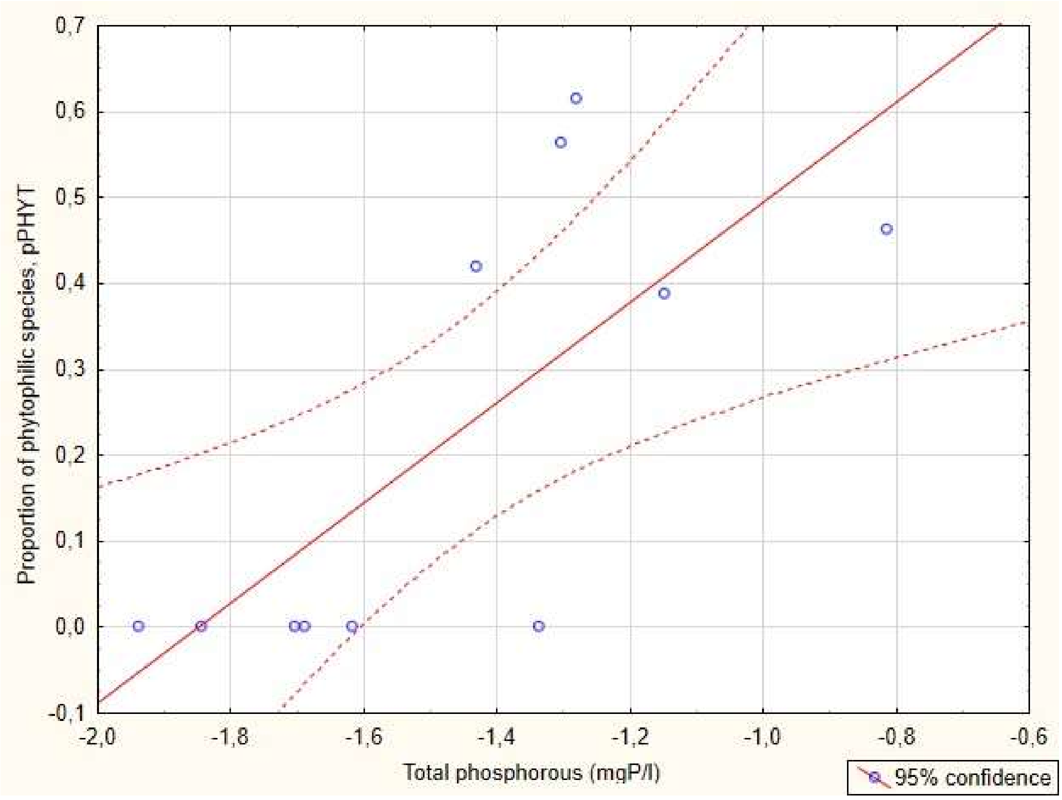

Figure 6. Scatterplot of the linear regression between the proportion of phytophilic species (pPHYT) and phosphorous concentration, based on the standardized values of metrics.

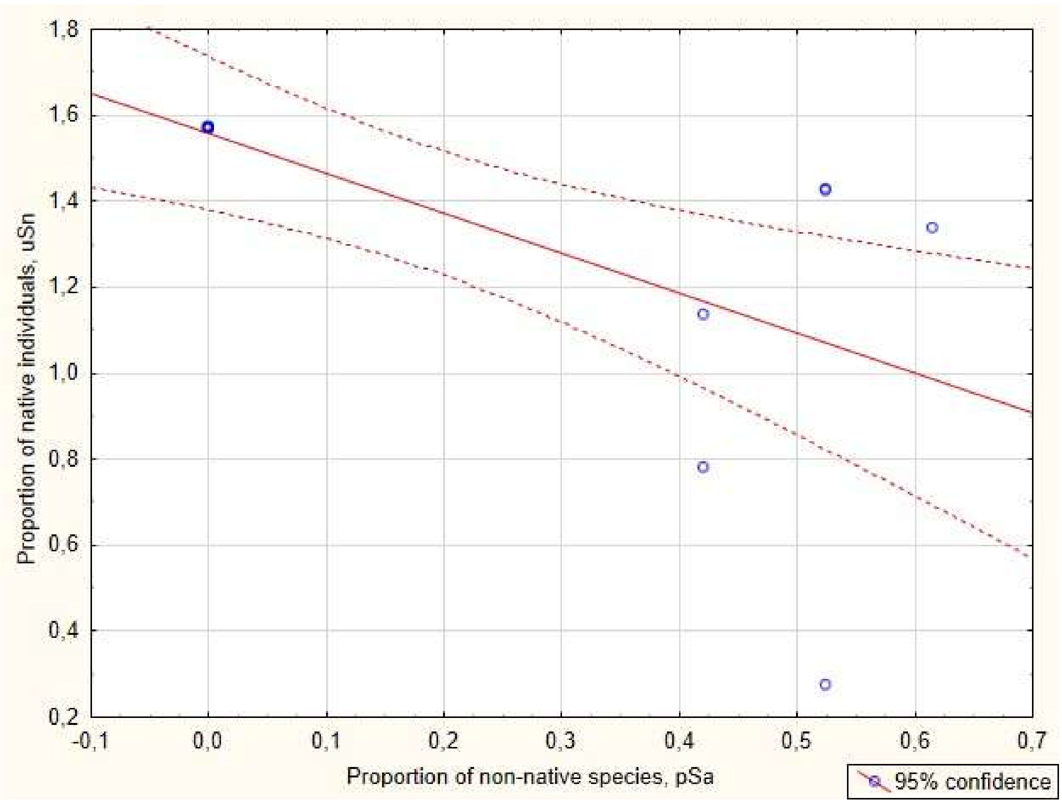

Figure 7. Scatterplot of the linear regression between the proportion of individuals belonging to native species (uSn) and proportion of non-native species (pSa), based on the standardized values of metrics.

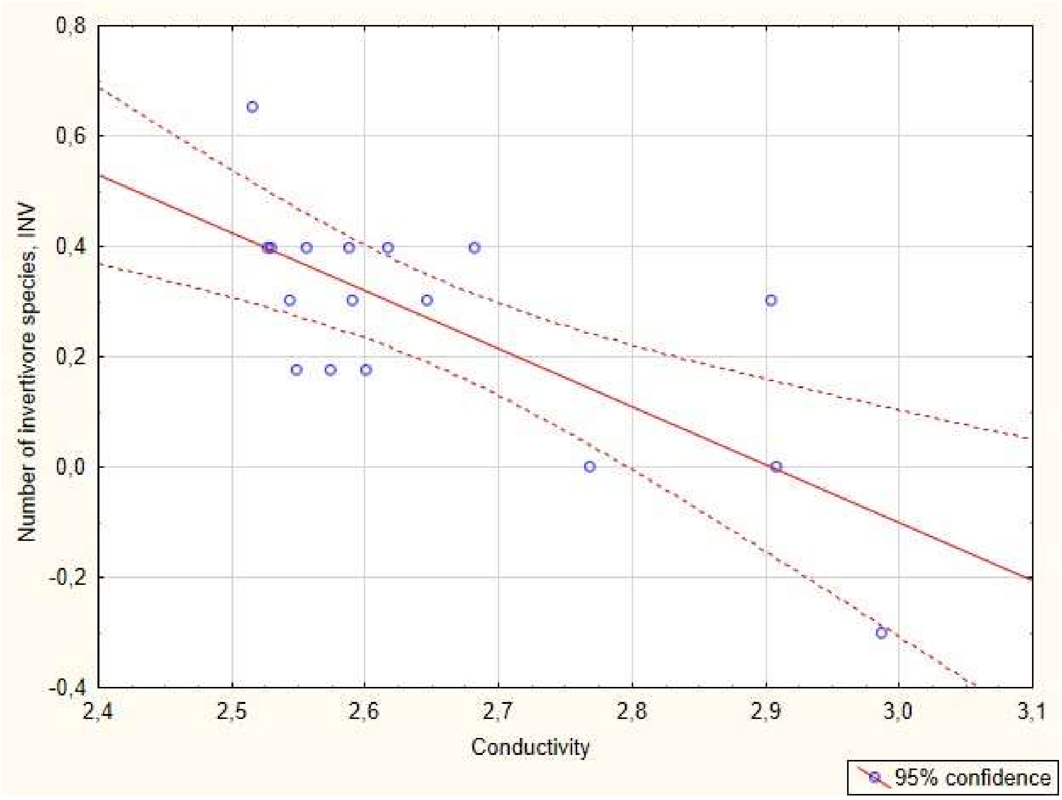

Figure 8. Scatterplot of the linear regression between the number of invertivore species (INV) and conductivity, based on the standardized values of metrics.

Descriptions of metrics of fish community to pressures in temporary rivers in the Dinaric coastal Ecoregion (types HR-R_16A and HR-R_16B)

- Proportion of individuals belonging to Cypriniformes (uCYPR) shows significant response to concentration of suspended particles in water (R^2=0,429, p=0,04651; Figure 9). Suspended particles of organic and inorganic origin end in water from different pollution sources – household wastewater, illegal landfills near or in watercourses, industrial wastewater, leaching of shores from which natural vegetation has been removed etc. On the other hand, increased concentration of suspended particles in water negatively affects fish. Regardless of the type of particles involved, suspended particles reduce the visibility and thus the efficiency of feeding fin a watercourse. The presence of organic particles leads to increased microbiological degradation, which provokes higher oxygen consumption and reduced concentration of dissolved oxygen in the water column. Inorganic particles cause even bigger problems - they often lead to clogging of the gills or blood vessels and to the death of fish.
- Proportion of individuals belonging to piscivorous species (uPISC) show significant response to concentration of ammonia in water (R^2=0,458, p=0,039; Figure 10). Similar situation was noted for mid-altitude and lowland small streams and lowland short streams with a slope, while in continental part it was recorded for invertivore and omnivore species. Molecular ammonium is the most toxic form of ammonia to fish, concentration of each form (molecule, ion) depends on environmental conditions and they shift from one to another.
- Relation of Shannon index based on native species and on all recorded species (Hrat) shows significant response to concentration of nitrates in water (R^2=0,465, p=0,03737; Figure 11). As

it was previously stated, nitrates can be eutrophication starters and they end in waters from agricultural areas.

Thereafter, Croatian fish index for rivers (CFIR) for the river types HR-R_16A and HR-R_16B (temporary rivers) in the Dinaric coastal ecoregion) shall be based on the following fish fauna metrics: uCYPR, uPISC and Hrat, that incorporate response of fish communities to suspended particles, ammonia and nitrates concentrations.

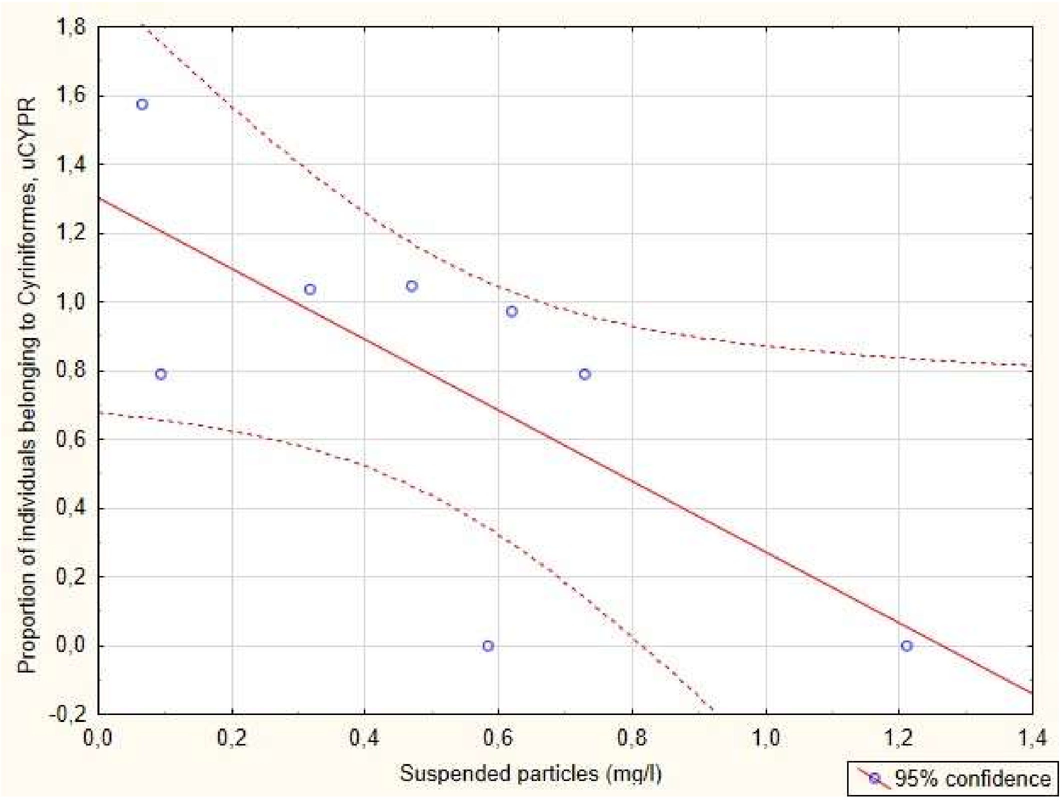

Figure 9. Scatterplot of the linear regression between the proportion of individuals belonging to Cypriniformes (uCYPR) and concentration of suspended particles, based on the standardized values of metrics.

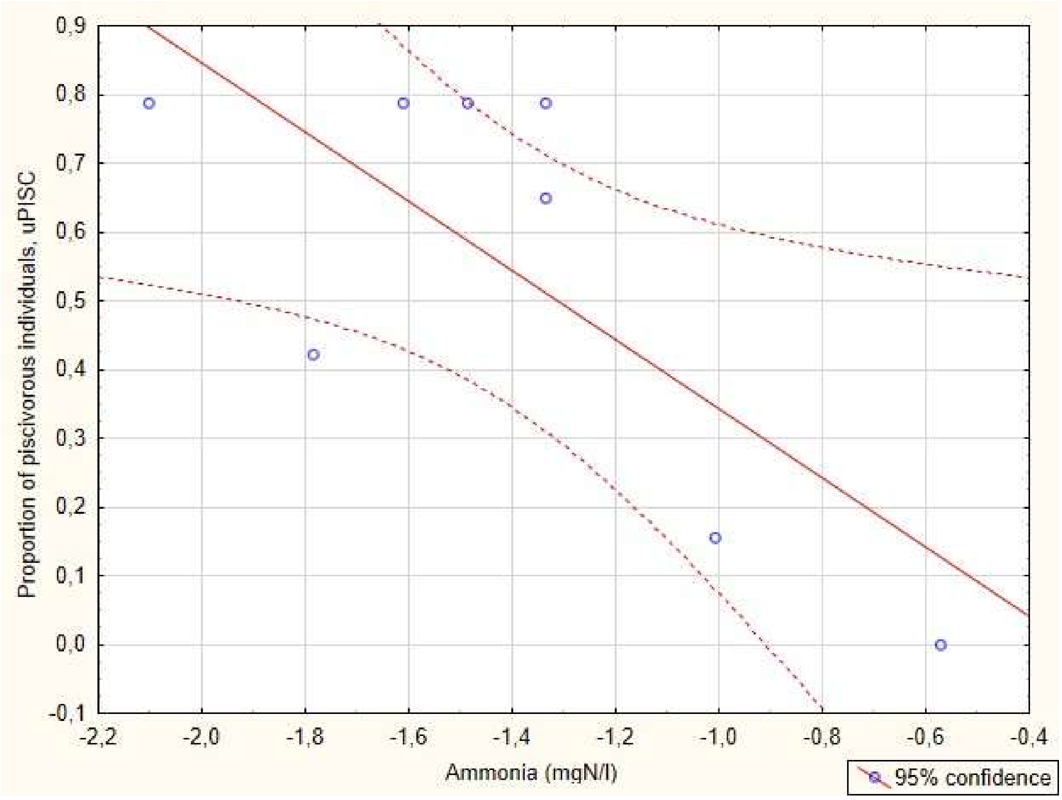

Figure 10. Scatterplot of the linear regression between the proportion of piscivorous species (uPISC) and concentration of ammonia, based on the standardized values of metrics.

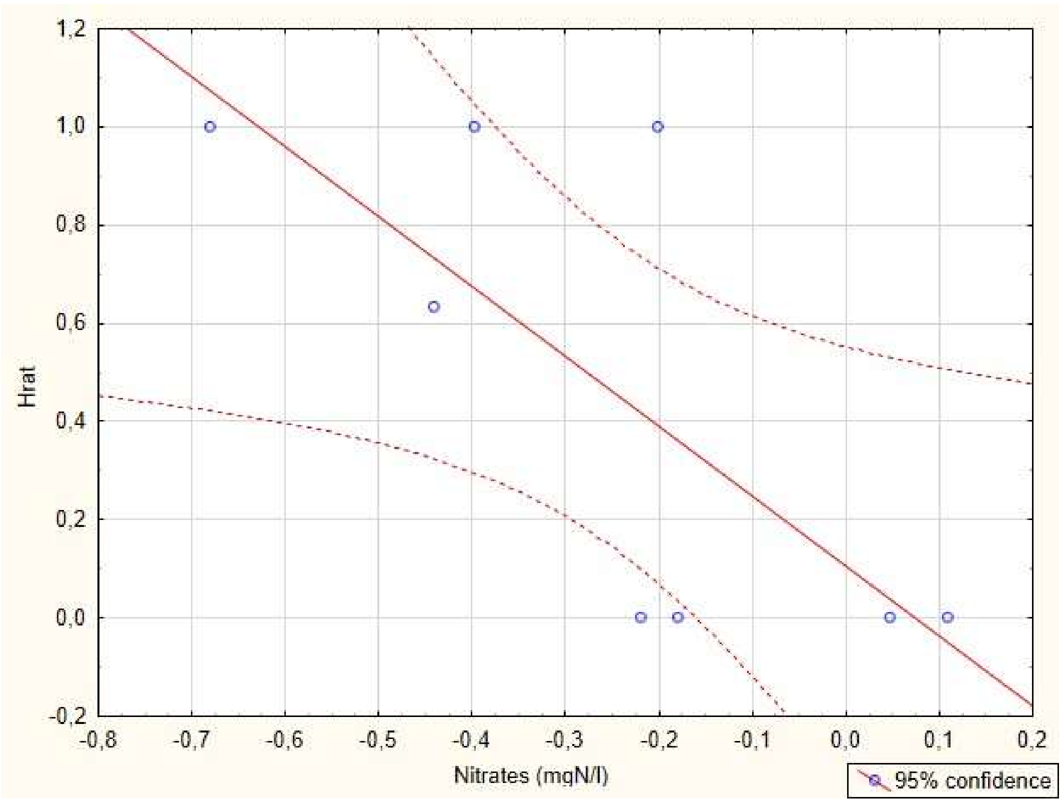

Figure 11. Scatterplot of the linear regression between the ratio of Shannon indices based on native species and the whole community (Hrat) and concentration of nitrates, based on the standardized values of metrics.

Descriptions of metrics of fish community to pressures in small, medium and temporary rivers of Istria (types HR-R_17, HR-R_18 and HR-R_19)

- Proportion of phytophilic species (pPHYT) shows significant response to concentration of phosphorus in water (R^2=0,489, p=0,01; Figure 12). Importance of phosphorus for eutrophication has already been explained, it was recorded in the previously described water types.
- Proportion of invertivore species (pINV) shows response to alkalinity (R^2=0,419, p=0,0186; Figure 13). Even though it is not usually listed as an indicator of eutrophication, increased alkalinity can stimulate it because it enhances the availability of nutrients and carbon dioxide to plants and thus stimulates photosynthesis (William and Wurts Durborow 1992). Furthermore, higher alkalinity enhances bioavailability of metals from sediments, affecting the toxicity of many metals to aquatic organisms (Andrew et al. 1977, Riethmuller et al. 2000). Changes of alkalinity can occur due to changes in the hydrological regime, but also due to chemical processes within the watercourse (e.g., reduction of nitrate or sulfur dioxide) (Smolders et al. 2006).
- Alpha index based on native species (Anat) shows response to electrical conductivity (R^2=0,678, p=0,00113; Figure 14). Increase of electrical conductivity is a result of pollution by untreated industrial and municipal wastewater, and also rising temperatures. Fish are sensitive to changes in salt concentration (which also causes changes in conductivity) and have problems with osmoregulation.
- Relation of Shannon's index based on native species and the same index based on all recorded species from one locality (Hrat) shows significant response to concentration of nitrates in water, same as it was established for previous river type (R^2=0,378, p=0,026; Figure 15).

Thereafter, Croatian fish index for rivers (CFIR) for river types HR-R_17, HR-R_18 and HR-R_19 (small, medium and temporary rivers in Istria) shall be based on the following fish fauna metrics: pPHYT, pINV, Anat and Hrat, that incorporate response of fish communities to phosphorous concentration, alkalinity, conductivity and nitrates concentrations.

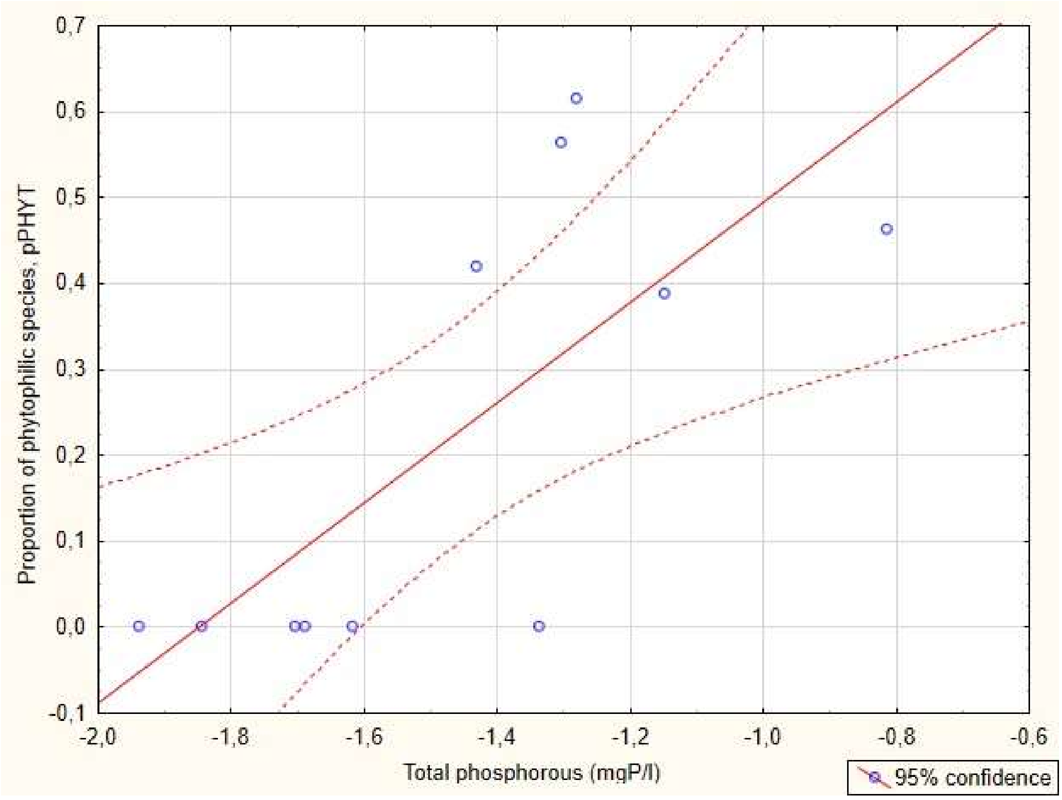

Figure 12. Scatterplot of the linear regression between the proportion of phytophilic species (pPHYT) and concentration of phosphorous, based on the standardized values of metrics.

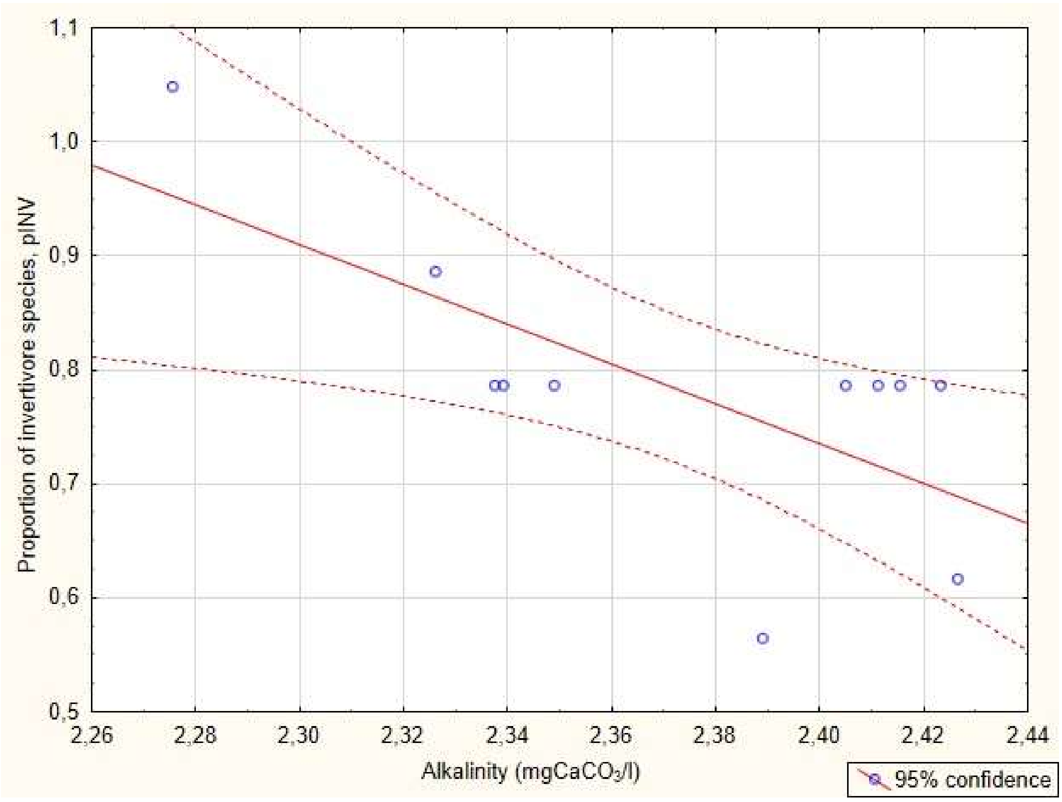

Figure 13. Scatterplot of the linear regression between the proportion of invertivore species (pINV) and alkalinity, based on the standardized values of metrics.

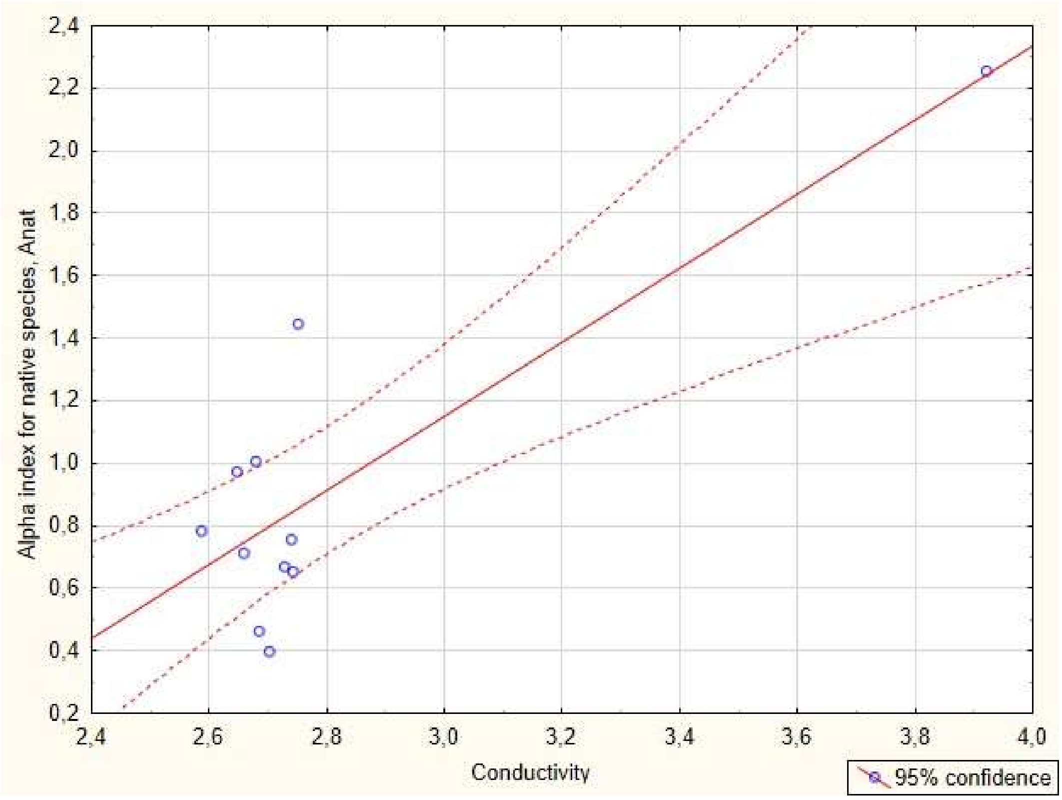

Figure 14. Scatterplot of the linear regression between the alpha index based on native species (Anat) and conductivity, based on the standardized values of metrics.

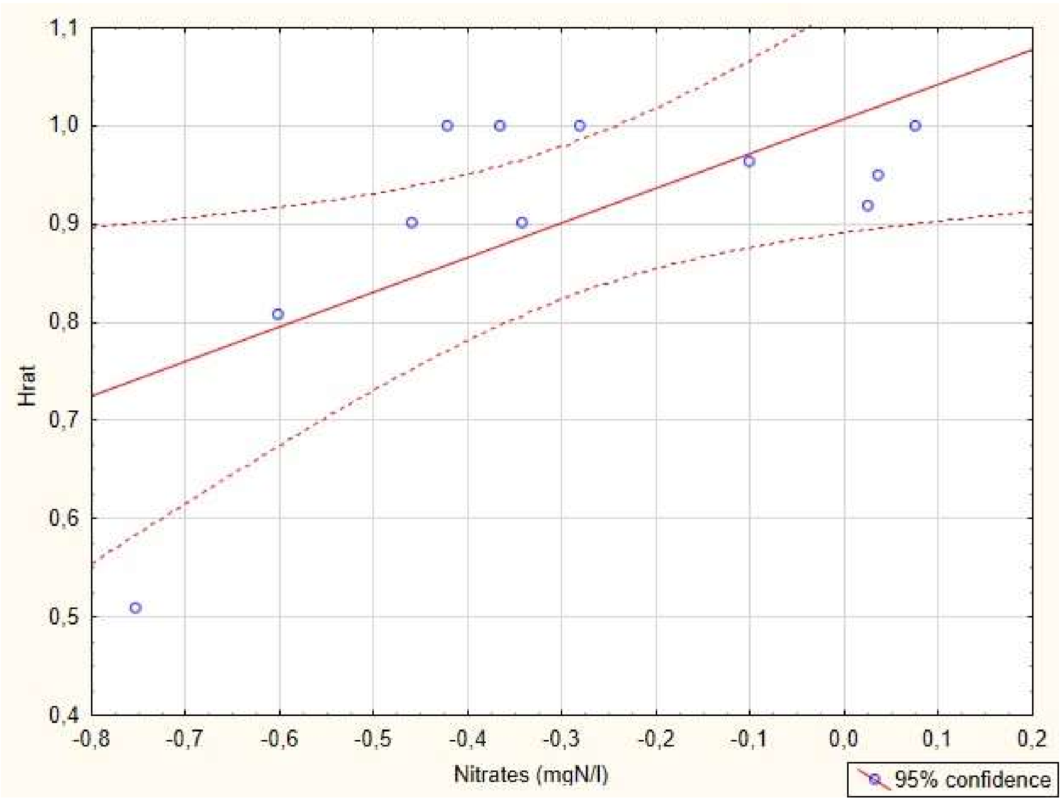

Figure 15. Scatterplot of the linear regression between the ratio of Shannon indices based on native species and the whole community (Hrat) and concentration of nitrates, based on the standardized values of metrics.

2.7. UPPER AND LOWER ANCHORS FOR ECOLOGICAL QUALITY RATIOS

Description of estimation of upper and lower anchors for Ecological Quality Ratios (EQRs)

Reference conditions were determined for each river type/group of types and responses to certain pressures were calculated. Since some river types comprised only a small number of locations that are influenced by various anthropogenic pressures, it was impossible to identify a satisfactory number of watercourses in which the fish community is in optimal condition, in order to determine the reference conditions for all metrics of the fish community for all national river types. Moreover, there is a lack of estimated reference conditions for some of the physico-chemical parameters, disabling extrapolation of reference conditions of fish metrics in cases where they show significant response to certain environmental parameter. Therefore, for determining The Ecological Quality Ratios we applied the approach proposed by Furse et al. (2006) for cases where it is not possible to determine reference locations – we set the upper and lower anchors for individual metrics of fish communities (which had shown pressure response). The upper anchor was defined as value observed or proposed for natural, stabile community (reference condition for certain river type) and the lower anchor as the worst possible value obtained for severely altered communities or expert assessment of the worst possible conditions (e.g. for the metrics tackling the proportion of non-native species, the worst possible situation is that only non-native species have been recorded on a locality).

Inside some national river types there are localities with undisturbed, or almost undisturbed fish communities and they present national reference conditions for certain river types (Table 5). In those communities chosen fish metrics have the best possible or almost the best possible values, yielding Ecological quality ratios (as described in the next chapter) of 1 or nearly 1.

Noteworthy, reference communities, in cases when they could be identified, are referent considering their structure. Particularly, they exhibit reference values of those fish community metrics that were, following the described procedure, chosen for inclusion in the Croatian fish index for rivers. Understandably, this does not mean that all fish communities belonging to a certain national river type should have, under natural type-specific conditions, the same composition or even comprise the same number of species. Namely, fish community belonging to a certain river type are very diverse concerning species composition and richness which is a natural consequence of ecological and evolutionary differences.

Table 5. National reference localities under river types where they could be identified, and the explanation for river types under which no reference localities could be identified, but reference values of fish community metrics were estimated.

<table>
<tr><th>NATIONAL RIVER TYPE</th><th>LOCALITY CODE</th><th>LOCALITY NAME</th><th>COMPOSITION OF FISH COMMUNITY</th></tr>
<tr><td>HR-R_11A & HR-R_11B</td><td colspan="3">On neither locality there is an undisturbed or nearly undisturbed fish community (all communities are more severely modified). Thereafter, identification of reference localities is not possible, yet reference conditions were estimated based on expert judgement and literature data.</td></tr>
<tr><td>HR-R_12</td><td>14006</td><td>Una, Loskun</td><td>Natural community composed of 4 native typical species.</td></tr>
</table>

HR-R_13 & HR-R_13A	40204	Zrmanja, Berberov buk	Natural community composed of 4 native typical species.
HR-R_14A, HR-R_14B & HR-R_14C	Neither locality can be considered reference – very small number of localities included in analyses (only 3) and they are very different considering fish communities.		
HR-R_15A & HR-R_15B	Only one locality analyzed, with disturbed fish community; thereafter, it cannot be considered reference.		
HR-R_16A & HR-R_16B	On neither locality there is an undisturbed or nearly undisturbed fish community (all communities are more severely modified). Thereafter, identification of reference localities is not possible, yet reference conditions were estimated based on expert judgement and literature data.		
HR-R_17	Only one locality analyzed, with disturbed fish community; thereafter, it cannot be considered reference.		
HR-R_18	On neither locality there is an undisturbed or nearly undisturbed fish community (all communities are more severely modified). Thereafter, identification of reference localities is not possible, yet reference conditions were estimated based on expert judgement and literature data.		
HR-R_19	On neither locality there is an undisturbed or nearly undisturbed fish community (all communities are more severely modified). Thereafter, identification of reference localities is not possible, yet reference conditions were estimated based on expert judgement and literature data.		

2.8. ECOLOGICAL QUALITY RATIOS CALCULATION

Description of Ecological Quality Ratios calculation

Ecological quality ratios (EQRs) were calculated separately for all chosen metrics of fish community (metrics that had shown significant response to a pressure, that are not intercorelated and meet the conditions of normality and linearity and are statistically significantly correlated with pressure) inside each type/group of types. For that purpose, formula form Furse et al. (2006) was used:

$EQR_{metrics}$ = (Metrics value – Lower limit) / (Upper limit – Lower limit), for metrics decreasing with increasing pressure

or

$EQR_{metrics}$ = 1 – (Metrics value – Lower limit) / (Upper limit – Lower limit), for metrics increasing with increasing pressure.

As already explained, upper and lower anchors for each metrics were determined as the best or the worst possible conditions, either observed based on reference localities, or estimated as reference values.

2.9. GENERATION OF CROATIAN FISH INDEX FOR RIVERS

Description of the methodology used to calculate Croatian fish index for rivers (CFIR)

As it was already described, in the previous steps we identified fish metrics that proved to be suitable for inclusion in the multimetric index, because they show significant responses to certain pressures, are significantly correlated with pressures, but are not intercorrelated. Finally, Croatian fish index for rivers (CFIR) was calculated separately for each river type/group of types as the sum of the ecological quality ratios determined for a certain river type/group of types divided by the number of ecological quality ratios included.

$$CFIR = \frac{EQR1 + EQR2 + \cdots + EQRn}{n}$$

CFIR is multimetric index which integrates multiple metrics and simplifies decision making because one value can be used to assess and monitor the quality of streams (Furse et al. 2006). Since the index combines the effects of different fish metrics, it combines responses to multiple pressures.

2.10. NATIONAL BOUNDARY SETTING

Ecological status classes were defined by dividing the EQR values to a five-class equidistant scale according to the prescriptions of the WFD (Table 6). Namely, CFIR expresses the relationship of the observed metrics in certain fish community with the same metrics under reference conditions (in natural, stabile, undisturbed fish community of certain river type) and ecological status of certain locality can be designated as belonging to one out of five classes, in accordance with the requirements of the WFD. CFIR values close to 1 imply undisturbed or only slightly disturbed fish communities, whereas values closer to 0 represent communities that are significantly modified as a consequence of anthropogenic pressures. The EQRs, and thereafter also CFIR, were developed in comparison with reference conditions (conditions in natural, undisturbed fish communities), class boundaries were set following the suggestion of Furse et al. (2006) – equidistal arrangement of class boundaries from 1 (reference conditions) to 0 for five ordinal rating categories for assessment of impairment in accordance with WFD requirements.

Table 6. CFIR classification – class boundaries setting for biological element fish.

EQR value intervals	Ecological Quality State (EQS)
0,80 – 1,0	high / very good
0,60 – 0,79	good
0,40 – 0,59	moderate
0,21 – 0,39	poor
0,0 – 0,20	bad

3. WFD COMPLIANCE CHECKING

The first step in the Intercalibration process requires checking whether national methods are in accordance with the WFD compliance criteria (Table 7).

Table 7. List of the WFD compliance criteria and the WFD compliance checking process and results.

Compliance criteria	Compliance checking
Ecological status is classified by one of **five classes** (high, good, moderate, poor and bad).	yes
High, good and moderate ecological status are set in line with the WFD's **normative definitions** (**Boundary setting procedure**)	yes
All relevant parameters indicative of the biological quality element are covered (see Table 1 in the IC Guidance). A **combination rule** to combine parameter assessment into BQE assessment has to be defined. If parameters are missing, Member States need to demonstrate that the method is sufficiently indicative of the status of the QE as a whole	yes, with the exception of age structure because it did not provide pressure-response answers
Assessment is adapted to **intercalibration common types** that are defined in line with the typological requirements of the Annex II WFD and approved by WG ECOSTAT	yes
The water body is assessed against **type-specific near-natural reference conditions**	yes
Assessment results are expressed as **EQRs**	yes
Sampling procedure allows for **representative** information about water body quality/ecological status **in space and time**	yes
All data relevant for assessing the biological **parameters** specified in the WFD's normative definitions are covered by the **sampling procedure**	yes
Selected taxonomic level achieves adequate **confidence and precision** in classification	yes (species level)

4. IC FEASIBILITY CHECKING

The intercalibration process ideally covers all national assessment methods within a GIG. However, the comparison of dissimilar methods ("apples and pears") has clearly to be avoided. Intercalibration exercise is focused on specific type / biological quality element / pressure combinations. The second step of the process introduces an "IC feasibility check" to restrict the actual intercalibration analysis to methods that address the same common type(s) and anthropogenic pressure(s), and follow a similar assessment concept.

4.1. TYPOLOGY

The Fish Cross GIG did not use the common intercalibration typology in its IC work (Mediterranean GIG, Eastern Continental GIG *etc.*). Instead, following regional groups were used:

Nordic Group
Lowland-Midland Group
Alpine-type Mountains Group
Mediterranean South-Atlantic
Danubian Group

Croatian rivers belong to the Mediterranean South-Atlantic Group (Adriatic River Basin) and to the Danubian Group (Danube River Basin).

Croatia fits national fish classification method to the results of the completed intercalibration exercise of the Fish Cross GIG Mediterranean Group, along with Spain, Portugal and Greece. Intercalibration common types are not used, and all the river sites were considered together for the IC exercise. In connection with the GIG agreed common data base included in the IC process, Croatian data are not divided according to their typological characteristics.
Thirteen (13) Croatian national river types are included in the common database of the Cross GIG Mediterranean Group. The national types correspond with the Mediterranean IC common river types, according to their abiotic characteristics, catchament area, altitude, geology and substrate (Table 8).

Table 8. Croatian river types, with the respective common IC river types, included in the Fish Cross GIG Mediterranean Group

ECOREGION	SUBREGION	NATIONAL TYPE NAME	NATIONAL TYPE	IC TYPE
DINARIC ECOREGION (5 DINARIC WESTERN BALKAN)	DINARIC COASTAL SUB-ECOREGION	Lowland and mid-altitude small river, calcareous geology	HR-R_11A	R-M1
		Mid-altitude medium river, calcareous geology	HR-R_12	R-M2
		Lowland medium river, calcareous geology	HR-R_13	R-M2
		Large lowland rivers with barage pools	HR-R_13A	R-M2
		Lowland short-flow small rivers with >5 ‰ slope, calcareous geology	HR-R_14A	R-M1
		Lowland short-flow medium rivers with >5 ‰ slope, calcareous geology	HR-R_14B	R-M2

ECOREGION	SUBREGION	NATIONAL TYPE NAME	NATIONAL TYPE	IC TYPE
		Small and medium rivers in karst fields	HR-R_15A	R-M1
		Medium rivers in karst fields	HR-R_15B	R-M2
		Mid-altitude small and medium temporary rivers, calcareous geology	HR-R_16A	R-M5
		Lowland small temporary rivers, calcareous and siliceouscalcareous geology	HR-R_16B	R-M5
	DINARIC LITTORAL SUB-ECOREGION – ISTRIA	Lowland and mid altitude small spring rivers of Istria, calcareous-flysch geology	HR-R_17	R-M1
		Lowland medium rivers of Istria, calcareous-flysch geology	HR-R_18	R-M2
		Temporary small lowland rivers of Istria, calcareous-flysch geology	HR-R_19	R-M5

4.2. PRESSURES ADDRESSED

The most important common pressures for IC are considered: water quality alteration, hydro-morphological modifications, connectivity disruption. The hydromorphological alteration scale ranges from 1 (no) to 5 (high) and consists of multiple smaller indices. The three main indices: hydrology regime, morphology and flow continuity ranged from 1 to 5, whereas the mean hydromorphological score ranged from 1 to 4,4.

The ranges for the chemical variables tested are:

Chemical variable	range
BOD_5 [mg L^{-1}]	0,52 – 3,5
COD [mg L^{-1}]	0,5 – 3,241
PO_4-P [mg L^{-1}]	0,002 – 0,092
NO_3-N [mg L^{-1}]	0,215 – 1,306
NH_4-N [mg L^{-1}]	0,0 – 0,632
Total P [mg L^{-1}]	0,008 – 0,138

The following pressure-response relationships have been derived:

1. <u>ORGANIC ENRICHMENT</u>

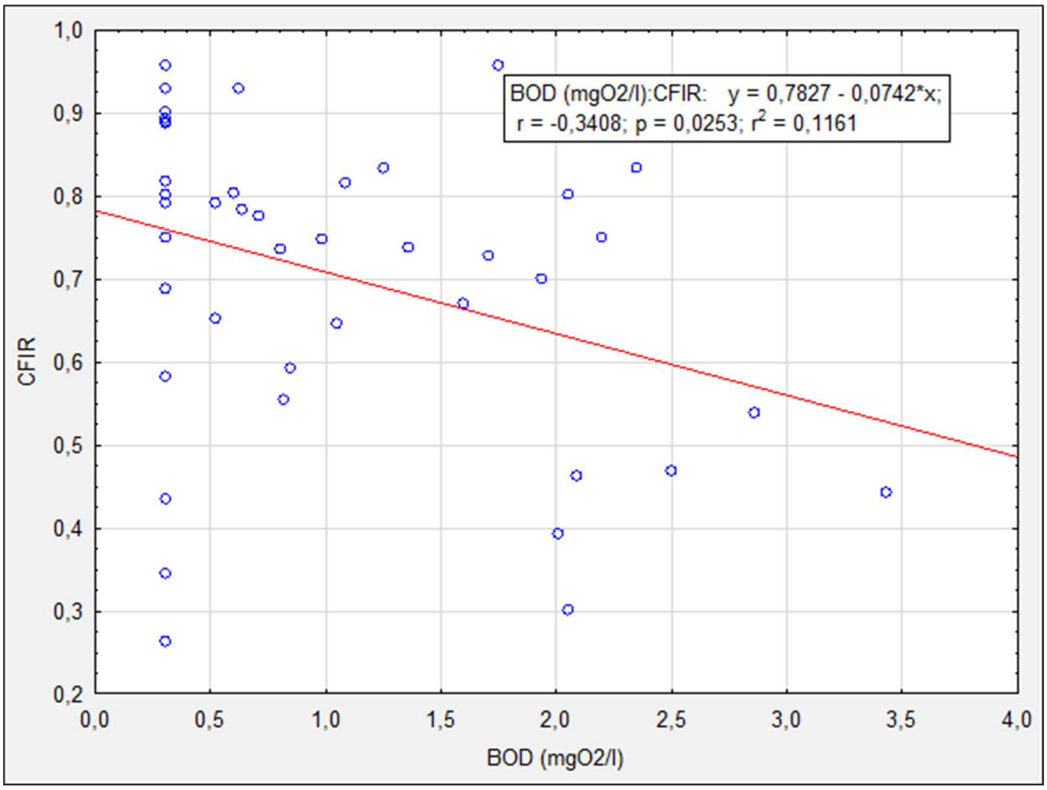

Figure 16. Pressure-response relationship between biological oxygen demand (BOD) and the EQR_{CFIR} values in the Mediterranean region of Croatia

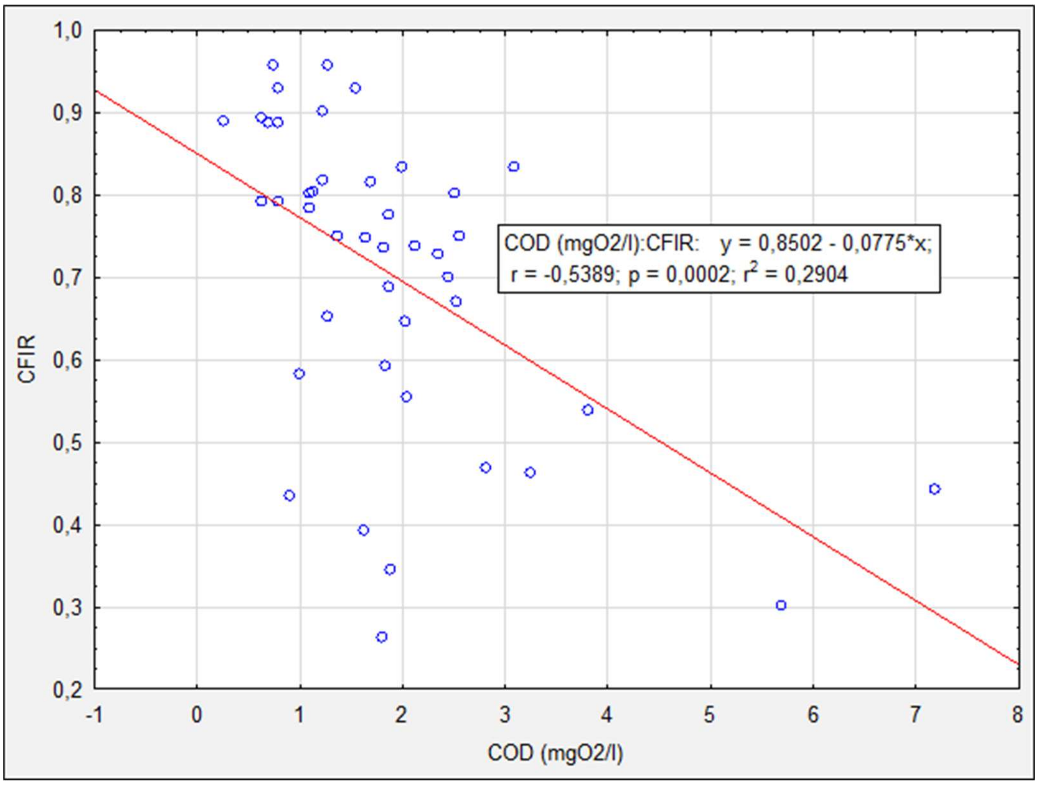

Figure 17. Pressure-response relationship between chemical oxygen demand (COD) and the EQR_{CFIR} values in the Mediterranean region of Croatia

2. <u>HYDRO-CHEMISTRY</u>

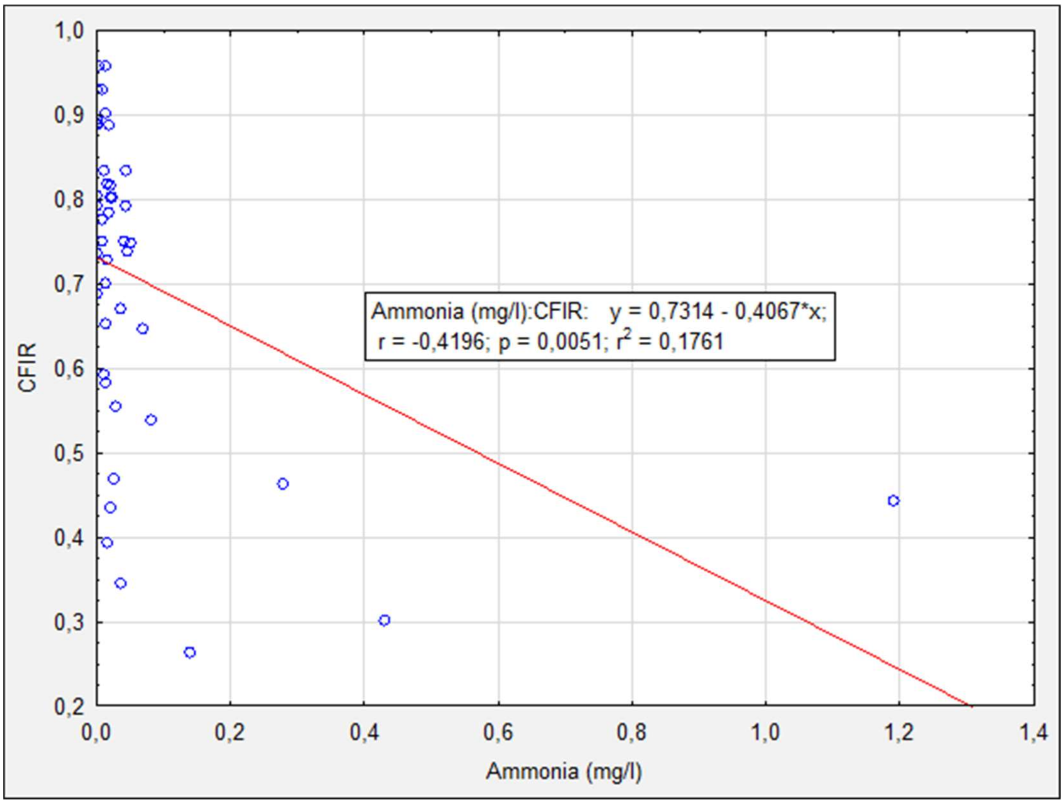

Figure 18. Pressure-response relationship between ammonia and the EQR_{CFIR} values in the Mediterranean region of Croatia

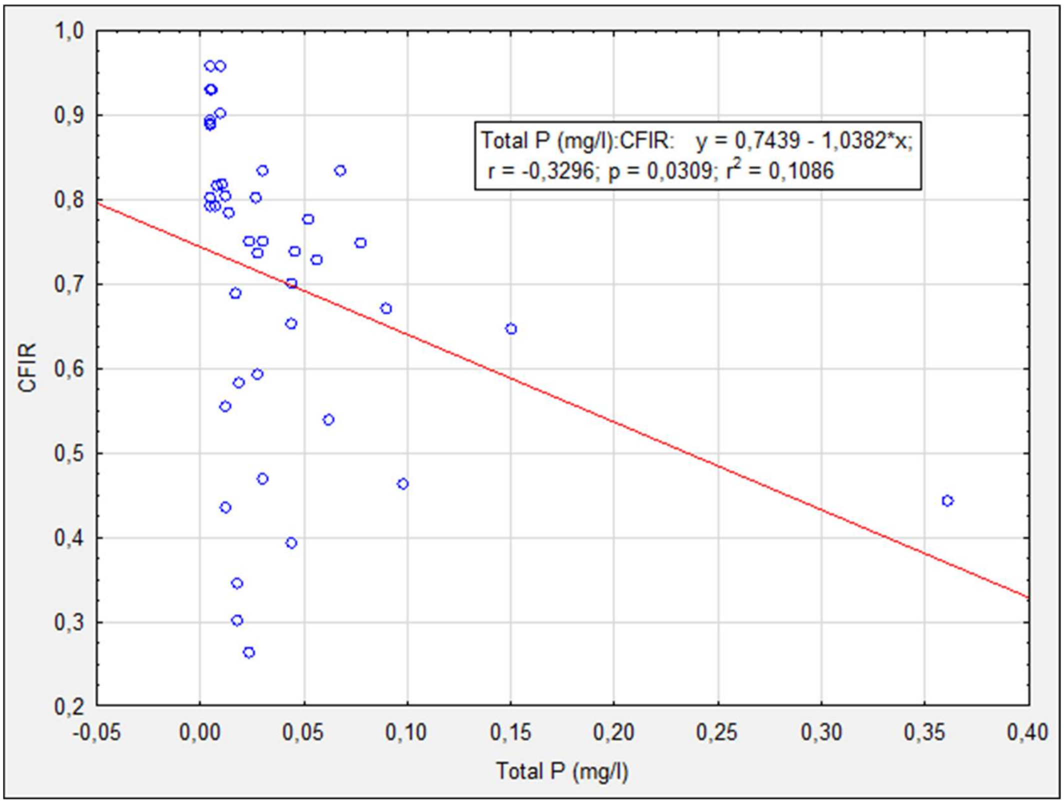

Figure 19. Pressure-response relationship between total phosphorous and the EQR_{CFIR} values in the Mediterranean region of Croatia

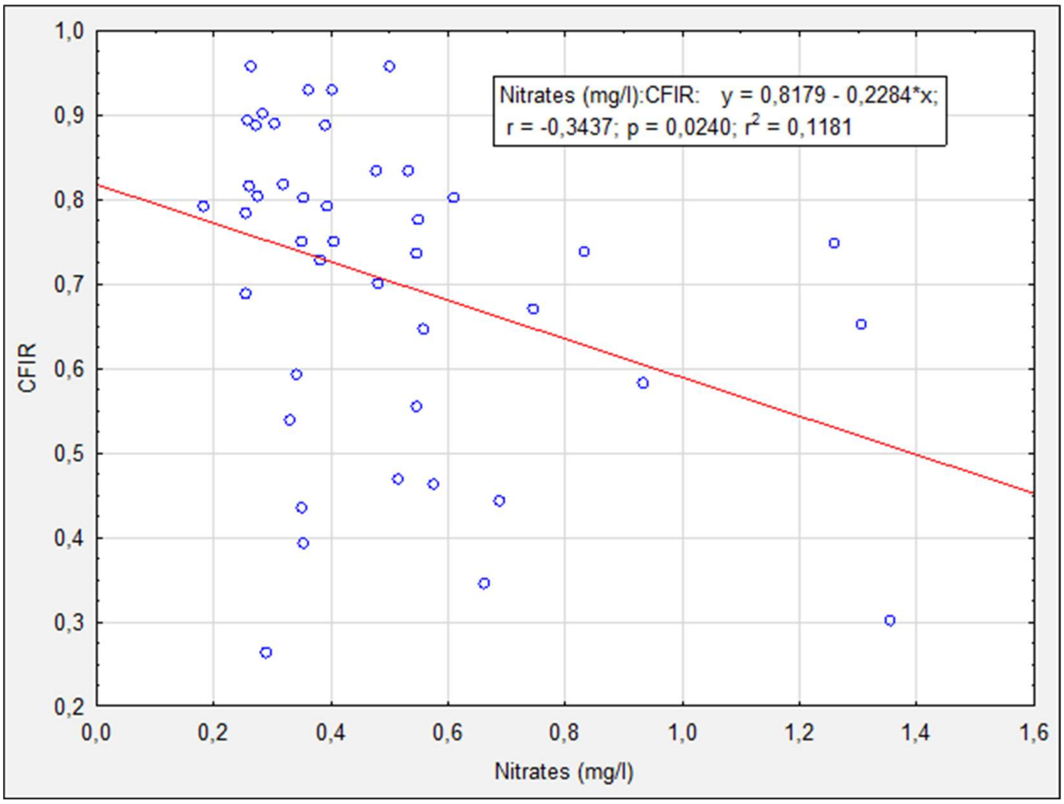

Figure 20. Pressure-response relationship between nitrates and the EQR_{CFIR} values in the Mediterranean region of Croatia

3. <u>LAND USE</u>

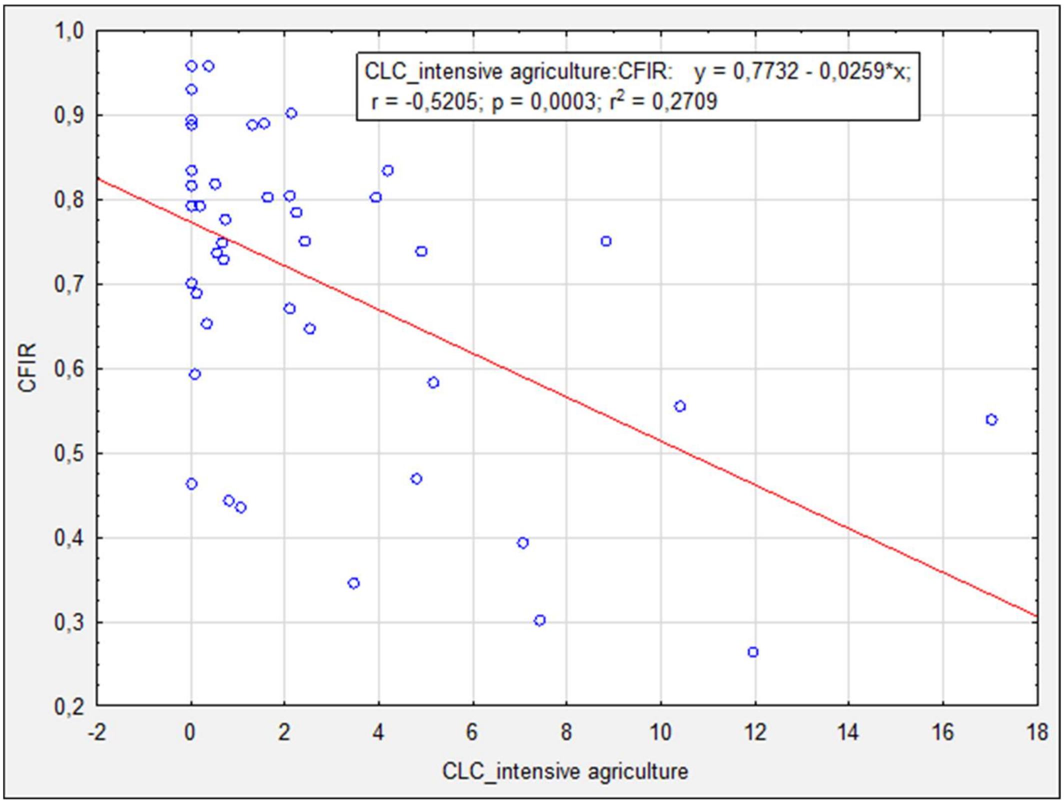

Figure 21. Pressure-response relationship between the Corine Land Cover- CLC (intensive agriculture) and the EQR_{CFIR} values in the Mediterranean region of Croatia

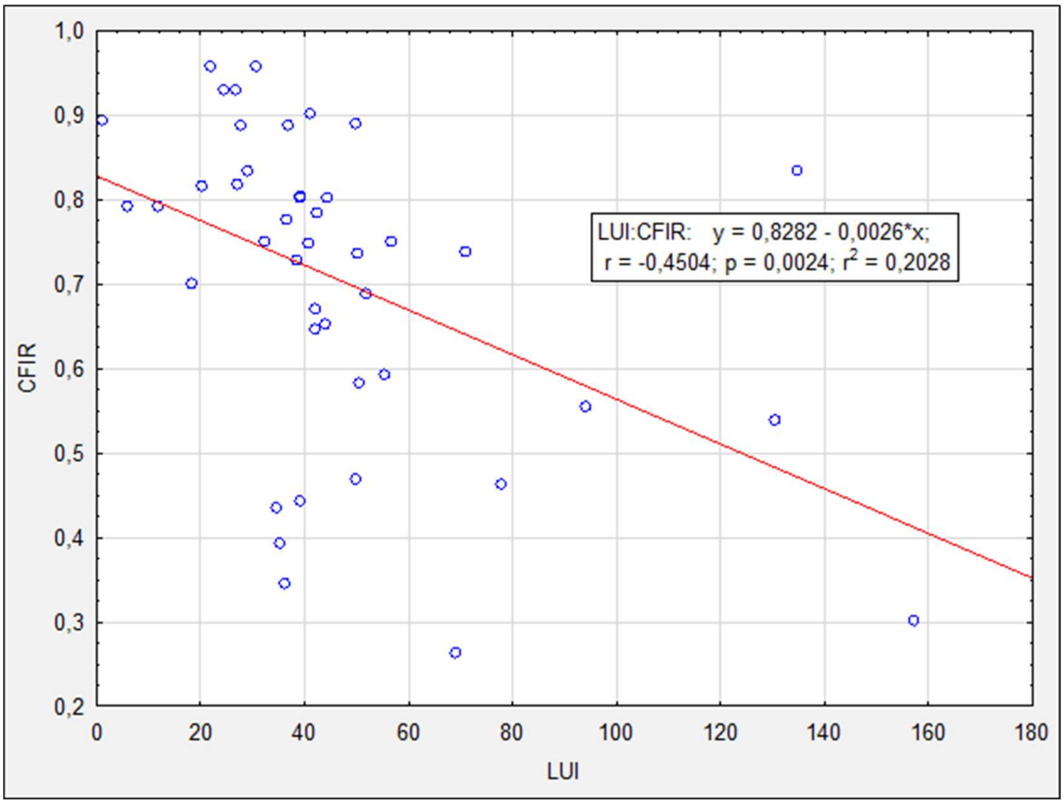

Figure 22. Pressure-response relationship between the Land Use Index-LUI and the EQR_{CFIR} values in the Mediterranean region of Croatia

4. HYDROMORPHOLOGY

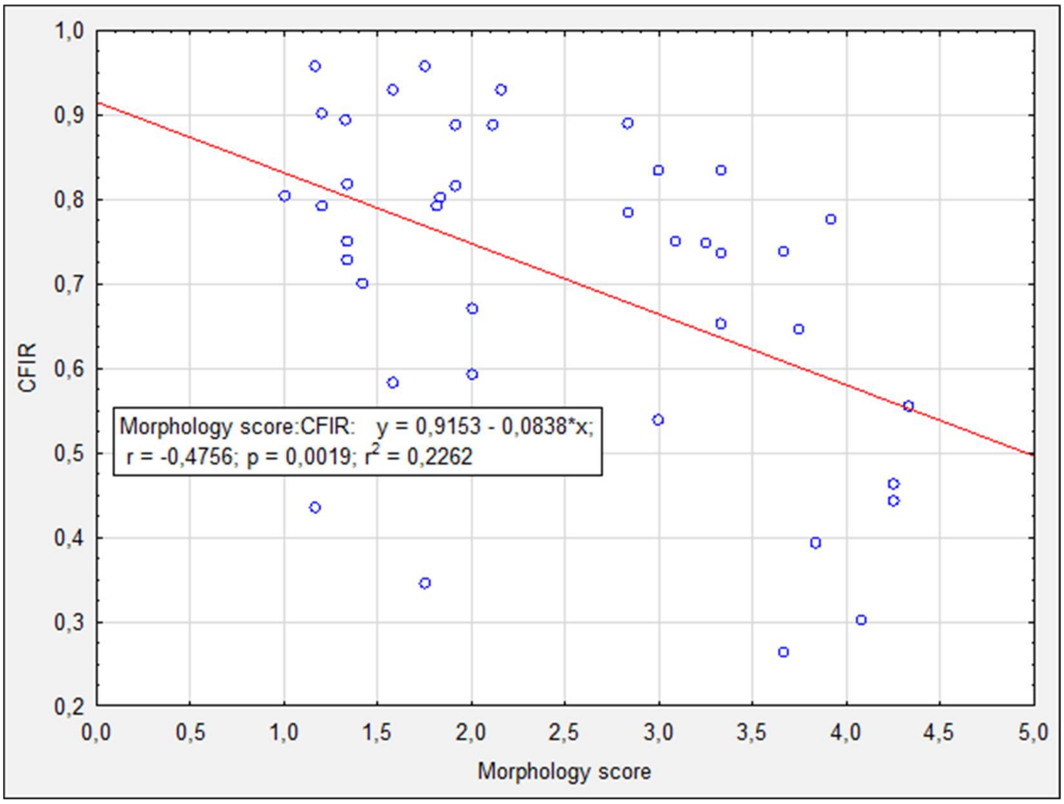

Figure 23. Pressure-response relationship between the River Morphology Score and the EQR_{CFIR} values in the Mediterranean region of Croatia

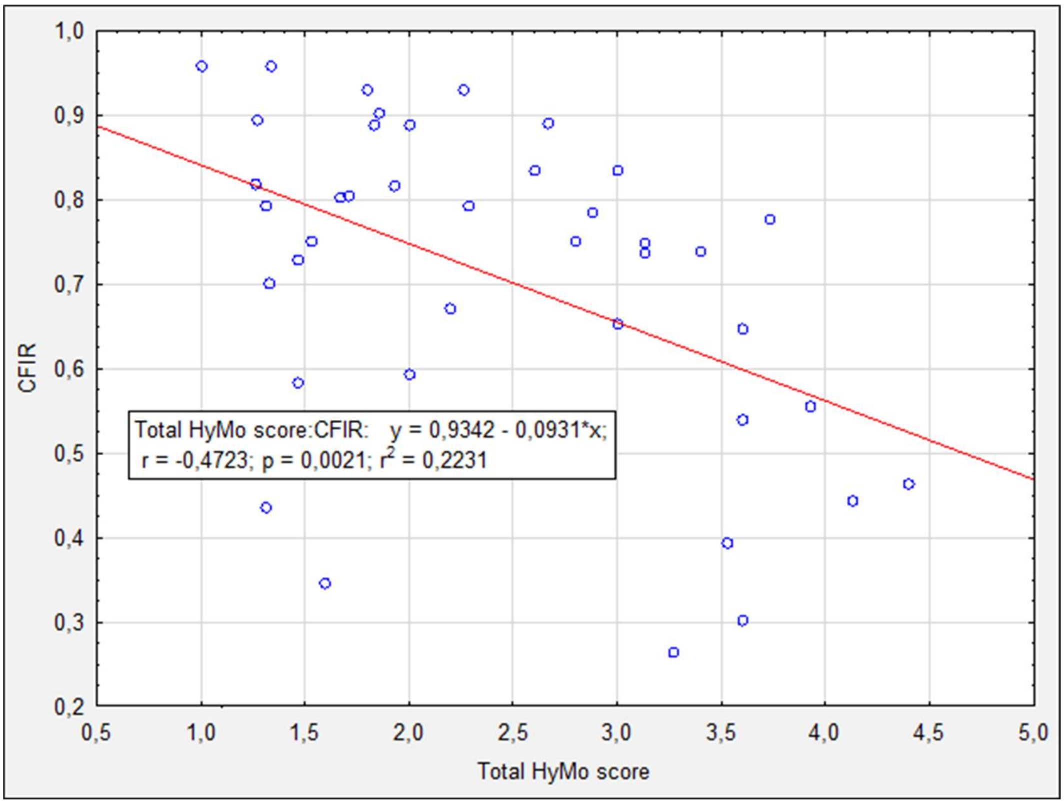

Figure 24. Pressure-response relationship between the mean (total) Hydromorphological Score and the EQR_{CFIR} values in the Mediterranean region of Croatia

5. RESUME

For four groups of pressures (organic enrichment, hydro-chemistry, land use and hydromorphology), significant regressions could be found. It is concluded that both the national fish based index clearly responds to anthropogenic impacts and can be used for the assessment of the ecological status.

4.3. ASSESSMENT CONCEPT

The Croatian national method under IC process is the Croatian fish index for rivers (CFIR). This index is a multimetric index that compares fish communities metrics to reference conditions, as described above. The Croatian national method follows the same assessment concept as other methods in the intercalibration group.

4.4. CONCLUSION ON THE INTERCALIBRATION FEASIBILITY

The number of sites fully complying in terms of the type criteria is high enough for carrying out the IC exercise.

5. DEMONSTRATING THE COMPLIANCE WITH THE COMPLETED INTERCALIBRATION EXERCISE

5.1. BACKGROUND

- **Description of the IC option and benchmark standardization used in the completed IC exercise;**

The comparison of reference conditions between countries is completed. Reference sites defined using common criteria at the European scale were used.

The common metrics (Table 9) are based on ecological and biological traits and are standardized in terms of natural environmental conditions, which means that they are independent of biogeographical conditions and environmental gradients.

Table 9. The metrics used to calculate the Salmonid and the Cyprinid Fish Index (EFI+ Manual 2009, WFD Intercalibration Phase 2: Milestone report, 2011)

Zone / Index	Metric name	Detailed name - guild
Salmonid	Ni.O2.Intol	Density (number of individuals per 100 m^2 in the 1 run of a sample site) of species **intolerant to oxygen depletion**, always more than 6 mg/l O_2 in water.
	Ni.Hab.Intol.150	Density (number of individuals per 100 m^2 in the 1. run of a sample site) ≤ 150 mm (total length) of species **intolerant to habitat degradation**.
Cyprinid	Ric.RHt.Par	Richness (number of species in the 1. run of a sample site) of species requiring a **rheophilic reproduction habitat**, i.e. preference to spawn in running waters.
	Ni.LITHO	Density (number of individuals per 100m^2 in the 1. run of a sample site) of species requiring **lithophilic** reproduction habitat, species which spawn exclusively on gravel, rocks, stones, cobble or pebbles. Their hatchlings are photophobic.

For each zone, the index value is the mean of the two metric values:

Salm.Fish.Index = (Ni.Hab.150 + Ni.O2.Intol) / 2

Cypr.Fish.Index = (Ric.RH.Par + Ni.LITHO) / 2

A second version of common metrics has been developed in 2010 (WFD Intercalibration Phase 2: Milestone report – October 2011).

This second version does not consider anymore the two river zones. Only two metrics are used to compute the fish index for all sites (see Table 5).

Fish.Index = (Ni.O2.Intol + Ric.RHt.Par) / 2

This second version is only applicable in the Lowland, Nordic and Danubian Group, but not in the Mediterranean Group because of the rarity of oxygen-intolerant species in this area. Croatia is located in the Mediterranean Group and the intercalibration process was done according to the scheme adopted in this group (version 1).

- **Selection of the correct procedure to use for intercalibrating new classification method**

IC Option 2 without piecewise transformation was chosen for intercalibration of the Croatian national method in the Mediterranean Group. Total number of reference sites for Croatia is 7. It is sufficient for benchmarking process according to Guidance Document No. 30 (European Union 2015).

5.2. DESCRIPTION OF IC DATASET

For intercalibration of the Croatian method, the dataset consisted of 43 sites sampled. These sites represented all of the abiotic river types present in Mediterranean Region of Croatia (except intermittent rivers classified as M-5) that are assessed with the national assessment method and have a wide pressure gradient. We took over 60 sites located in Mediterranean Group into consideration, but only 43 of them had all the necessary pressure-impact data to proceed with analysis.

5.3. DESCRIPTION OF INTERCALIBRATION PROCEDURE

- Benchmark standardization

  Benchmark standardization was conducted according to the scheme used in completed IC exercise (WFD Intercalibration Phase 2: Milestone report, 2011) and in other IC guiding documents. The Intercalibration Common Metrics (ICM) calculated for all sites in the Croatian national dataset were divided by a median value of ICM for 7 benchmark sites.

- Calculation of Intercalibration Common metrics (ICM) or Best-Related Intercalibrated National Classification (BRINC);

  Intercalibration Common metrics (ICM) were calculated for all sites in the Croatian national dataset with the original EFI+ software (EFI+ Manual 2009). According to the second version of CM adopted in the completed IC exercise for Mediterranean Group (WFD Intercalibration Phase 2: Milestone report, 2011), the final value of ICM was calculated using the formula described in chapter 5.1. ICM values were than benchmark standardized, as described above, into ICM_{bm}. These ICM_{bm} values were than related to the national EQR-s using OLS regression.

- Translation of national boundaries to ICM or BRINC:

  The approach used for the Fish River IC exercise in the Mediterranean Group is the case A1 (IC Option 1 or 2 using reference/benchmark sites) without piecewise transformation. Before checking the correlation between common metrics and the national indices, values are transformed in EQR. The results of OLS regression between ICMbm and national EQRs for a total of 43 sites of four different index types from the Croatian national dataset are presented in Figures 25-28. The regression slope should lie between 0,5 and 1,5 and the correlation coefficient should exceed 0,5 The correlation was sufficient (with R being 0,600, 0,501, 0,680 and 0,555 for four index types) as well as the slope value of all the OLS regressions which was within the range of these values for other countries in the Mediterranean Group of completed IC exercise (Table 10). For the Croatian national method, classes are as follows H/G=0,8; G/M=0,6; M/B=0,4 and B/P=0,2. The regression formula was used to translate the national boundaries to ICMbm scale and lower and upper class bias, as 0,25 class width, was calculated. These data were compared with values obtained for Mediterranean Group of completed IC exercise (Table 10) and the finalized Technical report by Greece (Tachos et al., 2017).

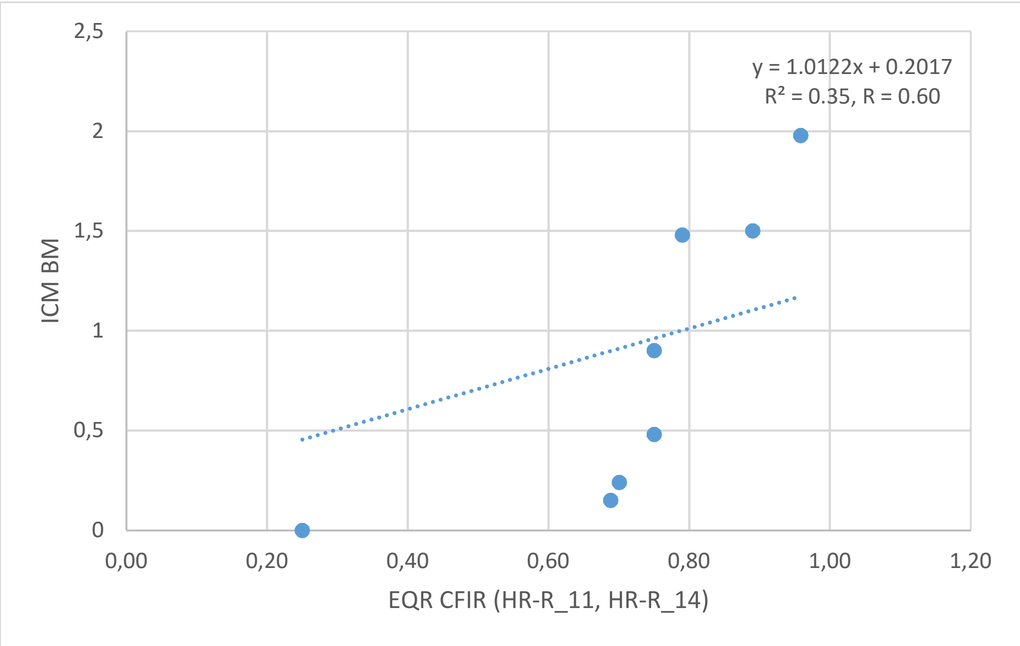

Figure 25. OLS regression between ICMbm and Croatian national index for types HR-R_11 and HR-R_14.

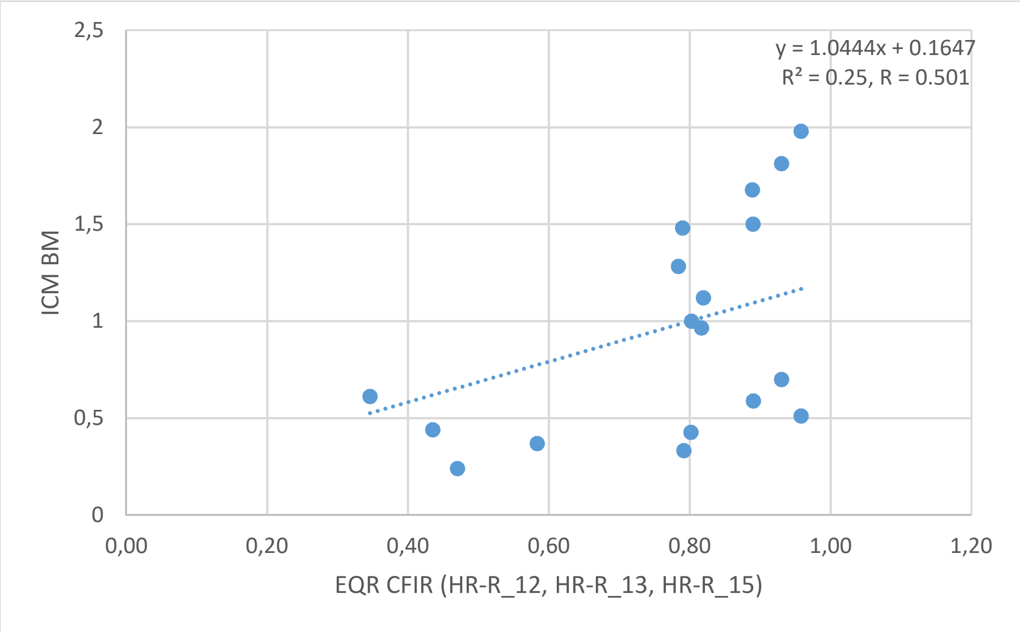

Figure 26. OLS regression between ICMbm and Croatian national index for types HR-R_12, HR-R_13 and HR-R_15.

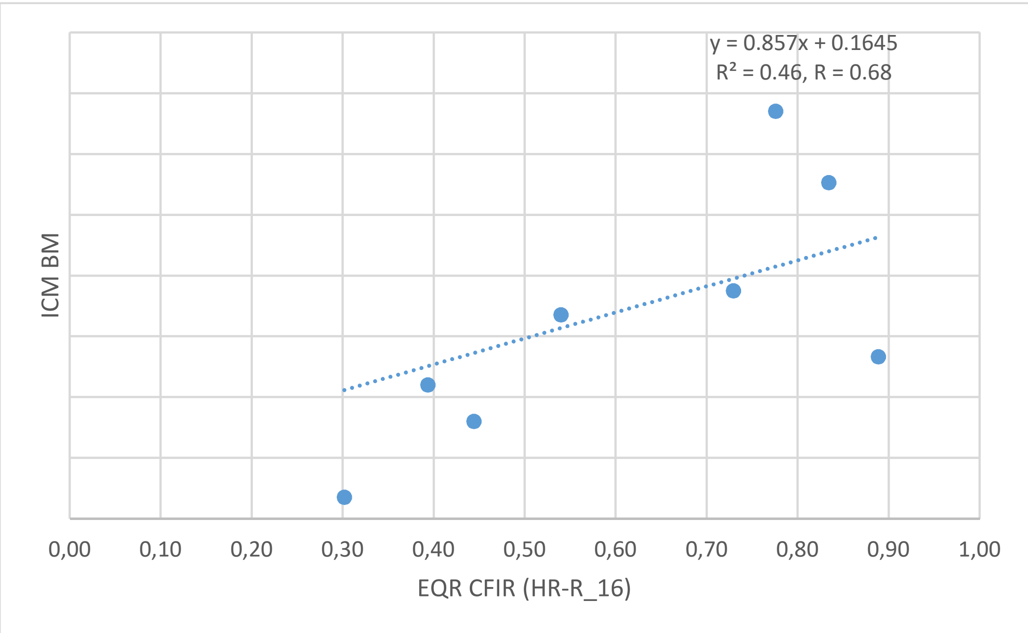

Figure 27. OLS regression between ICMbm and Croatian national index for type HR-R_16.

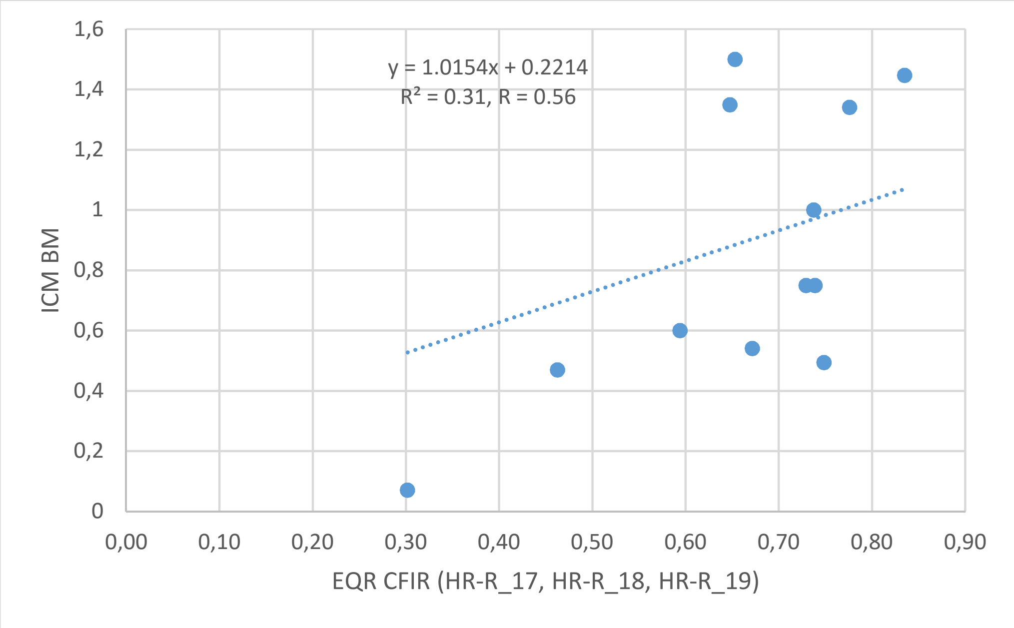

Figure 28. OLS regression between ICMbm and Croatian national index for types HR-R_17, HR-R_18 and HR-R_19.

- Calculating boundary bias:

  The Croatian National EQR boundaries for HRIR in the Mediterranean region of Croatia are as follows:

High	0,8-1
Good	0,6-0,79
Moderate	0,4-0,59
Bad	0,2-0,39
Poor	<0,2

According to Guidance Document No. 30 (European Union 2015) the boundary bias was calculated by subtracting the mean value of G/M or H/G boundary on CM_{bm} scale from Croatian EQR boundaries transposed to this scale with OLS regression. G/M boundary of Croatian index was located below the mean value, with a biases of -0,21, -0,24, -0,18 and -0,05 for four indices types and H/G boundary of Croatian method was placed above the mean value with a biases of 0,07, 0,08, 0,15 and 0,25 for four indices types (Table 10).

Table 10. Raw values (raw) and predicted values (fit) of the High-Good (H_G) and the Good-Moderate (G_M) boundaries (and the associated intervals: lower lwr) and upper (upr) values, which correspond to one fourth of a class; expressed in common metrics for 3 countries in Mediterranean Group of completed IC exercise (WFD Intercalibration Phase 2: Milestone report, 2011) and for Croatia before and after harmonization process.

Before harmonization										
Method_country Type	**H/G raw**	**G/M raw**	**H/G fit**	**H/G lwr**	**H/G upr**	**Bias**	**G/M fit**	**G/M flwr**	**G/M upr**	**Bias**
ES_IBIMED_T2	10,580	9,300	0,9990	0,9750	1,0070		0,8900	0,8770	0,9260	
ES_IBIMED_T3	16,930	13,340	0,9080	0,8530	0,9200		0,7560	0,7110	0,7790	
ES_IBIMED_T4	11,230	9,850	0,9470	0,9240	0,9730		0,8540	0,8010	0,8780	
ES_IBIMED_T5	7,470	5,610	0,9990	0,9490	1,0520		0,7990	0,7490	0,8490	
ES_IBIMED_T6	11,900	9,920	0,9580	0,9260	0,9660		0,8310	0,8120	0,8620	
PT_F_IBIP_index no	0,900	0,675	1,0030	0,9620	1,0220		0,8360	0,7940	0,8780	
HeFI_index	0,800	0,600	1,0700	1,0320	1,1070		0,9180	0,7940	0,8690	
Mean			0,9834				0,8406			
Median			0,9990				0,8360			
CFIR (HR-R_11 and HR-R_14)	0,8	0,6	0,99738	0,94765	1,04711	0,0140	0,79846	0,74873	0,84819	-0,0421
			Proportion bias/ HR_index HIGH			7%	Proportion bias/ HR_index Moderate			-21%
CFIR (HR-R_12, HR-R_13 and HR-R_15)	0,8	0,6	1,00022	0,948	1,05244	0,0168	0,79134	0,73912	0,84356	-0,0492
			Proportion bias/ HR_index HIGH			8%	Proportion bias/ HR_index Moderate			-24%
CFIR (HR-R_16)	0,8	0,6	1,0153	0,962125	1,068475	0,0319	0,8026	0,749425	0,855775	-0,0380
			Proportion bias/ HR_index HIGH			15%	Proportion bias/ HR_index Moderate			-18%
CFIR (HR-R_17, HR-R_18 and HR-R_19)	0,8	0,6	1,03372	0,98295	1,08449	0,0503	0,83064	0,77987	0,88141	-0,0099
			Proportion bias/ HR_index HIGH			25%	Proportion bias/ HR_index Moderate			-5%

5.4. FINAL BOUNDARIES

According to Guidance Document no. 30 (European Union 2015) the boundary bias of the Croatian method was assessed as not significant for all four indices types, because it was less or equal to the acceptable level of 0.25 class width (Table 10).

The Croatian National EQR boundaries for CFIR in the Mediterranean region of Croatia remain as follows:

High	0,8-1
Good	0,6-0,79
Moderate	0,4-0,59
Bad	0,21-0,39
Poor	0-0,2

POSSIBLE FURTHER DEVELOPMENTS

We acknowledge that the lack of bad sites and values therein in the method is not substantially reasoned. It is possible that an extended analysis may result in different pressure-impact relationships in the MED GIG. This may result in differentiation of reference values for the CFIR metric and additional differentiated normalization of CFIR in the future. As the monitoring efforts are ongoing in this region, a greater data set will surely give a more accurate setting of the reference values, as well as the pressure response relationships.

6. DESCRIPTION OF THE BIOLOGICAL COMMUNITIES

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT HIGH STATUS

In general, fish assemblages in high status contain most type specific species, characteristic for natural community of certain national river type. Proportion of non-native species and individuals belonging to non-native species is very low or they are not present. Ecological Quality Ratios important for certain river type are mostly above 0,8, indicating small or absent anthropogenic modifications.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT GOOD STATUS

Assemblages indicating good ecological status of rivers comprise high proportion of native species characteristic for natural communities of the certain river type. Nevertheless, non-native species are often present with low or moderate proportion of species and/or individuals. Ecological Quality Ratios are slightly to moderately (0,2-0,4) reduced in comparison to high ecological status. Small to moderate negative consequences of anthropogenic pressures can be noticed.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT MODERATE STATUS

Moderate ecological status is estimated based on the communities with a significantly reduced proportion of native species, higher proportion and sometime even dominance of non-native species and disrupted composition of fish communities. Ecological Quality Ratios are about half (0,4-0,6) of EQRs in natural communities characteristic for certain river type. Negative consequences of anthropogenic pressures led to significant disturbances in structures and compositions of fish communities.

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Report on lake phytoplankton classification method in the case where the Intercalibration exercise is not possible (Gap 3)

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Template for reporting the MS assessment method in the case where the Intercalibration exercise is not possible (Gap 3)

1. INTRODUCTION

The goal of this report is to declare that the present Croatian assessment method of the ecological status of lakes based on phytoplankton is compliant with the WFD normative definitions and has good pressure-impact relationship.

2. DESCRIPTION OF NATIONAL ASSESSMENT METHODS

Croatia has agreed to use the Hungarian classification method for lake phytoplankton assessment, which was intercalibrated for the Eastern Continental lakes GIG (Borics et al., 2018). Development of this intercalibrated method (setting of boundaries) was carried out with the involvement of national experts of the following countries: Bulgaria, Hungary and Romania (*Table 1*).

Table 1. Overview of Eastern Continental GIG lake phytoplankton assessment methods (Borics et al., 2018).

MS	Method	Status
BG	Hungarian lake phytoplankton index (HLPI)	Formally agreed national method
HU	Hungarian lake phytoplankton index (HLPI)	Formally agreed national method
RO	Hungarian lake phytoplankton index (HLPI)	Formally agreed national method

Phytoplankton based methods for Mediterranean reservoirs have already been intercalibrated (Poikane 2009), and from these, the methodology was adapted.
All natural lakes in Croatia are situated in the Dinaric Ecoregion. Lakes are classified according to national typology (Official Gazette 96/19), where each lake (except for Lakes Crniševo and Oćuša which belong to the same type) is considered a distinct type due to various abiotic factors - climatic, hydrologic, morphologic and geologic specificities. Besides the national typology classification, the lakes were grouped based on depth profile into shallow and deep lakes (*Table 2*).

Table 2. General characteristics of Croatian natural lakes

Lake	Maximum depth (m)	Ecoregion/subregion	National type	Lake description	Depth profile
Kozjak (Plitvička jezera)	48	DINARIC Continental	HR-L_1A	carbonate substrate, dimictic, barrage lake	deep
Prošće (Plitvička jezera)	38	DINARIC Continental	HR-L_1B	carbonate substrate, dimictic, barrage lake	deep

Vransko jezero (Cres)	78	DINARIC Mediterranean	HR-L_2	carbonate substrate, monomictic, cryptodepression	deep
Crniševo	31	DINARIC Mediterranean	HR-L_3	carbonate substrate, monomictic, cryptodepression	deep
Oćuša	20	DINARIC Mediterranean	HR-L_3	carbonate substrate, monomictic, cryptodepression	deep
Vransko jezero (Biograd)	4-5	DINARIC Mediterranean	HR_L_4	carbonate substrate, polymictic, cryptodepression	shallow
Visovačko jezero	28-30	DINARIC Mediterranean	HR-L_5	carbonate substrate, monomictic, barrage lake	deep

2.1. SAMPLING AND DATA PROCESSING

The sampling strategy and data processing techniques follow the standard well-acknowledged protocols (*Table 3*).

Table 2. Overview of the sampling and data processing of the national phytoplankton assessment methods.

Sampling strategy	Data processing
Integrated phytoplankton sample at the deepest point of the lake once a month during the vegetation period (April – September) according to standard EN 16698:2015. Depth profile of the integrated sample is dependent on lake type and stratification. a) Phases of water mixing (no thermal stratification) In shallow (polymictic) lakes (with maximum depth ≤ 10 m): integrated sample from the entire water column down to the depth of 1 m above the bottom. In stratified deep lakes: integrated sample to a maximum depth of 20 m or down to the depth of 1 m above the bottom. b) Summer stagnation phase In shallow (polymictic) lakes: down to the depth of 6 m or to the depth of 1 m above the bottom. In stratified deep lakes: depending on lake turbidity: 1) Turbid lakes where euphotic zone depth is less than epilimnetic depth ($Z_{eu}<Z_{epi}$): sample from the entire epilimnetic column. 2) Clear lakes where euphotic zone depth is larger than epilimnetic depth ($Z_{eu}>Z_{epi}$): sample from the entire euphotic column.	Phytoplankton samples: inverted microscopy of Lugol-preserved samples according to standard EN 15204:2006; phytoplankton biovolume determination based on the calculation of the volume of each unit from appropriate geometric formulae according to standard EN 16695:2015. Chlorophyll-*a* samples processed according to standard ISO 10260:1992.

The national dataset utilized for intercalibration comprises data coming from a total of 151 samples coming from 7 lakes (*Table 4*). Hydrochemical data, including basic physico-chemical data (total phosphorus, orthophosphates, total nitrogen, nitrates, nitrites, oxygen saturation, conductivity, alkalinity, total organic carbon (TOC), biological oxygen demand (BOD_5), suspended matter) and land-use data in catchment (urban and artificial areas, intensive and non-intensive agriculture, semi-natural areas), as well as biological data (Q_k, Q_k_stand, HLPI, phytoplankton taxa list with biomass) are available for all samples concerned (*Table 5)*.

Table 4. List of data available in the national dataset included in the intercalibration

Lake	Year	Physico-chemical data	Hydro-morphological data	Biological data	Complete dataset
Kozjak	2014	6	-	6	6
	2016	6	-	6	6
	2017	6	1	6	7
Prošće	2014	6	-	6	6
	2016	6	-	6	6
	2017	6	1	6	7
Vransko jezero (Cres)	2014	6	-	6	6
	2016	6	-	6	6
	2017	6	1	6	7
Crniševo	2014	6	-	6	6
	2016	6	-	6	6
	2017	6	1	6	7
Oćuša	2014	6	-	6	6
	2016	6	-	6	6
	2017	6	1	6	7
Vransko jezero (Biograd)	2014	12	-	12	12
	2016	12	-	12	12
	2017	12	1	12	13
Visovačko jezero	2014	6	-	6	6
	2016	6	-	6	6
	2017	6	1	6	7

Table 5. Range of values of different environmental variables at lake sites included in the method description.

(N=151)	MIN	MAX
TP [mg L^{-1}]	0.0015	0.0460
P-PO_4^{3-} [mg L^{-1}]	0.0005	0.0015

TN [mg L^{-1}]	0.1000	1.7900
$N-NO_3^-$ [mg L^{-1}]	0.0050	0.9600
$N-NO_2^-$ [mg L^{-1}]	0.0005	0.5900
O_2 %	66.9	179.0
Conductivity [µS cm^{-1}]	366	5300
Alkalinity [mg $CaCO_3$ L^{-1}]	40.0	368.8
TOC	0.59	27.89
BOD_5	5.9	14.8
Suspended matter	0.8	20.0
Artificial areas [%]	0.0	9.3
Intensive agriculture [%]	0.0	45.2
Extensive agriculture [%]	0.0	22.3
Semi-natural areas [%]	33.8	100.0

The proposed phytoplankton based method for assessment of Croatian deep and shallow lakes consists of three metrics (Poikane, 2009; Borics et al., 2018): 1) biomass metric, which is based on the chlorophyll-*a* concentration, 2) composition metric, which is calculated from the relative abundance of functional groups of algae and their sensitivity and tolerance to environmental stressors (e.g. TP and TN), and 3) algal bloom metric.

The method is not applicable if the salinity exceeds 5 ‰.

1) Biomass metric

Chl-*a* concentration is used as a biomass metric. For the boundary setting the proposed H/G and G/M chlorophyll-*a* values were adopted and adjusted from the intercalibrated MED-GIG IC reservoir types report (Poikane 2009, *Table 6*). The 3rd order polynomial regression equations are used to convert the measured chlorophyll-*a* values into the normalized scale with equal class widths and standardized class boundaries, where the H/G, G/M, M/P, and P/B boundaries are 0.8, 0.6, 0.4, 0.2, respectively. These converted values are considered as normalised EQR values (*Table 7*).

$EQR_{Chl\text{-}a}$: ecological quality ratio calculated from the Chl-*a* values

Table 6. Boundary values of chlorophyll-*a* and $EQR_{Chl\text{-}a}$ for Croatian lakes.

	Class categories	Chl-*a* boundary values (µg L^{-1})	$EQR_{Chl\text{-}a}$ values
DEEP LAKES	HIGH	≤2.0	0.8
	GOOD	≤5.3	0.6
	MODERATE	≤25.0	0.4
	POOR	≤50.0	0.2
	BAD	>50.0	<0.2
SHALLOW LAKES	HIGH	≤11.0	0.8
	GOOD	≤23.0	0.6
	MODERATE	≤35.0	0.4
	POOR	≤50.0	0.2
	BAD	>50.0	<0.2

Table 7. Equations for converting Chl-*a* to EQR values for Croatian lakes.

	DEEP LAKES		SHALLOW LAKES	
National type	HR-J_1A, HR-J_1B, HR-J_2, HR-J_3, HR-J_5		HR-J_4	
Equation for $EQR_{Chl\text{-}a}$	**If Chl-*a* < 5.3 µg L^{-1}**	**If Chl-*a* > 5.3 µg L^{-1}**	**If Chl-*a* < 50 µg L^{-1}**	**If Chl-*a* > 50 µg L^{-1}**
	$EQR_{Chl\text{-}a} = 0{,}0074x^2 - 0{,}1149x+1$	$EQR_{Chl\text{-}a} = 0{,}00005x^2 - 0{,}0118x+0{,}6617$	$EQR_{Chl\text{-}a} = -0{,}0161x + 0{,}9826$	$EQR_{Chl\text{-}a} = -0{,}004x + 0{,}4$

x: concentration of Chl-*a* (µg L^{-1})

The proposed boundary values of chlorophyll-*a* (*Table 6*) were compared to the Chl-*a* range and mean values for each lake (*Table 8*) to strengthen the benchmark justification.

Table 8. Range and mean values of Chl-*a* for Croatian lakes.

Lake	Chl-*a* range (µg L^{-1})	Chl *a* mean (µg L^{-1})
Kozjak	0.4 – 2.7	1.3
Prošće	0.5 – 8.1	3.8
Vransko (Cres)	0.4 – 2.1	0.7
Oćuša	0.2 – 9.9	2.3
Crniševo	0.2 – 9.9	2.2
Visovac	1.2 – 7.2	3.2
Vransko (Biograd) - station Motel	0.5 – 36.3	6.6
Vransko (Biograd) - station Prosika	0.5 – 34.8	6.8

2) Composition metric (Q_k)

Assessment is based on the quantitative phytoplankton data. The applied composition metric is based on the "Assemblage index" (Q) published by Padisák et al. (2006). This metric is based on the evaluation of functional groups (FG) of algae. Each FG is given a factor number (or F value) by considering the distribution of algae along with the stressor values. The factor numbers of the functional groups were set to reflect the nutrient pressures (*Table 9*), according to the EC-GIG Lakes Phytoplankton report (Borics et al., 2018). After the appropriate parametrization of the functional groups of algae in the various types of waters, Q_k is applicable to the ecological state assessment.

Q_k is given as:

$$Q_k = \sum_{i=1}^{s} (p_i F),$$

p_i: the relative contribution of the i^{th} assemblage to the total biomass,
F: is a factor number that evaluates the given assemblage in the given lake type.

Table 9. Proposed factor numbers (F) of the functional groups (FG).

FG	S1	S2	SN	XPh	H1	G	J	M	C	P	T	X1	LM	W1	W2	Q
F	1	1	1	1	1	3	3	3	5	5	5	5	5	5	5	5
FG	D	Y	E	K	LO	WS	MP	A	B	N	Z	X3	X2	F	U	V
F	7	7	7	7	7	7	7	9	9	9	9	9	9	9	9	9

The standardization of Q_k is achieved by dividing the calculated Q_k values of each phytoplankton sample with the maximum value of the index (9) according to the formula:

$$Q_{k}\_stand = Q_k/9$$

The boundaries of the composition metric were set with regards to Croatian national lake typology, where specific boundary values were assigned to each lake type. The suggested H/G boundary was set as the 75th percentile of all calculated Q_k_stand from all 18 samples for each lake type. The remaining degradation of continuum was divided equidistally into four width classes (*Table 10*).

H/G boundary = 75th percentile of Q_k_stand
G/M boundary = H/G * 0.75
M/P boundary = H/G * 0.50
P/B boundary = H/G * 0.25

Table 10. Boundary values of composition metric (Q_k_stand) and EQR_Q for Croatian lakes.

Class categories	Boundary values of Q_k_stand						EQR_Q values
	DEEP LAKES					SHALLOW LAKES	
	HR-J_1A	HR-J_1B	HR-J_2	HR-J_3	HR-J_5	HR-J_4	
HIGH	≥0.92	≥0.89	≥0.86	≥0.87	≥0.82	≥ 0.81	0.8
GOOD	≥0.69	≥0.67	≥0.65	≥0.65	≥0.62	≥ 0.61	0.6
MODERATE	≥0.46	≥0.45	≥0.43	≥0.44	≥0.41	≥ 0.41	0.4
POOR	≥0.23	≥0.22	≥0.22	≥0.22	≥0.21	≥ 0.20	0.2
BAD	<0.23	<0.22	<0.22	<0.22	<0.21	<0.20	<0.2

The proposed boundaries of Qk_stand (*Table 10*) are more precautionary than those proposed by the EC-GIG (*Table 11*), thus supporting their further use.

Table 11. Composition metric and EQR boundaries of the JRC EC-GIG report (Borics et al., 2018).

Class categories	Composition metric (Q) boundaries	EQR values
HIGH	≥0.82	0.8
GOOD	≥0.52	0.6
MODERATE	≥0.40	0.4
POOR	≥0.20	0.2
BAD	<0.20	<0.2

The type-specific 3rd order polynomial regression equations were used for conversion of the composition metric (Q_k_stand) into the normalized scale with equal class widths and standardized class boundaries, where the H/G, G/M, M/P, and P/B boundaries are 0.8, 0.6, 0.4, 0.2, respectively. These converted values are considered as normalised EQR_Q values (*Table 12*).

Table 12. Equations for converting the composition metric (Q_k_stand) to EQR_Q values for Croatian lakes.

	National type	Equation for EQR_Q
DEEP LAKES	HR-J_1A	$y = 7e^{-13}x^3 - 9e^{13}x^2 + 0.8696x - 2e^{-14}$
	HR-J_1B	$y = 0.8989x - 4e^{-15}$

	HR-J_2	$y = -2e^{-13}x^3 - 8e^{-14}x^2 + 0.9302x - 2e^{-14}$
	HR-J_3	$y = 7e^{-13}x^2 + 0.9195x - 8e^{-15}$
	HR-J_5	$y = -2e^{-13}x^3 - 9e^{-14}x^2 + 0.9756x - 8e^{-14}$
SHALLOW LAKES	HR-J_4	$y = 7e^{-13}x^3 - 9e^{-13}x^2 + 0,9877x - 6e^{-14}$

x: value of Q_k_norm

Combination of metrics

In the final EC-GIG report Borics et al. (2018) have shown that when comparing the strength of relationships between the composition and biomass metrics, the biomass metric can be a better predictor of the ecological state. Therefore, they proposed the Hungarian lake phytoplankton index (HLPI), which is composed of the two aforementioned metrics (biomass and composition metric) as the weighted average of the EQR values. HLPI is given as:

$$HLPI = \frac{EQR_Q + 2 \times EQR_{Chl-a}}{3}$$

HLPI: Hungarian lake phytoplankton index
EQR_Q: normalized EQR of the composition metric
$EQR_{Chl\text{-}a}$: normalized EQR of the biomass (Chlorophyll-*a*) metric

3) Bloom metric

The WFD requires that the frequency and intensity of algal blooms are considered in phytoplankton-based quality assessment. For the measure of water bloom several approaches have been proposed, of which the absolute abundance of cyanobacteria was found to be the most applicable. The boundaries proposed by Carvalho et al. (2012) suggested to apply the Cyanobacteria biomass = 2 mg L^{-1} as the boundary of low risk of harmful development and Cyanobacteria biomass = 10 mg L^{-1} as high risk of harmful algal development. Since some of the lakes in the Dinaric region are used for drinking water supply and as exceptional natural amenities, the proposed boundary for the bloom metric was set to be more strict. In this report we propose to use the bloom metric as follows:

If Cyanobacteria biomass <2 mg L^{-1}: the values of national metrics should be applied
If Cyanobacteria biomass >2 mg L^{-1}:
National EQR >0.6 The EQR should be reduced by 0.2
National EQR <0.6 No change

2.3. NATIONAL REFERENCE CONDITIONS

The criteria for selecting the lake reference sites were based on the intercalibrated Eastern Continental lakes GIG reference condition criteria (*Table 13*). Since there were no sites that match the given criteria, alternative benchmark approach had to be applied.

Table 13. Reference criteria for selection of lake reference sites in the EC-GIG (Borics et al., 2018).

Pressure type	Criterion
Diffuse source pollution	Reference" threshold <20% of intensive agriculture in the catchment area. "Rejection" threshold >50% of intensive agriculture in the catchment area (estimated from Corine data). Intensive agriculture between 20% and 50%: Validation with physico-chemical parameters at the site scale.
Point source pollution	No known point source discharge, or very localized impact with self-purification. If point sources are present, a validation with chemical and biological parameters is necessary.
Water abstraction	Only very minor reductions in flow level changes having no more than very minor effects on the quality elements.
Littoral vegetation modification	Only minor modification of the shoreline. Ratio of the natural littoral vegetation >90%. Complete zonation of the macrophytes in the littoral zone.
Biological pressures	No biomanipulation No invasive species, but alien species which are not at the invasive stage are tolerated.
Chemical pressures	TP: 76 µg L^{-1} (defined as 25 th percentile of TP values in the benchmark lake population) TN: 400 µg L^{-1} (defined as 25th percentile of TN values in the benchmark lake population) BOD: 2.5 mg L^{-1} If values are higher validation with chemical and biological parameters is necessary
Other pressures	No nearby intensive recreational use at the site scale: No regular bathing activities or motor boating. Occasional recreational uses (such as camping, swimming, boating, etc.) should lead to no or very minor impairment of the ecosystem.

2.4. NATIONAL BOUNDARY SETTING

SELECTION OF ALTERNATIVE BENCHMARK SITES

Since no lake matched all reference criteria, alternative benchmark criteria relevant for the BQE phytoplankton were chosen in the second step.

The used alternative benchmark criteria were based on the intercalibrated Eastern Continental lakes GIG (Borics et al., 2018):

- no major point sources in catchment, complete zonation of the macrophytes in the littoral zone,
- no (or insignificant) artificial modifications of the shore line,
- no mass recreation (camping, swimming, rowing)
- low/moderate fishing (fish standing stock <50 kg ha^{-1})
- Vegetation period mean TP <115 µg L^{-1}
- Vegetation period mean TN <1550 µg L^{-1}

Furthermore, rejection and reference limits for selection of lake benchmark sites from the JRC Mediterranean Lake Phytoplankton report were also applied (*Table 14*).

Table 14. Rejection and reference limits for selection of lake benchmark sites in the MED-GIG (de Hoyos et al., 2014).

	Artificial Land Use (%)	Intensive Agriculture (%)	Natural and Semi-natural Land Use (%)	Population density (hab km^2)	Total Phosphorus (µg L^{-1})
Rejection limits	< 4	< 20	> 70	< 30	< 30
Reference limits	< 1	< 10	> 80	< 10	< 12

2.5. PRESSURES ADDRESSED

Statistical analyses were performed to explore the responsiveness of the national phytoplankton-based assessment method to various anthropogenic stressors.

The pressure-response relationships were tested via:
(1) non-parametric Spearman rank correlations of the national metric (HLPI index) with environmental parameters (TP, P-PO_4^{3-}, TN, N-NO_3^-, N-NO_2^-, conductivity, alkalinity, suspended matter, total organic carbon (TOC), oxygen saturation, BOD_5,) and general land-use and hydrology parameters

(2) linear regressions of the national metric (HLPI) with pressure variables.

The results of Spearman correlation of HLPI with pressure variables are shown in *Table 15*. The coefficient showed statistically significant relationships ($p<0.05$) between national metric and several different pressures. Some of the pressures that present the strongest relationships with the national metric are presented in *Figure 1*. In general, lakes responded well to several nutrient pressures, in particular to total phosphorus (TP) and nitrites (N-NO_2^-), as well as to conductivity, alkalinity, suspended matter and total organic carbon. Furthermore, relationships between EQR (HLPI) and chl-*a* and TP were explored in the context of EC-GIG dataset (*Figures 2* and *3*). For the EQR - Chl-*a* regression a statistically significant relationship was found ($p<0.001$). No statistically significant relationship for TP was found.

Table 15. Summary of the Spearman correlations of the HLPI with different hydro-chemical and environmental pressures. Correlations marked in red are significant at $p < 0.05$.

	HLPI
TP	-0.356 $p=0.147*10^{-5}$
P-PO_4^{3-}	-0.036 p=0.672
TN	0.002 p=0.984
N-NO_3^-	-0.105 p=0.214
N-NO_2^-	0.240 p=0.004
Conductivity	-0.172 p=0.041

Alkalinity	-0.253 p=0.002
Suspended matter	-0.199 p=0.018
TOC	-0.356 p=0.023
O2 [%]	-0.135 p=0.110
BOD_5	-0.019 p=0.819

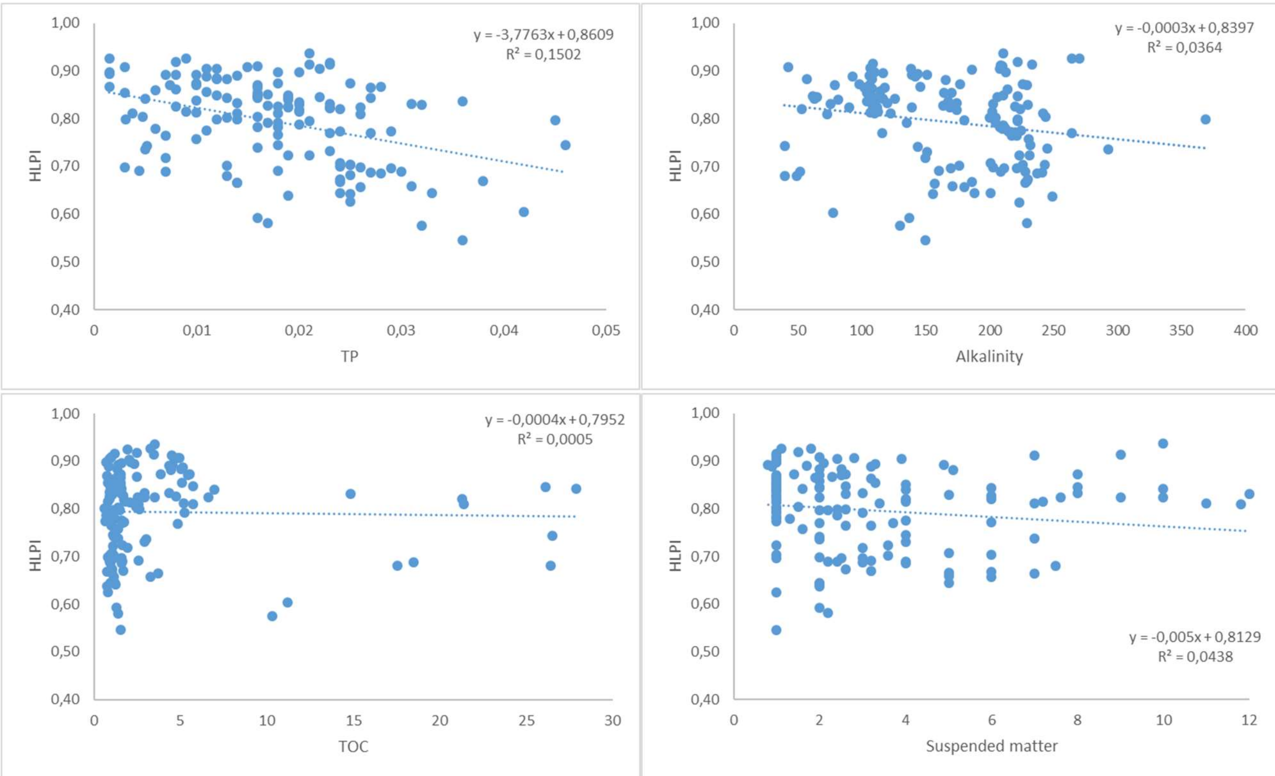

Figure 1. Pressure-response relationship between the most important pressures against the HLPI in Croatian lakes.

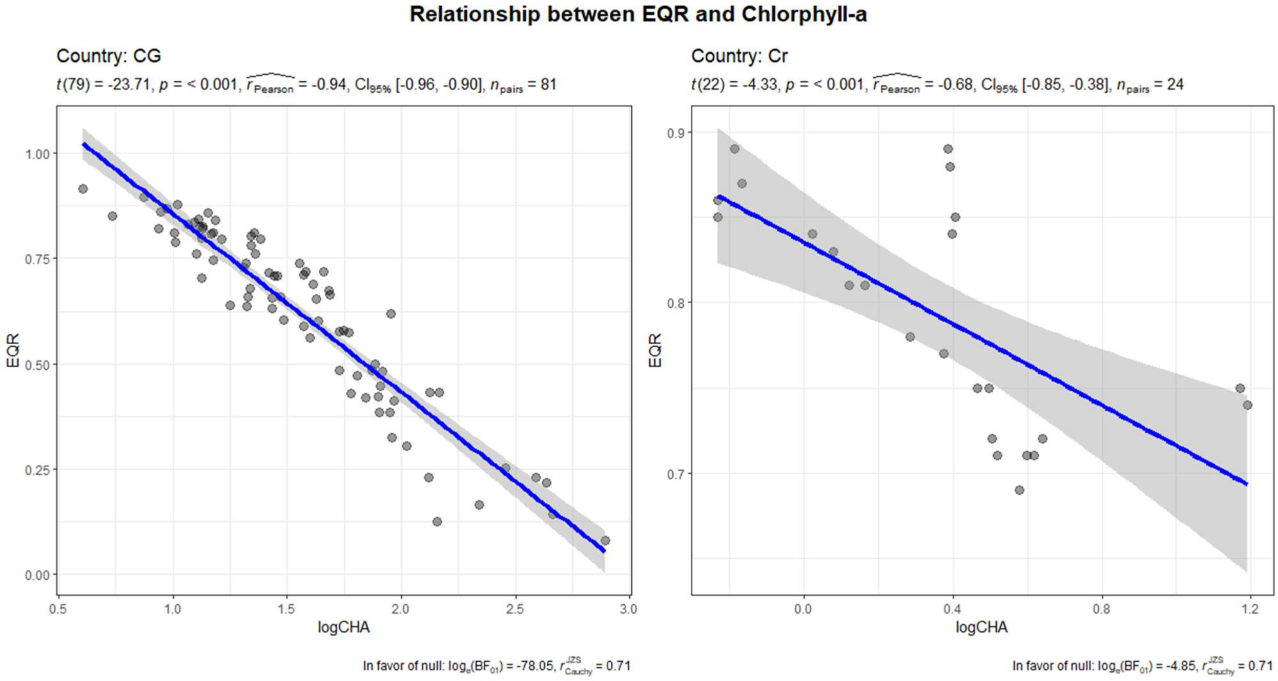

Figure 2. Relationship between EQR (HLPI) and Chl-*a*: a) whole dataset, b) Croatian data.

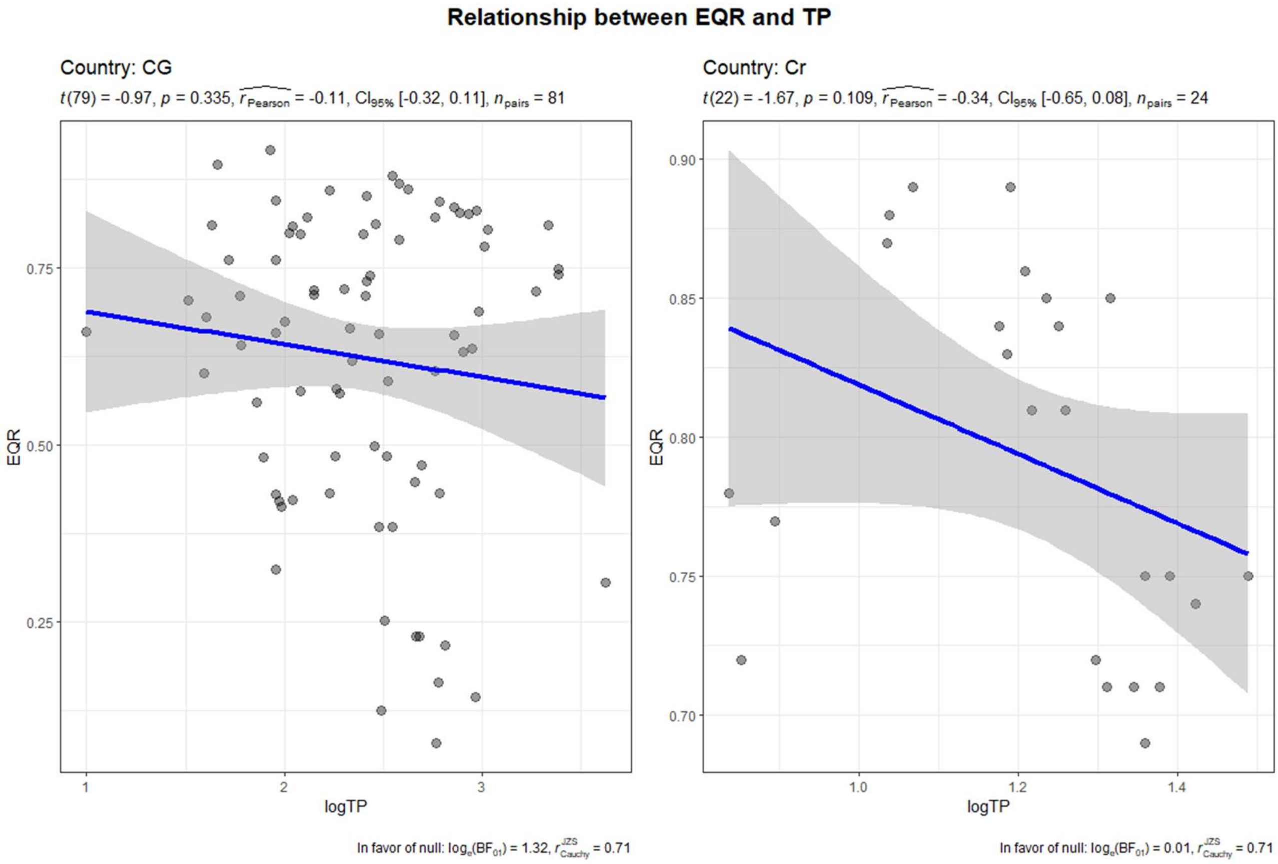

Figure 3. Relationship between EQR (HLPI) and TP: a) whole dataset, b) Croatian data.

3. WFD COMPLIANCE CHECKING

The first step in the Intercalibration process requires the checking of national methods considering the following WFD compliance criteria.

Table 16. List of the WFD compliance criteria and the WFD compliance checking process and results.

Compliance criteria	Compliance checking
Ecological status is classified by one of **five classes** (high, good, moderate, poor and bad).	YES
High, good and moderate ecological status are set in line with the WFD's **normative definitions** (**Boundary setting procedure**)	YES
All relevant parameters indicative of the biological quality element are covered (see Table 1 in the IC Guidance). A **combination rule** to combine parameter assessment into BQE assessment has to be defined. If parameters are missing, Member States need to demonstrate that the method is sufficiently indicative of the status of the QE as a whole	YES
Assessment is adapted to **intercalibration common types** that are defined in line with the typological requirements of the Annex II WFD and approved by WG ECOSTAT	YES
The water body is assessed against **type-specific near-natural reference conditions**	YES
Assessment results are expressed as **EQRs**	YES
Sampling procedure allows for **representative** information about water body quality/ecological status **in space and time**	YES
All data relevant for assessing the biological **parameters** specified in the WFD's normative definitions are covered by the **sampling procedure**	YES
Selected taxonomic level achieves adequate **confidence and precision** in classification	YES

IC FEASIBILITY CHECKING

The intercalibration process ideally covers all national assessment methods within a GIG. However, the comparison of dissimilar methods ("apples and pears") has clearly to be avoided. Intercalibration exercise is focused on specific type / biological quality element / pressure combinations. The second step of the process introduces an "IC feasibility check" to restrict the actual intercalibration analysis to methods that address the same common type(s) and anthropogenic pressure(s), and follow a similar assessment concept.

There are no common intercalibration types for both EC-GIG (Dinaric Western Balkan) and MED-GIG natural lakes yet. Croatia decided to classify the ecological quality of natural lakes using the Hungarian classification method for lake phytoplankton assessment, which was intercalibrated for the Eastern Continental lakes GIG (Borics et al., 2018), with some adaptations from the Mediterranean lakes GIG (Poikane, 2009; de Hoyos et al., 2014).

4.1. TYPOLOGY

Does the national method address the same common type(s) as other methods in the Intercalibration group? Provide evaluation if IC feasibility regarding common IC types.

There are seven natural lakes in Croatia with surface area larger than 0,5 km^2. All of them are located in Dinaric ecoregion (5. Dinaric Western Balkan): two of them (Plitvice lakes: Lake Kozjak and Lake Prošće) in Dinaric Continental sub-ecoregion (EC-GIG) and five of them in Dinaric Mediterranean sub-ecoregion (MED-GIG).

4.2. PRESSURES ADDRESSED

Does the national method address the same pressure(s) as other methods in the Intercalibration group? Provide evaluation if IC feasibility regarding pressures addressed.

In the Mediterranean GIG all national methods were calibrated to address eutrophication pressure.

4.3. ASSESSMENT CONCEPT

Does the national method follow the same assessment concept as other methods in the Intercalibration group? Provide evaluation if IC feasibility regarding assessment concept of the intercalibrated methods.

All assessment methods included in the IC Mediterranean exercise focus on the deepest zone of the lake, by sampling the euphotic zone and evaluating the biomass of the different taxa in biovolume. The Croatian assessment method follows the same assessment concept.

4.4. CONCLUSION ON THE INTERCALIBRATION FEASIBILITY

Provide conclusions on the IC feasibility.

Reasons for not doing the intercalibration was lack of appropriate comparable data, i.e. comparable lake types and reference conditions.

5. DESCRIPTION OF THE BIOLOGICAL COMMUNITIES

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT HIGH STATUS

Reference community of phytoplankton in high status deep karstic lakes of Dinaric ecoregion is characterized by the coexistence of chrysophytes (Chrysophyceae), dinoflagellates (Dinophyceae), and diatoms (Bacillariophyta). Chrysophytes are mainly represented with high biomass of *Dinobryon* spp. Ehrenberg, as well as a smaller proportion of *Kephyrion* spp. Pascher, *Mallomonas* spp. Perty, *Chrysocapsella planctonica* (West & G.S.West) Bourrelly, *Bitrichia chodatii* (Reverdin) Chodat etc. Dinoflagellates are represented with *Peridinium* Ehrenberg/*Peridiniopsis* Lemmermann/*Parvodinum* Carty group and *Ceratium hirundinella* (O.F.Müller) Dujardin. Centric diatom taxa (*Pantocsekiella* (*Cyclotella*) *costei* (J.C.Druart & F.Straub) K.T.Kiss & E.Ács, *Stephanodiscus parvus* Stoermer & Håkansson, *S. minutulus (*Kützing) Cleve & Möller, *S. medius* Håkansson, *Pantocsekiella* (*Cyclotella*) *ocellata* (Pantocsek) K.T.Kiss & Ács, *Cyclotella distinguenda* Hustedt, *Pantocsekiella* (*Cyclotella*) *comensis* (Grunow) K.T.Kiss & E.Ács , *Cyclotella plitvicensis* Hustedt, *Cyclotella radiosa* (Grunow) Lemmermann) are dominant throughout the investigated period, supported by pennate taxa belonging to *Fragilaria* spp. Lyngbye/*Synedra* spp. Ehrenberg. Cryptophytes (Cryptophyta) appear with lower biomass but high frequency, thus typifying the phytoplankton communities of deep karstic lakes.

Due to its proximity to Adriatic sea, the shallow Lake Vransko (Biograd) has a different phytoplankton community which is showing yearly changes, depending on the water salinity. In years with lower salinity, taxa like *Cosmarium tenue* W.Archer, *Synedropsis roundii* Torgan, Menezes, & Melo, *Fragilaria* spp., *Cyclotella meneghiniana* Kützing, *Kirchneriella contorta* (Schmidle) Bohlin and *Merismopedia* spp. Meyen can appear in high biomass. During more brackish years, high biomass of taxa like *Kirchneriella contorta*, *Thalassiosira* sp. Cleve and *Elakatothrix gelatinosa* Wille appear. No matter the salinity, shallow lake phytoplankton community is always abundant with benthic species that are resuspended from the benthic community. The most common tychoplanktonic species during the low salinity period is *Envekadea hedinii* (Hustedt) Van de Vijver, Gligora, Hinz, Kralj & Cocquyt. During the higher salinity period *Tetramphora croatica* Gligora Udovic, Caput Mihalic, Stankovic & Levkov becomes one of the dominant species in benthic community, as well as in the plankton.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT GOOD STATUS

In deep karstic lakes functionally well adapted phytoplankton groups interchange in dominance during the seasonal cycle from April to September and tolerate the constraining conditions of nutrients more successfully as an aggregated group than as taxonomic units. As was the case with the high status communities, the coexistence of chrysophytes (Chrysophyceae), dinoflagellates (Dinophyceae), and diatoms (Bacillariophyta) is also evident at good status. Dominant centric diatoms are represented with the following species: *Pantocsekiella costei*, *Stephanodiscus parvus, S. minutulus*, *S. medius*, *Pantocsekiella ocellata* and *Cyclotella distinguenda*. Furthermore, the appearance and notable biomass contribution of *Fragilaria crotonensis* Kitton*, Nitzschia* sp. Hassall, *Nitzschia acicularis* (Kützing) W.Smith, *Asterionella formosa* Hassall, *Synedra* sp. and *Ulnaria acus* (Kützing) Aboal conform to featured ecological properties of lakes. Such community composition can therefore be used as an indicator of transient ecological conditions towards good status. Considering dinoflagellates higher biomass contribution of *Ceratium hirundinella* is noted. The ecological preferences of karstic lakes favour mixotrophy of *Dinobryon* spp., allowing chrysophytes to effectively dominate the phytoplankton community at different status. Moreover, motile cells can actively control their appearance in water column, thus adapting to availability of nutrients, favourable light conditions or predator avoidance. Several green algae are observed in the community at high ecological status, mostly *Oocystis* spp. Nägeli ex A.Braun and *Elakathotrix* spp. Wille.

In the shallow Vransko Lake (Biograd) phytoplankton community at good status usually tends to shift to cyanobacteria, mostly *Microcystis* spp. Lemmermann as representative species. Some filamentous cyanobacteria also appear, such as *Chrysosporum bergii* (Ostenfeld) E.Zapomelová, O.Skácelová, P.Pumann, R.Kopp & E.Janecek*, Chrysosporum minor* (Kiselev) Komárek and *Phormidium* spp. Kützing ex Gomont.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT MODERATE STATUS

At moderate status the percentage of chrysophytes, dinoflagellates and diatoms, represented by different centric diatoms, pennate group *Ulnaria/Synedra/Fragilaria*, chrysophyte *Dinobryon*, and dinoflagellate *Ceratium/Peridinium* species, decrease and the coexistence is less than 50% of phytoplankton biomass in all lakes. Green algae (Chlorophyta) and cyanobacteria describe phytoplankton community of deep lakes at moderate status. The most prominent genera are *Ankistrodesmus* Corda, *Dictyosphaerium* Nägeli, *Sphaerocystis* Chodat, *Radiococcus* Schmidle, *Elakatothrix* and *Tetraselmis* F.Stein. During the conditions of fluctuating pH and low salinity *Botryoccocus braunii* Kützing appears in the community.

In the shallow Vransko Lake (Biograd) phytoplankton community at moderate status is usually shifted to cyanobacteria with *Woronichinia compacta* (Lemmermann) Komárek & Hindák*, Snowella lacustris* (Chodat) Komárek & Hindák*, Microcystis aeruginosa* (Kützing) Kützing and *Microcystis natans* Lemmermann ex Skuja as representative species.

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Report on fitting of phytobenthos classification method with the results of the completed intercalibration of the Lakes cross-GIG (HA, L-M1)

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Report on fitting of phytobenthos classification method with the results of the completed intercalibration of the lakes (L-M1)

1. INTRODUCTION

The official intercalibration exercise of phytobenthos-based methods of ecological status assessment of lakes within the cross-Geographical Intercalibration Group (cross-GIG) was successfully finalized in 2014 by eleven European countries. Lakes were classified into three broad common types: low, medium and high alkalinity lakes (*Table 1*). Due to the short gradient and confounding factors, it was not possible to perform a full intercalibration on low alkalinity lakes (Kelly et al. 2014). Croatia did not participate in the cross-GIG intercalibration exercise with phytobenthos data during the official exercise.
The goal of this report is to declare that the present Croatian assessment method of ecological status of Mediterranean natural lakes of the IC type (L-M1) based on benthic diatoms is compliant with the WFD normative definitions and its class boundaries are in accordance with the results of the completed intercalibration exercise.
In particular, the classification method was verified for WFD compliance and IC feasibility and the class boundaries were compared with agreed boundaries from the cross-GIG intercalibration exercise following the instructions of the CIS Guidance Document n°30: "Procedure to fit new or updated classification methods to the results of a completed intercalibration exercise" (Willby et al. 2014).

Table 1. Common intercalibration water body types and list of the MS sharing each type (from Kelly et al. 2014)

Common type	Common type characteristics, contributing types, region	MS sharing IC common type
HA	High alkalinity lakes CB-GIG: L-CB1, L-CB2 MED-GIG: L-M1 ALP-GIG: L-AL3	BE-F, DE, HU, IE, IT, PL, SE, SI, UK, **HR**
MA	Moderate alkalinity lakes CB-GIG: L-CB3 N-GIG: L-N8	BE-F, DE, FR, FI, IE, IT, SE, UK
LA	Low alkalinity lakes N-GIG: L-N2, L-N3	FI, IE, SE, UK

2. DESCRIPTION OF NATIONAL ASSESSMENT METHODS

The Croatian national method for ecological status assessment of lakes considers benthic diatoms as proxies for phytobenthos. It is compliant with normative definitions of WFD used by other MS and takes into account both taxonomic composition and species' relative abundance of benthic diatom assemblages. Sampling, sample treatment, diatom identification and data processing are based on the European standards EN 13946: 2014 and EN 14407: 2014. Ecological status is evaluated using the two multimetric diatom indices (MIL and MIB).

All natural lakes in Croatia are situated in the Dinaric Western Balkan ecoregion (ER5; sensu Illies 1978). Lakes are classified according to national typology (Official Gazette 96/19), where each lake (except for Lakes Crniševo and Oćuša which belong to the same type) is considered a distinct type due to various abiotic factors - climatic, hydrologic, morphologic and geologic specificities. Besides the national typology classification, the lakes were grouped based on depth profile into shallow and deep lakes (*Table 2*). According to the IC typology the lakes are classified into high alkalinity (HA) lakes of the MED-GIG group L-M1, EC-GIG group L-EC4 and some doesn't have existing IC type.

The lakes are influenced mostly by Mediterranean and partly Continental climate.

Table 2. General characteristics of Croatian natural lakes

Lake	Maximum depth (m)	Ecoregion/subregion/ altitude	IC Type	National type	Lake description	Depth profile
Kozjak (Plitvička jezera)	48	DINARIC Continental High altitude	L-EC4	HR-L_1A	carbonate substrate, dimictic, barrage lake	deep
Prošće (Plitvička jezera)	38	DINARIC Continental High altitude	L-EC4	HR-L_1B	carbonate substrate, dimictic, barrage lake	deep
Vransko (Cres)	78	DINARIC Mediterranean Lowland	Not existing	HR-L_2	carbonate substrate, monomictic, cryptodepression	deep
Crniševo	31	DINARIC Mediterranean Lowland	Not existing	HR-L_3	carbonate substrate, monomictic, cryptodepression	deep
Oćuša	20	DINARIC Mediterranean Lowland	Not existing	HR-L_3	carbonate substrate, monomictic, cryptodepression	deep
Vransko (Biograd)	4-5	DINARIC Mediterranean Lowland	L-M1	HR_L_4	carbonate substrate, polymictic, cryptodepression	shallow
Visovačko	28-30	DINARIC Mediterranean Lowland	Not existing	HR-L_5	carbonate substrate, monomictic, barrage lake	deep

2.1. SAMPLING AND DATA PROCESSING

Sampling method: Littoral diatoms are sampled from the natural (type specific) bottom, preferably at 0.5 - 1.5 m depth. Stones are preferred (minimum of five), but sampling on emergent or submersive macrophytic vegetation, rocks, artificial substrate or sand and mud is allowed, if stones are absent.

Sampling time and frequency: Sampling is performed once a year, principally in spring time during favourable and stable water level. A preferred number of sampling points is one per lake.

Sample treatment/data processing: Diatom samples in the laboratory are treated according to European standard EN 13946: 2014, where the hydrochloric acid is used to remove inorganic material, and sulphuric acid or hot hydrogen peroxide are used to remove all the organic material. Permanent slides are prepared by mounting clean diatom suspension with Naphrax on the microscopic slides.

Identification level: 400 valves are counted and identified to the lowest taxonomic level possible on each slide using light microscope with Differential Interference Contrast at 1000 x magnification.

2.2. DESCRIPTION OF NATIONAL METHODOLOGY

Metric calculation: Methods for lake phytobenthos, which were successfully intercalibrated during the professional checking processes of the EU (Kelly et al. 2014), consist of indices like IBD and EPI-D, which can be calculated using the OMNIDIA software (Lecointe et al. 2003, 2008), and index $TDIL_{1\text{-}20}$ developed by Stenger-Kovács et al. (2007). The calculation process of these three indices (*Table 3*) is based on the weighted average equation of Zelinka and Marwan (1961) modified by Coste (1982):

$$Index = \frac{\sum_{j=1}^{n} p_j \times s_j \times v_j}{\sum_{j=1}^{n} p_j \times v_j}$$

Where:
p_j: relative abundance of counting units of "j" taxon in sample
s_j: sensitivity of "j" taxon (optimum)
v_j: tolerance/indicator value of "j" taxon

Table 3. Diatom indices used as metrics for lake phytobenthos.

Index	Final calculation	References
EPI-D	$EPI\text{-}D = 20 - 4.75 \times \sum EPI\text{-}D$	Dell'Uomo 1996
IBD	$IBD = 4.75 \times \sum IBD - 8.5$	Prygiel & Coste 1999
$TDIL_{1\text{-}20}$	$TDIL_{1-20} = 3.8 \times \sum TDIL_{1-20} + 1$	Stenger-Kovács et al. 2007

Croatia has agreed to use two multimetric diatom indices proposed by the Lake Phytobenthos JRC Technical report (Kelly et al. 2014) and Ács et al. (2015). The use of multimetric indices was chosen due to stronger correlations with stressors, in contrast to the use of diatom indices on their own (Kelly et al. 2014). The theoretical values of these multimetric indices vary between 1-19 (*Table 4*).

Table 4. Proposed multimetric indices for the ecological assessment of Croatian lakes of the IC type L-M1.

Lake type	Indices used for calculation of multimetric index	Multimetric index	Reference value of multimetric index
SHALLOW LAKES	IBD EPI-D	$MIB = \frac{IBD + EPI - D}{2}$	17
DEEP LAKES	IBD EPI-D $TDIL_{1\text{-}20}$	$MIL = \frac{IBD + EPI - D + TDIL_{1-20}}{3}$	19

Ecological status assessment: Assessment is based on EQR values of MIB (shallow lakes) or MIL (deep lakes). Further details in Section "2.4. National boundary setting".

2.3. NATIONAL REFERENCE CONDITIONS

In the Dinaric ecoregion of Croatia, the landscape is mostly dominated by karst deposits, which have historically been seen as harsh habitats for agricultural and urban development. This means that very few lakes and reservoirs are affected by high levels of nutrient enrichment and are mostly in good and high ecological status (or good ecological potential in the case of reservoirs).
The criteria for selecting the lake reference sites were based on the intercalibrated Eastern Continental lakes GIG reference condition criteria (*Table 5*). Since there were no sites that match the given criteria, alternative benchmark approach had to be applied.

Table 5. Reference criteria for selection of lake reference sites in the EC-GIG (Borics et al. 2018).

Pressure type	Criterion
Diffuse source pollution	Reference" threshold <20% of intensive agriculture in the catchment area. "Rejection" threshold >50% of intensive agriculture in the catchment area (estimated from Corine data). Intensive agriculture between 20% and 50%: Validation with physico-chemical parameters at the site scale.
Point source pollution	No known point source discharge, or very localized impact with self-purification. If point sources are present, a validation with chemical and biological parameters is necessary.
Water abstraction	Only very minor reductions in flow level changes having no more than very minor effects on the quality elements.
Littoral vegetation modification	Only minor modification of the shoreline. Ratio of the natural littoral vegetation >90%. Complete zonation of the macrophytes in the littoral zone.
Biological pressures	No biomanipulation No invasive species, but alien species which are not at the invasive stage are tolerated.
Chemical pressures	TP: 76 µg L^{-1} (defined as 25^{th} percentile of TP values in the benchmark lake population) TN: 400 µg L^{-1} (defined as 25^{th} percentile of TN values in the benchmark lake population) BOD: 2.5 mg L^{-1} If values are higher validation with chemical and biological parameters is necessary
Other pressures	No nearby intensive recreational use at the site scale: No regular bathing activities or motor boating. Occasional recreational uses (such as camping, swimming, boating, etc.) should lead to no or very minor impairment of the ecosystem.

SELECTION OF ALTERNATIVE BENCHMARK SITES

Since no lake matched all reference criteria, alternative benchmark criteria were chosen in the second step. The used alternative benchmark criteria were based on the intercalibrated Eastern Continental lakes GIG (Borics et al. 2018):

- no major point sources in the catchment, complete zonation of the macrophytes in the littoral zone,
- no (or insignificant) artificial modifications of the shoreline,
- no mass recreation (camping, swimming, rowing)
- low/moderate fishing (fish standing stock <50 kg ha^{-1})
- vegetation period mean TP <115 µg L^{-1}
- vegetation period mean TN <1550 µg L^{-1}

Furthermore, rejection and reference limits for selection of lake benchmark sites from the JRC Mediterranean Lake Phytoplankton report (de Hoyos et al. 2014) were also applied (*Table 6*).

Table 6. Rejection and reference limits for selection of lake benchmark sites in the MED-GIG (de Hoyos et al. 2014).

	Artificial Land Use (%)	Intensive Agriculture (%)	Natural and Semi-natural Land Use (%)	Population density (hab km²)	Total Phosphorus (µg L^{-1})
Rejection limits	< 4	< 20	> 70	< 30	< 30
Reference limits	< 1	< 10	> 80	< 10	< 12

2.4. NATIONAL BOUNDARY SETTING

The national dataset utilized for intercalibration comprises data coming from a total of 35 samples coming from 7 lakes (*Table 7*). Of the total number of samples, 21 are biological data (MIL, MIB, diatom taxalist with relative abundances) which represent replicates taken during the same year (2019). Unfortunately, no samples from prior years were available for the IC fitting process, as the new methodology for sampling was adopted and exercised in the same year. The physico-chemical data (total phosphorus, orthophosphates, total nitrogen, nitrates, nitrites, ammonium, dissolved oxygen, conductivity, alkalinity, pH, biological oxygen demand (BOD_5), suspended matter) and land-use data in catchment (urban and artificial areas, intensive and non-intensive agriculture, semi-natural areas) are also included (*Table 8)*.

Table 7. List of data available in the national dataset included in the intercalibration.

Lake	Year	Physico-chemical data	Land-use data (catchment)	Biological data	Complete dataset	Benchmark
Kozjak	2019	1	1	3	5	Yes
Prošće	2019	1	1	3	5	Yes
Vransko (Cres)	2019	1	1	3	5	Yes
Crniševo	2019	1	1	3	5	No
Oćuša	2019	1	1	3	5	Yes
Vransko (Biograd)	2019	1	1	3	5	Yes
Visovačko	2019	1	1	3	5	Yes

Table 8. Range of values of different environmental variables at lake sites included in the method description.

(N=7)	MIN	MAX
TP [mg L^{-1}]	0.003	0.015
$P\text{-}PO_4^{3-}$ [mg L^{-1}]	0.002	0.005
TN [mg L^{-1}]	0.100	0.850
$N\text{-}NO_3^-$ [mg L^{-1}]	0.010	0.660
$N\text{-}NO_2^-$ [mg L^{-1}]	0.001	0.011
$N\text{-}NH_4^+$ [mg L^{-1}]	0.004	0.015
Conductivity [µS cm^{-1}]	359	1946
Alkalinity [mg $CaCO_3$ L^{-1}]	136.3	383.5
Suspended matter [mg L^{-1}]	1.0	8.6
COD-Mn [mg O_2 L^{-1}]	0.8	3.6
BOD_5 [mg O_2 L^{-1}]	0.25	1.80
Water temperature [°C]	9.5	19.6
DO [mg L^{-1}]	8.9	11.0
pH	7.3	8.7
Artificial areas [%]	0.0	9.3
Intensive agriculture [%]	0.0	45.2
Extensive agriculture [%]	0.0	22.3
Semi-natural areas [%]	33.8	100.0

From a total of seven lakes in the L-M1 type, six met the given benchmark criteria. Only Lake Crniševo did not fully comply to the benchmark criteria.

The multimetric index MIL was developed for Hungarian lakes and was successfully intercalibrated. In this report, we propose the use of MIL for Croatian natural lakes in Dinaric ecoregion. The proposed multimetric index MIB was developed, and successfully intercalibrated, for assessment of ecological status of Lake Balaton, which has comparable physico-chemical, geomorphologic, trophic and properties to shallow Lake Vransko (Biograd) in the Dinaric ecoregion. In the present report, we propose the use of MIB for ecological status assessment of shallow Lake Vransko (Biograd) in the Dinaric ecoregion of Croatia.

The two different multimetric indices for shallow and deep lakes were adopted from the official report for the assessment of the ecological potential of Croatian reservoirs based on benthic diatoms (Mihaljević et al. 2018a, b). The methods used in the report to assess the ecological status of reservoirs and lakes in the Pannonian Ecoregion of Croatia have previously been intercalibrated (JRC Technical Report, 2014) and published (Kelly, 2014). For assessing diatom-based quality of standing waters in the Dinaric Ecoregion of Croatia, intercalibrated indices were also proposed. As demonstrated in the JRC Technical Report (2014), the correlations between stressors and diatom indices were stronger when multimetric indices were used, in contrast to the use of indices on their own (JRC Technical Report, 2014). In the same report (Mihaljević et al. 2018a, b), four characteristic types of reservoirs could be separated in Pannonian and Dinaric Ecoregions of Croatia: very shallow standing water, shallow standing water, deep standing water and a river type. For each reservoir type, a different multimetric diatom index has been proposed, according to JRC Technical Report (2014) and Ács et al. (2015). This methodology was also used for the intercalibration process in the characterization of the natural lakes in the Dinaric Ecoregion of Croatia, where only two lake types are present: deep lakes and shallow lakes.

Setting of reference values and calculation of EQR:

The reference values of MIL and MIB are set as the theoretical maximum values for the Croatian dataset (*Table 4*).

The calculation of EQR follows the equations:

For deep lakes:

$$EQR = MIL/MIL\_ref$$

For shallow lakes:

$$EQR = MIB/MIB\_ref$$

Setting of EQR boundaries:

The High/Good EQR boundary was derived as the 25th percentile of EQR variability at available spatial based benchmark sites. The remaining degradation continuum was divided into four equal width classes. The boundary values of multimetric indices and corresponding EQR values are shown in *Table 9*.

H/G boundary = 25th percentile of benchmark sites
G/M boundary = H/G * 0.75
M/P boundary = H/G * 0.50
P/B boundary = H/G * 0.25

Table 9. Boundary values of multimetric indices and corresponding EQR values.

	Class categories	MIL boundary values	EQR values
DEEP LAKES	HIGH	≥ 16.01	≥ 0.84
	GOOD	12.01-16.00	0.63-0.83
	MODERATE	8.01-12.00	0.42-0.62
	POOR	4.00-8.00	0.21-0.41
	BAD	<4.00	<0.21
	Class categories	**MIB boundary values**	**EQR values**
SHALLOW LAKES	HIGH	≥ 15.70	≥ 0.92
	GOOD	11.80-15.69	0.70-0.91
	MODERATE	7.80-11.79	0.46-0.69
	POOR	3.90-7.79	0.23-0.45
	BAD	<3.90	<0.23

Since the EQR boundaries should be harmonized across the EU countries, the boundaries of both indices were modified according to the suggestion of the cross-GIG Intercalibration group (*Table 10*; Kelly et al. 2014, Ács et al. 2015).

Table 10. Modified boundary values of multimetric indices and corresponding EQR values.

	Class categories	MIL boundary values	EQR values	Equation for EQR normalization
DEEP LAKES	HIGH	≥ 15.21	≥ 0.80	$EQR = 0.0526 \times MIL$
	GOOD	11.41-15.20	0.60-0.79	$EQR = 0.0526 \times MIL$
	MODERATE	7.61-11.40	0.40-0.59	$EQR = 0.0526 \times MIL$
	POOR	3.80-7.60	0.20-0.39	$EQR = 0.0526 \times MIL$

	BAD	<3.80	≤0.19	$EQR = 0.0526 \times MIL$
	Class categories	**MIB boundary values**	**EQR values**	**Equation for EQR normalization**
SHALLOW LAKES	HIGH	≥ 13.60	≥ 0.80	$EQR = 0.0588 \times MIB$
	GOOD	10.20-13.59	0.60-0.79	$EQR = 0.0588 \times MIB$
	MODERATE	6.80-10.19	0.40-0.59	$EQR = 0.0588 \times MIB$
	POOR	3.40-6.79	0.20-0.39	$EQR = 0.0588 \times MIB$
	BAD	<3.40	≤0.19	$EQR = 0.0588 \times MIB$

After the corrections EQR values are used as the national metric in comparison with the intercalibration common metric (ICM).

2.5. PRESSURES ADDRESSED

The different national methods of the MS of the completed intercalibration exercise were reported to mainly address eutrophication, since nutrients are acknowledged as the key factor determining outcomes in lakes (Kelly et al. 2014). Statistical analyses were performed to explore the responsiveness of the national diatom-based assessment method to various anthropogenic stressors.

The pressure-response relationships were tested via:
(1) non-parametric Spearman rank correlations of the national diatom metric (MIL) with environmental parameters (TP, TN, $N\text{-}NO_3^-$, $N\text{-}NO_2^-$, conductivity, alkalinity, suspended matter, COD-Mn, BOD_5, water temperature, dissolved oxygen, pH) and general land-use parameters (artificial areas, intensive agriculture, extensive agriculture, semi-natural areas).

(2) linear regressions of the national diatom metric (MIL) with pressure variables.

Table 11. Summary of the Spearman correlations of the national diatom metric (MIL) with different hydro-chemical, environmental and land-use pressures. Correlations marked in red are significant at $p < 0.05$.

	MIL
TP	-0.5161 p=0.2946
TN	0.6957 p=0.1248
$N\text{-}NO_3^-$	0.5429 p=0.2657
$N\text{-}NO_2^-$	-0.4414 p=0.3809
Conductivity	-0.1429 p=0.4247
Alkalinity	0.5429 p=0.2657
Suspended matter	0.1309 p=0.8047
COD-Mn	0.8804 p=0.0206
BOD_5	0.6000

	p=0.2080
Water temperature	-0.8286 p=0.0416
DO	0.6179 p=0.1911
pH	-0.4058 p=0.4247
Artificial areas [%]	-0.3479 p=0.4993
Intensive agriculture [%]	-0.3928 p=0.4411
Extensive agriculture [%]	0.1429 p=07872
Semi-natural areas [%]	0.1429 p=0.7872

The results of Spearman correlation of multimetric index MIL with pressure variables are shown in *Table 11*. The coefficient showed statistically significant relationships ($p<0.05$) between national metric and several different pressures. In general, lakes responded well to several pressures, in particular to water temperature and chemical oxygen demand (COD-M). Although no statistically significant relationships were found between MIL and some nutrient pressures (TN, $N\text{-}NO_3^-$), BOD_5 and alkalinity, they are as well shown due to relatively high R^2 values in linear regressions. The pressures that present the strongest relationships with the national metrics are presented in *Figures 1* and *2*.

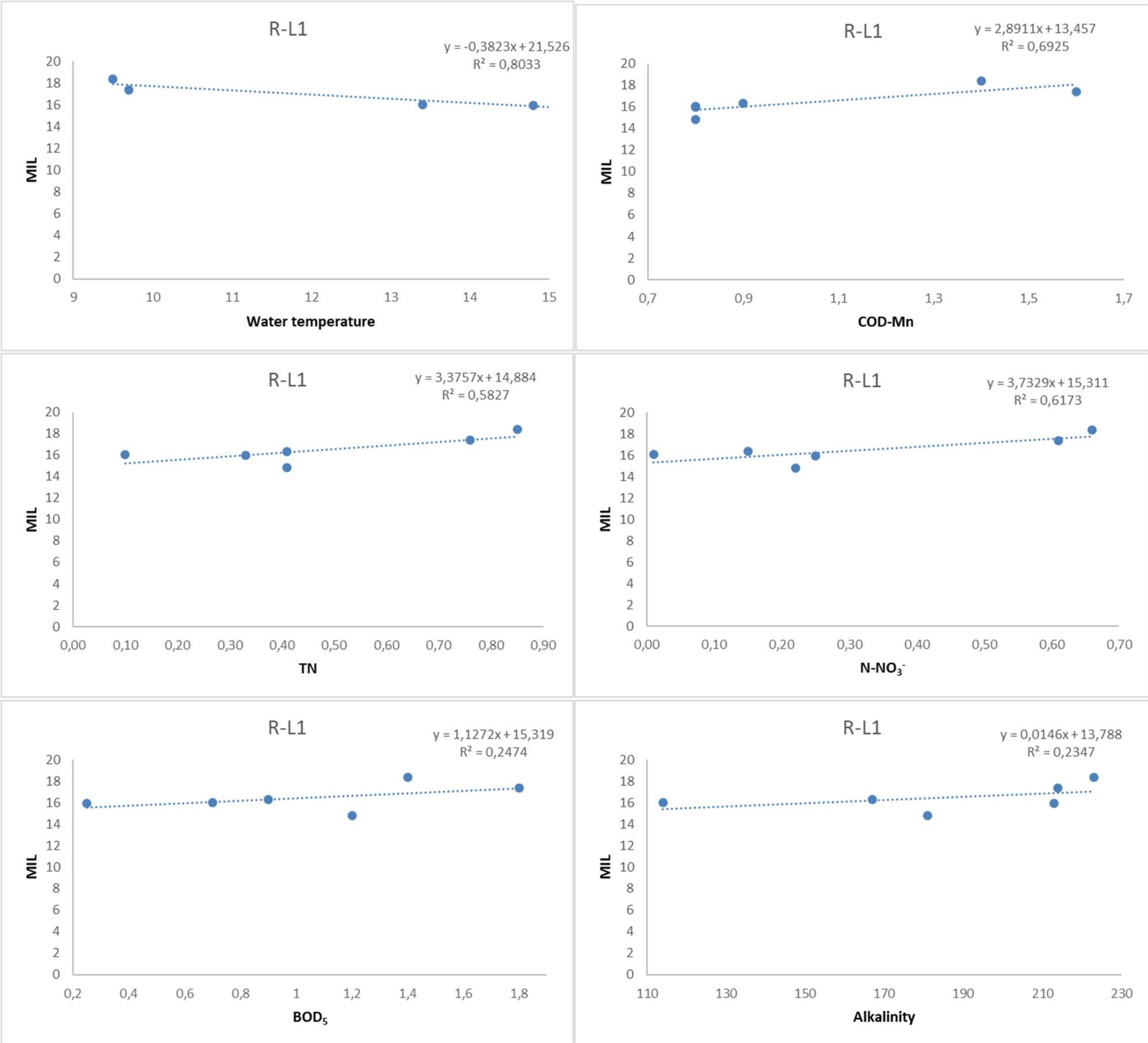


Figure 1. Pressure-response relationship between the most important pressures against the national metric (MIL).

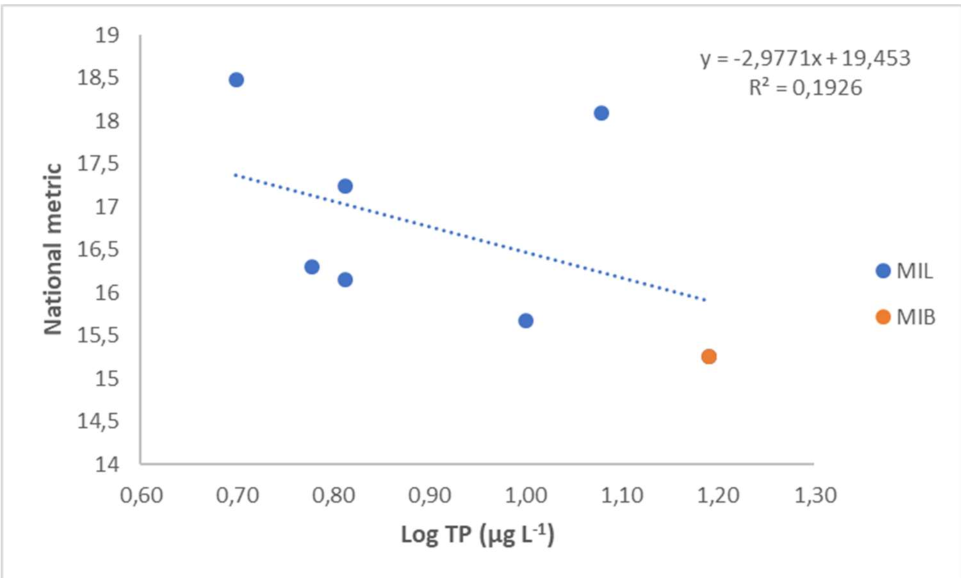
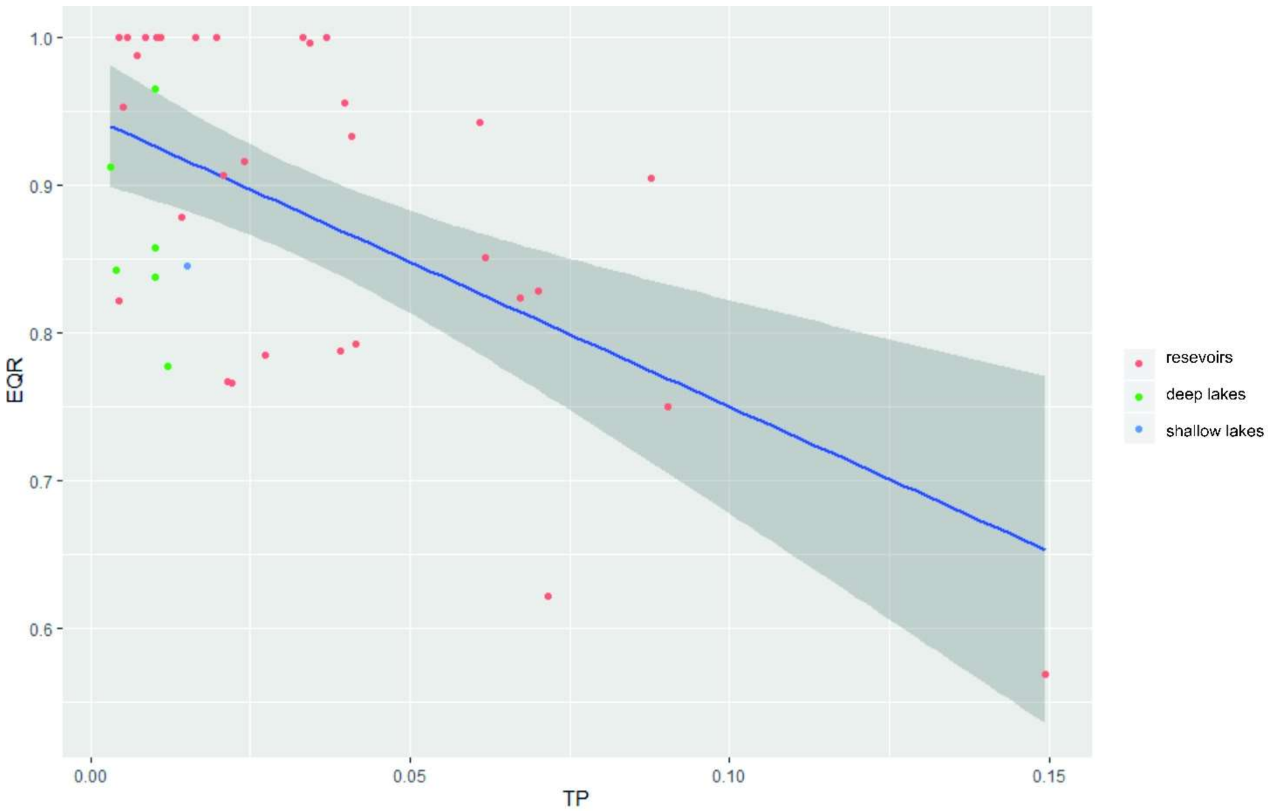


Figure 2. Pressure-response relationship between total phosphorus (Log TP) against the national metric (MIL for deep lakes; MIB for shallow lakes).

Furthermore, data from all Croatian lakes were treated together when building a linear regression. Most of the natural lakes in Croatia are in a very good or near natural states, so to give a gradient of pressure variables (total phosphorus, total nitrogen), man-made lakes (reservoirs) from the same geographic region were used in the regression by utilizing the Toolkit Nutrient software (Várbíró et al. 2018; *Figure 3*). For the pressure-response relationship between nutrients (total phosphorus and total nitrogen) against the national EQR (*Figure 3*), data from Croatian natural lakes (7) were treated together with data from Croatian reservoirs (31) included in the assessment of ecological potential (Mihaljević et al. 2018a, b).



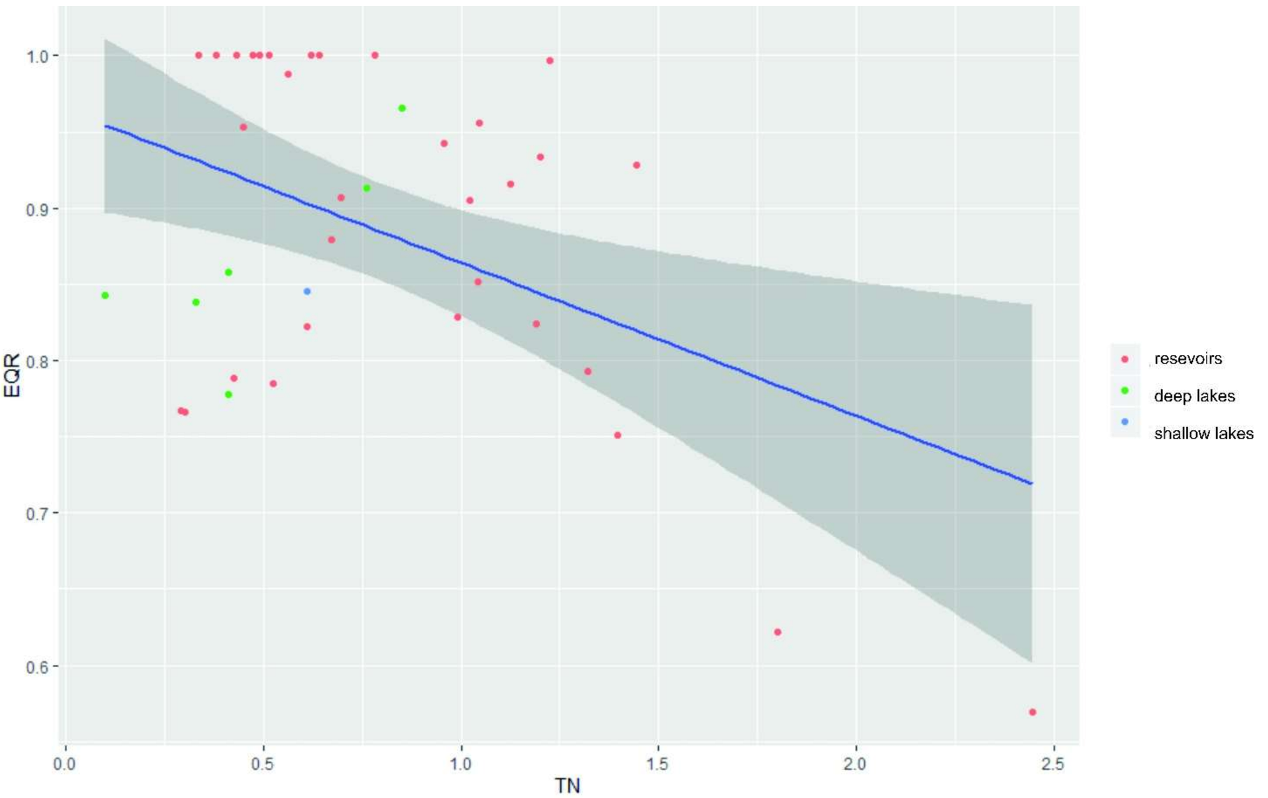


Figure 3. Pressure-response relationship between nutrients (total phosphorus and total nitrogen) against the national EQR.

Due to the small number of natural lakes in Croatia and limited availability of data for intercalibration (see *Table 7*), all Croatian lake data were incorporated into the Lake Phytobenthos cross-GIG dataset to get more representative and stronger pressure-response gradient (*Figure 4*).

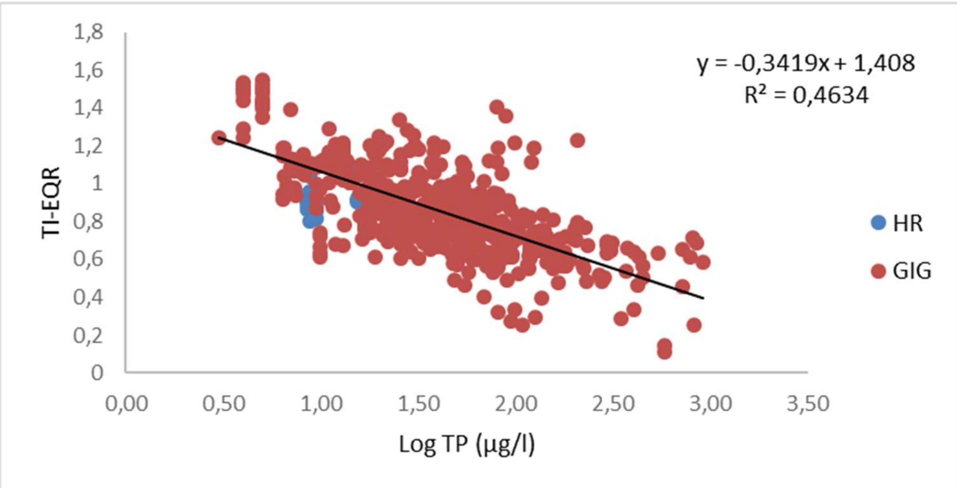


Figure 4. Pressure-response relationship between total phosphorus against the ICM (TI-EQR).

3. WFD COMPLIANCE CHECKING

The data acceptance criteria defined in the Lake Phytobenthos cross-GIG JRC Technical Report (Kelly et al. 2014) are listed in *Table 12*. The Croatian data fulfilled the listed criteria in all aspects except for the number of water quality classes represented in the dataset (last criterion). This criterion, however, is not so strictly relevant for the fit-in procedure. The dataset can be therefore considered sufficient for intercalibration.

Table 12. List of the WFD compliance criteria and the WFD compliance checking process and results

Compliance criteria	Compliance checking
Ecological status is classified by one of **five classes** (high, good, moderate, poor and bad).	Yes
High, good and moderate ecological status are set in line with the WFD's **normative definitions** (**Boundary setting procedure**)	Yes. Equidistant division of the EQR gradient. High-good boundary derived from metric variability at near-natural benchmark sites.
All relevant parameters indicative of the biological quality element are covered (see Table 1 in the IC Guidance). A **combination rule** to combine parameter assessment into BQE assessment has to be defined. If parameters are missing, Member States need to demonstrate that the method is sufficiently indicative of the status of the QE as a whole	Yes; both taxonomic composition and species relative abundance are taken into consideration. Diatom multimetric indices (MIL and MIB) - product of diatom indices which include relative abundance x sensitivity x tolerance/indicator value
Assessment is adapted to **intercalibration common types** that are defined in line with the typological requirements of the Annex II WFD and approved by WG ECOSTAT	Yes; common lake intercalibration type is used: high alkalinity (HA), L-M1
The water body is assessed against **type-specific near-natural reference conditions**	Yes
Assessment results are expressed as **EQRs**	Yes
Sampling procedure allows for **representative** information about water body quality/ecological status **in space and time**	Yes; 1 sampling per year during favourable and stable water level. Using brush/scraper for sampling. Single habitat(s) preferably epilithic phytobenthos – mesolithal (5 stones/cobbles from different points of streamline) or submerged parts of emerged macrophytes (e.g. *Phragmites australis*, *Scirpus lacustris*, *Cladium mariscus*) where there are no stones.
All data relevant for assessing the biological **parameters** specified in the WFD's normative definitions are covered by the **sampling procedure**	Yes.
Selected taxonomic level achieves adequate **confidence and precision** in classification	Yes; identification in species level or lower.

4. IC FEASIBILITY CHECKING

The intercalibration process ideally covers all national assessment methods within a GIG. However, the comparison of dissimilar methods ("apples and pears") has clearly to be avoided. Intercalibration exercise is focused on specific type / biological quality element / pressure combinations. The second step of the process introduces an "IC feasibility check" to restrict the actual intercalibration analysis to methods that address the same common type(s) and anthropogenic pressure(s), and follow a similar assessment concept.

4.1. TYPOLOGY

The intercalibration typological system of Lake Phytobenthos cross-GIG group was found to be the most appropriate for describing Croatian lakes, where common type: high alkalinity (HA), region: MED-GIG L-M1 is applicable for Croatia (*Tables 1, 2*).

4.2. PRESSURES ADDRESSED

Diatom assemblages as summarized by the national metrics (MIL and MIB) respond to several pressures, especially temperature, oxygen related pressures (namely COD-Mn) and nitrogen compounds. However, the feasibility check recognises that strong causal relationships with TP have not been demonstrated, even when treating Croatian natural lakes (7) together with Croatian reservoirs (31) in an attempt to give a gradient of pressure variables (TP and TN). Namely, according to the official report for the assessment of ecological potential of Croatian reservoirs (Mihaljević et al. 2018a, b):

- Only one sampling site was assigned in the Pannonian Ecoregion as "deep type reservoir". Thus, it is meaningless analysing correlations based on data of this site.

- In the shallow lakes of Pannonian Ecoregion, phytobenthic EQR correlated negatively to chemical oxygen demand (EQR – COD: r^2=0.2673). Besides, there was a slightly negative correlation between EQR and conductivity (EQR – COND: r^2=0.2139). In contrast, there was no correlation between EQR values and inorganic nitrogen and orthophosphate phosphorus (EQR – inorganic N: r^2=0.0237; EQR – PO_4^{3-}-P: r^2=0.0789).

- In the case of very shallow reservoirs of Pannonian Ecoregion, the correlations were relatively high between the algal EQR values and physical and chemical parameters. Strong negative relation could be observed only between EQR values and COD (EQR – COD: r^2=0.691; EQR – inorganic N: r^2=0.6573; EQR – PO_4^{3-}-P: r^2=0.3508). In contrast, strong, but a positive correlation was found between both EQR and conductivity (EQR – COND: r^2=0.1619).

- Because of the high EQR values in deep reservoirs of Dinaric Ecoregion (EQR_{min} = 0.997, EQR_{max} = 1), it is meaningless to analyse the correlations between these values and physical and chemical parameters.

- In the case of shallow lakes of Dinaric Ecoregion, there was weak or even positive correlation between EQR and physical and chemical parameters (weak: EQR – COD: r^2=0.0076; EQR – inorganic N: r^2=0.0219; positive: EQR – PO_4^{3-}-P: r^2=0.5429). The EQR correlated negatively only with COND (EQR – COND: r^2=0.3845).

- Because only three water bodies were assigned in the Dinaric Ecoregion into the "very shallow type reservoirs", and the differences between min and max EQR values and also between the min and max values of physical and chemical parameters were low, there were either weak or strong positive correlations between EQR and the parameters (weak: EQR – COD: r^2=0.0164; EQR – COND: r^2=0.0056; positive: EQR – inorganic N: r^2=0.9944; EQR – PO_4^{3-}-P: r^2=0.9878).

As shown above, no clear pressure-response relationship between nutrients (N and P) and the EQR values was shown for the reservoirs (Mihaljević et al. 2018a, b). Moreover, the EQR values at reservoirs range between 0.6-1 with no representation of lower water quality classes. Extending the lakes dataset with reservoirs from the Dinaric Ecoregion only, as they have similar conditions to those in natural lakes, gives us only five more points (samples) for deep lakes and 4 more points for shallow lakes. Also, the problem is very high EQR values of deep reservoirs (EQR_{min} = 0.997, EQR_{max} = 1). At present, the alternative approach (i.e. using a several years dataset from natural lakes) is not feasible, since the new methodology for sampling was adopted and exercised only starting from 2019. Even if more data were available, the same problem with short gradient would likely restrain us from achieveing significant pressure-response relationships, as well as water quality classes gradient.

4.3. ASSESSMENT CONCEPT

The national diatom-based assessment system consists of two multimetric indices – MIL (for deep lakes and MIB (for shallow lakes), which are based on diatom indices IBD, EPI-D and $TDIL_{1-20}$. These indices take into consideration tolerance and sensitivity of diatom species present in the assemblage and their relative abundances. The indices responded to several pressures addressed (see above Section "2.4. Pressures addressed").

4.4. CONCLUSION ON THE INTERCALIBRATION FEASIBILITY

The LM typology was chosen. The multimetric indices MIL (for deep lakes) and MIB (for shallow lakes) consist of diatom indices IBD, EPI-D and $TDIL_{1-20}$, which take into consideration tolerance and sensitivity of diatom species present in the assemblage and their relative abundances. The proposed indices have already been intercalibrated (Kelly et al. 2014). It is concluded that the fitting of MIL and MIB indices to the results of the Croatian MED-GIG lakes intercalibration was feasible.

5. DEMONSTRATING THE COMPLIANCE WITH THE COMPLETED INTERCALIBRATION EXERCISE

For the fit-in procedure, the original X-GIG phytobenthos exercise used “Case A2” from the CIS Guidance No. 30 (Willby et al. 2014), where continuous benchmarking was applied. The “Case A2” requires pressure data in the same format as that used in the completed exercise, with an assumption (though not explicitly stated in the Guidance document) that there is a significant pressure-response relationship between pressure (TP) and EQR. However, in the HR feasibility check of pressures addressed (Section 4.2.) the biological samples were strongly influenced by COD, and no strong causal relationships with TP have been demonstrated. Therefore, “Case A1” has been applied instead for fitting the HR assessment method using phytobenthos to the results of the Lake Phytobenthos cross-GIG. We fully acknowledge that this may not be the best fit-in procedure, but, in the light of problems identified in the feasibility check, it provides an approximate evaluation of HR boundaries in relation to those of other countries.

The requirements for case A1 are:

- *Full details of the common metric*

  The IC common metric applied in the Lake Phytobenthos cross-GIG is composed of one diatom metric (according to Kelly et al. 2014):

  - The Trophic index (TI) (Rott et al. 1999): a trophic index based on a weighted average equation: all taxa are given a sensitivity score, depending on the optimum nutrient concentration under which they are found in nature. The TI is the average of the sensitivities of all taxa present, “weighted” by their relative abundance (so a common nutrient-sensitive taxon will have more influence on the final index value than a nutrient-tolerant taxon that is only sparsely represented in the sample)

    ICM = TI-EQR

- *A suitable site x biology dataset covering a range of environmental quality from which the national EQR and common metric can be calculated*

  A total of 21 biological samples were available (see Section "2.3. National boundary setting")

- *Accompanying pressure data in the same format as that used in the completed exercise.*

  All accompanying pressure data are available (see *Table 9*).

- *Information on the specific thresholds already used in the completed exercise to define reference or alternative benchmark sites*

  The benchmark criteria of abiotic parameters (land-use and hydrochemical criteria) were adopted and adapted from the the Mediterranean lakes GIG (de Hoyos et al. 2014) and the Eastern Continental lakes GIG (Borics et al. 2018) (see Section "2.4. National reference conditions").

- *Details of exactly how benchmarking was undertaken in the complete exercise. If the completed exercise concluded that benchmarking was not necessary the mean value of the benchmark sites from each country must be provided so that the joining Member State can also judge the need to benchmark its own method.*

  Given benchmark criteria were applied by each MS in order to identify benchmark sites within each national dataset. The 25th percentile of MIL (for deep lakes) and MIB (for shallow lakes) of the national benchmark dataset were used for setting the H/G boundary and translation to the national EQR H/G boundary. Likewise, the 25th percentile of TI (Rott et al. 1999) of the benchmark sites was set as a reference value for the calculation of national TI-EQR. Linear regression was established between values of the national method (EQR) and the ICM (TI-EQR) so that the national boundaries could be translated to ICM using the equation.

- *Values of the global mean view of the HG and GM boundaries on the common metric scale for Member States who participated in the completed exercise.*

  The average boundaries, as TI-EQR, are 0.965 (high/good) and 0.790 (good/moderate).

The process of fitting the HR method to the completed IC exercise:
According to the Willby et al. (2014), the following steps should be followed:

i. *Calculate the common metric (CM) on the national dataset.*

The ICM applied in the cross-GIG is composed of one diatom metric (according to Kelly et al. 2014):

- TI (Rott et al. 1999): a trophic index based on a weighted average equation: all taxa are given a sensitivity score, depending on the optimum nutrient concentration under which they are found in nature. The TI is the average of the sensitivities of all taxa present, “weighted” by their relative abundance:

  TI-EQR = (4-observed value) / (4-reference value)

ICM = TI-EQR

ii. *Use the associated pressure data to identify sites in the national dataset that meet the criteria established by the GIG for the selection of benchmark or reference sites.*

Benchmark sites have been identified based on environmental pressures above (see Section "2.3 National boundary setting")

iii. *Standardize the common metric (CM_bm) against the benchmark according to the approach used in the completed exercise.*

The common metric was calculated for the benchmark sites in the national dataset. For the IC lake type L-M1 the median was ICM_LM1=0.92. These values were inside the range of the median values of the MS who took part in the intercalibration exercise.

iv. *Use OLS regression to establish the relationship between CM_bm (y) and the EQR of the joining method (x).*

Since Croatian deep and shallow lakes belong to the same IC type (L-M1), they were treated together. Therefore, for the translation of reference and boundary positions of the national method onto the ICM scale the presented linear regression equation was used. Relationship between national EQR and ICM (TI-EQR) for the Croatian shallow and deep lakes in the HA L-M1 lake type is shown in *Table 13* and *Figure 5*.

Table 13. OLS equations for the relationship between ICM and national EQR.

IC Lake type	No of samples	No of sites	Linear regression	R^2
L-M1	21	7	TI-EQR = 0,9222 x EQR + 0,1735	R^2 = 0,3588

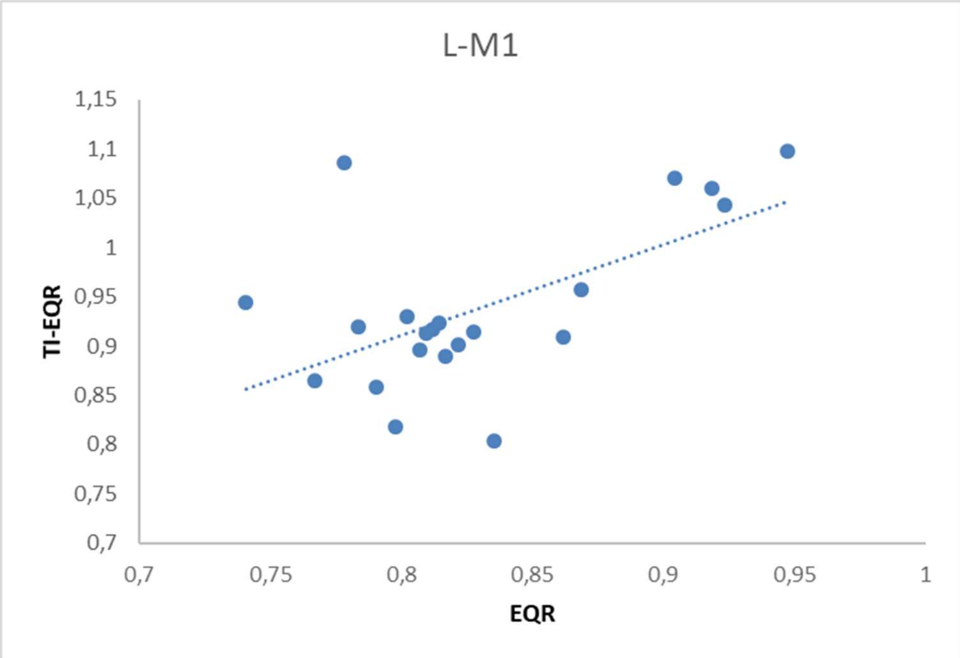


Figure 5. OLS regressions to establish the relationship between ICM (TI-EQR) and the national EQR for Croatian lake type L-M1.

As was the case with pressure-response, in checking the relationship between the national metric and the ICM (TI-EQR), the similar procedure of incorporating the Croatian results into the cross-GIG group dataset was also done (*Figure 6*).

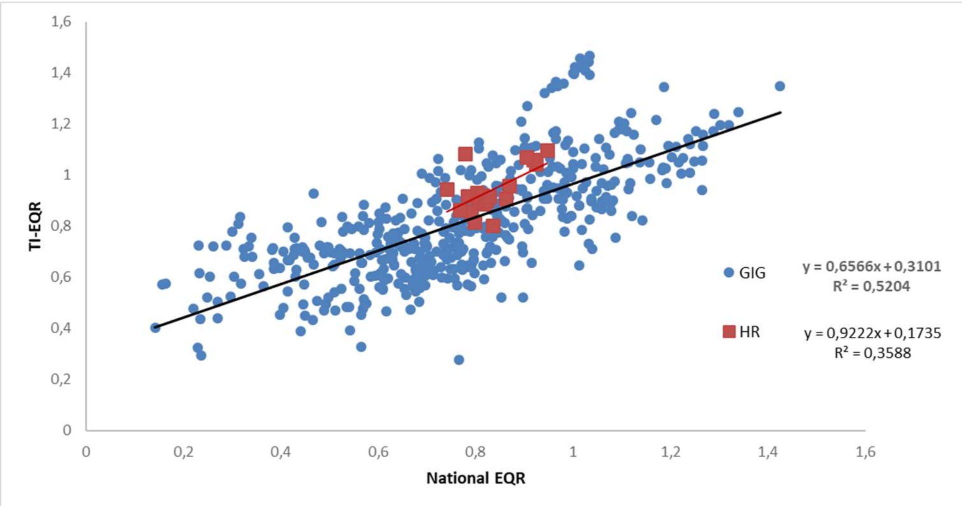


Figure 6. OLS regressions of the relationship between ICM (TI-EQR) and the national EQR together with the cross-GIG dataset.

v. *Predict the position of the national class boundaries (MP, GM, HG and reference) on the CM_bm scale.*

The prediction of the class boundaries on the CM scale was made using the OLS equations of the relationship between the national and the common metric (*Table 14*).

Table 14. Translation of the reference and boundary positions of the national method on the basis of OLS regression (see *Figure 5, Table 13*) into ICM.

IC Type	**L-M1**	
Boundary	EQR	Predicted boundaries on ICM scale
Reference	1.00	1.096
High / good	0.80	0.911
Good / moderate	0.60	0.727
Moderate / poor	0.40	0.542
Poor / bad	0.20	0.358

Table 15. Typological codes used in the boundary bias analysis.

Code	***Member State***
BE	BELGIUM
DE	GERMANY
IE	IRELAND
SE	SWEDEN
UK	UNITED KINGDOM
SI	SLOVENIA
HR	CROATIA

Table 16. Relationship between national metric and common metric (TI_EQR) for HA lakes.

	BE	**DE**	**IE**	**SE**	**UK**	**SI**	**HR**
Intercept (c)	0.152	0.529	0.303	-0.187	0.320	0.320	0.173
Slope (m)	1.017	0.504	0.748	1.252	0.717	0.858	0.922
Pearson's r	0.877	0.774	0.887	0.634	0.936	0.940	0.599
R^2	0.769	0.598	0.786	0.403	0.876	0.884	0.359

The explanation of the typological codes used is given in *Table 15*. Boundaries were compared using IC option 2 with a boundary translation to common metric (TI-EQR). The relationship between national metric and common metric (TI-EQR) for HA lakes is given in *Table 16*.

The outcomes of the regression complied with the following characteristics according to the IC Guidance (Kelly et al. 2014):

- All relationships were highly significant p<=0.001;
- Assumptions of normally distributed error and variance (homoscedasticity) of model residuals were met;
- Common metric represented all methods (r>0.5);
- Observed minimum r^2 > half of the observed maximum r^2 – this criterion is not fulfilled as min r^2 0.36< max r^2 0.88/2, but maximum r^2 may be artificially high as some MS use the intercalibration metric (TI) as their national metric;
- Slopes of the regression lie between 0.5 and 1.5.

The comparison of H/G and G/M original boundary values for the HA Croatian lakes of the L-M1 type with other HA lakes of the cross-GIG intercalibration is presented in *Figures 7* and *8*.

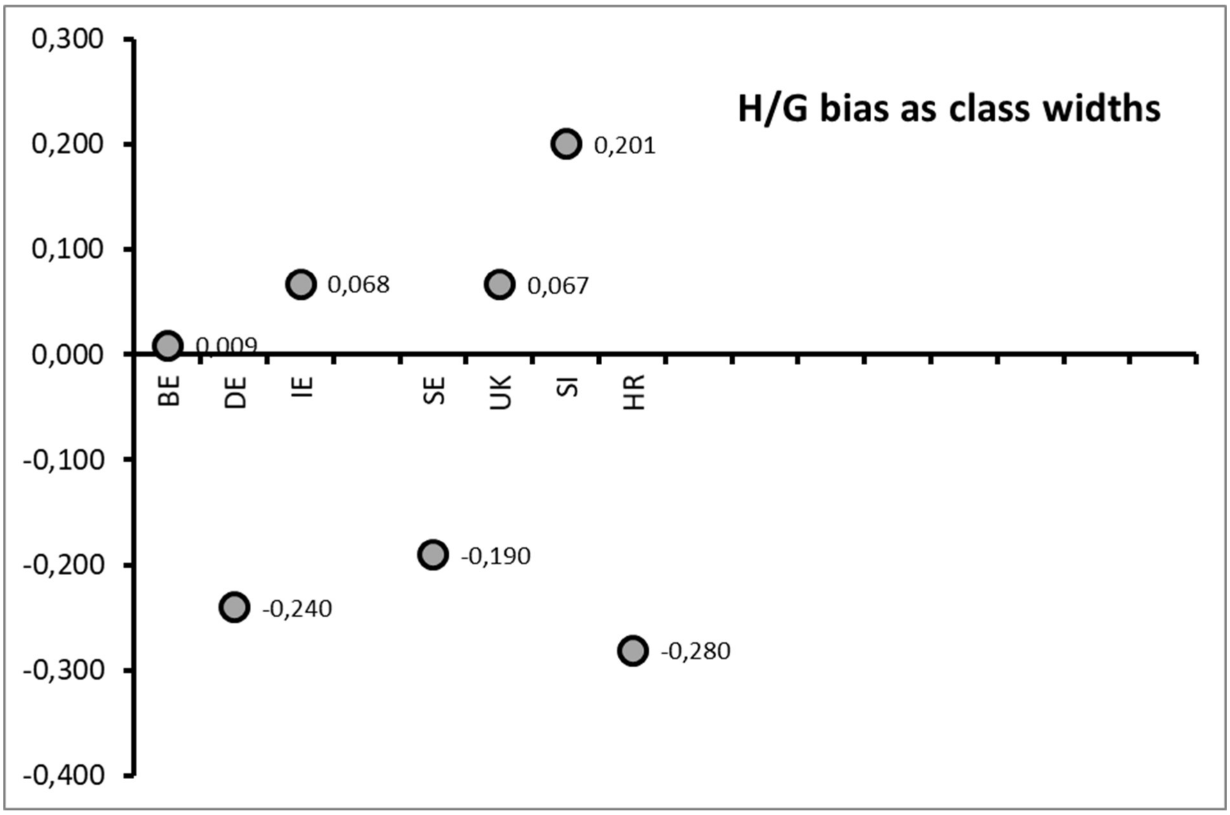


Figure 7. Comparison of H/G original boundaries for the HA lakes.

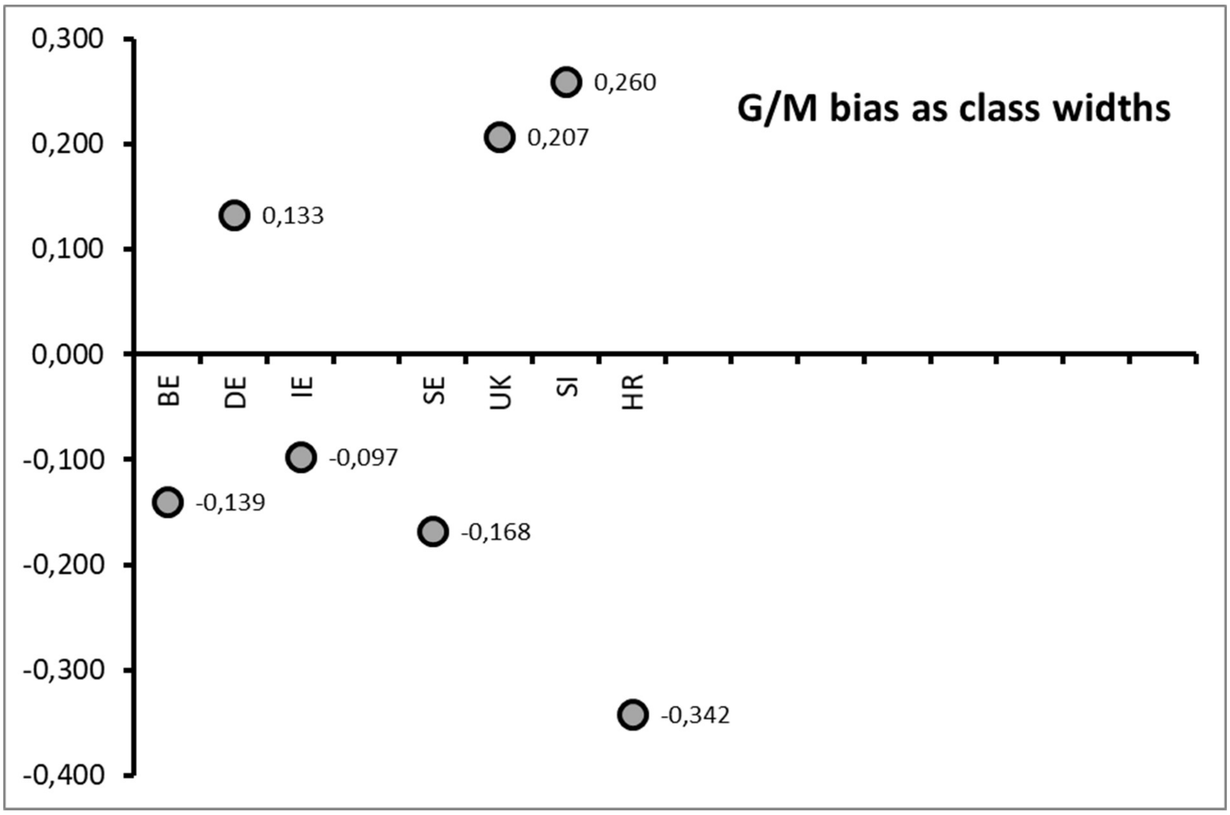


Figure 8. Comparison of G/M original boundaries for the HA lakes.

vi. Apply the comparability criteria as summarized in Chapter 6.

The adjustment of the boundaries follows the fit according to the guidance of chapter 6 (Willby et al. 2014). The main principle is that H/G or G/M statistic must not be >|0.25|. Both H/G and G/M boundary biases in HR L-M1 lake types were >|0.25| (*Table 17* in red) and thus adjustment in these boundaries was required by adding a value to the respective H/G and G/M boundaries until they reached the appropriate limit. The final boundaries adopted after the harmonization are presented in *Tables 18* and *19*. The comparison of H/G and G/M adjusted boundary values for the HA Croatian lakes of the L-M1 type with other HA lakes of the cross-GIG intercalibration is presented in *Figures 9* and *10*.

Table 17. High/Good and Good/Moderate class boundary derived from the OLS regression for HA lake types of the cross-GIG group and Croatian HA lakes of the L-M1 type. Red color represents statistic bias >|0.25|.

	BE	DE	IE	SE	UK	SI	HR
Max	1.000	1.000	1.170	1.000	1.283	1.050	1.000
H/G	0.800	0.800	0.900	0.890	0.920	0.800	0.800
G/M	0.600	0.550	0.630	0.740	0.700	0.600	0.600
M/P	0.400	0.330	0.420	0.500	0.460	0.400	0.400
P/B	0.200	0.100	0.210	0.250	0.230	0.200	0.200
CM_Max +Offset	1.168	1.034	1.179	1.065	1.241	1.220	1.096
CM_H/G +Offset	0.965	0.933	0.977	0.927	0.980	1.006	0.911
CM_G/M +Offset	0.762	0.807	0.775	0.739	0.823	0.834	0.727
CM_M/P +Offset	0.558	0.696	0.617	0.439	0.650	0.663	0.542
CM_P/B +Offset	0.355	0.580	0.460	0.126	0.485	0.491	0.358
H width to Max	0.203	0.101	0.202	0.138	0.261	0.214	0.184
G width	0.203	0.126	0.202	0.188	0.158	0.172	0.184
M width	0.203	0.111	0.157	0.300	0.172	0.172	0.184
H/G bias	0.002	-0.030	0.014	-0.036	0.017	0.043	-0.052
G/M bias	-0.028	0.017	-0.015	-0.050	0.033	0.045	-0.063
H/G bias_CW	0.009	-0.240	0.068	-0.190	0.067	0.201	-0.280
G/M bias_CW	-0.139	0.133	-0.097	-0.168	0.207	0.260	-0.342

Table 18. Harmonized High/Good and Good/Moderate class boundary derived from the OLS regression for HA lake types of the cross-GIG group and Croatian HA lakes of the L-M1 type. Green color represents statistic bias of HR boundaries after the harmonization.

	BE	DE	IE	SE	UK	SI	HR
Max	1.000	1.000	1.170	1.000	1.283	1.050	1.000
H/G	0.800	0.800	0.900	0.890	0.920	0.800	0.810
G/M	0.600	0.550	0.630	0.740	0.700	0.600	0.620
M/P	0.400	0.330	0.420	0.500	0.460	0.400	0.400
P/B	0.200	0.100	0.210	0.250	0.230	0.200	0.200
CM_Max +Offset	1.168	1.034	1.179	1.065	1.241	1.220	1.096
CM_H/G +Offset	0.965	0.933	0.977	0.927	0.980	1.006	0.920
CM_G/M +Offset	0.762	0.807	0.775	0.739	0.823	0.834	0.745
CM_M/P +Offset	0.558	0.696	0.617	0.439	0.650	0.663	0.542
CM_P/B +Offset	0.355	0.580	0.460	0.126	0.485	0.491	0.358

H width to Max	0.203	0.101	0.202	0.138	0.261	0.214	0.175
G width	0.203	0.126	0.202	0.188	0.158	0.172	0.175
M width	0.203	0.111	0.157	0.300	0.172	0.172	0.203
H/G bias	0.002	-0.030	0.014	-0.036	0.017	0.043	-0.042
G/M bias	-0.028	0.017	-0.015	-0.050	0.033	0.045	-0.045
H/G bias_CW	0.009	-0.240	0.068	-0.190	0.067	0.201	-0.242
G/M bias_CW	-0.139	0.133	-0.097	-0.168	0.207	0.260	-0.220

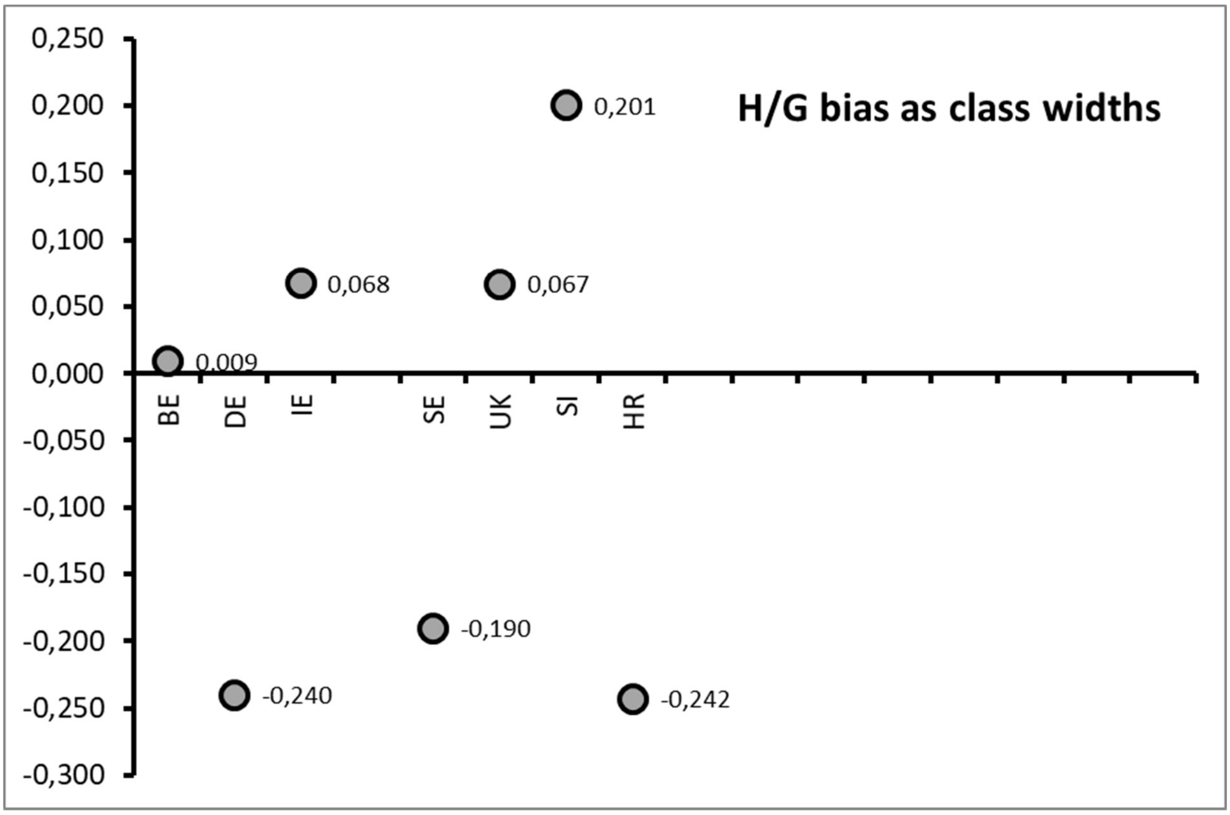


Figure 9. Comparison of H/G harmonized boundaries for the for the HA lakes.

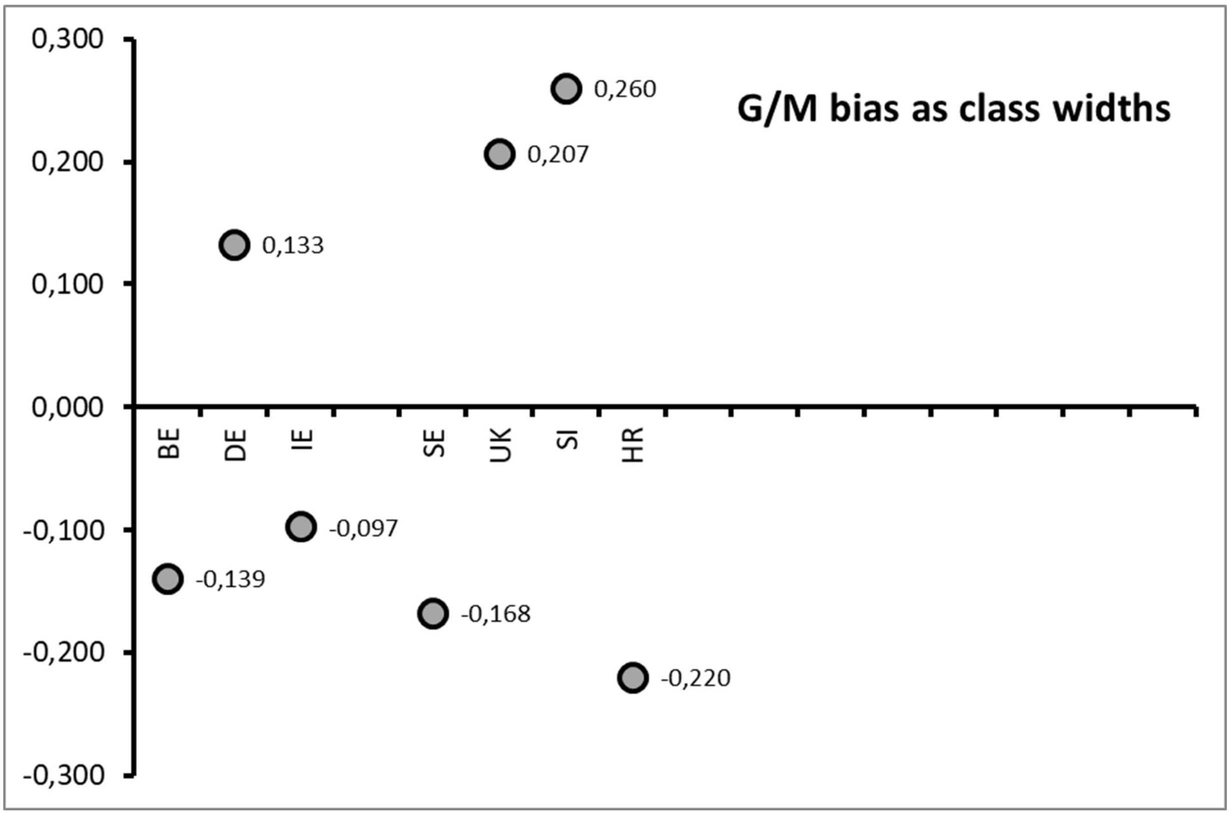


Figure 10. Comparison of G/M harmonized boundaries for the for the HA lakes.

Table 19. Final class boundaries adopted for the national metric and the ICM.

	Boundary	ICM Original	ICM Harmonized	National Original	National Harmonized
HR L-M1	Reference	1.096	1.096	1.00	1.00
	H/G	0.911	0.920	0.80	0.81
	G/M	0.727	0.745	0.60	0.62

Concluson

This report documents the fitting procedure of the Croatian phytobenthos-based assessment method for the HA lakes of the L-M1 type to the results of the completed cross-GIG lakes phytobenthos intercalibration.

We documented IC feasibility and compliance of the presented assessment method. Following the criteria and steps defined in the fit-in-procedure of Willby et al. (2014), both the high-good boundary and the good-moderate boundary in the lake types L-M1 required adjustments. After adjustment of the aforementioned boundaries, the national assessment method is considered comparable with the already intercalibrated methods and meets the comparability criteria. It is recommended to submit the method to the ECOSTAT group to recognize that the HR lake method cannot be intercalibrated and is asked to accept the revised boundaries (Table 19).

6. DESCRIPTION OF THE BIOLOGICAL COMMUNITIES

Diatom communities dissimilarity in different ecological status conditions was evaluated similarly to the rivers EC-GIG and rivers MED-GIG intercalibration exercise. The SIMPER analysis (log transformation of abundance data, Bray-Curtis similarity; up to 90% of contribution to av. similarity, Primer v7; Clarke & Gorley 2015) was used to determine the diatom species contributing the most (up to 90% of cumulative contribution) to the average dissimilarity between the sites classified as high and good and to the average similarity of the different status classes.

Three species are contributing the most in the observed similarity, while the rest significantly contributing species presented a low contribution (*Table 20*). Group similarities were relatively low, indicating a high within ecological status level variability. *Achnanthidium minutissimum* (Kützing) Czarnecki, *Encyonopsis microcephala* (Grunow) Krammer and *Fragilaria delicatissima* (W.Smith) Lange-Bertalot were mainly responsible for the within group similarity for high and good ecological status, and to some extent *Gomphonema lateripunctatum* E.Reichardt & Lange-Bertalot. Dissimilarity between different ecological groups is also presented (*Table 21*). The two groups differed by the contribution of *Denticula tenuis* Kützing, *Cymbella subhelvetica* Krammer and *Delicata delicatula* (Kützing) Krammer to high status group, and *Navicula cryptotenelloides* Lange-Bertalot, *Brachysira* sp. Kützing and *Achnanthidium exile* (Kützing) Heiberg contributing to good status group. The contribution of *A. minutissimum* as a strong indicator of high status, though also contributing at good and moderate status, for HA lakes has been reported in the cross-GIG intercalibration (Kelly et al. 2014).

Table 20. Species contribution to similarity within and dissimilarity between ecological status levels. The most contributing species (up to 90% contribution) are presented.

Group High Status Average similarity: 38.35 **Species**	**Average Abundance**	**Contribution %**	**Cumulative contribution %**
Encyonopsis microcephala	3.96	15.20	15.20
Achnanthidium minutissimum	3.98	15.08	30.28
Fragilaria delicatissima	3.23	11.21	41.49
Cymbella vulgata	1.78	5.65	47.14
Gomphonema lateripunctatum	1.91	5.42	52.56
Navicula cryptotenella	1.33	3.88	56.43
Nitzschia sp.	1.27	3.62	60.05
Navicula subalpina	1.20	2.95	63.00
Denticula tenuis	1.40	2.13	65.13
Cymbella subhelvetica	1.21	1.97	67.10
Cymbella lange-bertalotii	1.08	1.87	68.96
Delicata delicatula	1.41	1.84	70.80
Encyonopsis krammeri	1.46	1.79	72.59
Amphora pediculus	1.04	1.73	74.32
Navicula cryptotenelloides	1.22	1.67	75.99
Encyonopsis cesatii	0.87	1.62	77.60
Cyclotella pseudostelligera	1.09	1.46	79.06
Brachysira neglectissima	1.20	1.32	80.38
Cocconeis placentula var. placentula	0.60	1.28	81.66
Achnanthidium caledonicum	1.06	1.19	82.86
Encyonema ventricosum	0.69	1.08	83.93
Cyclotella plitvicensis	0.85	1.07	85.00
Gomphonema sp.	0.81	0.92	85.92
Adlafia bryophila	0.65	0.74	86.66
Cyclotella distinguenda	0.65	0.72	87.39
Navicula dealpina	0.35	0.72	88.11
Fragilaria pinnata	0.71	0.71	88.82
Fragilaria vaucheriae	0.58	0.64	89.46
Cymbella cymbiformis	0.52	0.64	90.09

Group Good Status Average similarity: 41.66 **Species**	**Average Abundance**	**Contribution %**	**Cumulative contribution %**
Achnanthidium minutissimum	4.20	16.99	16.99

Fragilaria delicatissima	4.26	16.95	33.95
Encyonopsis microcephala	3.90	15.43	49.38
Cyclotella distinguenda	1.72	4.76	54.14
Gomphonema lateripunctatum	1.63	4.44	58.58
Navicula cryptotenelloides	1.76	4.06	62.64
Cyclotella pseudostelligera	1.70	3.73	66.37
Brachysira sp.	1.92	3.70	70.07
Nitzschia sp.	1.26	2.85	72.91
Cymbella vulgata	1.19	2.78	75.70
Navicula subalpina	1.18	2.73	78.43
Cymbella lange-bertalotii	0.97	2.39	80.82
Navicula cryptotenella	1.42	1.97	82.79
Gomphonema sp.	0.90	1.63	84.42
Fragilaria vaucheriae	1.03	1.25	85.67
Achnanthidium exile	1.16	0.77	86.44
Amphora pediculus	0.88	0.68	87.12
Cymbella affiniformis	0.79	0.68	87.8
Nitzschia recta	0.55	0.65	88.44
Fragilaria tenera	0.86	0.64	89.08
Achnanthes sp.	0.70	0.63	89.71
Encyonopsis cesatii	0.67	0.62	90.33

Table 21. Species contribution to dissimilarity between ecological status levels. The 23 most contributing species are presented.

Groups High & Good Status Average dissimilarity = 61.82 **Species**	**Group Good Average Abundance**	**Group High Average Abundance**	**Contribution %**	**Cumulative contribution %**
Navicula cryptotenelloides	1.76	1.22	2.34	2.34
Brachysira sp.	1.92	0.59	2.33	4.68
Cyclotella pseudostelligera	1.70	1.09	2.32	7.00
Cyclotella distinguenda	1.72	0.65	2.17	9.17
Encyonopsis krammeri	0.5	1.46	2.10	11.27
Delicata delicatula	0	1.41	1.93	13.20
Denticula tenuis	0	1.40	1.90	15.10
Achnanthidium caledonicum	0.62	1.06	1.90	17.00
Fragilaria delicatissima	4.26	3.23	1.86	18.86
Navicula cryptotenella	1.42	1.33	1.79	20.65
Gomphonema lateripunctatum	1.63	1.91	1.77	22.42
Brachysira neglectissima	0.20	1.2	1.75	24.17
Achnanthidium exile	1.16	0	1.72	25.89
Cymbella subhelvetica	0.16	1.21	1.65	27.54
Amphora pediculus	0.88	1.04	1.63	29.17
Cyclotella pseudocomensis	0.66	0.73	1.61	30.77
Gomphonema calcareum	0.66	0.78	1.55	32.33
Fragilaria pinnata	0.84	0.71	1.54	33.86
Encyonopsis subminuta	1.05	0.38	1.51	35.37
Cyclotella ocellata	1.02	0.36	1.48	36.85
Fragilaria tenera	0.86	0.62	1.48	38.33
Gomphonema sp.	0.90	0.81	1.45	39.79
Cymbella vulgata	1.19	1.78	1.45	41.24

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Report on Croatian classification method for macrophytes in lakes in the case where the Intercalibration exercise is not possible (Gap 3)

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Report on Croatian classification method for macrophytes in lakes in the case where the Intercalibration exercise is not possible (Gap 3)

1. INTRODUCTION

- Croatia
- Macrophytes
- Lakes

2. DESCRIPTION OF NATIONAL ASSESSMENT METHODS

For assessment of ecological status based on biological element macrophytes Biocenological index (BM_{HR}) is used. The metric is based on degradation level determination of assumed referent type specific macrophyte community.

2.1. METHODS AND REQUIRED BQE PARAMETERS

Table 1. Overview of the metrics included in the national method

BM_{HR}	Taxonomic composition	Abundance	Additional
Coverage of colonized bottom with stonewort meadows (%)	*	*	Important habitat and key stone species for Croatian lakes
Number of characteristic species	*		Diversity important indicator of lake condition
Number of characteristic vegetation structures (communities, belts)**	*	*	Structure of macrophyte community important indicator of lake condition
Mean macrophyte depth limit (m)		*	Indicative of light climate, key indicator of lake condition with respect to eutrophication

Each component is assigned a BM score 1 – 5 representing one of the WFD classes (1 = Bad to 5 = High)

Scores are summarised by averaging and converted to an EQR by dividing by a reference BM score of 5, the value of BM if all components were considered to be at High status and thus a clear indicator of reference state.

EQR of Biocenological index (BM_{HR}) is used for BQE assessment.

The method is WFD compliant, in terms of the indicative parameters included.

2.2. SAMPLING AND DATA PROCESSING

Description of sampling and data processing:

- Sampling time and frequency

  Macrophyte sampling should be conducted during summer and early autumn when macrophytes are optimally developed, i.e. from June to September, with July and August being optimal for sampling. Premature sampling may cause the following difficulties:
  - since the plants are still not optimally developed or they have only started developing, their estimated numbers will be lower,
  - identification of incompletely developed plants is very difficult or even impossible.

  Delayed sampling is also not recommended, since the vegetative parts of many species disappear before winter, and the plant survives in the form of its permanent organs.

  In the optimal sampling period, sampling must not be performed during floods. A period of at least four weeks must pass between a flood occurrence and macrophyte sampling.
- Sampling method

  Macrophytes are estimated from a boat or by means of diving along 2 - 6 m wide transects, i.e. 1 - 3 m on each boat side.
  Transects are vertical to the bank, spreading from it to the macrophyte depth limit.
  Depending on the lake size and diversity of macrophyte vegetation, sampling is performed every 5 - 10 m, while the coordinate of each point is recorded by a GPS device. In the case of lakes whose enitire bottom surface is overgrown with macrophyte vegetation, transects are defined transversally over the entire lake, and the number of sampling points is determined as 15% of the total lake width.
  Transects can also be divided into different depth zones that correspond with different macrophyte communities, the appearance or disappearance of certain species or a more significant change in their abundance.
  Transects must be selected in a homogenous area which is representative of the general lake conditions.
  At least three samples are taken in each point to collect all species, if possible.
  To estimate abundance, a tube with a glass or a similar device must be used to enable underwater viewing.
  In each point, depth must be measured with an echo-sounding device and transparency with a Secchi disc, while the complete list of macrophyte and macroalgae species must be made and their abundance estimated using Kohler's scale (Table 2.). Samples must be identified to the species level if all identifying characteristics are present. Abundances of individual species estimated using Kohler scale should be summarized in total cover of macrophyte (charophyte) vegetation, what is asked in particular tables.
  Sampling is carried out by means of bottom grabbers, rakes attached to a rope or a handle (depending on water depth) or directly by means of diving. The number of transects depends on the lake size (Table 3.).

Table 2. Kohler's scale (1978) to assess aquatic macrophyte abundance

Level	Description	Explanation
1	Very rare, scattered	Only individual plants, up to 5 units
2	Rare	Approx. 6-10 units, loosely distributed over the surveyed area or up to 5 individual stands
3	Common	Cannot be foreseen, but not an abundant species; "can be found without being specifically looked for"
4	Abundant	Abundant species, but not in masses; incomplete coverage with large gaps (25 - 50%)
5	Highly abundant, in masses	Dominant species, present more or less everywhere; coverage significantly higher than 50%

Table 3. Transect number for macrophyte sampling depending on the lake surface

Lake surface (km^2)	No. of transects	Lake type	Lake name and surface
0,5	1 - 6	HR-J_3	Crniševo – 0,877 km^2
0,5 - 2	4 - 8	HR-J_1A HR-J_1B	Kozjak - 0,815 km^2 Prošće - 0,697 km^2
2 - 5	5 - 10		
5 - 10	10 - 20	HR-J_2 HR-J_5	Vrana/Cres - 5,75 km^2 Visovac – 5,72 km^2
> 10	15 - 25	HR-J_4	Vransko/Biograd – 30 km^2

- Data processing

Taxonomic and ecological groups that are sampled

In terms of taxonomic groups, aquatic macrophytes include higher (or vascular) plants, mosses (*Bryophyta*) and stoneworts (*Charophyceae*).

Ecologically, the spieces to sample are those which are fully submerged in water, whose leaves and flowers float on the water or those that are totally free floating as well as plants which are mostly submerged in water, with only a minor part emerging above the water. In a separate part of the list, it is recommendable to further list the species with only a minor part submerged in water (so called helophytes) and those that form riparian vegetation. These species must be clearly separated because they are not directly used in water status assessment, but may provide additional useful information on the status and ecological conditions in the lake.
Macrophyte species which are more difficult to determine (mosses, buttercups (*Ranunculus* spp.), narrow-leaved pondweeds (*Potamogeton* spp.), starworts (*Calitriche* spp.) and stoneworts (Charophyceae) must be stored for a subsequent laboratory identification.

Macrophyte identification

Laboratory analysis of macrophytes includes only the indentification of species which could not be identified in the field (mosses, stoneworts, etc.). Macrophytes are identified to the species level. If their development phase lacks the necessary taxonomic characteristics and identification to the species level is not possible, identification to the genus level is implemented.

Macrophytes are identified by means of identification keys, a stereo microscope and microscope, which facilitate observing plant parts necessary for identification. Plant parts or whole plants originating from habitats with a carbonate substrate are frequently calcified. In this case, plant parts or whole plants are submerged in 5% hydrochloric or acetic acid to remove inorganic carbonate cover and observe the structures needed for identification. This is usually performed with mosses and stoneworts from karst rivers and lakes.

Storage of plant material
Higher plants are generally stored in a herbarium, with the exception of some tender, tiny plants which should be stored, for easier identification, in a preservative (e.g. narrow-leaved species of genus *Potamogeton*, species of genus *Callitriche*). Mosses are best air-dried without pressing and stored in paper envelopes. It is recommended that stoneworts should be stored in a preservative because they may lose some identification characteristics when stored in a herbarium.

Each sample should be separetely labeled, kept in a cold place and examined in the shortest time period possible. As much water (preservative) should be added into plastic bags or containers in which macrophytes are stored as is necessary to keep the plants covered.

Based on the list of taxa and the assessment of total covering of macrophyte vegetation, Biocenological index (BM_{HR}) according to modified method of Weyer (2006) is calculated.

The method, along with the composition and structure of the communities, also includes the relationship between the trophic level and macrophyte depth limit (Table 4) as well as the relationship between the trophic level, Secchi disc transparency and lowermost macrophyte limit (Table 5).

Table 4. Relationship between the trophic level and macrophyte depth limit (Hoesch & Buhle 1996)

Trophic level	Max. macrophyte depth limit (m)	Intermediate macrophyte depth limit (m)
Oligotrophic	>12	>9
Mesotrophic	> 5,3	>3,6
Eutrophic	>1,3	>0,6
Polytrophic	<1,3	<0,6
Hypertrophic	0	0

Table 5. Relationship between the trophic level, Secchi disk transparency and lowermost macrophyte limit (Mauersberger & Mauersberger 1996)

Trophic level	Secchi disc transparency (summer mean, m)	Lowermost macrophyte limit (m)
Oligotrophic	>6	>8
Mesotrophic	3-6	4,2-8
Eutrophic	1,5-3	2,4-4,2
Highly eutrophic	1-1,5	1,8-2,4
Polytrophic	0,5-1	1,2-1,8
Hypertrophic	>0,5	<1,2

The status of macrophyte community is calculated on the basis of bottom inhabited by meadows of Characeae (%), number of characteristic species, number of characteristic vegetation structures (communities, zones) and macrophytes medium depth limit (m). The 1-5 scale scoring system is used for the calculation of five class biocenological values.

The biocenotic index value is the mean value of assessment elements listed in Tables 6. to 11. and ranges from 1 to 5.

$$BM_{HR} = \frac{\Sigma \text{ values of individual assessment elements}}{4}$$

The ecological quality ratio for the degradation level determined by the biocenotic method is calculated according to the following formula:

$$EQR_{BM_{HR}} = \frac{Value\ of\ BM_{HR}}{5}$$

If no macrophyte species could be found, the $EQR_{BM_{HR}}$ is considered to be 0.

The BM_{HR} value 5 represents the reference condition. This class is defined according to literature considering flora and vegetation of each lake. It does not represent necessarily the present state of lake.

Descriptions of lakes are mainly based on historic records, the oldest existing (1950-1990) with detailed description of macrophyte vegetation. We added some current data, especially concerning occurrence of type specific species.

Table 6. BM_{HR} values for the lake type HR- J_2

Assessment elements	BM_{HR} value				
	5	4	3	2	1
Coverage of colonized bottom with stonewort meadows (%)	> 50	25 - 50	10 - 25	5 - 10	< 5
Number of characteristic species*	> 6	4 - 5	2 - 3	1, common	0 - 1, rare
Number of characteristic vegetation structures (communities, belts)**	4	3	2	1	0 - 1
Mean macrophyte depth limit (m)	> 25	25 - 15	15 - 9	9 - 2,5	< 2,5

*Characteristic species: *Chara polycantha*, *Ch. fragilis*, *Ch. virgata*, *Ch. aspera*, *Ch. globularis, Ch. hispida*, *Nitella hyalina*, *Nitella opaca*, *Nitella confervacea*, *Najas marina*

**Characteristic communities (deph belts): community with *Najas marina* and several species of stonewort in shallower water; meadows of species *Chara polyacantha*; meadows of species *Chara virgata*; meadows of species *Nitellopsis opaca* and *Nitella confervacea*.

Table 7. BM_{HR} values for the lake type HR- J_1A

Assessment elements	BM_{HR} value				
	5	4	3	2	1
Coverage of colonized bottom with stonewort meadows (%)	> 50	25 - 50	10 - 25	5 - 10	< 5
Number of characteristic species *	> 10	10 - 7	7 - 3	< 3,1 common	0 - 1, rare
Number of characteristic vegetation structures (communities, belts)**	4	3	2	1	0

Assessment elements	BM$_{HR}$ value				
	5	4	3	2	1
Mean macrophyte depth limit (m)	> 19	19 - 9	9 - 4,2	4,2 - 2,4	< 2,4

Characteristic species: *Chara contraria*, *Ch. hispida*, *Ch. delicatula, Ch. globularis, Ch. vulgaris, Nitellopsis opaca, Potamogeton natans, P. perfoliatus, P. fluitans P. pusillus, P. pectinatus, Myriophyllum spicatum, M. verticillatum, Ranuculus trichophyllus*

** Characteristic communities (deph belts): community of vascular macrophytes in the shallowest belt; meadows of species *Chara contraria*; meadows of species *Chara globularis*; meadows (or fragmented stands) of species *Nitellopsis opaca.*

Table 8. BM$_{HR}$ values for the lake type HR- J_1B

Assessment elements	BM$_{HR}$ value				
	5	4	3	2	1
Coverage of colonized bottom with stonewort meadows and submersed macrophytes (%)	> 50	25 - 50	10 - 25	5 -10	< 5
Number of characteristic species *	> 12	12 - 8	8 - 4	< 4, 1 common	0 - 1, rare
Number of characteristic vegetation structures (communities, belts)**	4	3	2	1	0
Mean macrophyte depth limit (m)	> 11	11 - 9	9 - 5	5 - 2,5	< 2,5

* Characteristic species: *Chara contraria*, *Ch. globularis*, *Ch. hispida*, *Ch. rudis*, *Ch. vulgaris*, *Ch. virgata*, *Nitelopsis opaca*, *Myriophyllum verticillatum*, *Ranunculus trichophyllus*, *R. fluitans*, *Potamogeton natans*, *P. perfoliatus*, *Potamogeton pusillus*, *Mentha aquatica*, *Myriophyllum spicatum*, *Veronica beccabunga*

** Characteristic communities (deph belts): stands of stonewort and vascular macrophytes in coves; stands of species *Myriophillum verticilatum* with other macrophytes; meadows of species *Chara contraria*; meadows or stands of species *Nitellopsis opaca*

Table 9. BM$_{HR}$ values for the lake type HR- J_5

Assessment elements	BM$_{HR}$ value				
	5	4	3	2	1
Coverage of colonized bottom with stonewort meadows (%)	> 50	25 - 50	10 - 25	5 - 10	< 5
Number of characteristic species*	> 10	10 - 7	7 - 3	< 3, 1 common	0 – 1 rare

Number of characteristic vegetation structures (communities, belts)**	4	3	2	1	0
Mean macrophyte depth limit (m)	> 9	4,2 - 9	2,4 -4,2	1,2 - 2,4	< 1,2

* Characteristic species: *Potamogeton lucens*, *P. perfoliatus*, *P. pusillus*, *Myriophyllum spicatum*, *M. verticillatum*, *Ranunculus trichophyllus*, *Potamogeton pectinatus*, *Chara vulgaris*, *Ch. visianii*, *Ch. contraria, Nitella syncrpa, Nitella opaca, Nitelopsis obtusa, Lychonthamnus barbatus, Berula erecta, Nasturtium officinale, Callitriche spp., Mentha aquatica, Nuphar lutea, Nymphaea alba, Oenanthe fistulosa, O. aquatica, Veronica beccabunga, Veronica anagalis-aquatica, Hippuris vulgaris*

** Characteristic communities (deph belts): community of species *Berula erecta* and *Nasturtium officinale* with other species of vascular macrophytes, community of floating macrophytes with water lilies and yellow water lilies, communities of broad-leaved pondweeds (*Potamogeton lucens, P. perfoliatus*) with other species of vascular macrophytes and stoneworts, stonewort meadows

Table 10. BM_{HR} values for the lake type HR- J_3

Assessment elements	BM_{HR} value				
	5	4	3	2	1
Coverage of colonized bottom with stonewort meadows (%)	> 50	25 - 50	10 - 25	5 - 10	< 5
Number of characteristic species*	> 5	4 - 5	2 - 3	1, common	0-1, rare
Number of characteristic vegetation structures (communities, belts)**	2	1	1, fragmentary	only individual plants	0
Mean macrophyte depth limit (m)	> 7	7 - 4,2	4,2 - 2,4	2,4 - 1,2	< 1,2

* Characteristic species: *Chara corfuensis, Chara virgata, Ch. globularis, Najas marina, Myriophyllum spicatum, Potamogeton perfoliatus, P. natans, Nymphaea alba, Nuphar lutea*

** Characteristic communities (deph belts): belt of floating vegetation with species *Potamogeton natans* and/or *Nynphaea alba*; stonewort meadows with dominant species *Chara corfuensis*

Eutrophication indicators: *Potamogeton pectinatus, P. crispus, Ceratophyllum demersum*; appearance of the last three species indicates eutrophication.

Table 11. BM_{HR} values for the lake type HR - J_4

Assessment elements	BM_{HR} value				
	5	4	3	2	1
Coverage of colonized bottom with stonewort meadows (%)	> 50	25 - 50	10 - 25	5 - 10	< 5
Number of characteristic species*	> 5 abundance of species Potamogeton pectinatus is not higher than the abundance of stoneworts	4 - 5	2 - 3	1, common	0-1, rare
Number of characteristic vegetation structures (communities, belts)**	2, with a share of species P. pectinatus lower than 50%	2, with a share of species P. pectinatus higher than 50%	1	1, fragmentar y	0
Mean macrophyte depth limit (m)	> 5	5-4,2	4,2-2,4	2,4-1,2	<1,2

*Characteristic species: *Chara tomentosa, Ch. aspera, Ch. contraria, Nitellopsis obtusa, Najas marina, Myriophyllum spicatum, Myriophyllum verticillatum, Hippuris vulgaris, Berula erecta, Nasturtium officinale, Mentha aquatica, Utricularia australis, Potamogeton lucens, Potamogeton perfoliatus, Potamogeton trichoides, Nymphaea alba*.

**Characteristic communities (depth belts): communities of species *Najas marina* with other vascular macrphytes and stoneworts; meadows of species *Chara tomentosa* and other stoneworts with different shares of species *Potamogeton pectinatus*.

- Identification level.
  Determination goes to the species level.

2.3. NATIONAL REFERENCE CONDITIONS

For Biocenological index (BM_{HR}) reference value for EQR calculation is 5, and the poorest value is 0. The class 5 is defined according to literature considering flora and vegetation of each lake. It does not represent the present state of lake. It is based on comprehensive macrophyte vegetation descriptions published between 1950-ies, and 1980-ies. However, data for some lakes were not published (Crniševo) and we used field data records from 1980-ies compiled by original researchers.
Reference conditions are established for each national lake type, based on expert judgement based on existing literature and least disturbed sites, when they were available. Type specific reference macrophyte communities are used for BM_{HR} index.

Table 12. List of type specific reference macrophyte communities used for EQR calculation

HR Lake Type Code	HR_LAKE TYPE NAME
HR-J_2	OLIGOTROPHIC LAKE - reference community: meadows of Characeae (*Chara polycantha, Chara virgata and Nitella* sp.)
HR-J_1A	OLIGOTROPHIC LAKE - reference community: meadows of Characeae (*Chara contraria, Chara fragilis* and *Nitellopsis opaca*)
HR-J_1B	OLIGOTROPHIC-MESOTROPHIC LAKE -reference community: stands of *Myriophillum verticillatum* , meadows of Characeae (*Chara contraria* and *Nitellopsis opaca*)
HR-J_5	OLIGOTROPHIC-MESOTROPHIC LAKE - reference community: meadows of broadleaf Potamageton and meadows of Characeae
HR-J_3	MESOTROPHIC LAKE - reference community: meadows of Characeae and vascular macrophytes (*Chara corfuensis, Najas marina, Myriophyllum spicatum, Potamogeton spp.*)
HR-J_4	MESOTROPHIC LAKE - reference community: meadows of Characeae (*Chara intermedia*) and *Potamogeton pectinatus*

2.4. NATIONAL BOUNDARY SETTING

Ecological status is classified by one of five classes (high, good, moderate, poor and bad). The EQR calculated from Biocenological index (BM_{HR}) is considered to be the EQR for final assessment.

EQR class boundaries:
H/G boundary = 0.90
G/M boundary = 0.70
M/P boundary = 0.50
P/B boundary = 0.30

The Biocenological index (BM_{HR}) consists of four components each of which characterize aspects of a lake macrophyte community that are known to respond to eutrophication and other pressures that are likely to impact lakes. Each component is assigned a BM_{HR} score from one to five representing the WFD class (Bad to High) of that component (metric values), thus the quantitative descriptions represent the condition of that component for each class.

The above class boundaries were determined as they represent the EQR value obtained from the method when at least 50% of the components are classed as better than the metric boundary value.

The descriptions for BM class with a score of 5 represent High status and were based on historic information, supplemented by expert judgement (see section 2.3).

The first and third components of BM (cover of stoneworts, number of vegetation structures) have common description for each lake (type). The values used represent a transition from reference conditions to very damaged communities (<5% cover of chara and <1 vegetation structure), the intermediate values were determined using expert judgement, equal divisions between High and Bad status.

The second (number of characteristic species) and fourth components (depth of colonisation) of BM have different values for each lake (type) as the reference conditions for each lake differ.

For number of species (Table 13) it was considered appropriate that the boundary between Poor and Bad for all lakes to be the same and was set at 1, such that lakes with no macrophyte vegetation had a score of 0. The other values were set using expert judgement to reflect the normative definitions of slight and moderate change (table below).

Table 13. Boundary values expressed as number of macrophyte species

	N Species			
Lake	High/good 5	Good/mod 4	Mod/poor	Poor/bad
HR-J_J2	6	5	3	1
HR-J_1A	10	7	3	1
HR-J_1B	12	8	4	1
5HR-J_5	10	7	3	1
HR-J_3	5	4	3	1
HR-J_4	5	4	3	1

For the maximum depth of colonisation, high status was based on an assumption that macrophytes would colonise the entire water column of the littoral zone. The good/moderate boundary was based on the lower most limit for macrophytes given by Mauersberger & Mauersberger (1996), the remaining divisions being set by expert judgement (Table 14). Differences in values of maximum depth colonization should reflect different characteristics of lakes. Namely, Lakes Vrana Cres (HR-J_2), Kozjak (HR-J_1A) and Prošće (HR-J_1B) are deeper lakes (74 m, 47 m and 37 m) with lower conductivity (426. 3 µS cm^{-1}, 374.6 µS cm^{-1} and 418.8 µS cm^{-1}), since the last three: Visovac (HR-J_5), Crniševo (HR-J_3) and Vrana Biograd (HR-J_4) are shallower (27 m, 34 m and 4.7 m) with much higher conductivity (542.2 µS cm^{-1}, 1774.2 µS cm^{-1} and 1985.5 µS cm^{-1}). It should be stressed that Crniševo despite its deepness is composed of fresh water which lies above sea water and therefore more resembles to shallower lakes considering macrophyte vegetation. This general relation of lower deepness and higher conductivity is also reflected in higher concentrations of chlorophyll-a and amount of suspended particles, what all affect distribution and depth limits of macrophyte communities.

Table 14. Boundary values expressed as maximum depth of macrophyte colonization.

	Depth colonisation			
Lake	High/good 5	Good/mod 4	Mod/poor	Poor/bad
HR-J_J2	25	15	9	2.5
HR-J_1A	19	9	4.2	2.4
HR-J_1B	11	9	5	2.5
HR-J_5	9	4.2	2.4	1.2
HR-J_3	7	4.2	2.4	1.2
HR-J_4	5	4.2	2.4	1.2

In general the point good-moderate was defined as turnover point where referent macrophyte vegetation loses its integrity and diversity. This had to be defined theoretically due to the lack of natural gradients. In this procedure system proposed by WEYER VAN DE (2006) was followed.

2.5. PRESSURES ADDRESSED

Pressure addressed by the method is general degradation and eutrophication. The assessment is developed on the basis of expert knowledge and literature data respectively and could not be calibrated against general degradation and eutrofication gradients, because gradients are not available.
Descriptions of series of increasing status are based on WFD descriptions of quality classes and accorded to principles described by WEYER VAN DE (2006). Maximal depth of occurrence of macrophytes is accorded to HOESCH & BUHLE (1996) and MAUERSBERGER & MAUERSBERGER 1996. Physico-chemical parametres were considered for each lake. However, establishment of gradient was impossible since only 6 lakes of different types are included in the monitoring programme. Unfortunaltely, we do not have palaeolimnological data for those lakes at the moment. However, for oligotropic lakes dominated by charophites it is quite sure that it is their natural condition.

Although it is not possible to produce a conventional pressure response relationship for the method we provide the following information showing how the method performs and the current status of the lake (as assessed by the method) together with other indicators of pressure/impact (Table 15).

Table 15. Comparison between results of macrophyte based assessment method with phytoplankton based method and assessments based on total phosphorous and nitrates (for 2017) and CORINE land use data.

Lake	Macrophytes	Phytoplankt.	Total P	NO_3	Urban area %	Int. agricult. %
J_3 Crniševo	high	high	good	high	0	0
J_1A Kozjak	high	high	high	high	2.43	0
J_1B Prošće	high	high	good	high	0.01	0
J_2 Vrana (Cres)	high	high	good	high	0	0
J_5 Visovac	good	moderate	good	high	0.97	10.54
J_4 Vrana (Biograd)	good	poor	high	high	9.33	45.16

3. WFD COMPLIANCE CHECKING

The first step in the Intercalibration process requires the checking of national methods considering the following WFD compliance criteria.

Table 2. List of the WFD compliance criteria and the WFD compliance checking process and results

Compliance criteria	Compliance checking
Ecological status is classified by one of **five classes** (high, good, moderate, poor and bad).	YES
High, good and moderate ecological status are set in line with the WFD's **normative definitions** (**Boundary setting procedure**)	YES
All relevant parameters indicative of the biological quality element are covered (see Table 1 in the IC Guidance). A **combination rule** to combine parameter assessment into BQE assessment has to be defined. If parameters are missing, Member States need to demonstrate that the method is sufficiently indicative of the status of the QE as a whole	YES

Assessment is adapted to **intercalibration common types** that are defined in line with the typological requirements of the Annex II WFD and approved by WG ECOSTAT	YES
The water body is assessed against **type-specific near-natural reference conditions**	YES
Assessment results are expressed as **EQRs**	YES
Sampling procedure allows for **representative** information about water body quality/ecological status **in space and time**	YES
All data relevant for assessing the biological **parameters** specified in the WFD's normative definitions are covered by the **sampling procedure**	YES
Selected taxonomic level achieves adequate **confidence and precision** in classification	YES

4. IC FEASIBILITY CHECKING

The intercalibration process ideally covers all national assessment methods within a GIG. However, the comparison of dissimilar methods ("apples and pears") has clearly to be avoided. Intercalibration exercise is focused on specific type / biological quality element / pressure combinations. The second step of the process introduces an "IC feasibility check" to restrict the actual intercalibration analysis to methods that address the same common type(s) and anthropogenic pressure(s), and follow a similar assessment concept.

4.1. TYPOLOGY

Does the national method address the same common type(s) as other methods in the Intercalibration group? Provide evaluation if IC feasibility regarding common IC types.

There are six natural lakes in Croatia with a surface area larger than 0.5 km^2. All of them are located in the Dinaric ecoregion (5. Dinaric Western Balkans): two (Plitvice Lakes: Lake Kozjak and Lake Prošće) in the Dinaric Continental sub-ecoregion (EC-GIG) and four in the Dinaric Littoral sub-ecoregion (MED-GIG). According to abiotic factors, the lakes are divided into six types: high deep small calcareous oligotrophic lakes (Plitvice Lakes, Kozjak), high deep small calcareous oligotrophic-mesotrophic lakes (Plitvice Lakes, Prošće), lowland deep medium sized calcareous lakes, with cryptodepression (Vrana Lake, Cres Island), lowland deep small calcareous lakes, with cryptodepression (Baćina Lakes), lowland shallow large calcareous lakes, with cryptodepression (Vransko Lake) and lowland medium deep and medium sized calcareous lakes (Visovac Lake).

Since lakes are distributed through two biogeographical regions and are of different sizes and depths, it was not possible to unite them in reasonable groups. Therefore, we decided to consider each lake independently. Reasons for not doing the intercalibration was lack of appropriate comparable data, i.e. comparable lake types and reference conditions.

4.2. PRESSURES ADDRESSED

Does the national method address the same pressure(s) as other methods in the Intercalibration group? Provide evaluation if IC feasibility regarding pressures addressed.

The Croatian national assessment method addresses general degradation and eutrophication pressures, as well as Greek HeLM assessment method. French IBML and Italian assessment method are both focused on the assessment of the eutrophication pressure, while Spanish assessment method is mainly focused on the assessment of hydromorphological pressure. The addresses eutrophication and general degradation pressures.

4.3. ASSESSMENT CONCEPT

Does the national method follow the same assessment concept as other methods in the Intercalibration group? Provide evaluation if IC feasibility regarding assessment concept of the intercalibrated methods.

4.4. CONCLUSION ON THE INTERCALIBRATION FEASIBILITY

Intercalibration is not possible due to a lack of appropriate comparable data, i.e. comparable lake types and reference conditions.

5. DESCRIPTION OF THE BIOLOGICAL COMMUNITIES

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT HIGH STATUS

Descriptions of lakes are mainly based on historic records, the oldest existing (1950-1990) with detailed description of macrophyte vegetation. We added some current data, especially concerning occurrence of type specific species.

The lack of detaild description of communities at Good and Moderate status is a reflection of lack of impacted lakes, although current status was not used as reference status. As explained before, this was done mainly based on literature data. Five of six lakes are under some kind of protection (Vransko-Cres – strongly protected as water reservoir, Prošće – in National Park, Kozjak – in National Park, Visovac – in National Park, Vransko-Biograd – Nature Park) and consequently impacts are minimal. The only lake outside protected area is Crniševo and here changes since 1980-ies were obtained.

Degradation pressure gradient is not available. As already stated physico-chemical parameters were used, but they do not show any increase of nutrients. Since the lakes are situated inside protected areas catchment land-use is of little significance.

OLIGOTROPHIC AND OLIGOTROPHIC-MESOTROPHIC LAKES WITH STONEWORT COMMUNITIES (HR-J_1A, HR-J_1B, HR- J_2)

A) Oligotrophic lake – Type HR-J_2 (Lake Vransko on the island of Cres)

Reference community: stonewort meadows (*Chara polycantha, Ch. virgata and Nitella spp*.).

- Due to its exceptional transparency, macrophyte vegetation appears down to the depth of 30 m, and some green algae even to the depth of 50 m.
- Due to its steep banks, a continuous belt of wetland vegetation has not developed.

The colonisation of macrophyte communities according to depth belts (northern coast):

- to the depth of approx. 1 - 1.5 m: appearance of reed (*Phragmites australis*) and bulrush (*Scirpus lacustris*), among which also *Mentha aquatica* and *Juncus subnodulosus,* with significanly rarer *Scirpus holoschoenus*, *Scirpus triqueter* and *Typha angustifolia*;

- from 2.5 to 5 m depth: appearance of *Nitella hyalina*, *Najas intermedia*, *Chara aspera*, *Chara globularis*, *Ch. polyacantha*, *Ch. hispida*, *Potamogeton pectinatus*;
- from 5 to 23 m deph: meadows of species *Chara polyacantha;*
- from 23 to 29 m: meadows of species *Ch. virgata;*
- from 27 to 30 m: species *Nitellopsis opaca* and *Nitella confervacea*.

The colonisation of macrophyte communities according to depth belts (southeastern coast):

- to the depth of 3 m: monodominance of reed.

The colonisation of macrophyte communities according to depth belts (southern coast):

- to the depth of 3 to 3.5 m: appearance of *Potamogeton pectinatus*.

B) Oligotrophic lake – Type HR- J_1A (Lake Kozjak)

Reference community: stonewort meadows (*Chara contraria*, *Ch. fragilis* and *Nitellopsis opaca*) which extend from the shallowest parts to the depth of 20.5 m.

- The total number of species decreases with depth; however, their abundance increases.
- On the steep banks and in more intensely shaded spots a belt of wetland vegetation is completely absent.

The colonisation of macrophyte communities according to depth belts:

- to the depth of 1 m: the most frequent wetland community is that of swamp sawgrass (*Cladium mariscus*) which forms spatious, monodominant stands. The species *Typha latifolia*, *Phragmites communis, Scirpus lacustris, Carex elata* te *Carex rostrata, Carex vesicaria, Alisma plantago-aquatica, Mentha aquatica* and *Sparganium erectum* appear with less abundance;
- to the depth of approx. 6 m: appearance of species *Potamogeton natans, P. perfoliatus, P. fluitans, P. pusillus, P. pectinatus, Myriophyllum spicatum, M. verticillatum and Ranuculus trichophyllus*.
- in the depth belt from 1 - 9 (-10) m: start of appearance of stonewort (*Chara contraria*, which form the most aboundant meadows in the belt from 2 - 7 m, *Ch. hispida* grows in the belt from 2 - 8 m and *Ch. delicatula* from 6 - 9 m.
- in the depth belt from 3 - 19 m: spatious meadows of species *Ch. globularis*, which are dominant in the belt from 10 - 18 m and for the most part overlap with the distribution belt of species *Nitellopsis opaca* od 7-21 m. Dominant stonewort species: *Chara contraria*, *Ch. globularis* and *Nitellopsis opaca*.

C) Oligotrophic - mesotrophic lake - HR- J_1B (Lake Prošće)

Reference communities: stands of species *Myriophillum verticillatum*, stonewort meadows (*Chara contraria* and *Nitellopsis opaca*).
The most important communities are submerged macrophytes which are present from the shallowest bank parts to the depth of 13 m, but are the most abundant in the depth belt from 1.5 to 11 m. Characteristic species: stands of lakeshore bulrush (*Scirpus lacustris*), water horsetail (*Equisetum fluviatile*), Bur-reed (*Sparganium erectum*), tufted sedge (*Carex elata*), *Typha latifolia*, *Mentha aquatica*, *Oenathe fistulosa*, *Lythrum salicaria*, *Lysimachia vulgaris*, *Phragmites australis,* community of swamp sawgrass (*Cladium marisus*).

The colonisation of macrophyte communities according to depth belts:

- to the depth from 4 to 6 m: growth of stoneworts (*Chara contraria*, *Ch. hispida*, *Ch. rudis* and *Ch. vulgaris*) as well sa *Myriophyllum verticillatum*, *Ranunculus trichophyllus*, *R. fluitans*, *Potamogeton natans* and *P. perfoliatus;*
- to the depth of 7 m: growth of *Chara vulgaris;*
- to the depth of 8 m: growth of *Potamogeton pusillus;*
- to the depth of 9 m: growth of *Chara contraria;*
- to the depth from 9 to 13: growth of *Nitellopsis opaca*.

OLIGOTROPHYIC - MESOTROPHIC LAKES WITH COMMUNITIES OF BROAD-LEAVED PONDWEEDS AND STONEWORTS (HR-J_5)

Oligotrophyic - mesotrophic lake - HR-J_5 (Lake Visovac)

Reference community: meadows of broad-leaved pondweeds (broad-leaved species of genus Potamogeton) and stonewort meadows.

The riparian belt is formed by reed beds with dense, almost monodominant reed stands (*Phragmites australis*) which are towards higher depths replaced by stands of lakeshore bulrush (*Scirpus lacustris*).

Characteristic emergent vegetation: stands of galingale (*Cyperus longus*), *Typha latifolia*, *Sparganium erectum*, *Juncus compressus*, *Iris pseudacorus*, *Carex elata*, *Carex pendula*, *Cladium mariscus*, *Eleocharis palustris*, *Scirpus holoschoenus*, *Scirpus maritimus*, *Alisma plantago-aquatica*, *Galium palustre*, *Lythrum salicaria*, *Stachys palustris*, *Mentha aquatica*, *Hydrocotyle vulgaris*, *Oenanthe fistulosa*, *Oenanthe silaifolia*, *Equisetum paluste*.

Characteristic submerged vegetation: stands of mare's-tail (*Hippuris vulgaris*), *Callitriche cophocarpa*, *Berula erecta*, *Nasturtium officinale*, *Mentha aquatica*, *Veronica beccabunga, Agrsotis stolonifera*, white water lily (*Nymphaea alba*), yellow water lily (*Nuphar lutea*), common duckweed (*Lemna minor*), *Potamogeton lucens*, *P. perfoliatus, P. crispus*, *Myriophyllum spicatum*, *Ranunculus trichophyllus*, *Potamogeton pectinatus*, *Ceratophyllum demersum* and communities of stoneworts made of the following species: *Chara vulgaris*, *Ch. visianii*, *Ch. contraria* (which, apart from its typical form, also appears with f. denundata and f. dalmatica), *Nitella syncrpa*, *Nitella opaca*, *Nitelopsis obtusum*, *Lychonthamnus barbatus* and ***Chara visianii*** (endemic species of Lake Visovac).

MESOTROPHIC LAKES (SALINE AT THE BOTTOM) WITH COMMUNITIES OF STONEWORTS AND VASCULAR MACROPHYTES (HR-J_3)

Mesotrophic lake – Type HR-J_3 (Lake Crniševo/ Baćina Lakes)

Reference community: stonewort meadows and vascular macrophytes (*Chara corfuensis*, *Najas marina, Myriophyllum spicatum, Potamogeton spp.*)
Seawater intrudes into deeper parts of the lake preventing the development of macrophyte vegetation, therefore its depth limit is at approximately 8 m.

Characteristic species: *Phragmites australis*, *Typha angustifolia, Cladium mariscus*, *Sparganium erectum, Scirpus triqueter, Cyperus longus, Alysma plantago-aquatica*, *Potamogeton natans*, *Nymphaea alba*, *Chara virgata* and *Ch. globularis*, *Chara corfuensis* (endemic Balkan species), *Najas marina*, *Myriophyllum spicatum*, *Potamogeton perfoliatus*.

MESOTROPIC LAKES WITH A COMMUNITY OF STONEWORTS AND SAGO PONDWEED (HR-J_4)

Mesotrophic lake – Type HR-J_4 (Lake Vransko at Biograd)

Reference community: stonewort meadows and sago pondweed (Chara tomentosa and Potamogeton pectinatus).
Light penetration reaches the depth of 5 m, and therefore macrophyte vegetation is developed almost on the entire bottom surface.

Characteristic species: *Phragmites australis, Typha angustifolia, Scirpus lacustris, Najas marina, Potamogeton pectinatus, Chara tomentosa, Ch. aspera, Ch. contraria, Nitellopsis obtusa, Alisma lanceolatum, A. plantago-aquatica, Berula erecta, Hydrocotyle vulgaris, Nasturtium officinale, Ceratophyllum demersum, Myosotis scorpioides, Carex elata, Cladium mariscus, Cyperus fuscus, Cyperus longus, Eleocharis palustris, Schoenus nigricans, Scirpus holoschoenus, Scirpus litoralis, Scirpus maritimus, Myriophyllum spicatum, Myriophyllum verticillatum, Hippuris vulgaris, Iris pseudacorus, Juncua acutus, Juncus articulatus, Juncus gerardi, Juncus inflexus, Juncus maritimus, Juncus subnodulosus, Lycopus europaeus, Mentha aquatica, Stachys palustris, Utricularia australis, Lythrum salicaria, Nymphaea alba, Potamogetom crispus, Potamogeton lucens, Potamogeton perfoliatus, Potamogeton trichoides, Samolus valerandi, Ranunculus trichophyllus, Galium palustre, Veronica anagallis-aquatica, Sparganium erectum, Thelypteris palustris.*

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT GOOD STATUS

Species composition and their abundance only slightly differ from type-specific community. This difference do not include increased growth of algae and cyanobacteria or higher plants which could disturb community equilibrium and physical and chemical quality of water and/or sediment.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT MODERATE STATUS

Species composition differs in comparison to type-specific community, but characteristic species still dominates over disturbance indicators. Changes in species abundances are moderate.

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Report on Croatian lake benthic macroinvertebrates classification method in the case where the Intercalibration exercise is not possible (Gap 3)

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Report on benthic macroinvertebrates classification methods for natural lakes in Croatia in the case where the Intercalibration exercise is not possible (Gap 3)

1. INTRODUCTION

- **Member State:** Croatia;
- **BQE:** Macroinvertebrates;
- **Waterbody category (type):** Lakes.

Benthic invertebrates have been recognized as one of the most difficult biological quality element to use in ecological assessment of lakes, due to their heterogeneity in community composition, but also because of the very different sampling approaches used in Member States (Poikane et al., 2016). Some Member States take samples in the littoral zone, while others are taking samples of profundal benthic communities. Assessment metrics based on profundal invertebrate communities usually assess eutrophication and organic enrichment, while macroinvertebrates in the littoral zone are able to assess lake hydromorphological alterations. The present report discusses progress in the development of a national method for assessing the ecological status of Croatian natural lakes based on the biological quality element benthic macroinvertebrates of the littoral zone.

The macroinvertebrate classification method can be considered both type-specific and lake-specific, since almost all natural lakes in Croatia are considered different types except for two (Official Gazette 96/19): HR -L_1A (one lake), HR -L_1B (one lake), HR -L_2 (one lake), HR -L_3 (two lakes), HR -L_4 (one lake) and HR -L_5 (one lake). Reference values for each lake type were modeled on the basis of their natural abiotic and morphometric data and pressures (which were either minimized or set to zero).

Data from all lake types were treated together to create a stepwise multimetric linear model for hindcasting reference conditions. Most of the natural lakes in Croatia are in very good or near-natural conditions, so in order to give a gradient of pressure variables to the model, man-made lakes (reservoirs) from the same geographical region were used in the creation of the model.

The final assessment tool is a multimetric index.

The aim of this report is to declare that the macroinvertebrate classification methods for natural lakes in Croatia are compliant with the WFD normative definitions and have good pressure-response relationships.

2. DESCRIPTION OF NATIONAL ASSESSMENT METHODS

A benthic invertebrate – based index for the assessment of the ecological quality of natural Croatian lakes was developed. Seven natural lakes, representing the six lake types occurring in the country were analyzed (Table 1).

Table 1. General characteristics of Croatian natural lakes.

Lake	Maximum depth (m)	Ecoregion/ subecoregion	GIG	National type	Lake description	Depth profile
Kozjak (Plitvice Lakes)	48	Dinaric/ Continental	EC	HR-L_1A	carbonate substrate, dimictic, barrage lake	deep
Prošće (Plitvice Lakes)	38	Dinaric/ Continental	EC	HR-L_1B	carbonate substrate, dimictic, barrage lake	deep
Vrana Lake (on island Cres)	78	Dinaric/ Mediterranean	MED	HR-L_2	carbonate substrate, monomictic, cryptodepression	deep
Crniševo	31	Dinaric/ Mediterranean	MED	HR-L_3	carbonate substrate, monomictic, cryptodepression	deep
Oćuša	20	Dinaric/ Mediterranean	MED	HR-L_3	carbonate substrate, monomictic, cryptodepression	deep
Vransko Lake (near Biograd)	4-5	Dinaric/ Mediterranean	MED	HR-L_4	carbonate substrate, polymictic, cryptodepression	shallow
Visovac	28-30	Dinaric/ Mediterranean	MED	HR-L_5	carbonate substrate, monomictic, barrage lake	deep

Sampling was carried out in late spring of 2018 and 2019. Benthic macroinvertebrates were sampled from the littoral zone. Environmental parameters and anthropogenic pressures were assessed for each lake (Table 2). A stepwise linear regression of each metric (calculated with Asterics software, version 4.0.4, or manually calculated in the case of the percentage of Chironomini individuals in the community) against environmental parameters and anthropogenic pressures has been carried out to ensure pressure- response relationships. Reference conditions for each lake type were estimated by hindcasting procedure. The final index was expressed as the average of the EQRs of four (in the case of HR -L_2 only three) metrics.

Table 2. Environmental parameters and anthropogenic pressures used in the stepwise linear regression.

Environmental /morphometric conditions	Pressures
Lake Volume (in m^3*10^6)	Fish biomass (in kg/ha)
Altitude (in meters above sea level)	Non-natural land cover (NNLC, in %)
Retention time (in days)	Hydromorphology (morphological degradation scores 1-5)
Salinity (in g/kg)	Concentration of chlorophyll *a* (mg/L)

2.1. SAMPLING AND DATA PROCESSING

Description of sampling and data processing:

- **Sampling time and frequency:** mid to late spring
- **Sampling method:** Sampling site covered a length of 25 m of the lake shore, up to 10 m to the open water or to the point where the water depth exceeded 1 m, depending on the slope of the shore. At each sampling site, 10 samples were taken from a surface area of 25 × 25 cm with a hand net (mesh size, 500 µm) and four depth levels were defined: 0–0.25 m, 0.25–0.5 m, 0.5–0.75 m and 0.75–1 m. The samples were taken in microhabitats covering at least 10% of the area, proportional to their coverage at the sampling sites. The substrate categories were defined according to the AQEM Consortium (2002). The method is described in detail in Urbanič et al. (2012).
- **Data processing:** EPT [%] (abundance classes), Diversity (Margalef Index) and Number of Families are calculated using ASTERICS 4.04 software, whereas the percentage of Chironomini individuals was calculated as: N od Chironomini individuals/ N of all macroinvertebrates (%)
- **Identification level:** It is recommended that identification is conducted as detailed as possible, up to the level of species if possible. Required level of macroinvertebrate identification (Table 3):

Table 3. Level of identification required for the Croatian national assessment.

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		

2.2. DESCRIPTION OF NATIONAL METHODOLOGY

The data of all lake types were treated together to create a stepwise multimetric linear model for hindcasting reference conditions. Most natural lakes in Croatia are in very good or near-natural conditions. To obtain a gradient of pressure variables, man-made lakes (reservoirs) from the same geographical region were used to construct the model (Table 4).

Table 4. Number of sites and samples, range of independent and dependent variables.

	Reservoirs		Natural lakes	
Number of sites (datasets)	**21**		**43**	
Variable ranges	**min**	**max**	**min**	**max**
Pressures				
Chl a (µg/L)	0	0.77	0.35	6.33
NNLC (%)	0.05	99.98	0	66.19
Fish biomass (kg/ha)	10	240	30	225
HYMO	1.21	2.71	1	4
Environmental traits				
Volume ($m^3 * 10^6$)	0.4	25.7	7	220
Altitude (m a.s.l.)	9	733	-0.16	636
Retention time (days)	1	278	27	11680
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	0.002	0.109	0	0.194
Number of Families	10	36	9	51

A multimethric index was used. It consists of four (in one type three) metrics (Table 5): 1) percentage of Chironomini individuals in the community (% Chironomini); 2) Diversity (Margalef Index - this metric is not used in one lake type), 3) - EPT [%] (abundance classes) and 4) Number of Families. The multimetric index uses the same metrics for all lake types but with different reference values for each type. An exception is the lake type HR-L_2. This lake is a very deep (max. depth: 78 meters) cryptodepression, located on an island of Cres (surrounded by the lake). Due to this obvious isolation, this ultra-oligotrophic lake has a low diversity naturally. For this reason, the diversity index (Margalef diversity) is excluded from the evaluation of this lake's ecological status.

Table 5. Overview of the metrics included in the national method.

MS	Taxonomic composition and abundance/Major taxonomic groups	Diversity	Ratio tolerant/sensitive taxa
HR	EPT [%] (abundance classes) Number of Families.	Diversity (Margalef Index)	% Chironomini (tolerant taxa)

Combination rule used in the method: The final EQR is calculated as an average of the single EQRs of the four (three) metrics.
Conclusion on the WFD compliance (are all the indicative parameters included; if not, why): Method is compliant with the WFD normatives.

2.3. NATIONAL REFERENCE CONDITIONS

The reference conditions were predicted for each lake by the hindcasting procedure. The theoretical value of the metric was estimated after minimizing or setting pressure values to zero (Table 6). According to Poikane et al. (2011), chlorophyll a concentrations were set at 2.5 µg/L for shallow lakes and 1.8 µg/L for deep lakes. The maximum ratio of non-natural land cover was set at 8% according to Ntislidou et al. (2016), who also defined reference conditions for MED GIG lakes. The reference value for hydromorphological alteration was set at 1.5, which "corresponds totally, or nearly totally, to undisturbed conditions" (Poikane, 2009). The reference fish biomass was calculated from the reference values for total phosphorus concentration, which are 0.01 mg/L (10 µg/L) for deep lakes and 0.02 mg/L (20 µg/L) for shallow lakes (both values in accordance with de Hoyos et al., 2014). The biomass calculation follows Gassner et al. (2003) and is done using the formula
Fish biomass (kg/ha) = 3.8148*TP1.0940, where TP is the total phosphorus concentration in µg/L.

Table 6. Maximum pressure values determined for reference conditions.

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. (2009)
HR-L_1A	1.8	8	47.37	1.5
HR-L_1B	1.8	8	47.37	1.5
HR-L_2	1.8	8	47.37	1.5
HR-L_3	1.8	8	47.37	1.5
HR-L_4	1.8	8	47.37	1.5
HR-L_5	2.5	8	101.11	1.5

In the Dinaric ecoregion of Croatia, the landscape is mainly dominated by karst deposits, which in the past were considered harsh habitats for agricultural and urban development. This means that very few lakes and reservoirs are affected by high nutrient enrichment and are mostly in good and high ecological status (or good ecological potential in the case of reservoirs). All lakes have mean total phosphorus concentrations (TP) from the vegetation period below 30 µg/L, which theoretically makes them all suitable for reference sites with respect to this variable (de Hoyos et al., 2014 and Borics et al., 2018).

This means that the variable TP when correlated with macroinvertebrates or other biological metrics does not show typical stressor trends and, as concentrations are usually very low in some cases even acts as a promoter of biodiversity. Similar trends also occur for total nitrogen concentrations. For this reason, we have used chlorophyll a concentrations as "pressure" variables, which we believe best represent eutrophication pressure and correlate significantly with the macroinvertebrate metrics used.

The pressures addressed were Chl a (µg/L), NNLC (%), Fish biomass (kg/ha) and hydromorphological degradation. The parameters of the model (multiple regressions) for the macroinvertebrate metrics against environmental parameters and pressures are presented in Table 7. All metric regressions showed significant correlations with at least one pressure.

Table 7. Multimetric linear model for hindcasting reference conditions for four different metrics used in Croatian natural lakes. Significance levels marked as "*" for p<0.05; "**" p<0.01 and "***" for p<0.001.

		Environmental trait				Stressor				Multimetric linear model	
Metric	Intercept	Volume	Altitude	Retention time	Salinity	Fish biomass	NNLC	HYMO	chla 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	7.6927***	0.0124	-0.002	-0.2105***	-1.5398*	-0.0147**	-0.0131	0.1632	-0.0738	0.621	9.631
EPT (%)	12.2042***	-0.0049	0.0074*	0.0013**	-2.0193	0.0145	-0.0417	-0.7828*	0.9344**	0.548	7.135
N of fam	37.1813***	0.0512	-0.0005	-0.0022**	-7.8707	-0.0435	-0.1579*	0.2428	1.0566	0.579	8.068

2.4. NATIONAL BOUNDARY SETTING

The national boundaries of the ecological quality classes were set according to Hering et al. (2006).

Boundaries	Class
0.8-1	High
0.6-0.8	Good
0.4-0.6	Moderate
0.2-0.4	Poor
0-0.2	Bad

The reference values for all the metrics used in the equation are derived from the stepwise linear regressions presented in Chapter 2.3.

Table 8. Reference metrics values from six 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

National type	Metric	Reference value
	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

Lower anchors for all metrics were set as the worst metric value from the entire data set and corresponded to: % Chironomini -0.4; Diversity Margalef index -2.28; EPT (%) abundance classes - 1.02 and Number of families - 9.

2.5. PRESSURES ADDRESSED

Statistical analyses were performed to explore the responsiveness of the national macroinvertebrate-based assessment method to various anthropogenic stressors.
The pressure-response relationships were tested via:
(1) non-parametric Spearman rank correlations of the national EQR with environmental parameters
(2) linear regressions of the national metric with pressure variables.

The results of a Spearman correlation of the national macroinvertebrate-based assessment method with pressure variables are presented in Table 9. The coefficient showed statistically significant relationships ($p < 0.05$) between the national EQR and various pressure variables.

Table 9. Summary of the Spearman correlations of the national macroinvertebrate-based assessment method (EQR) with different pressures. Correlations marked in red are significant at $p < 0.05$.

Variable	Marked correlations are significant at p < 0,05; N=43 (Casewise deletion of missing data) EQR
Chl *a*	0,357
HYMO	0,150
Fish biomass	-0,729
Total P	-0,505
NNLC	-0,692

Some specific situations are present at two lake types (HR -J_1B and HR -J_5), where almost reference values of the EQR are calculated, whereas NNLC is present in the catchment area with ratios above 20%. Although the authors have set the reference criteria for this pressure at 8 %, the ecological characteristics of both lake types (i.e. lakes) support the high EQR of the sites:

The first lake type, represented by the Prošće Lake, is located within the Plitvice Lakes National Park. This barrage lake is surrounded by large forest areas, all of which are natural (no exploitation of forest resources). Within the parameters of National Park (i.e. the catchment area of the lake) there are also meadows and pastures, which make up about 22% of the catchment area. They represent the historical heritage of the landscape and, although they technically fall into the category of extensive agricultural land use, they have practically no livestock breeding or agricultural activity other than mowing the grass. Therefore, the authors support the high EQR values present at sites of this lake type.

The second lake type, represented by Visovac Lake, is located within Krka National Park. This lake is also a barge type (riverine) surrounded by a natural land cover and is located downstream of an anthropologically altered lake - Brljan. Brljan Lake is however 11 kilometers downstream of the town of Knin (which is also in the catchment area and accounts for 1%) and the surrounding agricultural areas (extensive and intensive, 12 and 11%). An increased inflow of organic matter from upstream mainly affects the first reservoir - Brljan, which in turn serves as a sedimentation tank for the inflow of organic matter and nutrients. This means that the environmental conditions in Visovac Lake are much more favorable (HR -J 5) and resemble the calculated EQR-s much more closely than the calculated NNLC, which can be misinterpreted.

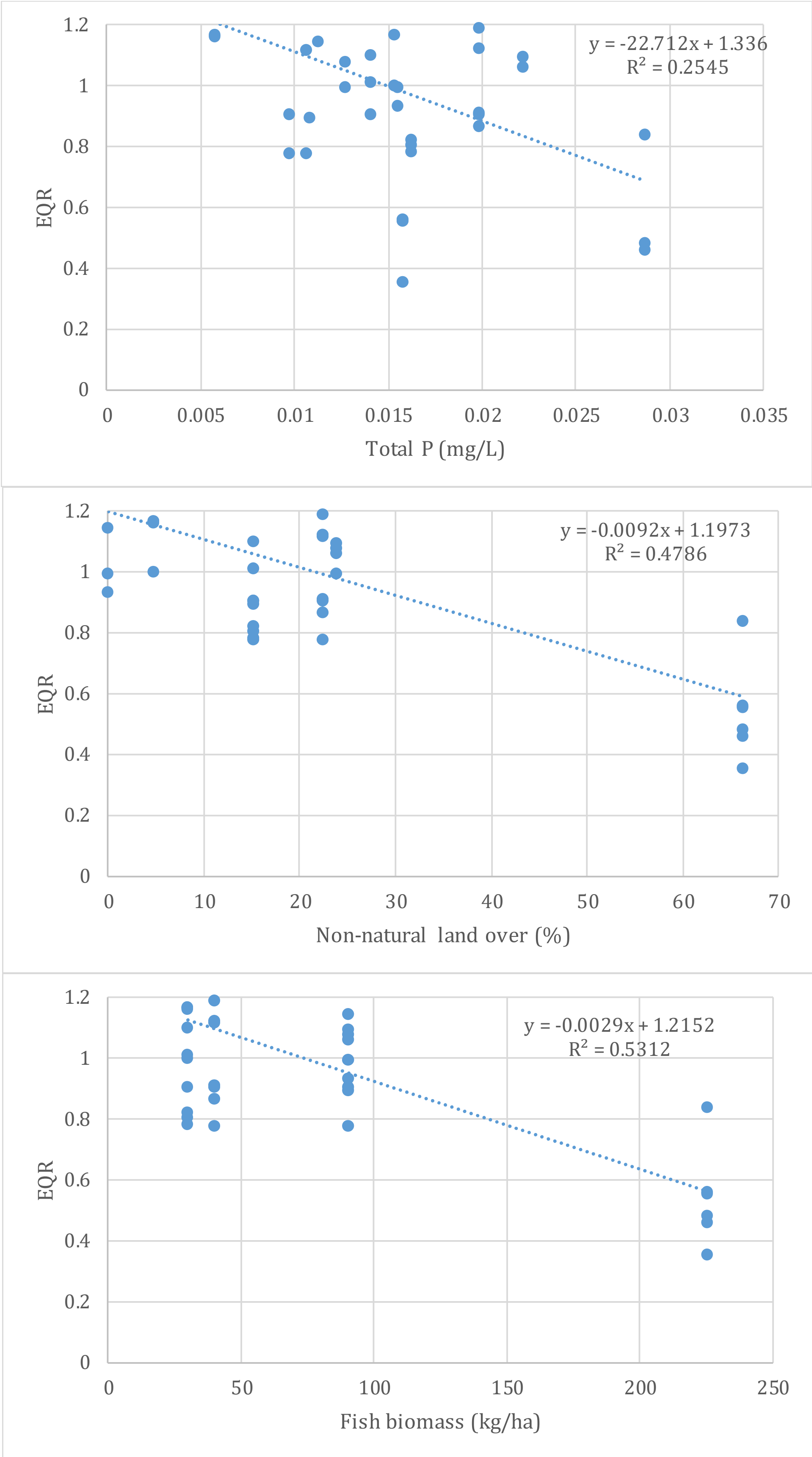

Figure 1. Pressure-response relationship between the most important pressures against the national macroinvertebrate-based assessment method (EQR) in Croatian lakes.

3. WFD COMPLIANCE CHECKING

Table 10. List of the WFD compliance criteria and the WFD compliance checking process and results

Compliance criteria	Compliance checking
Ecological status is classified by one of **five classes** (high, good, moderate, poor and bad).	YES
High, good and moderate ecological status are set in line with the WFD's **normative definitions** (**Boundary setting procedure**)	YES
All relevant parameters indicative of the biological quality element are covered (see Table 1 in the IC Guidance). A **combination rule** to combine parameter assessment into BQE assessment has to be defined. If parameters are missing, Member States need to demonstrate that the method is sufficiently indicative of the status of the QE as a whole	YES
Assessment is adapted to **intercalibration common types** that are defined in line with the typological requirements of the Annex II WFD and approved by WG ECOSTAT	no IC types defined
The water body is assessed against **type-specific near-natural reference conditions**	YES (hindcasting method for deriving reference conditions)
Assessment results are expressed as **EQRs**	YES
Sampling procedure allows for **representative** information about water body quality/ecological status **in space and time**	YES
All data relevant for assessing the biological **parameters** specified in the WFD's normative definitions are covered by the **sampling procedure**	YES
Selected taxonomic level achieves adequate **confidence and precision** in classification	YES

IC FEASIBILITY CHECKING

The intercalibration process ideally covers all national assessment methods within a GIG. However, the comparison of dissimilar methods ("apples and pears") has clearly to be avoided. Intercalibration exercise is focused on specific type / biological quality element / pressure combinations. The second step of the process introduces an "IC feasibility check" to restrict the actual intercalibration analysis to methods that address the same common type(s) and anthropogenic pressure(s), and follow a similar assessment concept.

There are still no common intercalibration types for both EC -GIG (Dinaric Western Balkan) and MED -GIG natural lakes. Croatia decided to classify the ecological quality of natural lakes according to stricter Mediterranean reference thresholds by de Hoyos et al (2014) and to adapt a certain type specificity to the assessments as described by Ntislidou et al (2016) in the evaluation of Greek natural lakes (MED GIG).

4.1. TYPOLOGY

Does the national method address the same common type(s) as other methods in the Intercalibration group? Provide evaluation if IC feasibility regarding common IC types.

There are seven natural lakes in Croatia with an area of more than 0.5 km^2. All of them are located in the Dinaric ecoregion (Ecoregion 5 - Dinaric Western Balkan): two of them (Plitvice lakes: Kozjak Lake and Prošće Lake) in the Dinaric Continental sub-ecoregion (EC - GIG) and five of them in the Dinaric Mediterranean sub-ecoregion (MED - GIG).

4.2. PRESSURES ADDRESSED

Does the national method address the same pressure(s) as other methods in the Intercalibration group? Provide evaluation if IC feasibility regarding pressures addressed.

In the Mediterranean GIG all national methods were calibrated to address eutrophication pressure. The Croatian method addresses eutrophication pressure, fish biomass and land use in the catchment area.

4.3. ASSESSMENT CONCEPT

Does the national method follow the same assessment concept as other methods in the Intercalibration group? Provide evaluation if IC feasibility regarding assessment concept of the intercalibrated methods.

All assessment methods included in the IC Mediterranean exercise, focus on the littoral zone of the lake, which is in use in some MS, while others use the profundal zone, hence “the case where the Intercalibration exercise is not possible”.

4.4. CONCLUSION ON THE INTERCALIBRATION FEASIBILITY

Provide conclusions on the IC feasibility.

The reason for not doing the intercalibration was lack of appropriate comparable data, i.e. comparable lake types and reference conditions among MS-s.

5. DESCRIPTION OF THE BIOLOGICAL COMMUNITIES

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT HIGH STATUS

The macroinvertebrate community in the high status of Croatian type HR-L_2 (deep karstic lake) is characterized by almost no Chironomini individuals present in the macroinvertebrate community. Here, the ratio of Ephemeroptera, Plecoptera and Trichoptera individuals equals 25% or more of the total macroinvertebrate abundance, whereas the ratio of Tubificinae individuals is up to 4%, respectively.

The macroinvertebrate community in the high status of all other Croatian “deep” types is characterized by almost no Chironomini individuals present in the macroinvertebrate community. Here, the ratio of Ephemeroptera, Plecoptera and Trichoptera individuals equals 25% or more of the total macroinvertebrate abundance. A high level of local diversity is present with the number of families around 30 and Margalef index values of 5.5 (and more).

In the shallow Vransko Lake near Biograd (HR-J_4) the macroinvertebrate community in high status is characterized by the ratio of Ephemeroptera, Plecoptera and Trichoptera individuals equal 10% or more of the total macroinvertebrate abundance, whereas the ratio of Tubificinae individuals is around

14%, respectively. A high level of local diversity is present with the number of families around 30 and Margalef index values of 5.5 (and more).

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT GOOD STATUS

The macroinvertebrate community in good status of Croatian type HR-L_2 (deep karstic lake) is characterized by very few Chironomini individuals present in the macroinvertebrate community. Here, the ratio of Ephemeroptera, Plecoptera and Trichoptera individuals is around 20% of the total macroinvertebrate abundance, whereas the ratio of Tubificinae individuals is up to 7%, respectively.

The macroinvertebrate community in good status of all other Croatian “deep” types is characterized by few Chironomini individuals present in the macroinvertebrate community. Here, the ratio of Ephemeroptera, Plecoptera and Trichoptera individuals is around 20% or more of the total macroinvertebrate abundance. There is a high local diversity with a number of families around 25 and Margalef index values around 5.0.

In the shallow Vransko Lake near Biograd (HR-J_4) the macroinvertebrate community good status is characterized by regularly Chironomini individuals present in the macroinvertebrate community. Here, the ratio of Ephemeroptera, Plecoptera and Trichoptera individuals is around 8% of the total macroinvertebrate abundance, whereas the ratio of Tubificinae individuals is around 19%, respectively. There is a high local diversity with a number of families around 25 and Margalef index values around 5.0.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT MODERATE STATUS

The macroinvertebrate community in moderate status of Croatian type HR-L_2 (deep karstic lake) is characterized by around 4% Chironomini individuals present in the macroinvertebrate community. Here, the ratio of Ephemeroptera, Plecoptera and Trichoptera individuals is around 15% of the total macroinvertebrate abundance, whereas the ratio of Tubificinae individuals is up to 10%, respectively.

The macroinvertebrate community in moderate status of all other Croatian “deep” types in the Dinaric ecoregion is characterized by around 4% Chironomini individuals present in the macroinvertebrate community. Here, the ratio of Ephemeroptera, Plecoptera and Trichoptera individuals is around 15% or more of the total macroinvertebrate abundance. The local diversity is relatively high, the number of families is about 20 and the Margalef Index is about 4.

In the shallow Vransko Lake near Biograd (HR-J_4) the macroinvertebrate community moderate status is characterized by around 8% Chironomini individuals present in the macroinvertebrate community. Here, the ratio of Ephemeroptera, Plecoptera and Trichoptera individuals is around 6% of the total macroinvertebrate abundance, whereas the ratio of Tubificinae individuals is around 24%, respectively. The local diversity is relatively high, the number of families is about 20 and the Margalef Index is about 4.

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Report on the Croatian assessment method for fish in natural lakes in the case where the Intercalibration exercise is not possible (Gap 3)

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Report on the Croatian assessment method for fish in natural lakes in the case where the Intercalibration exercise is not possible (Gap 3)

1. INTRODUCTION

- Member state: Croatia;
- BQE: Fish;
- Water body category (type): Lakes.

Croatia has abundant water resources; however, it has only six natural lakes, which are divided into five types according to abiotic factors: high deep small calcareous lakes (Kozjak and Prošćansko Lakes in the Plitvice Lakes system), lowland deep medium sized calcareous lakes, with cryptodepression (Vrana Lake on the Cres Island), lowland deep small calcareous lakes, with cryptodepression (Baćinska Lakes), lowland shallow large calcareous lakes, with cryptodepression (Vransko Lake near Biograd) and lowland medium deep and medium sized calcareous lakes (Visovac Lake).

Kozjak and Prošćansko Lakes, which are parts of the Plitvice Lakes system, and Visovac Lake are lake-forming river sections. Vransko Lake near Biograd and Baćinska Lakes (Crniševo and Oćuša) are connected to the sea, through permeable karstic terrain and a man-made connections. There are no similar water systems in a conserved state to serve for comparison as sources of reference values for the assessment of their ecological status. Considering the biological element fish, there is marked human impact on all lake systems. Intentionally or not, species from the Danube River basin or other river basins outside Croatia have been introduced to the Adriatic River basin and non-native (introduced) species often significantly influence the present composition of the ichthyofauna in the lakes.

2. DESCRIPTION OF NATIONAL ASSESSMENT METHODS

For the assessment of the ecological quality of six natural lakes present in Croatia, a fish-based index was developed, as required by the Water Framework Directive (WFD) 2000/60/EC. The development of the **Croatian fish index for natural lakes (CFIL)** included procedures previously identified as the best practices (Hering et al. 2006, Argillier et al. 2013, Petriki et al. 2017), but also takes into account peculiarities of Croatian karstic lakes and exceptionally rich ichthyodiversity, with high portion of endemic species. The methodology for CFIL development included steps and methods described in Hering et al. (2006), but also implemented in Petriki et al. (2017) and other papers describing fish-based indices for the assessment of the ecological quality of lakes. Thereafter, the following procedures were implemented:

- Field sampling of fish in Croatian lakes
- Obtaining of all relevant environmental parameters
- Calculating fish fauna metrics
- Selection of relevant environmental parameters and pressure proxies, as well as fish fauna metrics that respond to at least one pressure proxy

- Ecological Quality Ratios calculations
- Multimetric index generation
- Ecological quality class boundaries implementation

The Croatian fish index for natural lakes documents the relationships between fish and pressures in their habitats, as requested by the WFD.

2.1. FISH FAUNA SAMPLING

Description of sampling and data processing

- Sampling time and frequency:

Sampling is carried out once in three years, from April to November, when fish is active in water bodies.

- Sampling method:

Sampling is carried out according to the standard HRN EN 14757:2015 Water quality – Fish sampling with nets of different mesh sizes (EN 14757:2015), in which the system of random placement of net sets depending on the lake surface and depth are elaborated in detail.
According to the above standard, depending on the lake surface and depth, an exact number (Table 1) of standard nylon nets of different mesh sizes of the "Nordic" type is used for collecting a quantitative fish sample of all age categories. In small, shallow lakes (≤ 10 ha), even 8 nets could be sufficient for overfishing, thus it should be adapted to habitat conditions, although fishing efforts should not include less than 4 nets within a fishing period of 12 hours during one night.

Table 1. Number of efforts with benthic gillnets required to allow detection of 50% changes between sampling occasions in relation to lake area and maximum depth

Depth (m)	≤ 20	21- 50	51 - 100	101 - 250	251 - 1000	1000 - 5000
0 - 5,9	8	8	16	16	24	24
6 - 11,9	8	16	24	24	32	32
12 - 19,9	16	16	24	32	40	40
20 - 34,9	16	24	32	40	48	56
35 - 49,9	16	32	32	40	48	56
50 - 74,9			40	40	56	64
≥ 75					56	64

Fishing in lakes is performed with single nylon nets of the Nordic type that have a length of 30 m and height of 1.5 m. Each such net consists of 12 different fields with mesh sizes from 5 to 55 mm and length of 2.5 m in the following order: 43; 19.5; 6.25; 10; 55; 8; 12.5; 24; 15.5; 5; 35 and 29 mm. Considering the lake hydromorphology and ichthyofauna, it is not necessary to use pelagic nets; benthos nets suffice for obtaining an insight of satisfactory quality into the community for the status assessment.
The guidelines stated in Table 2 are applied to fishing for inventory purposes with nets.

Table 2. Minimum effort benthic gillnets (# of gillnet-nights) used in an inventory sampling in relation to lake area

Lake surface (ha)	Number of nets with different mesh sizes / night		
	Total	In epi-/metalimnion	In hypolimnion
≤ 50	4	2	2
51 – 300	8	4	4
301 – 2000	16	8	8
> 2000	24	12	12

Each net is individually cast as an independent sample at a random angle with respect to the bank, while taking into account the lake depth and a proportional representation of individual depths, i.e. more nets are cast into a depth covering a larger surface. Nets are always cast above the thermocline layer.

Prior to sampling, it is necessary to obtain a map of the lake's hydromorphology to distribute nets evenly at all depths, depending on the representation of an individual depth. If it is a large lake with an uneven bottom, then it is necessary to cast the nets randomly at all depths, provided that two experienced ichthyologists are involved on a maximum of 8 nets per night in oligo- to mesotrophic lakes and that the nets do not remain placed in the water after 8:00 a.m. Generally, nets are cast into a lake between 18:00 and 20:00, and removed from water between 06:00 and 08:00. In more productive lakes, the number of nets must be definitely reduced. Each used net is treated as a separate sample. If the recorded mass of fish caught in a single net is larger than 6 kg, the results are not considered perfect, thus the number of hours the net is in water has to be shortened during the next sampling.

- Data processing:

Fish must be removed from the net as soon as possible, determined to the species and its standard length in mm and mass in grams measured. If a large fish quantity is caught in the net, it is important to transfer the fish into a cool area to prevent the drying of the caught fish, and thus also mistakes in the mass measurement.

Laboratories perform only the analyses of questionable samples, i.e. determination of fish samples whose taxonomic status is questionable (a species with an unusual combination of morphological characteristics, a hybrid, a new species, etc.). It is expected that a large majority of samples will have standard determining characteristics.

- Identification level:

Determination to the species level is conducted.

Description of fish fauna metrics used to describe fish communities in Croatian lakes

All sampled fish species were classified in groups according to their feeding preferences (omnivores, OMNI; invertivores, INV; and piscivores, PISC), preferences for reproductive substrate (lithophilic, LITH; phytophilic, PHYT; phyto-lithophilic, PHLI; pelagophilic, PEL; species that spawn in the sea, SEA; polyphilic, POLI) and habitat preferences (benthopelagic, WCOL and benthic, BENT) (Table 3).

Table 3. Ecological characteristics of fish species found in the Croatian lakes. As non-native species we consider all species that were introduced to a certain lake, even though they might be native to other water bodies in Croatia.

Species	Family	Feeding strategy	Spawning substrate	Habitat preferences	Native/ Non-native
Alburnus neretvae	Cyprinidae	OMNI	PHLI	WCOL	native
Alosa fallax	Clupeidae	OMNI	LITH	WCOL	non-native
Anguilla anguilla	Anguillidae	INV/PISC	SEA	WCOL	native
Atherina boyeri	Atherinidae	INV	PHLI	WCOL	non-native
Aulopyge huegelii	Cyprinidae	INV	LITH	WCOL	native
Carassius gibelio	Cyprinidae	OMNI	PHYT	WCOL	non-native
Chelon auratus	Mugilidae	OMNI	PEL	WCOL	non-native
Chelon labrosus	Mugilidae	OMNI	PEL	WCOL	non-native
Cobitis bilineata	Cobitidae	INV	PHYT	BENT	native
Cobitis illyrica	Cobitidae	INV	PHYT	BENT	native
Cyprinus carpio	Cyprinidae	OMNI	PHYT	WCOL	non-native
Delminichthys adspersus	Cyprinidae	INV	PHYT	WCOL	native
Esox lucius	Esocidae	PISC	PHYT	WCOL	non-native
Gasterosteus aculeatus	Gasterosteidae	OMNI	PHYT	WCOL	native
Knipowitschia croatica	Gobiidae	INV	PHYT	WCOL	native
Knipowitschia mrakovcici	Gobiidae	INV	PHYT	WCOL	native
Lepomis gibbosus	Centrarchidae	INV	POLI	WCOL	non-native
Liza ramada	Mugilidae	OMNI	PEL	BENTH	non-native
Phoxinus phoxinus	Cyprinidae	INV	LITH	WCOL	native
Pseudorasbora parva	Cyprinidae	OMNI	PHLI	WCOL	non-native
Rutilus basak	Cyprinidae	INV	PHYT	WCOL	native
Sabanejewia larvata	Cobitidae	INV	PHYT	BENT	native
Salaria fluviatilis	Blenniidae	INV	LITH	WCOL	native
Salmo labrax	Salmonidae	INV/PISC	LITH	WCOL	native
Salmo trutta	Salmonidae	INV/PISC	LITH	WCOL	non-native
Scardinius dergle	Cyprinidae	OMNI	PHYT	WCOL	native
Scardinius erythrophtalmus	Cyprinidae	OMNI	PHYT	WCOL	non-native
Silurus glanis	Siluridae	PISC	PHYT	WCOL	non-native
Squalius cephalus	Cyprinidae	OMNI	LITH	WCOL	non-native
Squalius squalus	Cyprinidae	OMNI/PISC	LITH	WCOL	native
Tinca tinca	Cyprinidae	OMNI	PHYT	WCOL	non-native

After field investigation, determination and measurement of all individuals, we have prepared a total of 84 metrics that describe fish assemblages. Metrics belonging to four metric types have been prepared (following Furse et al. 2006), but also several additional metrics, similarly as conducted in previous fish-based indices assessments (for example Petriki et al. 2017). Noteworthy, collocation of certain fish metrics under metric types (as defined by Furse et al. 2006) is sometimes arbitrary, because the same metric can sometimes be collocated under more than one metric type. For example, proportion of individuals and biomass of species belonging to certain feeding or habitat preferences type can be addressed as functional metrics, because they correspond with ecological functions of taxa, but also as sensitivity/tolerance metrics, since they will be changed as a response to certain stressors. Nevertheless, all metric types are well represented in the metrics that describe fish communities of Croatian lakes and pertinence of certain metric to metric type is less important, because response in all of them to all environmental parameters and pressure proxies has been investigated, as will be described later.

Of the indicative parameters from the IC Guidance, the age structure of the community was not taken as a parameter that, together with the selected parameters, could additionally contribute to the total assessment of the ecological quality of the lakes. Considering the conducted investigations in Central Europe (Šmejkal et al, 2015), which focused on larger specimens that could not be caught by nets according to the EU standard, it was concluded that the share of common bream specimens older that five years in the sample was significantly underestimated. The case was similar with other long-living species. This inadequately shows the actual status regarding the age of the population without a modification of the standard method of net fishing, and the age structure was omitted from the parameters used for the calculation for the Croatian fish index for natural lakes (CFIL).

Table 4. Overview of the metrics included in the analyses with their abbreviations in brackets. Metrics uLITH, pPISC, Adif, Hrat and uSn were eventually chosen for index generation (marked with bold letters).

Composition/ abundance metrics	Richness/ diversity metrics	Sensitivity/ tolerance metrics	Functional metrics	Other metrics
Proportion of native species (pSn)	Total number of species (S)	**Proportion of native individuals (uSn)**	Number of lithophilic species (LITH)	Total biomass (B)
Proportion of non-native species (pSa)	Number of native species (Sn)	Proportion of non-native individuals (uSa)	Number of phytophilic species, (PHYT)	Biomass of native individuals (Bnat)
Proportion of phytophilic species (pPHYT)	Number of non-native species (Sa)	**Proportion of litophilic individuals (uLITH)**	Number of phyto-lithophilic species (PHLI)	Biomass of non-native individuals (Balo)
Proportion of phyto-lithophilic species (pPHLI)	Proportion of Salmoniform species (pSALM)	Proportion of phytophilic individuals (uPHYT)	Number of pelagophilic species (PEL)	Total length of the most abundant species based on the number of individuals (TLmaxn)
Proportion of pelagophilic species (pPEL)	Proportion of Cypriniform species (pCYPR)	Proportion of phyto-lithophilic individuals (uPHLI)	Number of species spawning in the sea (SEA)	Total length of the most abundant species based on the biomass (TLmaxb)
Proportion of species spawning in the sea (pSEA)	pSALM/pCYPR	Proportion of pelagophilic individuals (uPEL)	Number of invertivorous species (INV)	
Proportion of invertivorous species (pINV)	pPERC (proportion of Perciform species)/pCYPR	Proportion of individuals spawning in the sea (uSEA)	Number of omnivourous species (OMNI)	
Proportion of omnivourous species p(OMNI)	Shannon index (H)	Proportion of invertivorous individuals (uINV)	Number of piscivourous species (PISC)	
Proportion of piscivourous species (pPISC)	Reciprocal Simpson index (1/S)	Proportion of omnivourous individuals (uOMNI)	Number of benthopelagic species (WCOL)	
pPISC/pINV	Margalef index (Ml)	Proportion of piscivourous individuals (uPISC)	Number of benthic species (BENT)	
Proportion of benthopelagic species (pWCOL)	Alpha index (A)	uPISC/uINV	Proportion of phytophilic species biomass (bPHYT)	
Proportion of benthic species (pBENT)	Berger-Parker index (d)	Proportion of benthopelagic individuals (uWCOL)	Proportion of phyto-lithophilic species biomass (bPHLI)	
	Shannon index based on native species (Hnat)	Proportion of benthic individuals (uBENT)	Proportion of pelagophilic species biomass (bPEL)	
	Reciprocal Simpson index for native species (1/S)	Proportion of Salmoniform individuals (uSALM)	Proportion of biomass of species spawning in the sea (bSEA)	
	Margalef index fr native species (Mlnat)	Proportion of Cypriniform individuals (uCYPR)	Proportion of invertivorous species biomass (bINV)	
	Alpha index for native species (Anat)	uSALM/uCYPR	Proportion of omnivourous species biomass (bOMNI)	
	Berger-Parker index for native species (dnat)	uPERC (proportion of Perciform individuals)/uCYPR	Proportion of piscivourous species biomass (bPISC)	
	Hnat-H (Hdif)	Proportion of native individuals biomass (bnat)	bPISC/bINV	
	1/Snat-1/S (1/Sdif)	Proportion of non-native individuals biomass (balo)	Proportion of benthopelagic species biomass (bWCOL)	
	Mlnat-Ml (Mldif)		Proportion of benthic species biomass (bBENT)	
	Anat-A (Adif)		Proportion of Salmoniform species biomass (bSALM)	
	dnat-d (ddif)		Proportion of Cypriniform species biomass (bCYPR)	
	Hnat/H (Hrat)		bSALM/bCYPR	
	1/Snat/1/S (1/Srat)			
	Mlnat/Ml (Mlrat)			
	Anat/A (Arat)			
	dnat/d (drat)			

2.3. ENVIRONMENTAL PARAMETERS AND PRESSURE PROXIES

Description of all environmental parameters and indicators of anthropogenic pressures investigated in Croatian lakes

Altogether 28 parameters describing habitat conditions and anthropogenic pressures were assessed, including the hydrological, morphological and physico-chemical components (alkalinity, conductivity, pH, transparency, temperature, concentrations of ammonia ions, molecular ammonium, nitrates, nitrogen, phosphorous, total organic carbon, dissolved organic carbon, fluorides, calcium, potassium, chlorides, magnesium, sodium, dissolved silicates, sulphates, dissolved oxygen, oxygen saturation, biological oxygen consumption and chemical oxygen consumption, chlorophyll alpha, total biomass of phytoplankton, as well as the percentage coverage of each lakes' drainage area by non-natural land use (NNLC, estimated by ArcGIS 10) and Lake Habitat Modification Score (LHMS). Average values of all physico-chemical parameters in the warmer part of the year (from April to September) were included into further analyses. The LHMS was calculated for each lake (following procedure of Rowan et al. 2006) as a proxy of the general degradation of the lake. The NNLC and concentration of total phosphorus in the water are usually considered as the most important indicators of eutrophication (Launois et al. 2011, Argillier et al. 2013, Petriki et al. 2017). However, other parameters can also indicate eutrophication, particularly dissolved silicates concentration, dissolved oxygen concentration etc., but also point to other human pressures in the lakes. LHMS can also represent a proxy of the morphological alterations and human pressures on lakes (Petriki et al. 2017). Besides all already mentioned parameters, we have also considered proportion of non-native fish species in each lake as a pressure. Namely, non-native species did not enter any of the Croatian lakes by their invasive colonization and then established stabile population because some environmental parameters were changed so that they suit them, yet they were intentionally introduced in lake by humans or have entered lakes through man-made channels. Non-native species have been recognized as one of the most dangerous threats to native fish species. Thereafter, they should be considered as a pressure provoking certain negative responses in native fish communities, rather than a response to some other pressures.

2.4. STATISTIC ANALYSES FOR METRIC SELECTION

Detailed description of statistical analyses employed for metric selection and pressure-response relationships

Upon preparation of two sets of parameters (one describing fish communities and the second one concerning environmental parameters and pressure proxies), metrics in both of them were subjected to similar procedures in order to choose the ones that are not correlated with each other, that have normal distribution and for which a clear pressure-response relationship can be confirmed.

Parameters were first standardized by log-transformation (used for count measures) or logistic model (used for proportions), whereas diversity indices and measures derived from them were not transformed, because they are standardized *per se*.

After standardization, Pearson's correlation coefficient was calculated among all metrics inside each data set and in cases where coefficient was higher than 0.7, one or more metrics were excluded and the one with better ecological interpretation was retained. In cases where ecological interpretation was not clear, both variables were included in the next step and the one with no or lower pressure-response relationship was excluded later.

Responses of fish fauna metrics on all environmental parameters and pressure proxies were analyzed by stepwise linear regression. Metrics that were significantly correlated with at least one pressure ($R^2>0.4$ and significance level, $p<0.05$) were checked for complying with linear regression assumptions (normal distribution, linearity and absence of multi-collinearity). If both conditions were met (significant correlation with at least one pressure and linear assumptions), those metrics were considered for the index development. Again, correlation coefficients were calculated among metrics of both data sets and, finally, in cases of significant correlation, metrics for which better pressure-response relationships were obtained, were included in the index calculation.

2.5. PRESSURE-RESPONSE RELATIONSHIPS AND SELECTED METRICS

Description of the pressure-response relationships

Clear pressure-response relationship has been established in the following cases, in which metrics also have normal distribution and comply with linear regression assumptions:

- Proportion of individuals belonging to lithophilic species (uLITH) shows response to dissolved silicates ($R^2=0.797$, $p=0.01$; Figure 1) and total biomass of phytoplankton ($R^2=0.655$, $p=0.032$; Figure 2), both being proxies of anthropogenic pressure and eutrophication. Several investigations have already found higher total biomasses of phytoplankton on localities that are under stronger anthropogenic impact (Bužanić et al. 2016), particularly localities where eutrophication has been noticed (Smith 2003, Chislock et al. 2013, Gonzales & Roldan 2019, Taipale et al. 2019). On the other hand, even though total biomass of phytoplankton is elevated in cases of eutrophication, there are evidences that its nutritional value for fish is reduced (Taipale et al. 2019). Silicates in the water are also often connected with eutrophication (Schelske & Stoermer 1971, Conley et al. 1993, Ittekkot et al. 2000). Noteworthy, those two parameters are not significantly correlated in Croatian lakes, but both of them invoked response of fish community, particularly its lithophilic component.
- Proportion of piscivorous species (pPISC) shows response to pH ($R^2=0.682$, $p=0.027$; Figure 3) in Croatian lakes. Changes in water pH values can be consequence and, thereafter, indicators of eutrophication, even though connection between pH and eutrophication is complex and not well understood yet (Chislock et al. 2013). It has been proposed that the change in pH is connected with eutrophication, because it is related to the availability and absorption of nutrients from solution (Yang et al. 2008). Lowering of pH in the sea water (higher acidity), as a consequence of eutrophication, has already been documented (Wallace et al. 2014).
- Difference between alpha index based on native species and alpha index based on the whole fish community (Adif), including non-native species, is a metric showing significant response to total phosphorous in the water ($R^2=0.92$, $p=0.002$; Figure 4). Elevated concentrations of total phosphorus are considered as one of the most powerful indicators and causes of eutrophication (Correll 1998, Yang et al. 2008) and are often used as an eutrophication proxy.
- Ratio between Shannon index based on native species and the same index based on the whole fish community (Hrat), including non-native species, shows response to concentration of potassium ($R^2=0.928$, $p=0.001$; Figure 5). Even though potassium is necessary for osmoregulation in fish, elevated levels of this element and its salts are toxic and the tolerance of a fish species is determined by its physiological valence. It is possible that invasive fish species,

generally having wider ecological valence, are also more tolerant to elevated levels of this element.

- Finally, as expected, proportion of individuals belonging to native species (uSn) show obvious response to proportion of non-native species in certain fish community in Croatian lakes (R^2=0.575, p=0.049; Figure 6).

Besides the abovementioned environmental parameters that can be considered as pressures and provoke responses in fish communities in Croatian lakes, changes in several fish fauna parameters as a response to alkalinity have also been established. However, we find differentiations of this parameter in Croatian lakes natural conditions of karstic watersheds and, even though they do affect the fish community, we consider that as a natural condition and not as consequence of anthropogenic pressures.

Thereafter, Croatian fish index for natural lakes (CFIL) , based on fish as biological element, shall be based on the following fish fauna metrics: uLITH, pPISC, Adif, Hrat and uSn, incorporating response of fish communities on dissolved silicates, total mass of phytoplankton, pH, total phosphorus, dissolved potassium and presence of non-native fish species. These metrics have strong correlation with pressures and have ecological interpretation, but are not significantly intercorrelated. Thereafter, all five metrics are eligible to be included in the index.

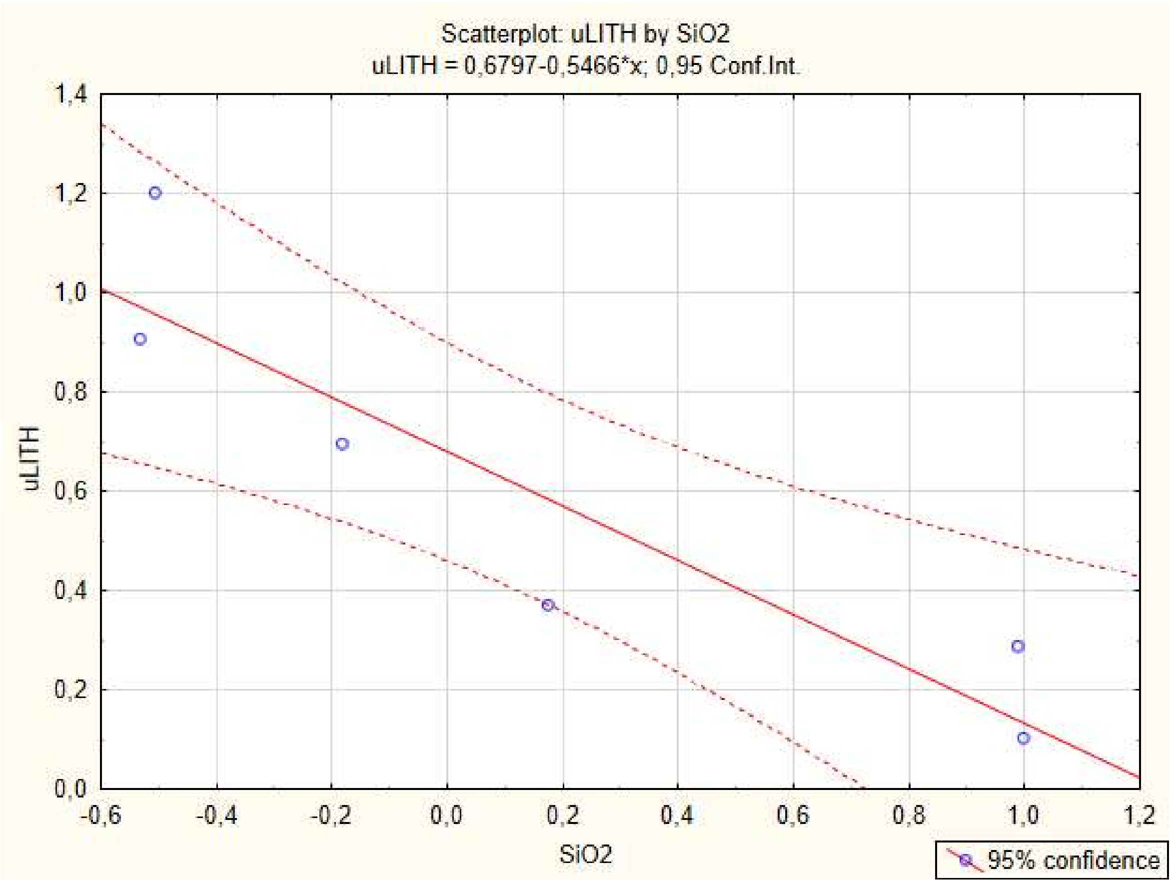

Figure 1. Scatterplot of the linear regression between proportion of individuals belonging to lithophilic species (uLITH) and concentration of dissolved silicates (SiO_2). The scatterplot is based on the standardized values of metrics.

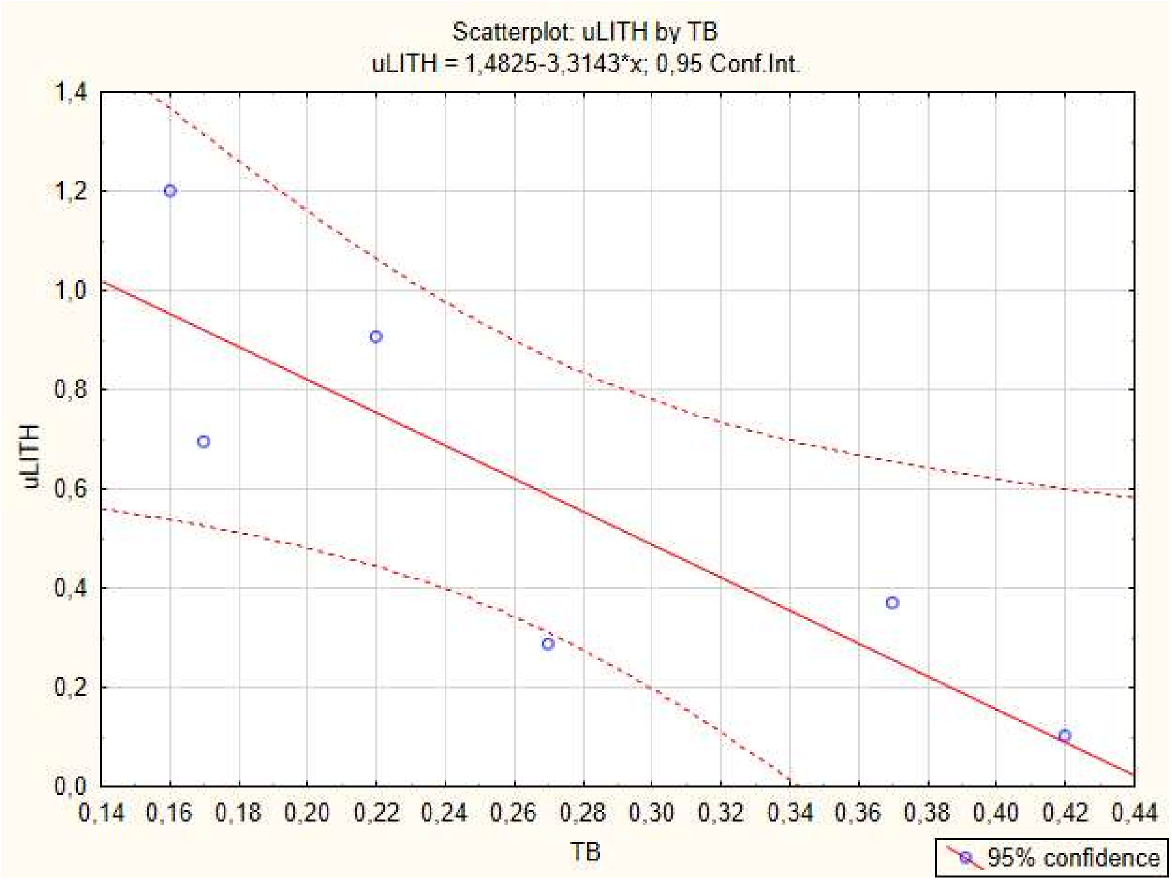

Figure 2. Scatterplot of the linear regression between proportion of individuals belonging to litophilic species (uLITH) and total biomass of phytoplankton (TB). The scatterplot is based on the standardized values of metrics.

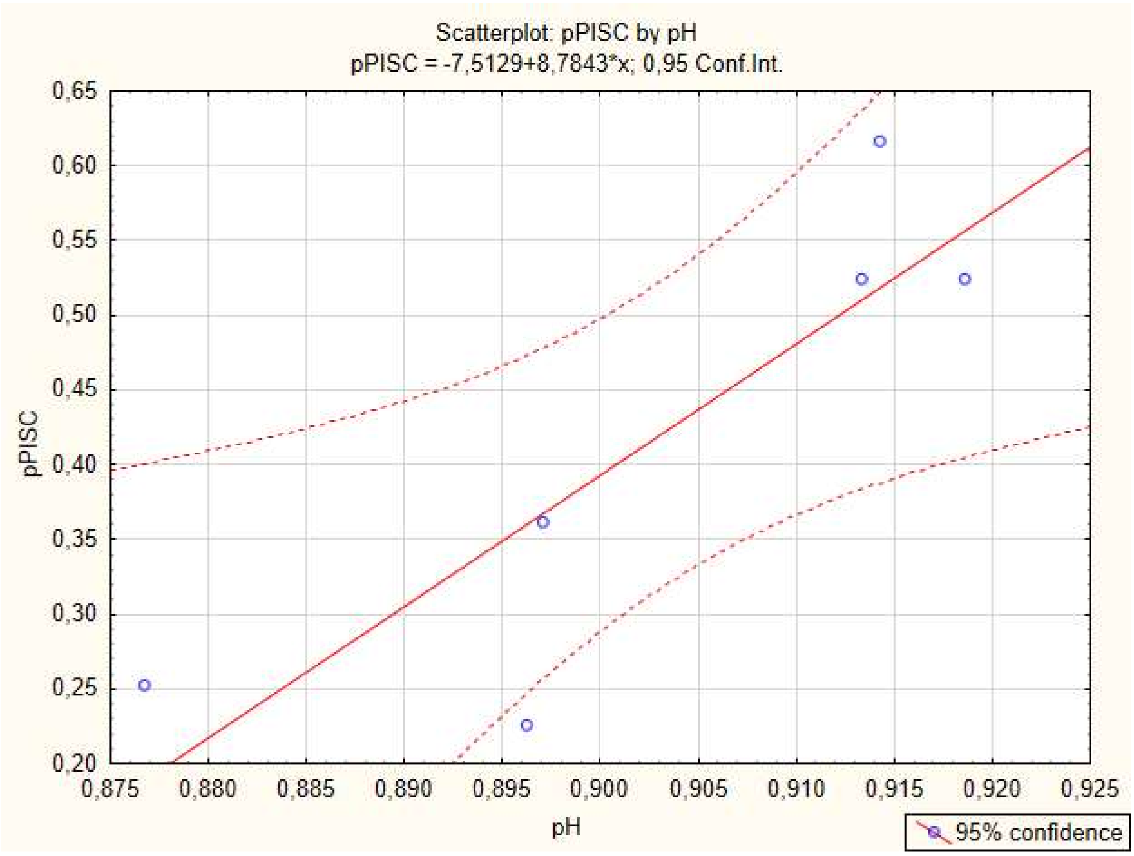

Figure 3. Scatterplot of the linear regression between proportion of piscivorous species (pPISC) and pH values. The scatterplot is based on the standardized values of metrics.

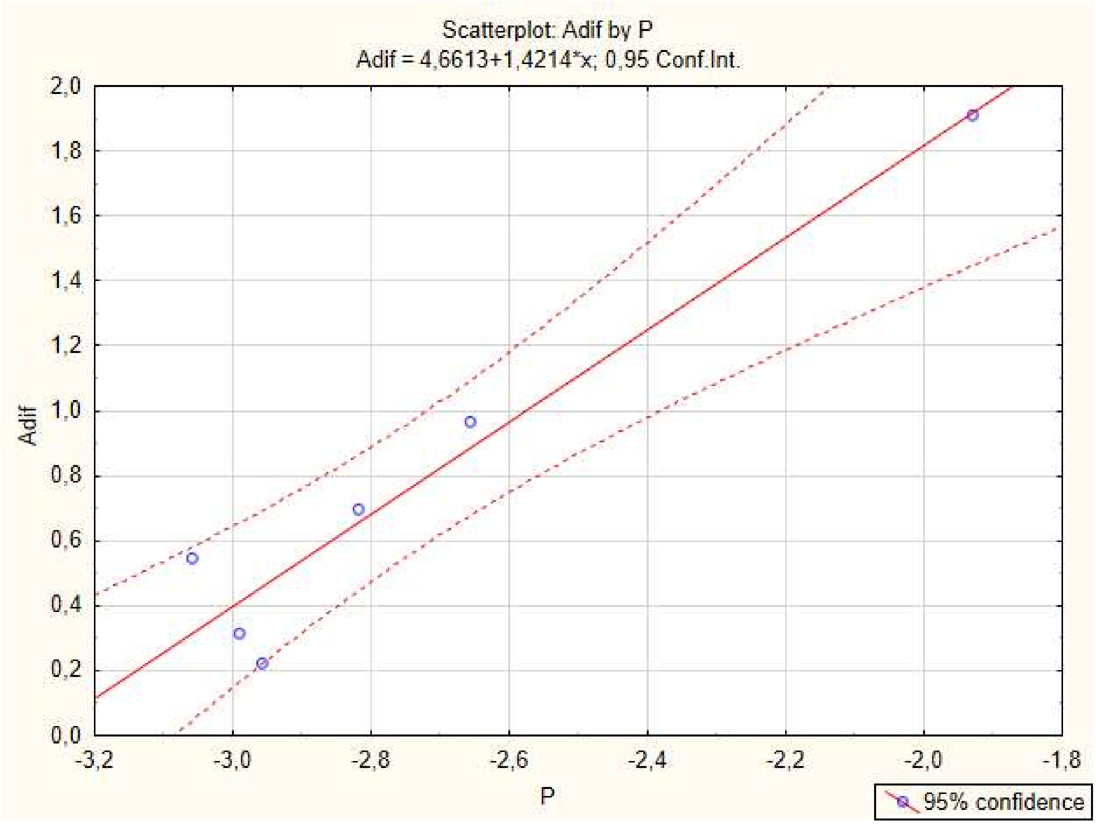

Figure 4. Scatterplot of the linear regression between difference between the alpha index based on the native species and on the whole fish communities (Adif) and concentration of phosphorus (P). The scatterplot is based on the standardized values of metrics.

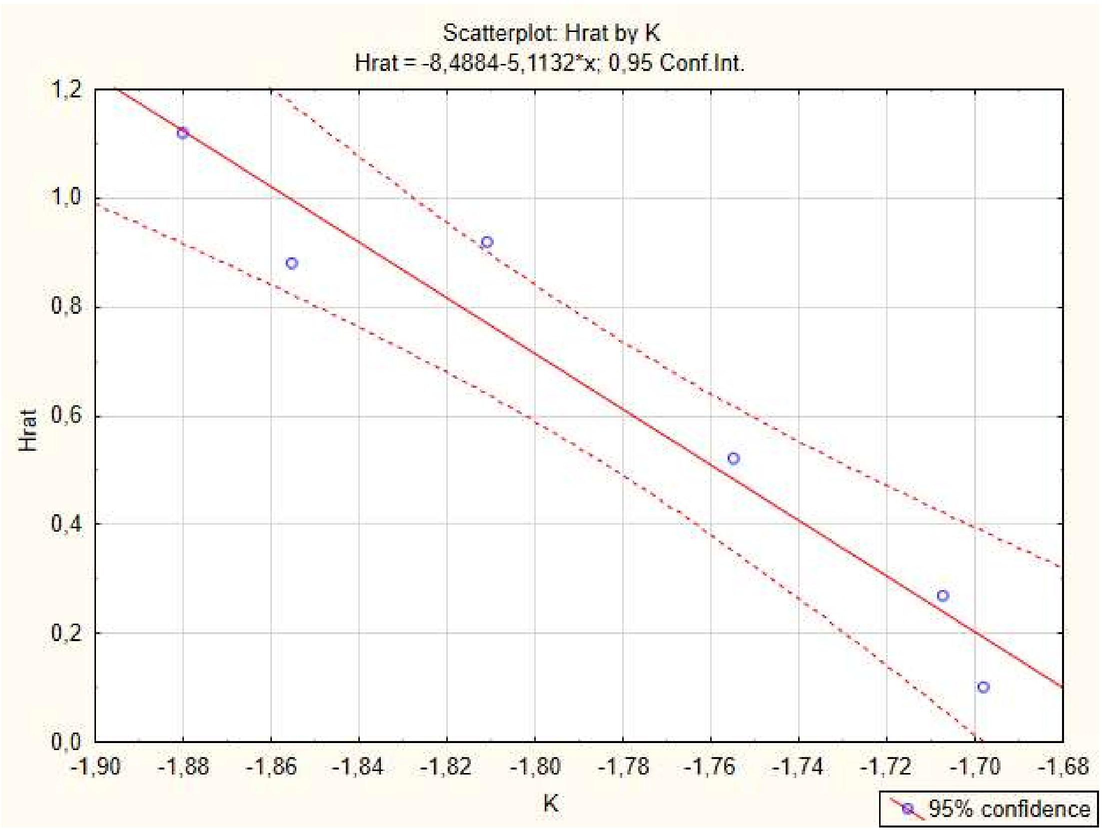

Figure 5. Scatterplot of the linear regression between ratio of the Shannon index based on native species and the same index based on the whole fish community (Hrat) and the concentration of dissolved potassium (K). The scatterplot is based on the standardized values of metrics.

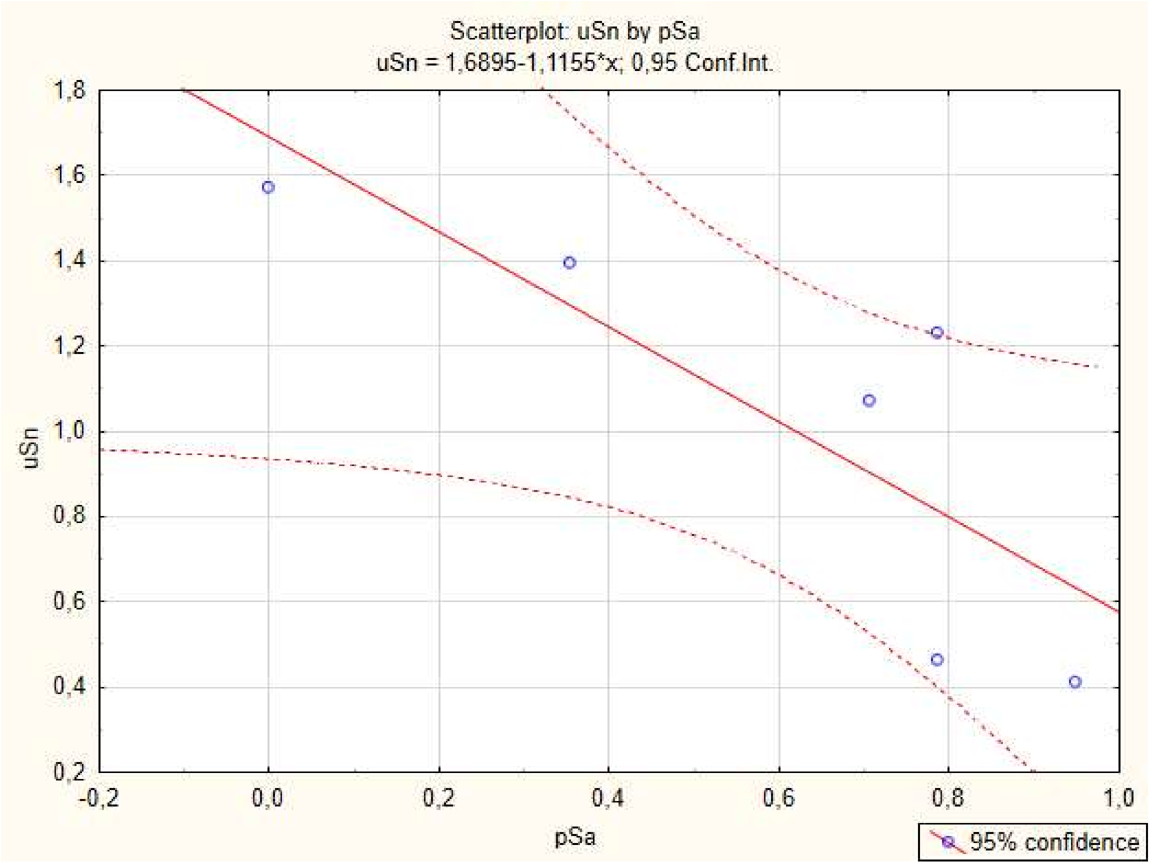

Figure 6. Scatterplot of the linear regression between proportion of individuals belonging to native species (uSn) and proportion of non-native species (pSa). The scatterplot is based on the standardized values of metrics.

2.6. NATIONAL REFERENCE CONDITIONS

Several problems embitter application of usually applied methods when estimating reference conditions in Croatian lakes. Low number of lakes that are all influenced by various anthropogenic threats disables identification of any of the lakes as expressing reference conditions regarding fish community, even though some other parameters (for example some physico-chemical parameters) might express reference conditions. Moreover, there is a lack of estimated reference conditions for some of the physico-chemical parameters, disabling extrapolation of reference conditions for fish metrics in cases where they show significant response to certain environmental parameter. And finally, extrapolations are also problematic because fish metrics do not necessarily follow the same pressure-response pattern, because some are influenced by more than one pressure. Moreover, some are influenced by natural, pronounced differences among fish communities between various lakes. Even though our final index is based on metrics for which a clear pressure-response relationship could be established and metrics connected with natural fluctuations among lakes were excluded, it is possible that pattern of the observed pressure-response relationship is connected with the community structure. Nevertheless, inclusion of five different metrics into the final index calculation enables yielding balanced index that incorporates responses to various pressures. Due to the described limitations, we did not base Ecological Quality Ratios on national reference conditions, but have estimated upper and lower anchors for all metrics, following recommendations of Furse et al. (2006), for assessments with no reference sites.

2.7. ECOLOGICAL QUALITY RATIOS CALCULATION

Detailed description of ecological quality ratios calculation, including anchors estimation

Due to the described limitations and since data on reference sites cannot be applied, we have estimated upper and lower anchors for Ecological Quality Ratios (EQRs) either by extrapolation (in cases where extrapolation was possible, as will be explained later) or as corresponding to metrics' values under the best (for the upper anchor) and the worst (for the lower anchor) attainable conditions, following recommendations of Furse et al. (2006).

Upper and lower anchors for calculations of EQR(uLITH) were obtained by extrapolation. Namely, concentration of dissolved SiO_2 is significantly correlated with the concentration of total phosphorous and anchors for this element are proposed for Croatian lakes (0,005 mgL^{-1} as the best state, and 0,1 mg L^{-1} as the worst state). Since uLITH is significantly and linearly correlated with SiO_2 concentration, upper and lower anchor values were estimated by extrapolation. Similarly, extrapolation of upper and lower anchors was conducted for EQR(Arat), that is significantly correlated with total phosphorus concentration.

Extrapolation of the anchors for pPISC was not possible, because reference conditions for pH (nor any of metrics to which pH is significantly correlated) are not established, so we had to apply another approach. From the pressure-response analysis it is obvious that proportion of piscivorous species in fish communities is lower in lakes where pH is also lower (acidification is higher, possibly due to eutrophication). Thereafter, the worst possible case (lower anchor) would be complete absence of piscivorous species. On the other hand, the best possible case would not be dominance of piscivourous species, but their proportion around 1/3 of the fish community (based on the historical data of fish communities in Croatian lakes).

For the metric Hrat, that is based on differences between Shannon index based on native community and the whole community, including non-native species, upper and lower anchors were set to 1 and 0, because those are values under best and worst possible conditions. The similar situation was for the proportion of individuals belonging to native species for which 1 is upper anchor (the best possible condition - case when pressure value is 0) and 0 is the lower anchor (the worst possible condition - case where the pressure value is 1).

Upon estimation of the anchors, EQRs were calculated following formulas of Furse et al. (2006):

EQR_{metric} = (Metric result – Lower anchor)/(Upper anchor – Lower anchor), for metrics decreasing with increasing pressure (uLITH, uPISC, Hrat and uSn)

EQR_{metric} = 1- (Metric result – Lower anchor)/(Upper anchor – Lower anchor), for metrics increasing with increasing pressure (Adif).

2.8. GENERATION OF CROATIAN FISH INDEX FOR NATURAL LAKES

Detailed description of methodology used to calculate Croatian fish index for natural lakes

As already mentioned, described procedure yielded five fish community metrics (uLITH, pPISC, Adif, Hrat, uSn) that are eligible for inclusion in the multimetric index, because they show significant response to certain pressures, are strongly correlated with pressures, but are not intercorrelated. Ecological

Quality Ratios (EQRs) based on the five metrics were calculated following described procedure and the final index named CFIL (Croatian Fish Index for Natural Lakes) was estimated as the average value of five EQRs:

$$CFIL = \frac{EQR(uLITH) + EQR(pPISC) + EQR(Adif) + EQR(Hrat) + EQR(uSn)}{5}$$

CFIL is a multimetric index, integrating several metric scores and, thereafter, simplifying decision making, because a single value can be used to determine and monitor the quality class of certain lake (Furse et al. 2006). Since it combines effects of different fish fauna metrics, it aggregates responses to several pressures: concentration of dissolved silicates and total biomass of phytoplankton, which both are indicators of eutrophication, lowering of the pH value (higher acidity), higher concentrations of phosphorus and potassium, as well as presence of non-native species. The advantage of CFIL is that, besides the response of fish community to eutrophication, it considers pressures related to non-native species and pollution. Namely, most of fish based indices reflect the eutrophication status of the lakes (reviewed in Petriki et al. 2017) and the response of fish to eutrophication has been well documented (e.g. Jeppesen et al. 2002, Donohue et al. 2009). On the other hand, based on previous reports and expert judgements, non-native species are the most problematic threat for fish communities in Croatian lakes, but also elsewhere. Inclusion of responses to eutrophication, but also to non-native species and pollution, enables CFIL to be a balanced index, integrating effects of all pressures acting on fish communities. Moreover, by integrating all pressures in CFIL, conservational measures acting on any of the pressures and lowering pressure effect will result in improvement of CFIL. The index is site-specific.

2.9. NATIONAL BOUNDARY SETTING

Since CFIL provides a score that represents the average value of EQRs, which present the relationship between values of fish metrics in certain lake and values of those parameters under reference conditions, the ecological status of lakes can be classified into five classes for assessment of impairment in accordance with the demands of the WFD.

Table 5. CFIL classification - class boundaries setting for biological quality element fish

ECOLOGICAL STATUS CATEGORY	**CFIL CLASS BOUNDARIES**
HIGH / VERY GOOD	**0.80-1.00**
GOOD	**0.60-0.79**
MODERATE	**0.40-0.59**
POOR	**0.21-0.39**
BAD	**0-0.20**

Using described protocol, the ecological status of six natural lakes in Croatia is assessed (Table 6).

Table 6. Assessment of ecological status of Croatian natural lakes, based on fish as biotic element.

	EQR(uLITH)	EQR(pPISC)	EQR(Adif)	EQR(Hrat)	EQR(uSn)	**CFIL**	**Ecological status**
Plitvice-Kozjak	1,00	0,83	0,82	1,00	0,2	0,77	GOOD
Plitvice-Prošćansko Lake	1,00	1,00	0,77	0,10	0,16	0,61	GOOD
Vrana (Cres)	0,84	1,00	0,90	0,52	0,89	0,83	VERY GOOD
Baćinska	0,81	0,33	0,68	0,88	0,93	0,73	GOOD
Vransko (Biograd)	0,78	0,57	0,36	0,27	0,6	0,52	MODERATE
Visovac	0,95	0,42	0,93	0,92	0,97	0,84	VERY GOOD

Even though anthropogenically induced pressures are present on the Croatian natural lakes, results of our assessment corroborate that they are still mostly in good and very good status, based on fish as biotic element. Two lakes, **Vrana** (on the Cres Island) and **Visovac lake** express very good (high) ecological status. Introductions of non-native species, pollution and eutrophication should be prevented in order to preserve such status.

Three Croatian lakes express good ecological status. The greatest problems in two lakes belonging to the Plitvice Lakes system (**Kozjak** and **Prošćansko Lakes**) is a high portion of non-native species in fish communities (EQR(uSn) for these two lakes are only 0.2 and 0.16). Employment of effective measures for non-native fish removal should enable raising of the ecological status for these two lakes, but also will help recovery and survival of the native fish community. Even though proportion of individuals belonging to non-native species is lower in the **Baćinska Lakes**, those species changed composition of fish community and have the strongest effect on the Adif metric. Those species entered Baćinska lakes mostly through an artificial channel that connects Baćinska lakes with sea and they present a problem for native fish community.

The proportion of individuals belonging to non-native species in the **Vransko Lake** (near Biograd) should also be lowered in order to improve its ecological status. However, environmental parameters in that lake are not optimal for native fish community and should be recovered first. Artificial passage enables see water coming into the Vransko Lake, provoking its salinization, but also allowing several non-native species to invade into the Vransko Lake. Furthermore, we believe that significant pollution from the nearby agricultural areas is also a cause of the impairment of the habitat conditions in the Vransko Lake. This lake is the only Croatian natural lake whose ecological status, based on the fish as biotic element, is moderate so actions aimed at enhancing environmental conditions (closing the connection with the sea water and diminishing pollution) are necessary. Once conditions in the lake are satisfactory, a native fish community should be restored, which will enable improvement of the ecological status of this lake.

Nevertheless, maximal efforts should be conducted (specified in the above text) not only to conserve, but to improve ecological status of Kozjak, Prošćansko and Baćinska Lakes, even though good ecological status is minimal requirement posed by WDF. Achieving very good status of those lakes is possible and will be very beneficial for the native fish communities. Particularly, efficient and urgent actions are required for the Prošćansko Lake, because it is currently near moderate/good status borderline and additional deterioration of the native fish community (due to either eutrophication or non-native species) could make its ecological status becomes moderate very fast.

3. WFD COMPLIANCE CHECKING

The first step in the Intercalibration process requires the checking of national methods considering the WFD compliance criteria (Table 7).

Table 7. List of the WFD compliance criteria and the WFD compliance checking process and results

Compliance criteria	Compliance checking
Ecological status is classified by one of **five classes** (very good, good, moderate, poor and bad).	YES
Very good, good and moderate ecological status are set in line with the WFD's **normative definitions**	YES
All relevant parameters indicative of the biological quality element are covered (see Table 1 in the IC Guidance). A **combination rule** to combine parameter assessment into BQE assessment has to be defined. If parameters are missing, Member States need to demonstrate that the method is sufficiently indicative of the status of the QE as a whole	YES for abundance and taxonomic composition but age structure is not reflected The multimetric Index results from the mean of the selected metrics
Assessment is adapted to **intercalibration common types** that are defined in line with the typological requirements of the Annex II WFD and approved by WG ECOSTAT	NO because of lack of the common intercalibration types
The water body is assessed against **type-specific near-natural reference conditions**	NO because of the lack of near-natural reference conditions. Hence, EQRs were calculated using upper and lower anchors following Furse et al. (2006) recommendations.
Assessment results are expressed as **EQRs**	YES
Sampling procedure allows for **representative** information about water body quality/ecological status **in space and time**	YES
All data relevant for assessing the biological **parameters** specified in the WFD's normative definitions are covered by the **sampling procedure**	YES
Selected taxonomic level achieves adequate **confidence and precision** in classification	YES

4. IC FEASIBILITY CHECKING

The intercalibration process ideally covers all national assessment methods within a GIG. However, the comparison of dissimilar methods ("apples and oranges") has clearly to be avoided. Intercalibration exercise is focused on specific type / biological quality element / pressure combinations. The second step of the process introduces an "IC feasibility check" to restrict the actual intercalibration analysis to methods that address the same common type(s) and anthropogenic pressure(s), and follow a similar assessment concept.

4.1. TYPOLOGY

There are six natural lakes in Croatia with a surface area larger than 0.5 km^2. All of them are located in the Dinaric ecoregion (5. Dinaric Western Balkans): two (Plitvice Lakes: Lake Kozjak and Prošćansko Lake) in the Dinaric Continental sub-ecoregion (EC-GIG) and four in the Dinaric Littoral sub-ecoregion (MED-GIG). According to abiotic factors, the lakes are divided into five types, showed in Table 8. All lake types have average annual alkalinity greater than 1 meq/l. Eastern Continental lake type HR-J_1 are deep mountain lakes, while common intercalibration type L-EC1 is shallow lowland. Mediterranean lake types HR-J_3, HR-J_4 and HR-J_5 have mean depth less than 15 m, hence they are not comparable with common intercalibration types L-M5/7 and L-M8 (Table 9). Only the Vrana Lake on Cres Island (HR-J_2) is deeper than 15 m, and could be classified to IC type L-M8.

Table 8. Lake typology in Croatia.

Type name	Type code	Lake	Lake surface area (km²)	Geology	Altitude (m)	Mean depth (m)	Trophic status	Thermal stratification	Dissolved oxygen stratification	Lake origin
DINARIC ECOREGION (5. DINARIC WESTERN BALKAN)										
DINARIC CONTINENTAL SUB-ECOREGION										
Mountain, deep, small lakes; carbonate bed; oligotrophic	HR-J_1A	Plitvice Lakes, Kozjak	0.5 - 1	carbonate	> 500	> 15	oligotrophic	dimictic	clinograde	carstic, barrage
Mountain, deep, small lakes; carbonate bed; oligotrophic - mesotrophic	HR-J_1B	Plitvice Lakes, Prošće	0.5 - 1	carbonate	> 500	> 15	oligotrophic	dimictic	clinograde	carstic, barrage
DINARIC MEDITERRANEAN SUB-ECOREGION										
Lowland, deep, medium lakes; cryptodepression, carbonate bed	HR-J_2	Vrana Lake, Cres Island	1 - 10	carbonate	< 200	> 15	oligotrophic	monomictic	orthograde to clinograde	cryptodepression
Lowland, medium deep, small lakes; cryptodepression, carbonate bed	HR-J_3	Baćinska Lakes (Crniševo & Oćuša)	0.5 - 1	carbonate	< 200	3 – 15	oligotrophic-mesotrophic	monomictic	clinograde	cryptodepression
Lowland, shallow, big lakes; cryptodepression, carbonate bed	HR-J_4	Vransko Lake, Biograd	10 - 100	carbonate	< 200	< 3	mesotrophic	polimictic	–	cryptodepression
Lowland, medium deep, medium lakes; carbonate bed	HR-J_5	Visovac Lake	1 - 10	carbonate	< 200	3 – 15	oligotrophic	monomictic	orthograde to clinograde	carstic, barrage

Table 9. Common intercalibration types in Eastern Continental and Mediterranean

Type	Lake characterisation	Annual mean precipitation (mm) and T (°C)	Altitude (m above sea level)	Mean depth (m)	Area (km^2)	Catchment (km^2)	Alkalinity (meq/l)	Conductivity (µS/cm)
L-EC1	Lowland very shallow hard-water		< 200	< 6			1 - 4	300 - 1 000
L-M5/7	Reservoirs, deep, large, siliceous, 'wet' areas	> 800 and/or < 15	< 1 000	> 15	0.5 - 50	< 20 000	<1	
L-M8	Reservoirs, deep, large, calcareous		< 1 000	> 15	0.5 - 50	< 20 000	>1	

French natural lakes do not belong to any Mediterranean intercalibration type. The Italian assessment method is site specific and not type specific, so no common types are addressed. Also due to the small number of lakes of the same type, the IC would not be possible. However, two "lake categories" based on fish fauna composition (shallow and deep lakes, respectively) are similar to the following intercalibration types: Mediterranean, small-large, calcareous/mixed. The Greek assessment method is site specific and not type specific as well. Greek deep lakes are mostly mesotrophic to eutrophic, dominated by cyprinids, while shallow lakes are characterized by high endemism and differences in fish fauna among lakes. Bulgarian methodology mainly focuses on the lake type L5 (riparian lakes). Spain does not use fish as BQE in natural lakes. In Portugal and Malta there are no natural lakes. Our closest neighbouring country Slovenia has only two deep lakes in alpine and subalpine region, which were compared with Italian lakes of the similar origin to pass the IC exercise.

4.2. PRESSURES ADDRESSED

Does the national method address the same pressure(s) as other methods in the Intercalibration group? Provide evaluation if IC feasibility regarding pressures addressed.

Italy, France, Bulgaria and Greece have developed their own assessment methods. The Greek method responds to pressure with respect to eutrophication and hydromorphological changes, while the French method responds only to eutrophication. The Italian assessment method addresses catchment land use and eutrophication, hydrology, water level, shoreline degradation, connectivity, fisheries exploitation. Spain does not use fish as BQE in natural lakes. In Portugal and Malta, there are no natural lakes.

The Croatian national assessment method incorporates pressures provoked by eutrophication, non-native species and pollution. Thereafter, some of the pressures addressed are the same or similar (eutrophication), but some pressures considered in other national assessment methods (hydromorphological changes, shoreline degradation, fisheries exploitation) are not integrated into Croatian assessment method. Namely, those pressures are not significantly affecting fish communities in Croatian lakes, as demonstrated also by LHMS, that are very low for all Croatian lakes. On the other hand, non-native species are significant threat for fish communities in Croatian natural lakes, so they are included into Croatian assessment methods, even though it is not considered by some other national assessment methods.

4.3. ASSESSMENT CONCEPT

Does the national method follow the same assessment concept as other methods in the Intercalibration group? Provide evaluation if IC feasibility regarding assessment concept of the intercalibrated methods.

No, due to the lack of common intercalibration types, both for EC-GIG (Dinaric Western Balkans) and MED-GIG natural lakes.

4.4. CONCLUSION ON THE INTERCALIBRATION FEASIBILITY

Provide conclusions on the IC feasibility.

Intercalibration is not possible due to the lack of common intercalibration types, both for EC-GIG (Dinaric Western Balkans) and MED-GIG natural lakes, as well as different pressures addressed.

5. DESCRIPTION OF THE BIOLOGICAL COMMUNITIES

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT HIGH STATUS

High/very good status is the status of lake fish community, whose composition and abundance of species is in line with natural or near natural status. The species sensitive to disturbances are present in the sample. The greatest portion of individuals belong to native fish species (at least 80% of all individuals). Piscivorous species comprise at least about quarter of all fish species in the community. Individuals belonging to lithophilic species comprise at least 10% of all individuals.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT GOOD STATUS

The abundance of the disturbance-sensitive species shows slight signs of distortion from type specific conditions attributable to anthropogenic impacts on physicochemical or hydromorphological or fish community quality elements. Between 60 - 79% of individuals belong to native species, but the proportion of non-native individuals is higher than in communities at moderate ecological status. Proportion of piscivorous species is also lower, they comprise around 18 – 24% of the total number of species. Lithophilic species are present in the community, but with less than 10% of individuals. If some of the index components are higher (for example satisfactory composition of fish community), others might be lower (for example, number of individuals belonging to native species might be lower than 60%).

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT MODERATE STATUS

Non-native species dominate at expense of the native species, there is a lack of native piscivourous species, and the proportion of individuals belonging to lithophilic species is also low.

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Template for reporting the MS assessment method in the case where the Intercalibration exercise is not possible (Gap 3)

1. INTRODUCTION

- Member State Croatia
- BQE Phytoplankton
- Water body category Transitional waters.

2. DESCRIPTION OF NATIONAL ASSESSMENT METHODS

MS has to provide the complete description of the method in the Annex. The main features should be given below

A Multimetric Phytoplankton Index (MPI) proposed by Facca *et al.* (2013) is used for water quality assessment in relation to eutrophication process in transitional waters. The MPI incorporates Hulburt's dominance index, bloom frequency and Menhinick's diversity index, calculated on the basis of phytoplankton species composition and concentration of chlorophyll *a* with the aim to include phytoplankton biomass. The MPI was set up using data obtained through 2014 and 2015. The MPI was calculated by averaging the Ecological Quality Ratio (EQR) values per site obtained through the sampling period. EQR values were calculated for each of 4 metrics:

- Hulburt's dominance index (δ2)
- bloom frequency (F)
- Menhinick's diversity index (D)
- Concentration of Chl *a* (Chl *a*).

The Multimetric Phytoplankton Index (MPI) was calculated by averaging 4 EQRs at each site:
MPI = mean EQR (100- δ2); EQR (100-F); EQR (D); EQR (Chl *a*)

Only organisms identifiable to species level (single indeterminate taxa, such as *Navicula* sp. 1, Cryptophyceae sp. 1, Taxa sp.1, can be included) by means of conventional inverted light microscopy (cells size >2 μm) have to be used to calculate the abundance (N) and hence Hulburt's dominance index, bloom frequency and Menhinick's diversity index.

Hulburt's dominance index (δ2)
Hulburt's index tests the species dominance and is calculated as:

$\delta 2 = 100(n1+n2)/N$

n1 represents abundance of dominant species; n2 represents abundance of the second most abundant species; N represents total abundance
The numerical values are calculated as 100- δ

Bloom frequency (F)

Bloom frequency is defined as the number of times in which the abundance of a single species exceeded 50% of total phytoplankton abundance at each sampling site. It is also necessary that its abundance exceeds at least $5x10^5$ cells/L. The numerical values are calculated as 100- F.

Menhinick's diversity index (D)

$D = S / \sqrt{N}$

where S represents the number of species and N represents total abundance

Menhinick diversity index for each sample is corrected by multiplying it with the correction factor in order to reduce the error due to omitting multiple species groups (identified to the genus level). Calculation of the correction factor is described in section 2.2.

Chlorophyll *a* concentration (Chl *a*)

Chl *a* data were log10-transformed and outliers (values outside the range |average ± 2.5 * std. dev.|) removed in order to create a more robust dataset. Based on the new dataset, average concentrations were calculated for each sampling site and the antilogs were calculated then.

2.1. METHODS AND REQUIRED BQE PARAMETERS

Conclusion on the WFD compliance (are all the indicative parameters included; if not, why)

Table 2.1. Overview of the metrics included in the national method - example given for phytoplankton. For other BQEs there will be other indicative parameters (see Table 1. Page 17, IC Guidance)

MS	Taxonomic composition	Abundance	Frequency and intensity of algal blooms
HR	Menhinick's diversity index	Hulburt's dominance index	Bloom frequency, Chl a

Combination rule used in the method

2.2. SAMPLING AND DATA PROCESSING

Description of sampling and data processing:

- Sampling time and frequency;
  Data set used in this study is obtained through seasonal samplings in 2014 and 2015. Samples were collected between 2 and 6 times per year, depending on the station. Sampling was performed at 24 sites, including the estuaries of 9 rivers (Mirna, Raša, Rječina, Zrmanja, Krka, Cetina, Jadro, Neretva and Ombla). A total of 256 samples were analyzed in this study.
- Sampling method;
  Sampling was performed by Niskin sampler at standard depths (0.5 m, 5 m and 10 m). In the case where the depth became less than 10 m, sampling was performed at standard depths and the bottom layer of 2 m above the bottom. Sub-samples of 250 and 500 ml were taken for determination of phytoplankton community composition and concentration of chlorophyll *a*, respectively. Water (500ml) for Chl *a* determination was filtered immediately on board or stored in the refrigerator until arrival at the laboratory
- Data processing;
  Chlorophyl *a* data where log10 transformed and averaged per site. Outliers (values outside the range |average ± 2.5 * std. dev.|) were removed. Average values per each site were then antilogged. PRIMER7 Software was used for calculation of Menhinick's diversity index and Hulburt's dominance index. Previous to statistical analysis, the distribution of each environmental variable was tested for normality using Kolmogorov–Smirnov and Lilliefors tests ($p < 0.05$), log-transforming non-normally distributed variables when necessary.
- Identification level

Phytoplankton were enumerated using inverted microscopes at 100–800×magnification. Species composition was determined to the lowest possible level (most often to species level). Multiple indeterminated species identified to the genus level (e.g. *Chaetoceros* spp.) and flagellates were not used in calculations of Hulburt's dominance index, bloom frequency and Menhinick's diversity index. To reduce the error caused by deletion of multiple indeterminate taxa, a correction factor was introduced. This factor was calculated as the ratio of the sum of the determinate taxa to the original total abundance (determinate + indeterminate taxa). For each sample Menhinick diversity indeks (D) was multiplied by the correction factor.

2.3. NATIONAL REFERENCE CONDITIONS

Detailed description of setting of national reference conditions

The highest values for Hulburt's index (100-δ2), bloom frequency (100-F) and Menhinick index with environmental conditions (LUSI and nutrient concentrations) on sites where they were obtained are taken into consideration to selected the reference values by expert judgment.

The 30th percentile of averaged and antilogged chlorophyll *a* data is taken as the reference value due to different hydrodynamic conditions of studied estuaries and different natural features of river basins. The Krka river (sites FP13, FP13a, FP13b) largely flows through the forest area, which is not under strong anthropogenic influence but nevertheless brings a large amount of nutrients that can support phytoplankton blooms. Those blooms could therefore be attributed to the natural eutrophication process (Figure 2.3.1). Most of the sites are under slight or medium pressure, except river Jadro sites (FP9a, FP10, FP10a) which are under strong anthropogenic pressure. Due to similar anthropogenic pressure on estuaries with different hydrodynamic and ecological features, the 30th percentile of averaged and antilogged chlorophyll *a* data is taken as the reference value for this metric.

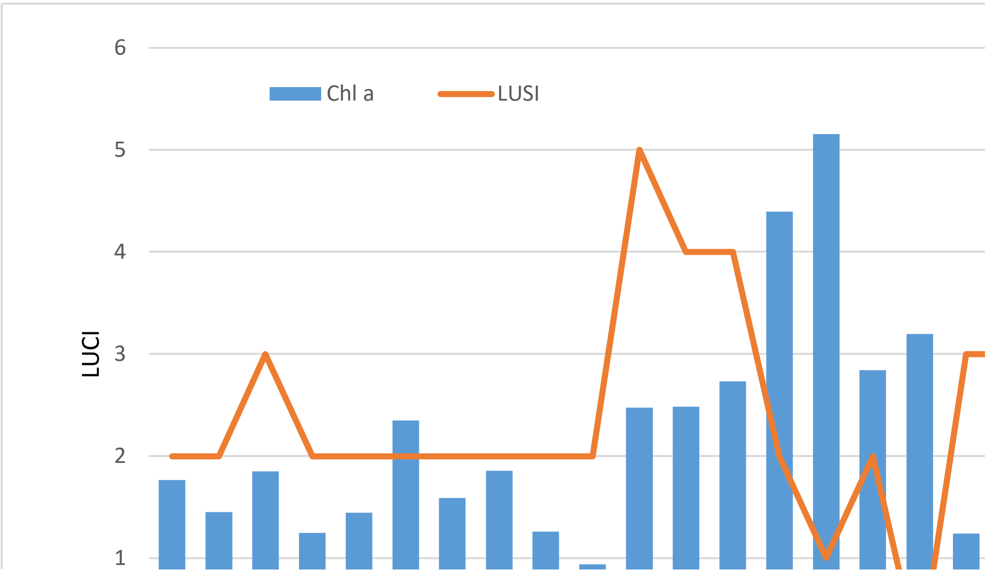

Figure 2.3.1. Averaged (*per site*) and antilogged Chl *a* concentration and LUSI at analysed sites

2.4. NATIONAL BOUNDARY SETTING

Detailed description of methodology used to derive ecological class boundaries.

After reference values were set up, EQRs for each metric are calculated and MPI per site is computed (Figure 2.4.1.). According to the Water Framework Directive (WFD), the index range was equally divided into 5 ecological status classes:

0–0.2 Bad (eutrophic conditions with dominance of opportunistic species); 0.21–0.4 Poor; 0.41–0.6 Moderate; 0.61–0.8 Good and 0.81–1 High (The composition and abundance of the taxa of phytoplankton are consistent with undisturbed conditions and blooms occur at a frequency and intensity which is consistent with the type specific physicochemical conditions).

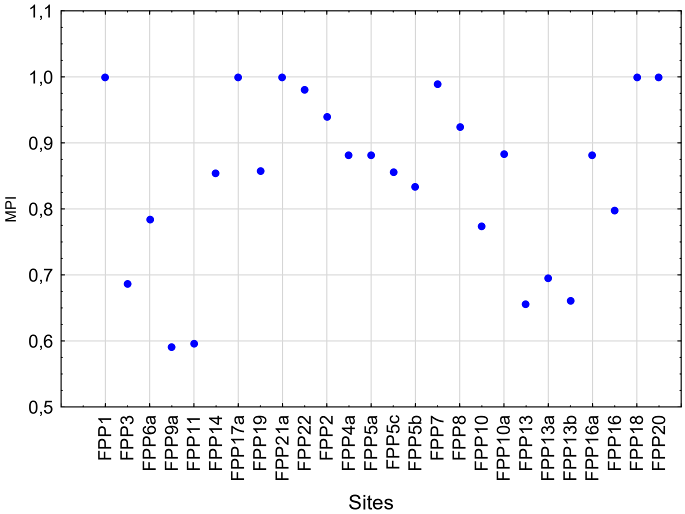

Figure 2.4.1. Multiple phytoplankton index (MPI) computed for sites of analyzed transitional waters

2.5. PRESSURES ADDRESSED

Please describe the pressures addressed by the method and provide pressure-response relationship (graph, equation)

Since the water quality assessment should be done in relation to anthropogenic pressure, **preliminary evaluations** of known pressures that could possibly affect the water quality within the study area were done using the land use pressures according to Corine Land Cover information system 2000–2006. Land uses simplified index **(LUSI)** was calculated according to Flo *et al.* (2011). Assessment of anthropogenic pressure on coastal zone by calculating the LUSI index using publicly available data is described in UNEP/MAP, 2011. The scoring system was slightly modified by adding the scoring for presence of discharge in the vicinity of site. We analyzed the area within 3 km radius from the sampling sites. Results are presented in Figure 2.3.1. and were used for the selection of reference values of metrics incorporated in MPI and description of biological communities under different trophic status.

Water nutrient concentrations and **oxygen saturation** were used in order to investigate the response of each metric to eutrophication.

The relationships between each metric and the parameters used as pressure indicators (mainly nutrients) measured in all samples were analyzed using Spearman rank correlation (statistical significance considered as $p < 0.05$) (Table 2.5.1). Computed MPI showed statistically significant linear correlation with nitrogen and silicates (Figure 2.5.1., 2.5.2.).

Table 2.5.1. Statistically significant ($p<0.05$) Spearman rank correlation of each metrics used for calculation of EQRs and Multiple phytoplankton index (MPI) with pressures indicators

	DIN	N total	PO4	P total	oxygen
Hulburt index δ2 (100- δ2)	-0.26	-0.25			-0.21
Bloom frequency (100-F)	-0,19	-0,16			-0.13
Menhinick diversity index	-0.23	-0.27	-0.17	-0.21	-0.33
Log10 Chl a	-0.20	-0.15			

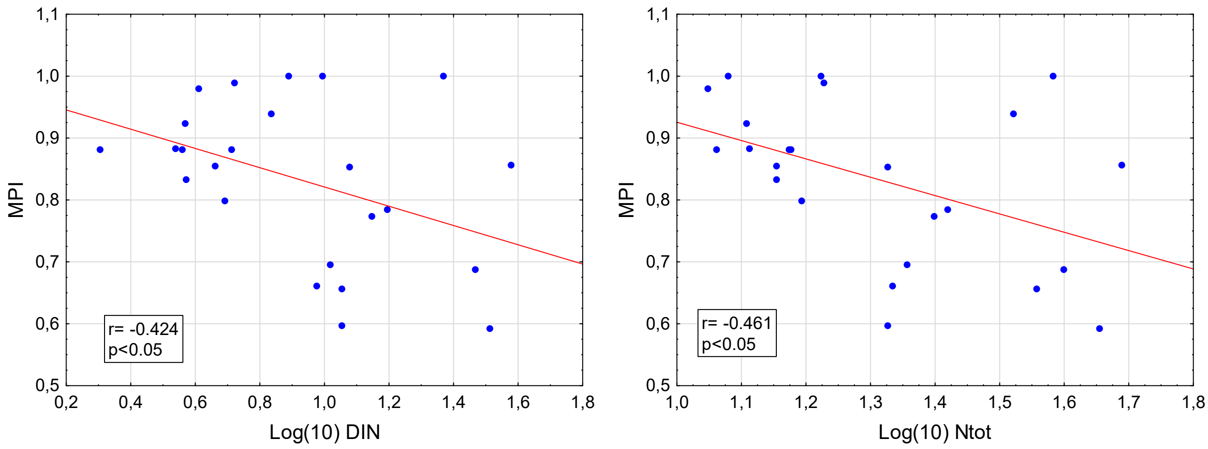

Figure 2.5.1. Linear regression between Multiple phytoplankton index (MPI) and concentrations of nitrogen

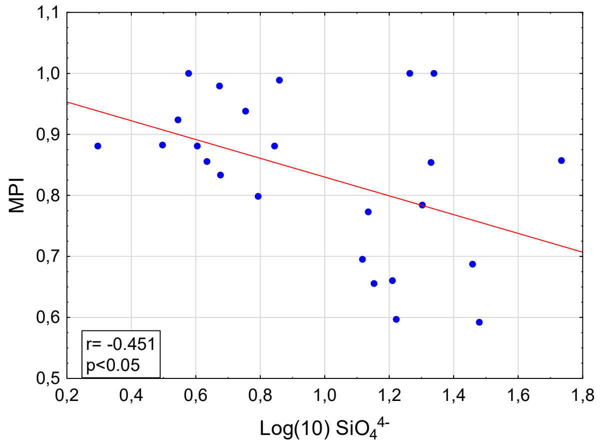

Figure 2.5.2. Linear regression between Multiple phytoplankton index (MPI) and concentrations of silicates

3. WFD COMPLIANCE CHECKING

The first step in the Intercalibration process requires the checking of national methods considering the following WFD compliance criteria.

Table 3.1. List of the WFD compliance criteria and the WFD compliance checking process and results

Compliance criteria	Compliance checking
Ecological status is classified by one of **five classes** (high, good, moderate, poor and bad).	Yes
High, good and moderate ecological status are set in line with the WFD's **normative definitions** (**Boundary setting procedure**)	Yes
All relevant parameters indicative of the biological quality element are covered (see Table 1 in the IC Guidance). A **combination rule** to combine parameter assessment into BQE assessment has to be defined. If parameters are missing, Member States need to demonstrate that the method is sufficiently indicative of	Yes

the status of the QE as a whole	
Assessment is adapted to **intercalibration common types** that are defined in line with the typological requirements of the Annex II WFD and approved by WG ECOSTAT	Yes
The water body is assessed against **type-specific near-natural reference conditions**	Yes
Assessment results are expressed as **EQRs**	Yes
Sampling procedure allows for **representative** information about water body quality/ecological status **in space and time**	Yes
All data relevant for assessing the biological **parameters** specified in the WFD's normative definitions are covered by the **sampling procedure**	Yes
Selected taxonomic level achieves adequate **confidence and precision** in classification	Yes

4. IC FEASIBILITY CHECKING

The intercalibration process ideally covers all national assessment methods within a GIG. However, the comparison of dissimilar methods ("apples and oranges") is clearly to be avoided. Intercalibration exercise is focused on specific type / biological quality element / pressure combinations. The second step of the process introduces an "IC feasibility check" to restrict the actual intercalibration analysis to methods that address the same common type(s) and anthropogenic pressure(s), and follow a similar assessment concept.

4.1. TYPOLOGY

Does the national method address the same common type(s) as other methods in the Intercalibration group? Provide evaluation if IC feasibility regarding common IC types.

The intercalibration is not feasible in terms of **common IC types**. There is only one common IC type of transitional waters in Croatia - Estuaries (salt wedge type), but Spain has only one waterbody in Andalusia region, and Croatia has 25 grouped waterbodies. Croatia decided to classify the ecological quality of transitional waters using the assessment method based on eutrophication: Multimetric Phytoplankton Index (MPI) proposed by Facca *et al.* (2013).

4.2. PRESSURES ADDRESSED

Does the national method address the same pressure(s) as other methods in the Intercalibration group? Provide evaluation if IC feasibility regarding pressures addressed.

The intercalibration is feasible in term of **pressures** addressed by the methods in other IC common types. Pressure targeted is eutrophication.

4.3. ASSESSMENT CONCEPT

Does the national method follow the same assessment concept as other methods in the Intercalibration group? Provide evaluation if IC feasibility regarding assessment concept of the intercalibrated methods

The Intercalibration is feasible in terms of **assessment concept** for methods in other IC common types. The set of metrics is combined and they include Chlorophyll *a* concentration, taxonomic composition and bloom frequency.

4.4. CONCLUSION ON THE INTERCALIBRATION FEASIBILITY

Provide conclusions on the IC feasibility.

At the moment, there is small possibility to check assessment method for its intercalibration feasibility, since in Spain there is only one waterbody belonging to IC common type Estuaries (salt wedge type).

5. DESCRIPTION OF THE BIOLOGICAL COMMUNITIES

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT HIGH STATUS

The abundance of phytoplankton is in order of magnitude of 10^5 cells L^{-1}. The most abundant are small flagellate organisms and diatoms. *Dynobrion* spp. (Chrysophyceae) is common in the community. Diversity is high. Contribution of dinoflagellate to total community abundance is low

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT GOOD STATUS

Community composition is similar as at the high status, with higher abundances of some diatom species as *Pseudonitzschia* spp., *Skeletonema marinoii*, *Cyclotella* spp., which are fast growing species.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT MODERATE STATUS

The abundance of the phytoplankton community could reach 10^7 cells L^{-1}. Intense bloom occurs in the warmer part of year (e.g, *Kryptoperidinium foliaceum*), particularly blooms of species which prefer eutrophicated area. In the community have been reported species belong to Euglenophyceae, (*Eutreptia* sp and *Eutreptiella* sp.) which are also an indicator of anthropogenic impacts. During the rest of the year, the community was common for this type of water and was dominated by pennate diatoms. The composition of the community indicates the occasional stronger anthropogenic pressure and increased levels of phosphate that results with blooms of dinoflagellates.

6. REFERENCES

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Template for reporting the MS assessment method in the case where the Intercalibration exercise is not possible (Gap 3)

1. INTRODUCTION

- Member State: Republic of Croatia
- BQE: macrophytes
- Water body category (type): transitional waters

2. DESCRIPTION OF NATIONAL ASSESSMENT METHODS

MS has to provide the complete description of the method in the Annex. The main features should be given below

ZonoMI index (*Zostera noltei* multivariate index)
ZonoMI method is based on the principles on which already published methods CYMOX, for the seagrass *Cymodocea nodosa* (Oliva et al. 2011) and ZoNI, for the seagrass *Zostera noltei* (García-Marín et al. 2013), are based.
Multivariate analysis of variables, that represent different levels of plant physiology, description of population and pollution, is used for calculation of ZonoMI index. Variables, which are considered to be able to respond on changes occurring in environment, are selected after analysis of previous researches on seagrass *Z. noltei*, and other seagrasses present in the Mediterranean Sea, for which ecological quality indices are already proposed (Plus et al. 2001; Brun et al. 2002, 2007, 2008; Peralta et al. 2005; Pergent-Martini et al. 2005; Cabaço et al. 2007, 2008; Machás 2007; Orfanidis et al. 2007, 2010; Romero et al. 2007; Leston et al. 2008; Martínez-Crego et al. 2008; Lopez y Royo et al. 2010; Oliva et al. 2012; García-Marín et al. 2013; Orlando-Bonaca et al. 2015).
Following variables were selected:
- total dried weight of roots, rhizomes and leaves,
- root weight ratio (ratio between dried weight of roots and sum of dried weights of leaves and roots),
- shoot density,
- N content (%) in dried weight of rhizomes,
- isotope δ15N ratio in dried weight of rhizomes,
- Cu content in dried weight of rhizomes,
- Pb content in dried weight of rhizomes,
- Cd content in dried weight of rhizomes,
- Zn content in dried weight of rhizomes.

These variables differently respond to changed ecosystem conditions. In the case of the increased degradation in environment, the total weight of roots, rhizomes and leaves, shoot density and ratio between weight of roots and sum of weights of leaves and roots decrease (Plus et al. 2001; Cabaço et al. 2008). Nitrogen content and isotope δ15N ratio increase (Machás 2007; Cabaço et al. 2008), and also increase metals' content (Cu, Pb, Cd, Zn) in plant tissues (Malea and Haritonidis 1999).

Results of measurements of these variables are expressed in different units. After transformation, ZonoMI method integrates them in the single index.
Multivariate Principal Component Analysis (PCA) is used for ZonoMI index calculation. Obtained values for the first axis (C1) represents values of ecological status of each site.

EQR values for each site are calculated as follows:

$$EQRx = \frac{C1x - C1\ worst}{C1\ best - C1\ worst}$$

where:
EQRx (0-1) – ecological quality ratio of the site x,
C1x – score on the first component (PC1) of the site x,
C1best – score on the first component (PC1) of the virtually best site,
C1worst – score on the first component (PC1) of the virtually worst site.

Validity of this method has been demonstrated by high correlation of obtained EQR values on individual sites with abiotic factors in the environment (r=0,9718; p<0,01). Environmental factors, that according to expert opinion and references have the greatest influence on plant condition, and that are also under the impact of anthropogenic activities, were considered. Concentrations of oxygen, nitrates, nitrites, ammonium, phosphate, silicate and total nitrogen and phosphorous in water were analysed.
Analysis shows significant correlation between ZonoMI index (calculated from the scores on the first component of PCA analysis applied on seagrass *Zostera noltei* variables on each site) and environmental quality in that areas (scores on the first component of PCA analysis of environmental factors) (Figure 2.1).

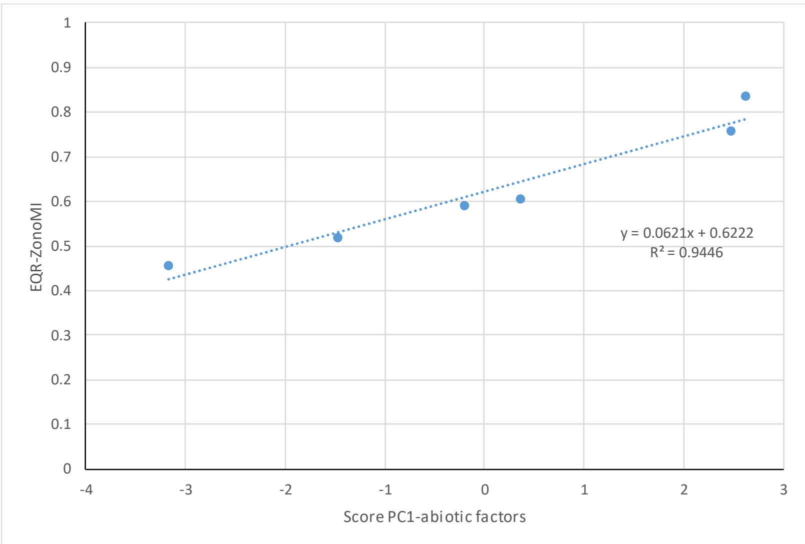

Figure 2.1. Linear regression between EQR values obtained by ZonoMI method and scores on the first component of PCA analysis of environmental factors.

2.1. METHODS AND REQUIRED BQE PARAMETERS

Table 1. Overview of the metrics included in the national method - example given for phytpoplankton. For other BQEs there will be other indicative parameters (see Table 1. Page 17, IC Guidance)

MS	Taxonomic composition	Abundance
HR	Yes	Yes

Combination rule used in the method
Since the ZonoMI method (*Zostera noltei* multivariate index) is based on the monitoring of the single species of seagrass *Zostera noltei*, it is not necessary to combine these parameters. Results of measurements of seagrass variables (9 variables) are integrated in single index. For calculation of ZonoMI index, multivariate Principal Component Analysis (PCA) is used.

Conclusion on the WFD compliance (are all the indicative parameters included; if not, why)
All indicative parameters are included, and the method is in accordance with WFD.

2.2. SAMPLING AND DATA PROCESSING

Description of sampling and data processing:

- Sampling time and frequency: July, once in four years
- Sampling method: Sampling is carried out at predetermined sites of the national monitoring network, at depths of 0.5 - 1 m. Samples of seagrass are collected by means of tooth edged corer, 15 cm in diameter, at five points 10 - 15 m distant from each other, at constant depth. Samples for chemical analysis of plant tissue are collected at three points 20 m distant from each other. Plant material is collected manually within 20 x 20 cm squares. The samples are frozen until further processing in laboratory.
- Data processing: The shoots of seagrass collected within corers are counted in the laboratory. The dry weight of individual plant parts is weighted. Average values of each variables for each site are calculated from obtained values. Collected plant material has to be prepare for chemical analysis that includes measuring of nitrogen content (%), isotope δ15N ratio, content (μg/g) of copper, lead, cadmium and zinc in dry weight of rhizomes.
  PCA analysis includes all data measured at all researched sites. For each site, one numerical value is obtained.
- Identification level: species level

2.3. NATIONAL REFERENCE CONDITIONS

Detailed description of setting of national reference conditions
Reference values represent the best recorded values of biomass and shoot density of seagrass *Zostera noltei* recorded during research from 2011 to 2018.
Shoot density: 7600 shoots per m^2
Biomass of seagrass: 650 g dry weight of seagrass (leaves, rhizomes and roots) per m^2

2.4. NATIONAL BOUNDARY SETTING

Detailed description of methodology used to derive ecological class boundaries.

According to instructions for implementation of WFD (European Commission, 2000), ecological quality ratio (EQR), from 0 to 1, is divided into five categories, which represent five ecological status: high, good, moderate, poor and bad. According to García-Marín et al. (2013), when the seagrass *Zostera noltei* is used as a biological quality element, bad ecological status includes values from 0 to 0.1, and the boundaries are set in a way to divide the rest of the scale on four equal parts. Scale division on equal parts is in accordance with Pollard and van de Bund's (2005) protocol.
Following scale is used:
1-0.775 (high)
0.774-0.550 (good)
0.549-0.325 (moderate)
0.324-0.1 (poor)
0.1-0 (bad)

2.5. PRESSURES ADDRESSED

Please describe the pressures addressed by the method and provide pressure-response relationship (graph, equation)

ZonoMI index generally refers to eutrophication and degradation in ecosystem. Inclusion of heavy metals in the method enables detection of pollution from industry and marine traffic.

Relationship between pressures from environment and seagrass response is established by high correlation of obtained EQR values (calculated from the scores on the first component of PCA analysis applied on seagrass *Zostera noltei* variables; ZonoMI index) at each site with measured environmental abiotic factors (scores on the first component of PCA analysis of environmental factors) ($r=0.9718$; $p<0.01$).
Relationship between total biomass and density of seagrass with scores on the first component of PCA analysis of environmental factors is established by linear regression (Figure 2.5.1).
Relationship between total biomass and density of seagrass with individual environmental factors, that according to expert opinion have the greatest influence on condition of seagrass, is also established by linear regression. Results are presented graphically (Figures 2.5.2, 2.5.3, 2.5.4).

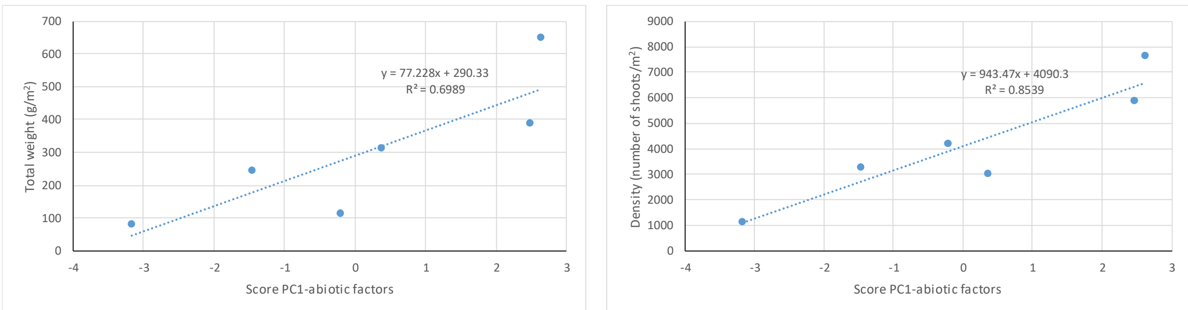

Figure 2.5.1. Linear regression between total dry weight of seagrass (g/m^2) and density of seagrass (number of shoots/m^2) *Zostera noltei* and scores on the first component of PCA analysis of abiotic factors.

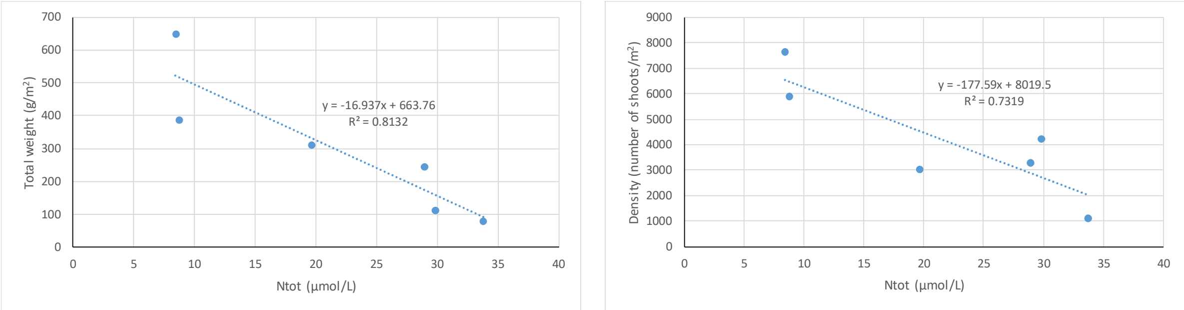

Figure 2.5.2. Linear regression between total dry weight of seagrass (g/m^2) and density of seagrass (number of shoots/m^2) *Zostera noltei* and concentration of nitrogen (μmol/L).

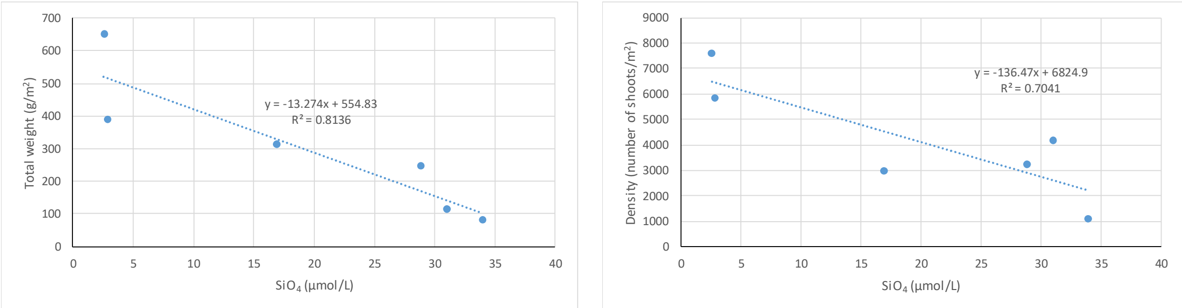

Figure 2.5.3. Linear regression between total dry weight of seagrass (g/m^2) and density of seagrass (number of shoots/m^2) *Zostera noltei* and concentration of silicates (μmol/L).

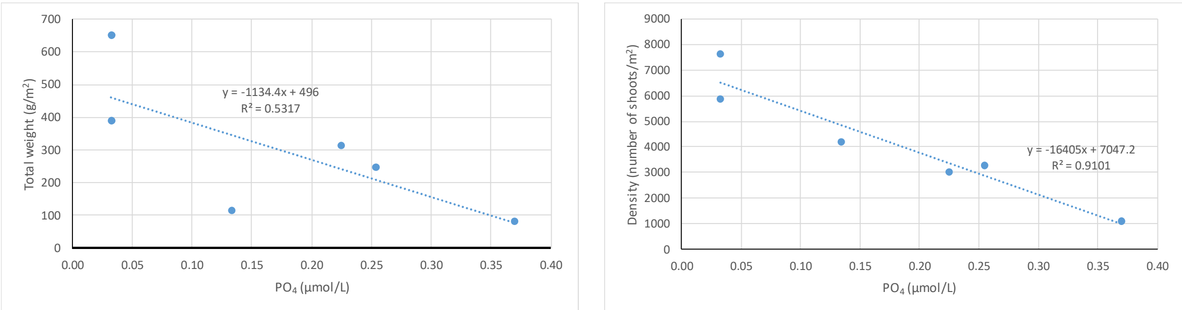

Figure 2.5.4. Linear regression between total dry weight of seagrass (g/m^2) and density of seagrass (number of shoots/m^2) *Zostera noltei* and concentration of phosphates (μmol/L).

3. WFD COMPLIANCE CHECKING

The first step in the Intercalibration process requires the checking of national methods considering the following WFD compliance criteria.

Table 2. List of the WFD compliance criteria and the WFD compliance checking process and results

Compliance criteria	Compliance checking
Ecological status is classified by one of **five classes** (high, good, moderate, poor and bad).	Yes
High, good and moderate ecological status are set in line with the WFD's **normative definitions** (**Boundary setting procedure**)	Yes

All relevant parameters indicative of the biological quality element are covered (see Table 1 in the IC Guidance). A **combination rule** to combine parameter assessment into BQE assessment has to be defined. If parameters are missing, Member States need to demonstrate that the method is sufficiently indicative of the status of the QE as a whole	Yes (only one species is included, seagrass *Zostera noltei*, and there is no need to combine parameters)
Assessment is adapted to **intercalibration common types** that are defined in line with the typological requirements of the Annex II WFD and approved by WG ECOSTAT	Yes
The water body is assessed against **type-specific near-natural reference conditions**	Yes
Assessment results are expressed as **EQRs**	Yes
Sampling procedure allows for **representative** information about water body quality/ecological status **in space and time**	Yes
All data relevant for assessing the biological **parameters** specified in the WFD's normative definitions are covered by the **sampling procedure**	Yes
Selected taxonomic level achieves adequate **confidence and precision** in classification	Yes (only one species is included, seagrass *Zostera noltei*)

4. IC FEASIBILITY CHECKING

The intercalibration process ideally covers all national assessment methods within a GIG. However, the comparison of dissimilar methods ("apples and pears") has clearly to be avoided. Intercalibration exercise is focused on specific type / biological quality element / pressure combinations. The second step of the process introduces an "IC feasibility check" to restrict the actual intercalibration analysis to methods that address the same common type(s) and anthropogenic pressure(s), and follow a similar assessment concept.

4.1. TYPOLOGY

Does the national method address the same common type(s) as other methods in the Intercalibration group? Provide evaluation if IC feasibility regarding common IC types.

Yes. ZonoMI index is appropriate for intercalibration type estuary (salt wedge).

4.2. PRESSURES ADDRESSED

Does the national method address the same pressure(s) as other methods in the Intercalibration group? Provide evaluation if IC feasibility regarding pressures addressed.

Yes. The data of environmental pressures (eutrophication) and measured parameters of seagrass *Zostera noltei* for water bodies of transitional waters are available.

4.3. ASSESSMENT CONCEPT

Does the national method follow the same assessment concept as other methods in the Intercalibration group? Provide evaluation if IC feasibility regarding assessment concept of the intercalibrated methods

This method uses the same concept of assessment as the other methods based on multivariate analysis of biological and chemical characteristic of seagrasses. It is based on *Zostera noltei* species.

4.4. CONCLUSION ON THE INTERCALIBRATION FEASIBILITY

Provide conclusions on the IC feasibility.
ZonoMI index includes biological and chemical parameters of seagrass *Zostera noltei*. The method is tested for transitional waters on the territory of Republic of Croatia. It is possible to intercalibrate this method with other methods based on the seagrass *Zostera noltei*, in the same types of transitional waters.

5. DESCRIPTION OF THE BIOLOGICAL COMMUNITIES

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT HIGH STATUS

Characteristics of *Zostera noltei* communities at high status, biomass and density of meadow, are very similar to the characteristics that are in accordance to the reference values. Expected densities are over 6000 shoots per m^2, and dry mass of seagrass (leaves, rhizomes and roots) more than 500 g per m^2.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT GOOD STATUS

Expected densities of seagrass *Zostera noltei* shoots in meadows that represents good environmental status are in range from 4400 to 6000 shoots per m^2, and values of dry mass of seagrass (leaves, rhizomes and roots) are in range from 370 g to 500 g per m^2.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT MODERATE STATUS

Expected densities of seagrass *Zostera noltei* shoots in meadows that represents moderate environmental status are in range from 2800 to 4400 shoots per m^2, and values of dry mass of seagrass (leaves, rhizomes and roots) are in range from 230 g to 370 g per m^2.

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Report on Croatian classification method for benthic invertebrates in transitional waters in the case where the Intercalibration exercise is not possible (Gap 3)

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Status of the document: Draft-version 2.0

Hrvatske vode

Zagreb, 3 December 2019

Report on Croatian classification method for benthic invertebrates in transitional waters in the case where the Intercalibration exercise is not possible (Gap 3)

1. INTRODUCTION

- Member State; **Croatia**
- BQE; **Benthic invertebrates** (macroinvertebrates)
- Water body category (type) **Transitional water**

2. DESCRIPTION OF NATIONAL ASSESSMENT METHODS

MS has to provide the complete description of the method in the Annex. The main features should be given below.
The AZTI's Marine Biotic Index (**AMBI)** is classification method selected for the assessment on the Ecological Quality Status (**EQS**) in the Croatian transitional waters (**TW**). Index was proposed by Borja *et al.* (2000) to establish the ecological quality of soft-bottom macrozoobenthos within European estuarine and coastal environments. AMBI is derived from the proportions of individual abundance of species pooled in five functional ecological groups (**EG**) which are related to the degree of sensitivity/tolerance to an environmental stress gradient as follows: EG I sensitive species, EG II indifferent species, EG III tolerant species, EG IV 2nd rank opportunistic species and EG V 1st rank opportunistic species (Pearson and Rosenberg, 1977; Borja *et al*., 2000).

AMBI is calculated according formula:

$$AMBI = \frac{[(0 \text{ x } \%GI) + (1{,}5 \text{ x } \%GII) + (3 \text{ x } \%GIII) + (4{,}5 \text{ x } \%GIV) + (6 \text{ x } \%GV)]}{100}$$

using freely available software: AMBI© V5.0 (AZTI' Marine Biotic Index software)

It has been validated and applied to different impact sources and geographical areas, demonstrating its usefulness as the indicator for general type disturbance (Borja *et al.*, 2000, 2004, Muxika *et al,* 2007). Following requirements of Water Framework Directive (**WFD**), Muxika *et al.* (2007) proposed integration of AMBI with species richness (S) and Shannon-Wiener diversity index (H') into new multiparametric index M-AMBI, and proposed equivalent ranking for AMBI pollution classification to $EQS_{M\text{-}AMBI}$ classification. It is compliant to WFD normative definition of five EQS, that describe High (H), Good (G), Moderate (M), Poor (P) and Bad (B) status, following continuous distribution of $M\text{-}AMBI_{EQR}$ values on 0-1 scale (Table 2.1.).

AMBI was applied to dataset of benthic macroinvertebrates obtained in transitional waters of Croatia. To achieve a compliance of EQR values derived by AMBI with Ecological Quality Ratio (**EQR**) prescribed by normative definitions of WFD, $\mathbf{EQR_{AMBI}}$ values in the Croatian classification system were normalized to 0-1 scale.
Table 2.1. Summary of the AMBI values and their equivalences (after Borja *et al.*, 2000). The last column shows the proposed equivalent Ecological Status (WFD), proposed by Muxika et al., 2007.

AMBI VALUES	DOMINATED EG	BENTHIC COMMUNITY HEALTH	SITE POLLUTION CLASSIFICATION	ECOLOGICAL STATUS
$0.0 < BC \leq 0.2$	I	Normal	Unpolluted	HIGH
$0.2 < BC \leq 1.2$	I	Impoverished		
$1.2 < BC \leq 3.3$	III	Unbalanced	Slightly polluted	GOOD
$3.3 < BC \leq 4.3$	IV-V	Transitional to pollution	Meanly polluted	MODERATE
$4.3 < BC \leq 5.0$	IV-V	Polluted		POOR
$5.0 < BC \leq 5.5$	V	Transitional to heavily pollution	Heavily polluted	
$5.5 < BC \leq 6.0$	V	Heavily polluted		BAD
Azoic (7.0)		Azoic	Extremely polluted	

2.1. METHODS AND REQUIRED BQE PARAMETERS

Table 2.1.1. Overview of the metrics included in the national method

MS	Taxonomic composition	Abundance	Disturbance sensitive taxa
HR	Not in strict sense (only composition of 5 preclassified sensitivity classes)	Not in strict sense (only relative abundance of 5 preclassified sensitivity classes)	5 sensitivity classes

MS	Diversity	Taxa indicative of pollution	Combination rule of metrics
HR	no	Specific opportunistic species	no

Combination rule used in the method:

- No combination rule used in the method proposed.

The combination rule that includes Shannon–Wiener diversity (H') into multiparametric AMBI (M-AMBI) has been initially selected for the assessment of EQS using Biological Quality Element Benthic Invertebrates (**BQE BI**) in the Croatian transitional waters. However, statistical analyses performed on the increasing Croatian TW data set, find no significant response of H' to the Land based **LUSI** index (Fig. 2.1.1) and Integrative pressure impacts "z" index (Fig. 2.1.2). A negligible relationship between **M-AMBI** and pressures index could be related to intermediate disturbance in estuaries, which can influence diversity values. Therefore, AMBI index was found to be more appropriate classification method for assessment of EQS in this type of transitional water bodies.

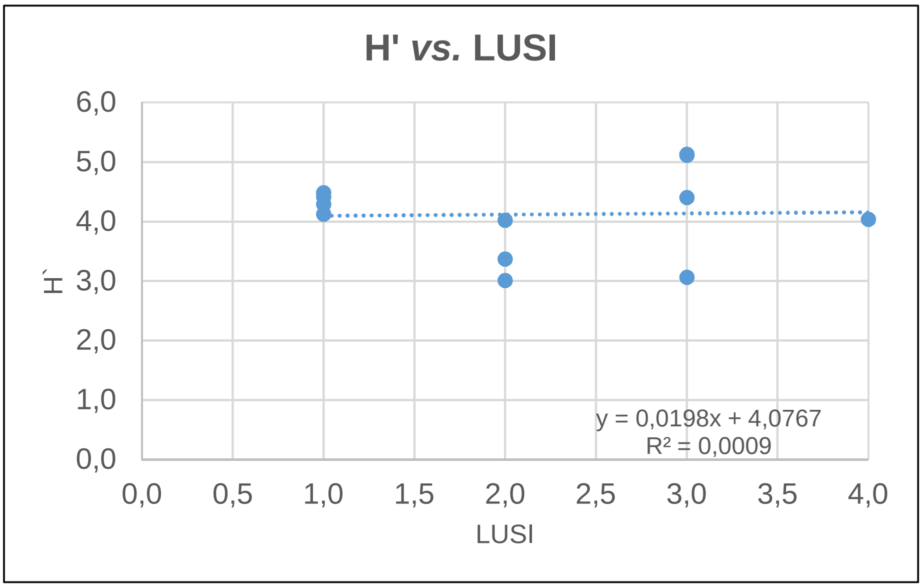

Figure 2.1.1. Linear regression between Shannon- Wiener diversity and LUSI indeks (N=12, R^2=0.0009, p>0.05).

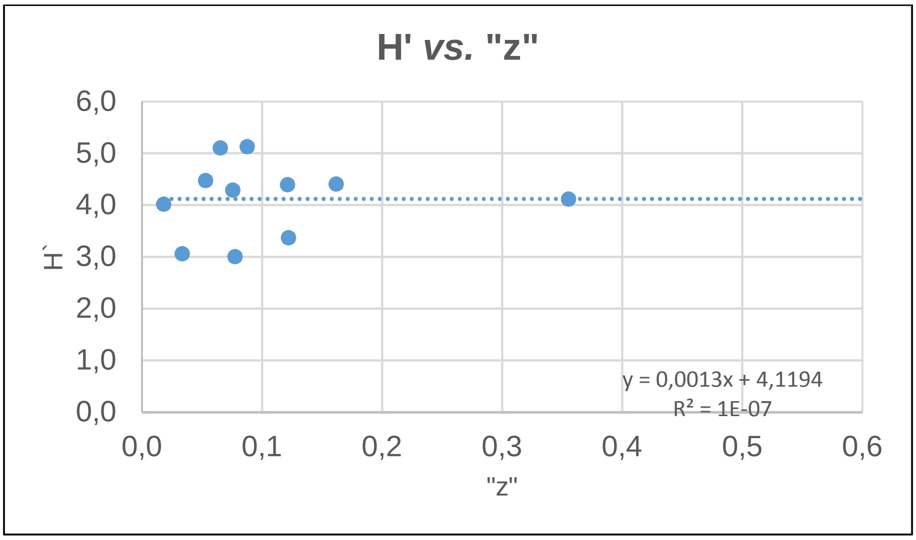

Figure 2.1.2. Linear regression between Shannon- Wiener diversity index and integrative measure of anthropogenic pressure “z” (N=12, R^2=1E-07, p>0.05).

Conclusion on the WFD compliance (are all the indicative parameters included; if not, why)

- Indicative parameters of diversity (Shannon–Wiener diversity index – H’) is not included in the national method for assessment of EQS in transitional waters. The explanation is given in the paragraph above.

According to suggestion to the coordinator of the intercalibration exercise in coastal and transitional waters we tested AMBI with available pressure indices, and find statistically significant correlation between AMBI and integrative measure of pressure “z”.

Here, instead of M-AMBI, we suggest the use of AMBI as the classification method for the assessment of EQS using BQE BI in the Croatian transitional waters.

2.2. SAMPLING AND DATA PROCESSING

Description of sampling and data processing:

- **Sampling time and frequency;**

Sampling frequency for BQE BI is scheduled once in year, triennially, preferably in a warm season. Data set used in this study is obtained in 2012-2017 period. Sampling was conducted once (operational monitoring) or twice per site (surveillance monitoring), in summer 2012, 2014, 2015 and 2016, and the early autumn 2017.

It performed in all but one River Estuaries (**R.E.**) distributed along Croatian coast, i.e. Estuary of Mirna, Raša, Rječina, Zrmanja, Krka, Jadro, Cetina, Neretva and Ombla rivers. Dragonja River is too small and too shallow for sampling benthic invertebrates by standard and comparable device (Van Veen grab), so it cannot be sampled for analysis of (BQE BI).

Sampling in the transitional water included 13 sampling sites within 13 Water Bodies (**WB**) P2_3 KRP, P2_3CE, P2_2NEP, P2_3LPP, P2_2OM, P2_2JAP, P1_3KR, P2_3KR, P2-2ZR, P2-3ZR, P2_2MI, P2_2RJP, P2_3RA (Fig. 2.2.2.). A few sites in WB P2_3LPP (BB-P5b, Neretva R.E. - Port of Ploče), P2_2 JAP (site BB-P10a, Jadro R.E.), P2_3 KR (BB-P13b, central part of the Krka R.E.) and P2_2 RJP (BB-P18, Rječina R.E) were sampled twice in biennial period, within surveillance monitoring. The other sampling sites in transitional waters were: BB-P22 (Mirna), BB-P20 (Raša), BB-P8 (Cetina), BB-P4a (Neretva), BB-P2 (Ombla), BB-P11 and BB-P13 (Krka), BB-P16 and BB-P16a (Zrmanja).

- **Sampling method;**

Sampling was performed from the Research vessel (using hydraulic winch) by Van Veen grab (0, 1 m^2). At each site, four replicate grab samples were taken on each occasion. On the board, sediment from each single grab was rinsed with seawater and sieved through 1mm mesh size. Rough separation (invertebrates > 5mm) and fixation (70% ethanol) were performed on board - parallel with sieving. Sediment remained on the sieve was fixed in 4% formaldehyde sea-water solution, labelled and stored until the next step (final separation, invertebrates < 5mm). In the laboratory, all invertebrates were sorted and classified to higher taxa level (basic operational units of macrozoobenthos), counted (total census method) and fixed in 70% ethanol. Two predominant phyla (Annelida and Mollusca) were identified to species level. All organisms were stored if there is a possibility for afterward reliable determination of unidentified species.

- **Data processing;**

Data set contains all replicate samples of identified species and their absolute abundances. Prior to statistical analysis replicate data was pooled on the site level. At sites sampling twice, samples from each year were analysed separately. AMBI method is based on the relative abundance of species belonging to one of five functional groups, according species-specific sensitivity to pollution. Statistical analyses of data set was performed using by AMBI© V 5.0 AZTI's software and accompanying Guidance (Borja et al., 2005; 2012). Software provides an abundant database of BI, with its affiliation to one of five Ecological Groups (EG I - EG V) with different sensitivity/tolerance to disturbance. AMBI analysis and resulted in:

- percentage share of each single EG
- calculation of BI and AMBI index
- ranking equivalent to EQR values associated with five EQS continuously distributed on the 0-1 scale

AMBI classification describes seven categories of benthic communities health associated with corresponding range of Biotic index (**BI**) on the 0-7 scale. The scale comprises five site pollution classes, which indicate gradient from unpolluted to extremely polluted conditions (Table 2.1). To achieve a compliance of EQR values derived by AMBI with EQR prescribed by normative definitions of WFD, $\mathbf{EQR_{AMBI}}$

have to be normalized to 0-1 scale. Normalization of AMBI metrics to 0-1 scale is obtained using formula (Boon *et al*., 2011):

$$EQR_{AMBI} = (7\text{-}AMBI)/7$$

- **Taxonomic composition and Identification level.**

Two predominant taxa (Annelida and Mollusca) mainly comprise >75% of total macrofauna and they are considered reliable indicators of environmental disturbance. Due to their high abundance, indicators' performance and reliability of identification, their identification to the species level should be sufficiently representative for assessment of EQS using BQE BI. Here, we need to stress that reliable identification of benthic invertebrates on the species level is possible exclusively by taxonomic experts for particular higher taxa. Since all methods and results for assessment of EQS based on BQI BI are highly dependent on the reliability of species identification, and since due to global crisis of taxonomist very few state have available experts for all invertebrate Phyla, non-expert analysis can easily lead to misidentification. In lack of experts for less abundant Phyla, we are limited to incomplete but credible and suitable solution, to avoid the risk of misidentification. In other words, taxonomic composition selected in this study, offers an adequate confidence and precision in species classification.

2.3. NATIONAL REFERENCE CONDITIONS

According to WFD, the Reference Conditions (**RC**) for a type-specific Water Body (**WB**) are description of the biological elements which corresponds totally, or nearly totally, to undisturbed (= pristine) conditions, i.e. a marine environment with no, or with only a very minor impact from human activities. Compliant to WFD normative definitions, such conditions are associated with two upper classes (High and Good) of the Ecological Quality Status (EQS) (EC, 2003a).

The WFD identifies four main options for deriving reference conditions: 1) comparison with an existing 'pristine'/undisturbed site (or a site with minor disturbance), 2) historical data and information, 3) models and 4) expert judgement. The alternative choice is certain combination of above-mentioned options (EC 2003a, 2003b).

In the initial phase of WFD implementation, reference condition in Croatian transitional waters were selected using historical data/information, corresponded to totally and/or near-totally undisturbed conditions. Historical data on the composition of soft-bottom benthic invertebrates, obtained in 1987 from transitional waters of Mirna River Estuary (five sites, 2 WB) were a baseline for the analysis of functional structure of benthic communities, calculation of biotic indices (AMBI, M-AMBI), assessment of ecological quality status and definition of national reference conditions. Due to low amount of historical data and the absence of full environmental gradient, classification was performed using originally identified intervals, with a boundary 0.83 between High and Good classes, and other boundaries set equidistantly to H/G boundary (REFCOND, 2003). All five sites from Mirna River Estuary were classified into categories of High or Good EQS, with median of 0.83 which corresponded to selected original boundary value of EQS classification. Based on that analysis, we described preliminary reference conditions (Hrvatske vode, 2015), in accordance with WFD normative definition.

Since the climate, land cover, and marine ecosystems vary naturally over periods relevant for WFD, characterization of WB and RC are not permanent and from 2013 have to be reviewed every six years (EC, 2003b). For that reason and the fact that the initial reference conditions were derived from small dataset, they are considered 1st generation RC, that should be adapted/updated following results obtained in all R.E./WB scheduled for WF monitoring. Dragonja River is too shallow for sampling of BI using standard methodology (Van Veen grab). The same worth for oligotrophic waters (Type P_1), except one site BBP-11 from the WB P1_3 (Krka R.E.). That site characterized by Good EQS (EQR_{AMBI}=0,751), but it was the only suitable site for sampling in WB P_1, and have no pressure data available.

Reference conditions must be formulated in a way to include natural variability over a period of at least six years (EC, 2003a). In the period 2012-2017 BQE BI monitoring were performed at 13 sites (Fig. 2.3.1.) within 13 WB (Fig. 2.3.2.). Sampling sites were selected due to: 1) geographical representativeness, 2) the strength of taxonomic lists, 3) the minor impact of human activities and 4) methodological approach (sampling, laboratory analyses) eligible for ecological quality assessment.

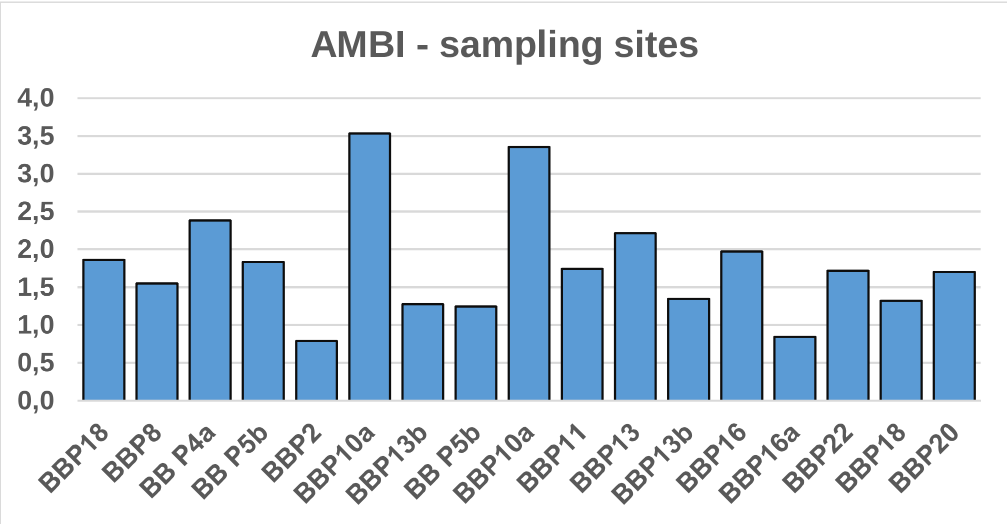

Figure 2.3.1. AMBI scores for Transitional Water sampling sites (2012-2017).

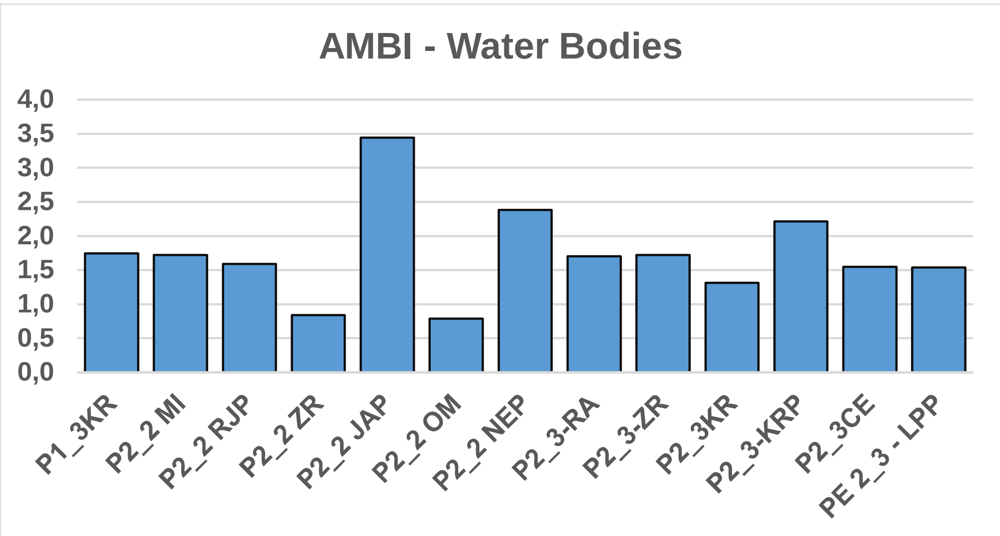

Figure 2.3.2. AMBI scores for Transitional Water Bodies (2012-2017).

Biological communities in High status (WB P2_2-OM, site BB-P2 and WB P2_2-ZR site BB-P16a), that corresponded to undisturbed conditions (nutrients, chl-a and bottom dissolved oxygen are in High status; integrative pressure metrics "z"=0,1) were selected for description of National Reference Conditions (**NRC**). Mean values of biological descriptors at selected sites were: EQR_{AMBI}=0.82, EG I=62%, EG II=26%, EG III=8%, EG IV and EG V=4%.

Reference criteria used for selection of RC were minor anthropogenic influence and biological criteria: lowest AMBI, highest $EQR_{AMBI,}$ dominance of sensitive taxa, high contribution of EGI and EG II species and low contribution of tolerant and opportunistic species. Accordingly, Reference conditions are set up as follows:

AMBI ≤ 1.2*, EQR_{AMBI} ≥ 0.80**, EG I and EG II ≥ 70%, EG III ≤ 20%, EG IV I EG V ≤ 10%

* upper boundary limit for unpolluted state in the AMBI classification (Borja *et al*., 2000)
** 12º Percentile of distribution EQR_{AMBI} values derived from H/G data

2.4. NATIONAL BOUNDARY SETTING

Detailed description of methodology used to derive ecological class boundaries.

The Republic of Croatia did not participate in IC exercises, due to lack of common WB in transitional waters with other MS involved in MED-GIG process. Over here, we report national results in the case where the IC exercises were not possible and suggest novelation of NRC in the Croatian part of the Adriatic Sea using data obtained during six year WFD monitoring (2012-2017). Updated NRC is compliant to WFD normative definitions, as follows in Description of the biological communities in High and Good status (chapter 5). Accordingly, data selected for establishment of NRC are compliant with WFD normative definition in terms of: 1) spatial coverage, 2) methodological approach and 3) requirement of minor impact from human activities.

National boundaries setting

According to WFD, the ecological status is preclassified into five classes (Bad, Poor, Moderate, Good, Very good/High), indicated differences in ecological status along a gradient of disturbance, based on BQE BI assessment.

Croatian TW dataset for National Boundary Setting (**NBS**) related to 13 WB monitored during 2012-2017. Results were unequally distributed between High and Moderate EC. The boundary H/G was derived calculating mean value of EQR scores associated with H classes (0.884), corrected for 12% of calculated natural variability. The H/G boundary is set up at 0.80, and other boundaries are set equidistantly:

H/G=0.80
G/M=0.60
M/P=0.40
P/B=0.20

EQR_{M-AMBI} intervals associated with each of five ecological class are presented in Table 2.4.1. This classification scheme is common for all WB in the Croatian TW.

Table 2.4.1. An EQR_{AMBI} classification for biological quality element benthic invertebrates, based on data from 2012-2017 monitoring of transitional waters.

ECOLOGICAL STATE CATEGRY (EQS)	EQR_{AMBI} CLASS BOUNDARIES
HIGH / VERY GOOD	0.80-1.00
GOOD	0.60-0.80
MODERATE	0.40-0.60
POOR	0.20-0.40
BAD	<0.20

Following novel RC and NBS, based on the existing data from nine River Estuaries, all but one results (VT P2_2-JAP, Jadro River Estuary) indicated High or Good EQS. Results obtained during six year monitoring indicated High EQS for 6, Good EQS for 9 and Moderate EQS for 2 sampling sites (Fig. 2.4.1.) and High EQS for 3, Good EQS for 10 and Moderate EQS for 1 WB (Fig. 2.4.2.).

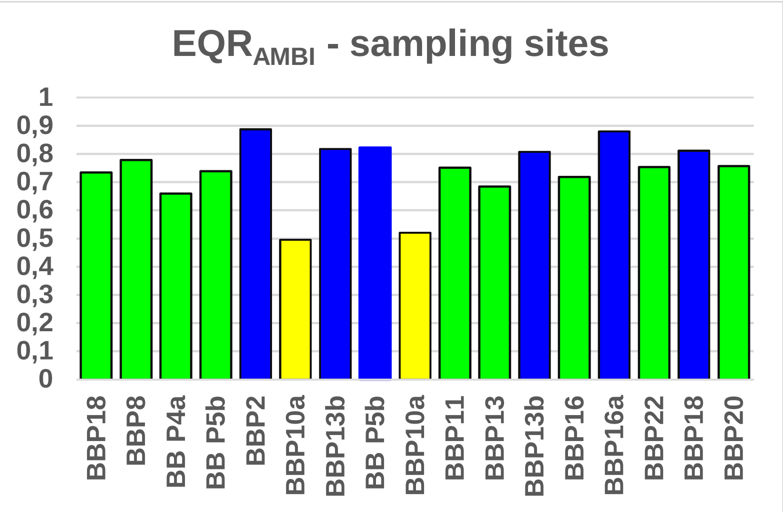

Figure 2.4.1. EQR_{AMBI} for Transitional Water sampling sites (2012-2017).

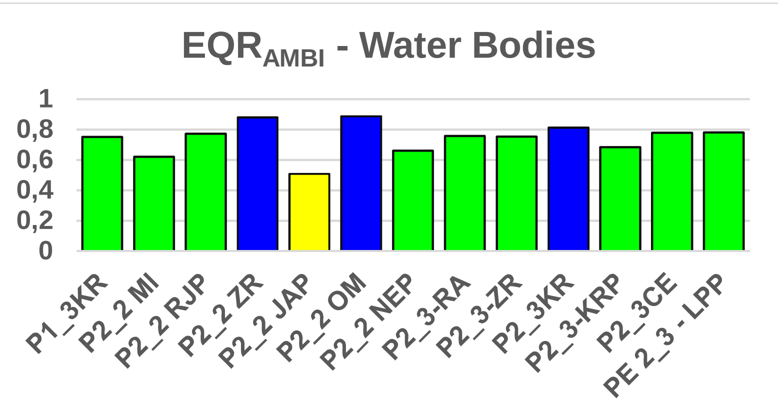

Figure 2.4.2. Type-specific water bodies in Transitional Waters. Ecological quality status (EQS) assessment using AMBI classification method (2012-2017).

2.5. PRESSURES ADDRESSED

Pressures considered ascertaining the relationship between anthropogenic pressures and BQE BI were land usage (urbanization, industry, agriculture) and sea usage (mariculture, ports, wastewater discharge). These pressures were preliminary evaluated through organic matter content in sediment and assessment of land use pressures according to Corine Land Cover information system - using "Land Uses Simplified Index" (**LUSI**) calculated according Flo et al. (2011) and EC (2013).

The linear regression plot for AMBI and EQR_{AMBI} with total organic matter content (TOM), have shown moderate positive corellation for AMBI (Figure 2.5.1.) and moderate negative corellation for EQR_{AMBI} (Figure 2.5.2.).

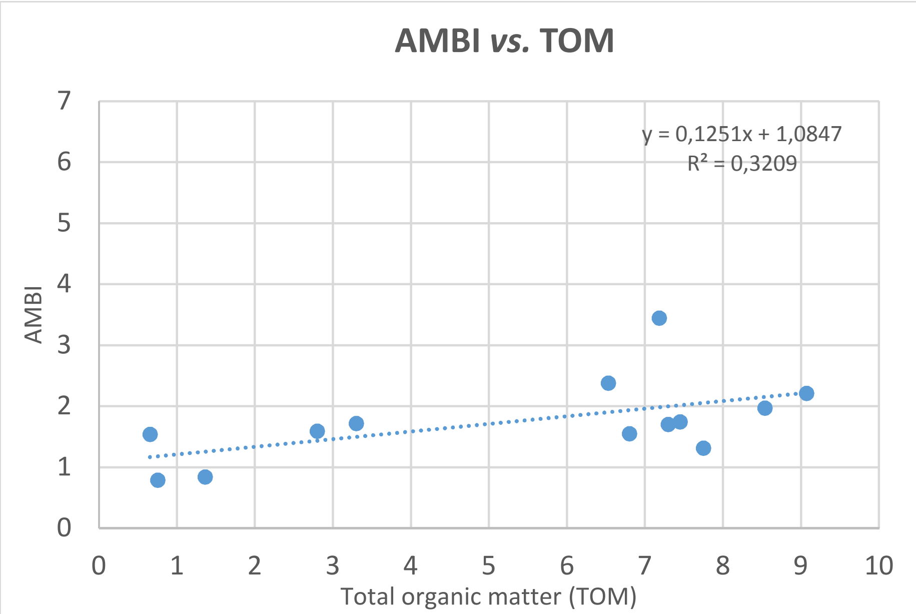

FIGURE 2. 5.1. Linear regression between AMBI and Total organic matter content (TOM %). Statistically significant positive correlation (R^2=0.3209, R=0.566, N=13, $p<0.05$) is established.

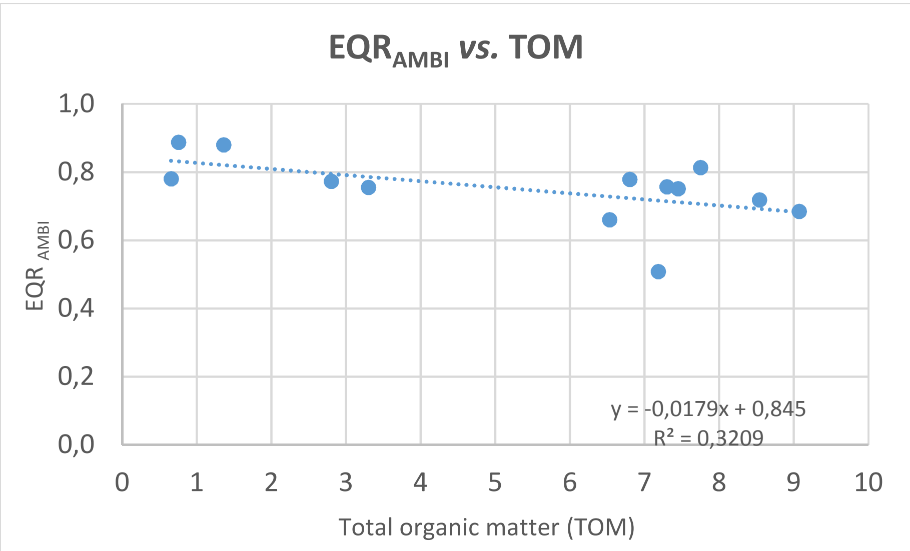

FIGURE 2.5.2. Linear regression between AMBI and Total organic matter content (TOM %) . Statistically significant negative correlation (R^2=0.3209, R=0.566, N=13, $p<0.05$) is established.

Land uses simplified index (LUSI) scoring system is slightly modified and presented in Table 2.5.1. Modification is provided using one additional score for hydrological pressures (rivers, channels, influence of adjacent water bodies) and/or harbor pressure, respectively (if it was significant).

TABLE 2.5.1. Land uses simplified index (LUSI) scoring system. LUSI quantifying potential pressure according to percent of land used in different anthropogenic activities.

URBAN	AGRICULTURAL	INDUSTRIAL	HARBOR	SCORE
<3	< 10	<10		0
3-33	10-40	> 10	1-10	1
33-66	> 40	> 30	> 10	2
>66		>60		3

Assessment of anthropogenic pressure in transitional waters using LUSI index was analyzed in the area within 3 km radius from the sampling site. Linear regression analysis found no relationship between EQR_{AMBI} and LUSI (R^2=0.01; $p>0.05$).
Then, we have tested AMBI against pressure variable “z”, that integrated eight partial pressures e.g. point source, untreated municipal waters, mariculture, land use (LUSI), non-indigenous and invasive species, shipping, fishing and hydromorphological pressures, and were already applied as an integrative measure of pressure in the Croatian transitional waters (Kušpilić *et al.*, 2016).

An integrative “z” metrics were calculated for each transitional water body using the formula:

$z=(x-\mu)/\sigma$ where x=single measurement, μ=average value, σ=standard deviation)

and calibrated with type-specific data on the established ecological status. Since “z” values can be positive (indicates over-average pressures) and negative (indicates sub-average pressures), prior to correlation analysis they were square transformed.
The linear regression analysis of AMBI and EQR_{AMBI} with integrative metrics of antrophogenic pressures (“z”), have shown moderate and significant relationship, positive for AMBI (Figure 2.5.3.) and negative for EQR_{AMBI} (Figure 2.5.4.). Therefore, AMBI can be considered as suitable metrics and “z” as suitable indicator of antrophogenic pressure in Croatian transitional waters.

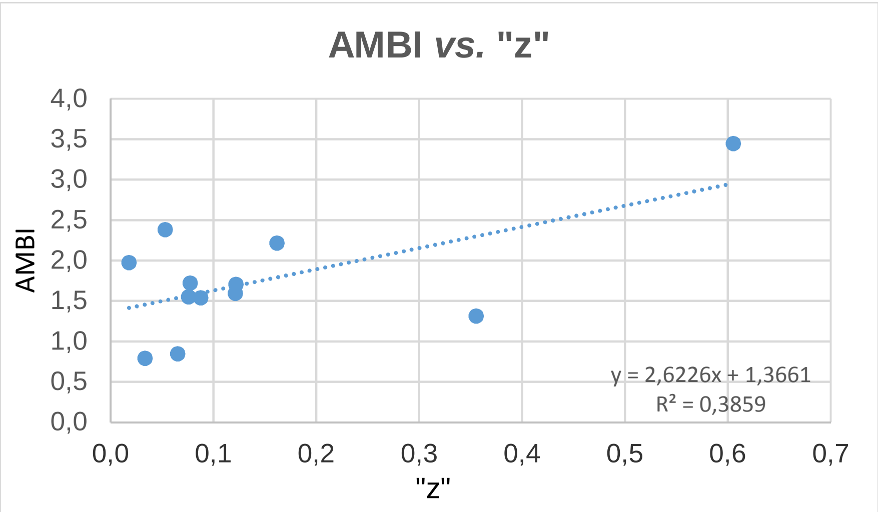

FIGURE 2. 5.3. Linear regression between AMBI and integrative measure of anthropogenic pressure ("z"). Statistically significant positive correlation (R^2=0.386, R=0.621, N=12, p<0.05) is established.

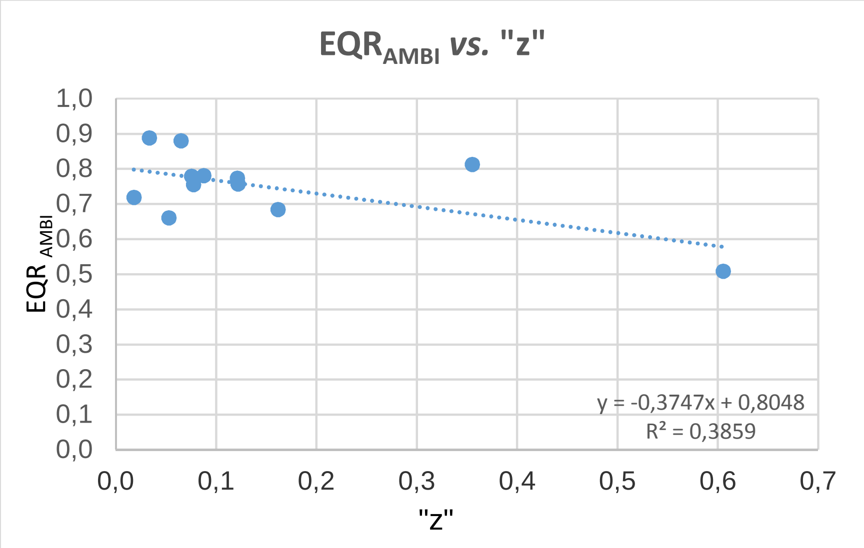

FIGURE 2.5.4. Linear regression between AMBI and integrative measure of anthropogenic pressure ("z"). Statistically significant negative correlation (R^2=0.386, R=0.621, N=12, p<0.05) is established.

3. WFD COMPLIANCE CHECKING

The first step in the Intercalibration process requires the checking of national methods considering the following WFD compliance criteria.

Table 2. List of the WFD compliance criteria and the WFD compliance checking process and results

Compliance criteria	Compliance checking
Ecological status is classified by one of **five classes** (high, good, moderate, poor and bad).	Yes
High, good and moderate ecological status are set in line with the WFD's **normative definitions** (**Boundary setting procedure**)	Yes
All relevant parameters indicative of the biological quality element are covered (see Table 1 in the IC Guidance). A **combination rule** to combine parameter assessment into BQE assessment has to be defined. If parameters are missing, Member States need to demonstrate that the method is sufficiently indicative of the status of the QE as a whole	No (explan. in chapt. 2.1.)
Assessment is adapted to **intercalibration common types** that are defined in line with the typological requirements of the Annex II WFD and approved by WG ECOSTAT	Yes
The water body is assessed against **type-specific near-natural reference conditions**	Yes
Assessment results are expressed as **EQRs**	Yes

Sampling procedure allows for **representative** information about water body quality/ecological status **in space and time**	Yes
All data relevant for assessing the biological **parameters** specified in the WFD's normative definitions are covered by the **sampling procedure**	Yes
Selected taxonomic level achieves adequate **confidence and precision** in classification	Yes

4. IC FEASIBILITY CHECKING

The intercalibration is not feasible. There is only one common IC type in transitional waters in Croatia - Estuaries (salt wedge type). Croatia has 25 grouped waterbodies in this common IC type and Spain has only one waterbody in Andalusia region, which is why the dataset is not enough to do the intercalibration.

5. DESCRIPTION OF THE BIOLOGICAL COMMUNITIES

DESCRIPTAT HIGH STATUS BQI BENTHIC INVERTEBRATES COMPRISES OFTEN HIGH NUMBER OF SPECIESION OF THE BIOLOGICAL COMMUNITIES AT HIGH STATUS

At High status AMBI for BQE Benthic Invertebrates ≤ 1.2 and EQR_{AMBI} ≥ 0.80. At sampling sites AMBI and EQR_{AMBI} range 0.79-0.84 and 0.88-0.89, respectively.

All the disturbance-sensitive taxa of the type-specific communities associated with undisturbed conditions are present. Relative abundances of EG assessed by AMBI indicate normal or slightly impoverished community and unpolluted site.
Sensitive taxa (EG I) are dominant; indifferent and/or tolerant taxa (EG II, EG III) are subdominant and opportunistic taxa (EG IV+EG V) are absent or have negligible abundance.

High status of BQE BI is described for communities from WT P2_2-OM (site BB-P2) - Ombla River Estuary, P2_2-ZR (site BB-P16a) – Zrmanja River Estuary and P2_3-KR (site BB-P13b)- Krka River Estuary.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT GOOD STATUS

At Good status AMBI for BQE Benthic Invertebrates ≤ 3.3 and EQR_{AMBI} ≥ 0.60. At sampling sites AMBI and EQR_{AMBI} range 1.54 -2.38 and 0.66-0.81, respectively.

Most of the sensitive taxa of the type-specific communities are present. Relative abundances of EG assessed by AMBI indicate slightly unbalanced community and slightly polluted site. Sensitive and indifferent taxa (EG I and EG II) are dominant, tolerant taxa (EG III) are mainly subdominant (except BB-P18, BB-P20, where dominated), while opportunistic taxa (EG IV and EG V) abundance may range from negligible or low share up to 25%.

Good status of BQE BI is described for most communities from TW, i.e. for communities from BB-P4a, BB-P5b (Neretva R.E.), BB-P8 (Cetina R.E.), BB-P11, BB-P13, BB-P13b (Krka R.E.), BB-P16 (Zrmanja R.E.) BB-P18 (Rječina R.E), BB-P20 (Raša R.E.), BB-P22 (Mirna R.E.).

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT MODERATE STATUS

At Good status AMBI for BQE Benthic Invertebrates ≤ 4.3 and EQR_{AMBI} ≥ 0.40. At sampling sites AMBI is 3.45 and EQR_{AMBI} range 1.2 -3.3 and 0.50-0.52.

Relative abundances of EG assessed by AMBI indicate communities transitional to pollution and moderately polluted site. Opportunistic taxa (EG IV+EG V) are dominant; sensitive, indifferent and tolerant taxa are subdominant and mainly co-dominate with abundances 10-20%.

Moderate status of BQE BI is described only for community from WB P2_2 JAP (site BBP-10a), Jadro River Estuary.

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Software used for calculation of National metrics: AZTI Technalia AMBI index software (Version 5.0), freely obtained from http://ambi.azti.es.

Template for reporting the MS assessment method in the case where the Intercalibration exercise

1. INTRODUCTION

- Member State Croatia;
- Biological quality element Fish fauna;
- Water body category Transitional waters.

2. DESCRIPTION OF NATIONAL ASSESSMENT METHODS

MS has to provide the complete description of the method in the Annex. The main features should be given below

The modified Estuarine Fish Index (M-EFI) consists of fifteen elements, which each aim to assess a different functional aspect of the estuarine fish assemblages and the integrated quality of the ecosystem (general degradation). It is based on an estuarine fish index (EFI) developed for the Scheldt estuary in Flanders (Belgium) (Goethals et al., 2002, Adriaenssens et al., 2002a, Adriaenssens et al., 2002b), that is further modified by few parameters (specific fish families for Mediterranean area) (Table 2; Table 3).

2.1. METHODS AND REQUIRED BQE PARAMETERS

Conclusion on the WFD compliance (are all the indicative parameters included; if not, why)

Table 1. Overview of the metrics included in the national method

MS	Taxonomic composition	Abundance	Disturbance sensitive taxa	Taxa indicative of pollution
M-EFI	X	X	X	X

For BQE assessment, scores of fifteen biological elements are calculated, and the average value of scores represents the value of M-EFI. Index is transformed to EQR's with formula: EQR = reference value / index value.
The method is WFD compliant, in terms of the indicative parameters included, with the exception of parameter bioaccumulation-bioassays. However, taxa indicative on several types of pollution are listed in Table 3. according to existing literature sources of their vulnerability related to different pollution.

2.2. SAMPLING AND DATA PROCESSING

Description of sampling and data processing:

- Sampling time and frequency:
  Sampling is conducted once to three times in a year, every three years, both for surveillance and operational monitoring. Sampling is done in warm part of the year (best in late spring period-June).
- Sampling method:
  Sampling stations should be chosen so as to include the maximum diversity of habitat types. Size sampling stations must be sufficient to include the living space of the dominant species and encompass all characteristic habitats, and to present the fish community in a satisfactory

manner. Measuring station should be selected on the basis of biological (vegetation, sediment), relief (placed position, slope) and hydrographic (depth, current speed) factors. In addition, the monitoring station must be large enough to be able to perform additional hauls (3x) of the sampling which represent subsamples. Selecting the number of stations within a water body shall be sufficient for the quality assessment of the structure, density and age structure of populations within a community fish. By the inclusion of a greater number of habitats in the selection of sampling stations, an easier access to the sampling site and prior knowledge of a particular station should be considered as well.
Samples are collected with special coastal seine nets (50 m long, 4mm mesh size). Data are based on averaging data collected during one day (3 hauls within one measuring station, which covers a higher number of specific microhabitats and assure sample representativeness), recalculated as average catch per day per haul for a particular month. Fishing haul, from the early closure of the location by net to its pull on the coast, typically lasts for 30 minutes. Averaging of data is done by averaging the number of species, the number of individuals and the biomass.

- Data processing

Fish abundance within the sample/survey is measured by individual counts, and abundance is related to area and fishing effort. Other biological data determined are: species, the total length and weight of the individual specimen.
For the calculation of M-EFI metric, following elements should be determined: % Flounder (Solea sp.), % Picarel (Spicara sp.), % Mullet (Mugilidae), % Seabream (Sparidae), % Seabass (Moronidae), % omnivores, % piscivores, Number of Estuarine resident species (ERS), % ERS, % diadromous species, % marine juvenile migrating species, Indicator species and New / alien species. Scores of these elements are calculated, and the average value of scores represents the value of M-EFI.

Table 2. Metrics, elements and scoring system for M-EFI

PARAMETER	SCORE				
	1	2	3	4	5
Total number of species	>= 4	5 - 14	15 - 19	20 - 24	> 24
Type Species					
% Flounder (*Solea* sp.)	<= 5	> 5 - 10 > 50 - 80			> 10 - 50
% Picarel (*Spicara* sp.)	<= 5	> 5 - 10 > 50 - 80			> 10 - 50
% Mullet (Mugilidae)	<= 5	> 5 - 10 > 50 - 80			> 10 - 50
% Seabream (Sparidae)	<= 5	> 5 - 10 > 50 - 80			> 10 - 50
% Seabass (Moronidae)	<= 5	> 5 - 10 > 50 - 80			> 10 - 50
Trophic composition *					
% omnivores	<= 1 > 80	> 1 - 2,5 > 20 - 80			> 2,5 - 20
% piscivores	<= 5 > 80	> 5 - 10 > 50 - 80			> 10 - 50
Tolerance**	< 1,20	1,20 - 1,59	1,60 - 1,99	2 - 3	> 3
Estuarine resident species (ERS)					
Number ERS	< 2	2	3	4	> 4
% ERS	< 5 > 50	> 5 - 10 > 40 - 50			> 10 - < 40
% diadromous species	< 5 > 80	5 - 10 > 70 - 80			> 10 - 70
% marine juvenile migrating species	<= 10 > 90	5 - 10 > 80 - 90	> 20 - 30 > 70 - 80		> 30 - 70
Indicator species	0	1	2 - 4	5 - 7	> 7
New / alien species	0	1	2 - 4	5 - 7	> 7

*Adding missing scores 3, 4 (and 5) would be of no ecological relevance, presence of extremely low as well as extreme high number reflect deterioration
**A tolerance score was attributed to each fish species present.

Table 3. Ecological characteristics of fishes for M- EFI index*

Species	Trophic category	Spawning area	Ecological requests	Indicator/ new/ alien species
Alburnus neretvae	ZOO/INV	Freshwater	ST	
Carassius auratus gibelio	OMNI	Freshwater	EU	
Gambusia hoolbroki	OMNI	Freshwater	ST	
Lepomis gibbosus	INS/INV	Freshwater	LI	Invasive
Leuciscus leuciscus	OMNI	Freshwater	RE	
Padogobius martensi	INS/INV	Freshwater	EU	
Phoxinus phoxinus	INS/INV	Freshwater	RE	
Rodeus amarus	INV/PISC	Freshwater	RE	
Rutilus basak	INS/INV	Freshwater	EU	
Salmo gairdneri	INV/PISC	Freshwater	RE	
Salmothymus obtusirostris salonitana	INV/PISC	Freshwater	RE	
Salmo trutta trutta	INV/PISC	Freshwater	RE	
Scardinius plotizza	OMNI	Freshwater	LI	
Squalius squalus	INV/PISC	Freshwater	EU	
Alosa fallax nilotica	ZOO/PISC	Marine	EU	
Engraulis encrasicolus	ZOO/INV	Marine	EU	
Sardina pilchardus	ZOO/INV	Marine	EU	
Sprattus sprattus	ZOO/INV	Marine	EU	
Belone belone	INV	Marine	ST	
Anguilla anguilla	INV/PISC	Marine	EU	
Conger conger	INV/PISC	Marine	EU	
Syngnathus abaster	ZOO/INV	Marine	EU	
Syngnathus acus	ZOO/INV	Marine	EU	
Syngnathus taenionotus	ZOO/INV	Marine	EU	
Syngnathus tenuirostris	ZOO/INV	Marine	EU	
Syngnathus typhle	INV/PISC	Marine	EU	
Hippocampus hippocampus	OMNI	Marine	EU/RE	
Nerophis maculatus	ZOO/INV	Marine	EU	
Nerophis ophidion	ZOO/INV	Marine	EU	
Gasterosteus aculeatus	OMNI	Marine	EU/RE	
Serranus hepatus	INV/PISC	Marine	EU	
Serranus scriba	INV/PISC	Marine	EU	
Dicentrarchus labrax	INV/PISC	River estuaries	EU	
Dicentrarchus punctatus	INV/PISC	River estuaries	EU	
Lichia amia	PISC	Marine	EU	
Mullus barbatus	INV	Marine (channels)	EU	Indicator
Mullus surmuletus	INV	Marine	ST	Indicator
Boops boops	ZOO	Marine	ST	
Diplodus annularis	OMNI	Marine	EU	
Diplodus puntazzo	OMNI	Marine	EU	Indicator
Diplodus sargus	INV	Marine	ST	Indicator
Diplodus vulgaris	INV	Marine	ST	Indicator
Lithognathus mormyrus	INV	Marine	EU	
Oblada melanura	INV	Marine	EU	
Pagellus acarne	INV	Marine	ST	
Pagellus erythrinus	INV	Marine	ST	
Sarpa salpa	HERB	Marine	ST	
Sparus aurata	INV/PISC	Marine	EU	
Centrachantus cirrus	INV	Marine	ST	
Spicara flexuosa	INV	Marine	ST	
Spicara smaris	INV	Marine	ST	
Chromis chromis	ZOO/INV	Marine	EU	
Coris julis	ZOO/INV	Marine	EU	
Symphodus cinereus	INV	Marine	EU	

Species	Trophic category	Spawning area	Ecological requests	Indicator/ new/ alien species
Symphodus mediterraneus	INV	Marine	EU	
Symphodus ocellatus	INV	Marine	EU	
Symphodus roissali	INV	Marine	EU	
Symphodus tinca	OMNI	Marine	EU	
Eichthys vipera	INV/PISC	Marine	EU/RE	
Gobius buchichi	OMNI	Marine	EU/RE	
Gobius cobitis	OMNI	Marine	EU/RE	
Gobius geniporus	OMNI	Marine	EU/RE	
Gobius niger	INV/PISC	Marine	EU/RE	
Gobius paganellus	INV/PISC	Marine	EU/RE	
Buenia affins	INV/INS	Marine	EU/RE	
Knipowitschia caucasica	INV/INS	Marine	EU/RE	Low oxygen level
Knipowitschia panizzae	INV/INS	Marine	EU/RE	Low oxygen level
Padogobius martensi	INV	Marine	EU/RE	
Pomatoschistus bathi	INV	Marine	EU/RE	
Pomatoschistus canestrini	INV	Marine	EU/RE	
Pomatoschistus marmoratus	INV	Marine	EU/RE	
Pomatoschistus minutus	INV	Marine	EU/RE	
Zebrus zebrus	INV	Marine	EU/RE	
Zosterisessor ophiocephalus	INV/PISC	Marine	EU/RE	Thermal and chemical pollution
Callionymus fasciatis	INV	Marine	EU/RE	
Callionymus pusillus	INV	Marine	EU/RE	
Callionymus risso	INV	Marine	EU/RE	
Aidablennius sphynx	OMNI	Marine	EU/RE	
Lypophrys dalmatinus	OMNI	Marine	EU/RE	
Lipophrys fluviatilis	OMNI	Freshwater/Brackish	RE	
Lypophrys pavo	OMNI	Marine	EU/RE	
Parablennius sanguinolentus	HERB/DETR	Marine	EU/RE	
Parablennius tentacularis	INV	Marine	EU/RE	
Centrolopus niger	ZOO/INV	Marine	ST	
Chelon labrosus	OMNI	Marine	EU	Communal sewage
Mugil cephalus	OMNI /DETR	Marine	EU	
Liza aurata	OMNI	Marine	EU	
Liza ramada	OMNI	Marine	EU	
Liza saliens	OMNI /DETR	Marine	EU	
Oedalechilus labeo	OMNI /DETR	Marine	EU	
Atherina boyeri	ZOO/INV	Marine	EU	Thermal and chemical pollution, Low oxygen level
Atherina hepsetus	INS/INV	Marine	EU	Thermal and chemical pollution, Low oxygen level
Trigla lucerna	INV/PISC	Marine	ST	
Arnoglossus laterna	INV/PISC	Marine	EU	
Buglossidium luteum	INV/PISC	Marine	EU	
Platichthys flesus	INV/PISC	Marine	EU	
Pleuronectes platessa	INV/PISC	Marine	EU	
Scophthalmus rhombus	INV/PISC	Marine	EU	
Solea kleinii	INV	Marine	EU	
Solea vulgaris	INV	Marine	EU	

Legend: OMNI (omnivores), PISC (piscivores), INV (invertebrata), INS (insects), HERB (herbivores), DETR (detrivores), ZOO (zooplanktivores), EU (eurihaline), ST (stenohaline), RE (rezident).
*the list is not completed, it will be complemented

- Identification level:
  Determination goes to the species level.

2.3. NATIONAL REFERENCE CONDITIONS

Detailed description of setting of national reference conditions

Nacional reference conditions are set using a combination of historical data, expert knowledge and recent data collections.

Table 4. Type-specific reference values of elements of M-EFI metric

Elements of M-EFI metric	P1_2 Oligohaline estuary, coarse substrate	P1_3 Oligohaline estuary, fine-grained substrate	P2_2 Meso- and Polyhaline estuary, coarse substrate	P2_3 Meso- and Polyhaline estuary, fine-grained substrate
Total number of species	0,540	0,560	0,680	0,760
% Flounder (*Solea* sp.)	0,250	0,430	0,620	0,470
% Picarel (*Spicara* sp.)	0,200	0,230	0,540	0,620
% Mullet (Mugilidae)	0,750	0,780	0,810	0,640
% Seabream (Sparidae)	0,230	0,200	0,660	0,830
% omnivores	0,500	0,520	0,590	0,430
% piscivores	0,300	0,280	0,880	0,810
Tolerance	0,450	0,500	0,680	0,640
Number ERS	0,810	0,810	0,780	0,690
% ERS	0,800	0,820	0,750	0,700
% diadromous species	0,520	0,530	0,630	0,610
% marine juvenile migrating species	0,210	0,290	0,620	0,850
Indicator species	0	0	0,350	0,300
New / alien species	0	0	0	0

2.4. NATIONAL BOUNDARY SETTING

Detailed description of methodology used to derive ecological class boundaries.

According to the Water Framework Directive (WFD), the index range was equally divided into 5 ecological status classes: 0–0.2 Bad; 0.21–0.4 Poor; 0.41–0.6 Moderate; 0.61–0.8 Good and 0.81–1 High:

Ecological state category	EQR class boundaries
Referent/Very good	EQR= >0.80
Good	EQR = 0.60 - 0.80
Moderate	EQR = 0.20 - 0.60
Bad	EQR= < 0.20
Very bad	No fish fauna

2.5. PRESSURES ADDRESSED

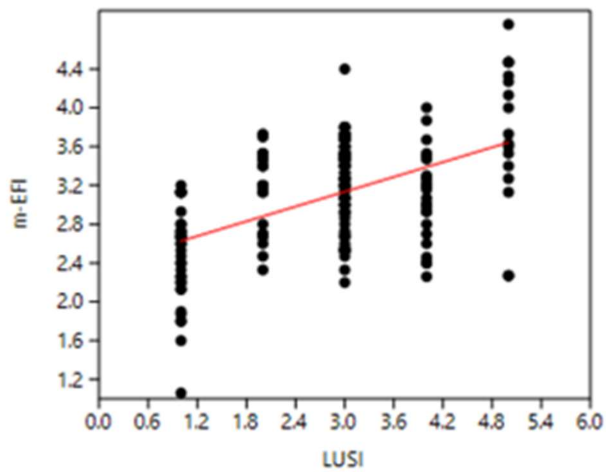
Please describe the pressures addressed by the method and provide pressure-response relationship (graph, equation)

Since the water quality assessment should be done in relation to anthropogenic pressure, preliminary evaluations of known pressures that could possibly affect the water quality within the study area were done using the land use pressures according to Corine Land Cover information system 2000-2006. Land uses simplified index (LUSI) was calculated according to Flo et al. (2011). Assessment of anthropogenic pressure on coastal zone by calculating the LUSI index using the publicly available data is described in UNEP/MAP (2011). The scoring system was slightly modified and Presented in Table 5. Harbor areas due to their direct influence on water quality were scored separately from other industrial influences. We analyzed the area within 3km radius from the sampling sites to assure the precision of land pressure information for stations, which are more distant from the coast.

Table 5. Slightly modified Land uses simplified index (LUSI) scoring system. Index quantifying potential pressure according to percent of land used in various anthropogenic activity.

Urban (%)	Agricultural (%)	Industrial (%)	Harbor (%)	Score
< 3	< 10	<10		0
3-33	10-40	>10	1-10	1
33-66	> 40	>30	>10	2
>66		>60		3

The pressure addressed by the assessment method is general degradation, although corresponds the most with component of eutrophication, as it is presented by equation y=0.254x+2.373 (Figure 1).



Ordinary Least Squares Regression: LUSI-m-EFI
Slope a: 0,25417 Std. error a: 0,033076
Intercept b: 2,3731 Std. error b: 0,10131
95% bootstrapped confidence intervals (N=1999):
Slope a: (0,18105, 0,33103)
Intercept b: (2,1492, 2,5837)
Correlation:
r: 0,51223
r2: 0,26238
t: 7,6843
p (uncorr.):1,2732E-12
Permutation p: 0,0001

Figure 1. Relationship between LUSI and m-EFI for the Croatian transitional waters data set

Obviously, the value of m-EFI is linearly proportional to LUSI value. With higher eutrophication, the transitional waters are more productive allowing more food both for resident and juvenile marine fishes. Also, productive waters offer more complex habitat that can provide micro niches and shelters for a number of fish species. The land impact, as sum of urban, agricultural and industrial pressures, at almost all sampling stations in specific water types is still low enough to encourage good ecological status. For sure, by attainment of a particular value, eutrophication will begin to negatively reflect on fish community in transitional waters. Low m-EFI values (characterized by low number of fish species in community), obtained for low LUSI values is rather reflection of existing hydrographic and meteorological situation (prolonged rain season, storms,) and seasonal variations than indicator of moderate or bad ecological status.

3. WFD COMPLIANCE CHECKING

The first step in the Intercalibration process requires the checking of national methods considering the following WFD compliance criteria.

Table 6. List of the WFD compliance criteria and the WFD compliance checking process and results

Compliance criteria	Compliance checking
Ecological status is classified by one of **five classes** (high, good, moderate, poor and bad).	YES
High, good and moderate ecological status are set in line with the WFD's **normative definitions** (**Boundary setting procedure**)	YES
All relevant parameters indicative of the biological quality element are covered (see Table 1 in the IC Guidance). A **combination rule** to combine parameter assessment into BQE assessment has to be defined. If parameters are missing, Member States need to demonstrate that the method is sufficiently indicative of the status of the QE as a whole	YES
Assessment is adapted to **intercalibration common types** that are defined in line with the typological requirements of the Annex II WFD and approved by WG ECOSTAT	YES
The water body is assessed against **type-specific near-natural reference conditions**	YES
Assessment results are expressed as **EQRs**	YES
Sampling procedure allows for **representative** information about water body quality/ecological status **in space and time**	YES
All data relevant for assessing the biological **parameters** specified in the WFD's normative definitions are covered by the **sampling procedure**	YES
Selected taxonomic level achieves adequate **confidence and precision** in classification	YES

4. IC FEASIBILITY CHECKING

The intercalibration process ideally covers all national assessment methods within a GIG. However, the comparison of dissimilar methods ("apples and pears") has clearly to be avoided. Intercalibration exercise is focused on specific type / biological quality element / pressure combinations. The second step of the process introduces an "IC feasibility check" to restrict the actual intercalibration analysis to methods that address the same common type(s) and anthropogenic pressure(s), and follow a similar assessment concept.

4.1. TYPOLOGY

Does the national method address the same common type(s) as other methods in the Intercalibration group? Provide evaluation if IC feasibility regarding common IC types.

Regarding intercalibration process, at MED GIG meeting in Rome (2014) it was concluded that it is not possible to intercalibrate the modified Estuarine Fish Index (M-EFI) assessment method with Italian and Greek methods due specific geographic characteristics of Croatian transitional waters (estuaries characterized by canyons or channels (like Mirna, Zrmanja, Krka, Cetina), instead of wide shallow delta estuaries (like Neretva) typical for Italy and Greece. The only solution is to run intercalibration exercise with Spain when they developed their method.

4.2. PRESSURES ADDRESSED

Does the national method address the same pressure(s) as other methods in the Intercalibration group? Provide evaluation if IC feasibility regarding pressures addressed.

The Croatian national assessment method addresses the general degradation pressure, as the other national methods in the Intercalibration group, although corresponds the most with component of eutrophication.

4.3. ASSESSMENT CONCEPT

Does the national method follow the same assessment concept as other methods in the Intercalibration group? Provide evaluation if IC feasibility regarding assessment concept of the intercalibrated methods.

Croatian method follows the same assessment concepts as other methods. It is consisted of 7 metrics (total number of species, type species, trophic composition, tolerance, estuarine residence, indicator species and new / alien species). Sampling strategies and frequency are comparable across GiG and take into account the possible seasonal variability of the taxonomic composition.

4.4. CONCLUSION ON THE INTERCALIBRATION FEASIBILITY

Provide conclusions on the IC feasibility.

In the Med GiG meeting in Rome (2014) it was not possible to intercalibrate the modified Estuarine Fish Index (M-EFI) as Croatian national method because of the lack of a sufficient number of similar estuaries in the Mediterranean group, or more precisely due to the specific geographic characteristics of Croatian transitional waters (estuaries characterized by canyons or channels like Mirna, Zrmanja, Krka, Cetina) that plunge into the sea instead of slow wide inflow making wide shallow delta estuaries (like Neretva) typical for Italy and Greece. The only solution is to run intercalibration exercise with Spain when they developed their method in the future.

5. DESCRIPTION OF THE BIOLOGICAL COMMUNITIES

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT HIGH STATUS

At high status the fish community comprises often high number of fish species that are sensitive on eutrophication. With higher productivity, community is characterized with higher diversity and more significant in term of abundance and biomass. Total number of species is ≥ 20 (3-4 predominant species, mostly Mugilidae). Genus Solea sp., Spicara sp. and families Mugilidae, Sparidae and Moronidae are represented by 10-50 %. Omnivores are represented by 2-20 %; piscivorius by 10-50 %. Tolerant species in community is > 3. Estuarine resident species are represented by 10-40 %. Diadromous species are present within range of 10-70%. Marine juvenile species are abundant (30-70%). Indicator or alien species are well presented >5.

Type specific fish communities are:

P1_2 Oligohaline estuary, coarse substrate		
Zrmanja:	Mirna	Neretva
Atherina boyeri	*Atherina boyeri*	*Alburnus neretvae*
Knipowitcchia panizzae	*Chelon labrosus*	*Atherina boyeri*

Liza ramada	*Engraulis encrasicolus*	*Gasterosteus aculeatus*
Pomatoschistus canestrini	*Gambusia hoolbroki*	*Knipowitcchia panizzae*
Pomatoschistus marmoratus	*Pomatoschistus canestrini*	
Rodeus amarus	*Squalius squalus*	
Syngnathus abaster		
Cetina	Jadro	Rječina
Atherina boyeri	*Atherina boyeri*	*Atherina boyeri*
Atherina hepsetus	*Knipowitcchia panizzae*	*Liza ramada*
Callionymus pusillus	*Liza aurata*	
Diplodus annularis	*Salmo trutta trutta*	
Gobius niger		
Lithognathus mormyrus		
Liza ramada		
Pomatoschistus marmoratus		
P1_3 Oligohaline estuary, fine-grained substrate		
Ombla	Krka	Raša
Atherina boyeri	*Atherina boyeri*	*Atherina boyeri*
Gobius niger	*Knipowitcchia panizzae*	*Knipowitcchia panizzae*
Knipowitcchia panizzae		*Liza ramada*
Liza ramada		*Squalius squalus*
Syngnathus taenionotus		
P2_2 Meso- and Polyhaline estuary, coarse substrate		
Ombla	Neretva	Cetina
Atherina boyeri	*Atherina boyeri*	*Atherina hepsetus*
Centracenthus cirrus	*Chelon labrosus*	*Diplodus vulgaris*
Chelon labrosus	*Diplodus annularis*	*Mullus barbatus*
Diplodus vulgaris	*Diplodus vulgaris*	*Pomatoschistus marmoratus*
Gobius cobitis	*Gobius cobitis*	*Sarpa salpa*
Lipophrys pavo	*Gobius niger*	*Sparus aurata*
Oedalechilus labeo	*Lichia amia*	
Pagellus acarne	*Lipophrys pavo*	
Symphodus ocellatus	*Liza aurata*	
Sarpa salpa	*Liza ramada*	
	Parablennius sanguinolentus	
	Sarpa salpa	
	Sparus aurata	
Jadro	Zrmanja	Rječina
Atherina boyeri	*Atherina boyeri*	*Aidablennius sphynx*
Dicentrarchus labrax	*Knipowitcchia panizzae*	*Chromis chromis*
Gobius niger	*Pomatoschistus canestrini*	*Dicentrarchus labrax*
Liza aurata	*Syngnathus abaster*	*Diplodus annularis*
Sparus aurata	*Zosterisessor ophiocephalus*	*Diplodus vulgaris*
Zosterisessor ophiocephalus		*Liza ramada*
		Parablennius sanguinolentus
		Sarpa salpa
		Symphodus tinca
Mirna:		
Atherina boyeri	*Gobius niger*	*Sarpa salpa*
Dicentrarchus labrax	*Lipophrys pavo*	*Sardina pilchardus*
Diplodus puntazzo	*Liza ramada*	*Sparus aurata*
Gambusia hoolbroki	*Parablennius sanguinolentus*	
Gobius cobitis	*Pomatoschistus marmoratus*	
P2_3 Meso- and Polyhaline estuary, fine-grained substrate		
Neretva	Cetina	Krka
Atherina boyeri	*Atherina boyeri*	*Atherina boyeri*
Dicentrarchus labrax	*Atherina hepsetus*	*Atherina hepsetus*
Diplodus annularis	*Callionymus pusillus*	*Chelon labrosus*
Diplodus vulgaris	*Coris julis*	*Dicentrarchus labrax*
Gobius niger	*Diplodus annularis*	*Diplodus annularis*
Gobius paganellus	*Diplodus sargus*	*Diplodus vulgaris*
Knipowitcchia panizzae	*Diplodus vulgaris*	*Gobius niger*
Lipophrys pavo	*Gobius geniporus*	*Knipowitcchia panizzae*
Liza ramada	*Knipowitcchia panizzae*	*Liza ramada*

Pomatoschistus marmoratus	*Liza aurata*	*Liza saliens*
Sparus aurata	*Mullus barbatus*	*Pomatoschistus canestrini*
Syngnathus taenionotus	*Mullus surmuletus*	*Sarpa salpa*
Zosterisessor ophiocephalus	*Pagellus acarne*	*Syngnathus abaster*
	Pagellus erythrinus	*Zosterisessor ophiocephalus*
	Pomatoschistus marmoratus	
	Sarpa salpa	
	Sardina pilchardus	
	Symphodus ocellatus	
	Trigla lucerna	
Zrmanja:	Raša	
Atherina boyeri	*Atherina boyeri*	
Callionymus pusillus	*Atherina hepsetus*	
Diplodus vulgaris	*Chelon labrosus*	
Gobius cobitis	*Dicentrarchus labrax*	
Gobius geniporus	*Diplodus vulgaris*	
Gobius niger	*Gobius cobitis*	
Lipophrys pavo	*Gobius niger*	
Liza aurata	*Liza aurata*	
Parablennius sanguinolentus	*Mullus surmuletus*	
Pomatoschistus marmoratus	*Sarpa salpa*	
Serranus scriba	*Sparus aurata*	
Symphodus cinereus		
Symphodus roissali		
Symphodus tinca		
Syngnathus typhle		

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT GOOD STATUS

At good status the fish community comprises often relatively high number of fish species that are sensitive on eutrophication. Total number of species is 10-20 (3 predominant species, mostly Atherinidae). Genus *Solea* sp., *Spicara* sp. and families Mugilidae, Sparidae and Moronidae are represented by <10 or >50 %. Omnivores are represented by <2 or >20 %; piscivorius by <10 or >50 %. Tolerant species in community is 2-3. Estuarine resident species are represented by <10 or >40 %. Diadromous species are present within range of 10-70%. Marine juvenile species are less abundant (usually 20-30%). Indicator or alien species are relativelly well presented 4-5.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT MODERATE STATUS

At moderate status the fish community comprises often relatively low number of fish species that are sensitive on eutrophication, they are low trophic, mostly planctivores and detrivores, resident fishes. Total number of species is 4-9 (1-3 predominant species). Genus *Solea* sp., *Spicara* sp. and families Mugilidae, Sparidae and Moronidae are represented by 10 %. Omnivores are represented by 2.5 %; piscivorius by 10 %. Tolerant species in community is < 2. Estuarine resident species are represented by 10 %. Diadromous species are present by 10%. Marine juvenile species are within range of 20-30% or very numerous in community (70-80 %) . Indicator or alien species are presented <4.

6. REFERENCES

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Member State: Croatia
BQE: Phytoplankton
Water body category (type): coastal IIIW Adriatic Sea.

The document WFD 3rd IC PHASE, MED GIG CW, BQE PHYTOPLANKTON, CROATIA, ITALY AND SLOVENIA WORKING DOCUMENT (Type I, Type II A "Adriatic" and "Thyrrenian", Type IIIW "Adriatic" and Thyrrenian") contains the final results of the elaborations that led to the definition of the ecological classification criteria for the CW BQE Phytoplankton.

These results were accepted by member states (Italy, Slovenia and Croatia) for assessing ecological status according to BQE phytoplankton. At the time of compiling data for the of the COMMISSION DECISION (EU) 2018/229 Croatia has not yet accepted the threshold values for G/M boundary for the type IIIW Adriatic proposed in the above document (table 1).

Table 1. Proposed thresholds for the assessment of ecological status based on chlorophyll a concentration, expressed as geometric mean and 90th percentile in type IIIW Adriatic

Geometric mean (µg/l)	90 th percentile (µg/l)	Ecological status
0,64	1,7	good/moderate

In the past period Croatia has conducted a statistical rechecking of the newly acquired data (2012 to 2019) to verify the threshold G/M boundary proposed in the above document using the concentration of chlorophyll a in the surface layer to a depth of 10 m as the sole parameter for evaluating water quality.

Considering statistical rechecking confirmed proposed threshold values for type IIIW Adriatic Croatia can officially accept them.

Report on fitting the Croatian classification method for benthic invertebrates in coastal waters with the results of the completed intercalibration of the MED GIG

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Hrvatske vode

Zagreb, March 2022

Report on fitting the Croatian classification method for benthic invertebrates in coastal waters with the results of the completed intercalibration of the MED GIG

1. INTRODUCTION

- Member State; **Croatia**
- BQE; **Benthic invertebrates** (macroinvertebrates)
- Water body category (type) **Coastal waters**

2. DESCRIPTION OF NATIONAL ASSESSMENT METHODS

MS has to provide the complete description of the method in the Annex. The main features should be given below.

The Multiparametric AMBI (**M-AMBI**) (Muxika *et al.*, 2007) is classification method selected for the assessment on the Ecological Quality Status (**EQS**) in the Croatian Coastal Waters (**CW**). It is based on the AZTI's Marine Biotic Index (**AMBI**), developed for soft-bottom macrozoobenthos of European estuarine and coastal environments (Borja *et al.*, 2000). M-AMBI is a multimetric biotic index that incorporates three metrics: 1) the number of species (S), 2) Shannon-Wienner diversity index (H') and 3) the AMBI index. The first one refers to the number of taxa identifiable to species level and/or to those identified to some higher taxonomic level, i.e. "Operational units" (e.g. *Turitella* sp.1, *Nucula* sp., Spionidae etc.), which are listed in AZTI AMBI database, and can be unambiguously attributed to one of five ecological groups (EG I-EG V). Shannon-Wienner diversity index incorporates two elements - the species richness and equitability. Unlike above-mentioned discreet biotic indices (S and H'), AMBI formula integrates continuous Biotic Coefficient – which makes it more suitable for statistical analysis and free from subjectivity (Borja *et al.*, 2000). AMBI is derived from the proportions of individual abundance in five functional Ecological Groups (EG I – EG V) which are related to the degree of sensitivity/tolerance to an environmental stress gradient (Pearson and Rosenberg, 1977; Borja *et al.*, 2000), hence it takes into consideration taxonomic and functional composition of benthic invertebrate species. The M-AMBI index is compliant to WFD normative definition of five EQS classes, that describe High (H), Good (G), Moderate (M), Poor (P) and Bad (B) EQS (Borja *et al.*, 2004), following continuous distribution of Ecological Quality Ratio values on 0-1 scale. It was selected as the national assessment method and applied to dataset of benthic macronvertebrates obtained in coastal waters of Croatia.

2.1. METHODS AND REQUIRED BQE PARAMETERS

Table 2.1.1. Overview of the metrics included in the national method

MS	Taxonomic composition	Abundance	Disturbance sensitive taxa
HR	Not in strict sense (only composition of 5 preclassified sensitivity classes)	Not in strict sense (only relative abundance of 5 preclassified sensitivity classes)	5 sensitivity classes

MS	Diversity	Taxa indicative of pollution	Combination rule of metrics
HR	Shannon-Wiener's index (H') , Species richness (S), linear relationship	Specific opportunistic species	M-AMBI (see below)

Combination rule used in the method
Multivariate factorial analysis performed on combination of AMBI index, Shannon_Wiener's diversity index (H') and species richness (S).

Conclusion on the WFD compliance (are all the indicative parameters included; if not, why)
All indicative parameters were included.

2.2. SAMPLING AND DATA PROCESSING

Description of sampling and data processing:

- **Sampling time and frequency;**

Sampling frequency for Biological Quality Element Benthic Invertebrates (**BQE BI**) is scheduled once in year, triennially, in a summer and/or autumn. Data set used in this study is obtained in 2012-2019 period. Sampling was conducted once per site in summer 2012, 2013, 2014, 2015, 2016 and 2017, in the autumn 2018, and summer (central and southern Adriatic Sea) and autumn (Northern Adriatic Sea) in 2019.
It was performed at thirty-nine sampling sites distributed within seventeen of twenty-five coastal Water Bodies (**WB**) along Croatian coast of the Adriatic Sea: O313-BAZ, O313-JVE, O313-KZ, O313-NEK, O313-MMZ, O313-ŽUC, O313-KASP, O412-ZOI, O412-PULP, O413-LIK, O413-RAZ, O413-PAG, O413-PZK, O413-STLP, O422-SJI, O422-KVV, O422-VIS, O423-BSK, O423-KOR, O423-KVA, O423-KVS, O423-RIZ, O423-RILP, O423-VIK and O423-KVJ.

- **Sampling method;**

Sampling was performed from the Research vessel (using hydraulic winch) by Van Veen grab (0.1 m^2). At each site, four replicate grab samples were taken on each occasion. On the board, sediment from each single grab was rinsed with seawater and sieved through 1mm mesh size. Rough separation (invertebrates > 5mm) and fixation (70% ethanol) were performed on board - parallel with sieving. Sediment remained on the sieve was fixed in 4% formaldehyde sea-water solution, labelled and stored until the next step (final separation, invertebrates < 5mm). In the laboratory, all invertebrates were sorted and classified to higher taxa level (basic operational units of macrozoobenthos), counted (total census method) and fixed in 70% ethanol. Two predominant phyla (Annelida and Mollusca) were identified to species level. All organisms were stored if there is a possibility for afterward reliable determination of unidentified species.

- **Data processing;**

Data set contains all replicate samples of identified species and their absolute abundances. Prior to statistical analysis data was pooled on the site level. At sites sampled twice, samples from each year were analysed separately. M-AMBI method is based on the relative abundance of species belonging to one of five functional groups, according to species-specific sensitivity to pollution. Statistical analyses of data set was performed using by AMBI© V 6.0 AZTI's software and Guidance (Borja *et al.*, 2012). Software provides an abundant database of BI, with its affiliation to one of five Ecological Groups (EG I - EG V) with different sensitivity/tolerance to disturbance. M-AMBI analysis and resulted in:

- percentage share of each single EG
- calculation of AMBI index
- calculation of species richness (S) and Shannon-Wiener diversity index (H')
- calculation of M-AMBI
- classification into one of five sensitivity classes indicated EQS categories: High, Good, Moderate, Poor and Bad

- **Taxonomic composition and Identification level.**

Above-mentioned taxa (Annelida and Mollusca) mainly comprised >75% of total macrofauna and they are considered reliable indicators of environmental disturbance. Due to their high abundance, indicators' performance and reliability of identification, their identification to the species level should be sufficiently representative for assessment of EQS using BQE BI. Here, we need to stress that reliable identification of benthic invertebrates on the species level is possible exclusively by taxonomic experts for particular higher taxa. Since all methods and results for assessment of EQS based on BQI BI are highly dependent on the reliability of species identification, and since due to global crisis of taxonomist very few state have available experts for all invertebrate Phyla, non-expert analysis can easily lead to misidentification. In lack of experts for less abundant Phyla, we are limited to incomplete but suitable solution, to avoid the risk of misidentification. In order to check compatibility of results obtained by analysis of complete *vs.* partial composition (Annelida and Mollusca) of macrobenthic species, we compared two appropriate sets of EQR_{M-AMBI} data from Slovenian database, using the original reference values. The linear regression analysis, have shown very high - statisticaly significant positive relationship for the compared data sets (R=0,979, p<0,01) (Figure 2.2.1.), supporting of results obtained by partial analysis .

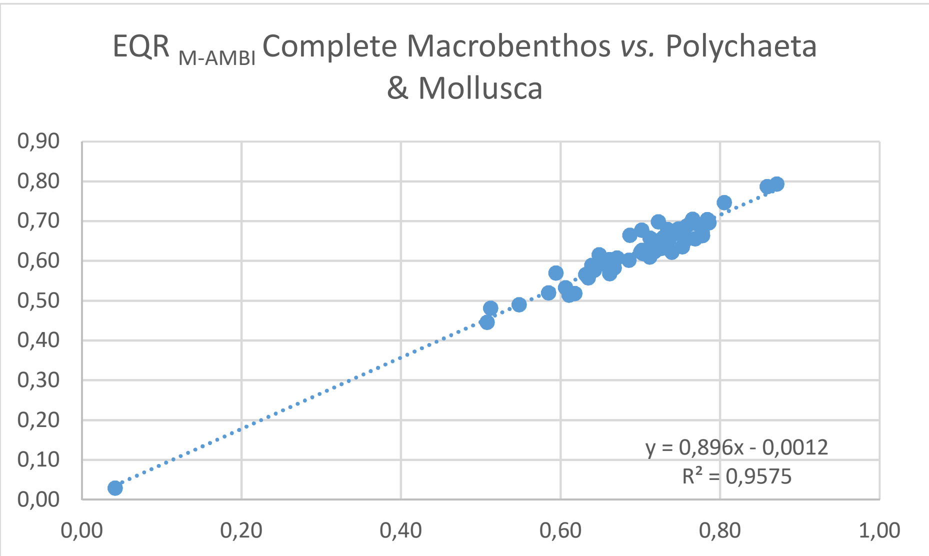


Figure 2.2.1. Linear regression between M-AMBI values based on complete composition of macrobenthos *vs.* composition of two dominant taxa (Polychaeta and Mollusca), derived from Slovenian

CW data-set. Statistically significant positive correlation (R^2=0.9575, R=0.979, N=54, p<0.01) is established.

Consequently, taxonomic composition selected in this study offers an adequate confidence and precision in species classification, as well as reliable EQR_{M-AMBI} values.

2.3. NATIONAL REFERENCE CONDITIONS (NRC)

According to Water Framework Directive (**WFD**), the reference condition for a type-specific **WB** is a description of the biological elements which corresponds totally, or nearly totally, to undisturbed (= pristine) conditions, i.e. a marine environment with no, or with only a very minor, impact from human activities. Compliant to WFD normative definitions, such conditions are associated with two upper classes (High and Good) of the EQS (EC 2003 a).

The WFD identifies four main options for deriving reference conditions: 1) comparison with an existing 'pristine'/undisturbed site (or a site with minor disturbance), 2) historical data and information, 3) models and 4) expert judgement. The alternative is certain combination of above-mentioned options (EC 2003a, 2003b).

In the initial phase of WFD implementation, Reference Condition (**RC**) in Croatian coastal waters were selected using historical data/information and corresponded to totally and/or near-totally undisturbed conditions. Historical data on the composition of soft-bottom benthic invertebrates were obtained between 1973 and 1986 from coastal waters of Limski zaljev, Rovinj coastal area, zaljev Raša and Kvarner region (seventeen sites, 5 WB). It was a baseline for the analysis of functional structure of benthic communities, calculation of biotic indices (AMBI, M-AMBI), assessment of ecological quality status and definition of national reference conditions. Due to low amount of historical data and the absence of full environmental gradient, classification was performed using original classification setting (REFCOND, 2003), with a boundary 0.83 between High and Good classes, and other boundaries set equidistantly to H/G. Out of seventeen sites analysed for $EQR_{M-AMBI,}$ ten were assigned to High and seven to Good EQS. Based on that analysis, we described preliminary/1st generation National Reverence Conditions (**NRC**, Hrvatske vode, 2015), in accordance with WFD normative definition.

Since the climate, land cover, and marine ecosystems vary naturally over periods relevant for WFD, characterization of WB and RC are not permanent and from 2013 have to be reviewed every six years (EC, 2003a, 2003b). Consequently, in the period 2012-2017 BQE BI monitoring were performed at 23 stations – twice at BB-O15a and BB-O38 (Fig. 2.3.1.) within 17 coastal WB (Fig. 2.3.2.). Sampling sites were selected according to: 1) geographical representativeness (data corresponded to majority of WB scheduled to implementation of the WFD, 2) the strength of taxonomic lists, 3) the minor impact of human activities and 4) methodological approach (sampling, laboratory analyses) eligible for EQS assessment.

Results obtained during six year monitoring indicated High EQS for 6, Good EQS for 15 and Moderate EQS for 4 sampling sites (Fig. 2.3.3.). Biological communities in High status that corresponded nearly totally to undisturbed conditions (nutrients, chl-a and bottom dissolved oxygen are in High status, LUSI=1) were selected for description of NRC. Reference criteria used for selection of National Reference Sites (**NRS**) was minor anthropogenic influence met the following biological criteria: low AMBI value, high S and H', dominance of sensitive and indifferent taxa (EGI and EG II) and minimal share of opportunistic taxa (EG IV and EG V).

Accordingly, reference condition values were set as follows:

M-AMBI ≥0,81**, AMBI<2; S>40; H'>4,5
EG I and EG II ≥ 79,6%, EG III ≤ 18,3%, EG IV I EG V ≤2,1%

** 90th Percentile of distribution EQR_{M-AMBI} values derived from H/G data

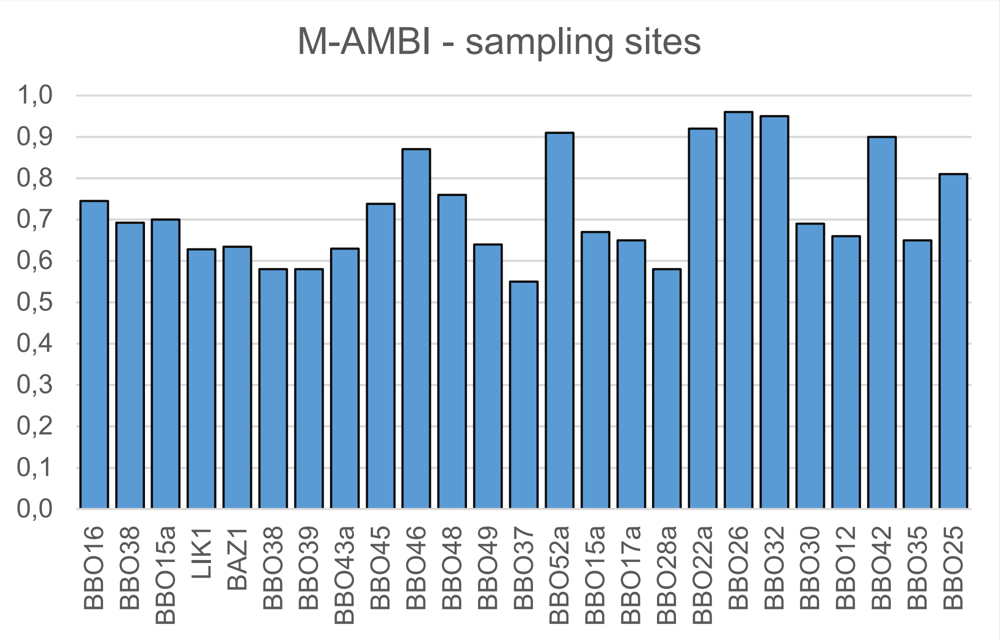


Figure 2.3.1. M-AMBI scores for Coastal Water sampling sites (2012-2017).

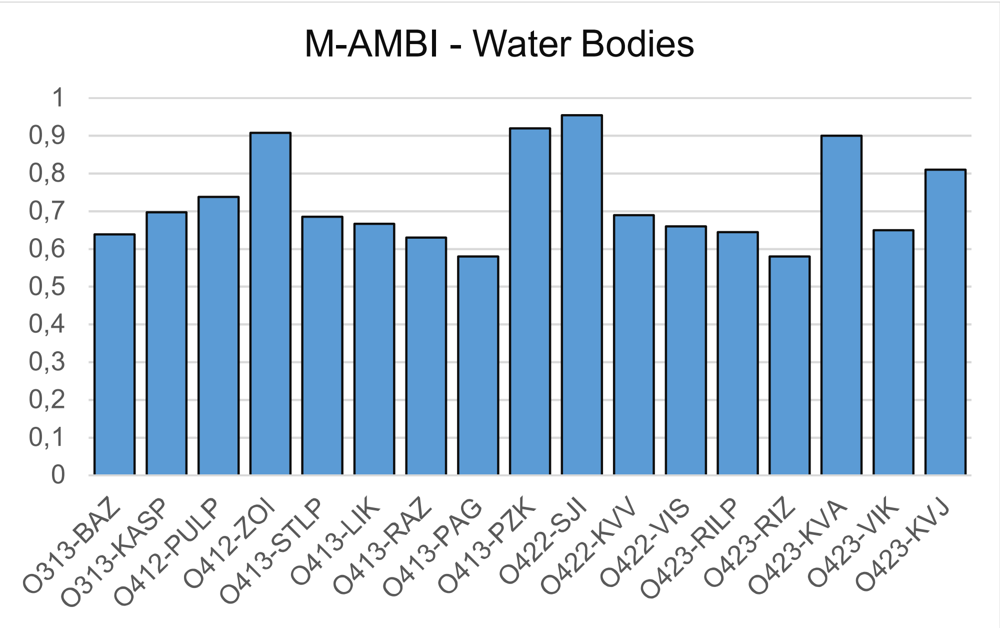


Figure 2.3.2. M-AMBI scores for Coastal Water Bodies (2012-2017).

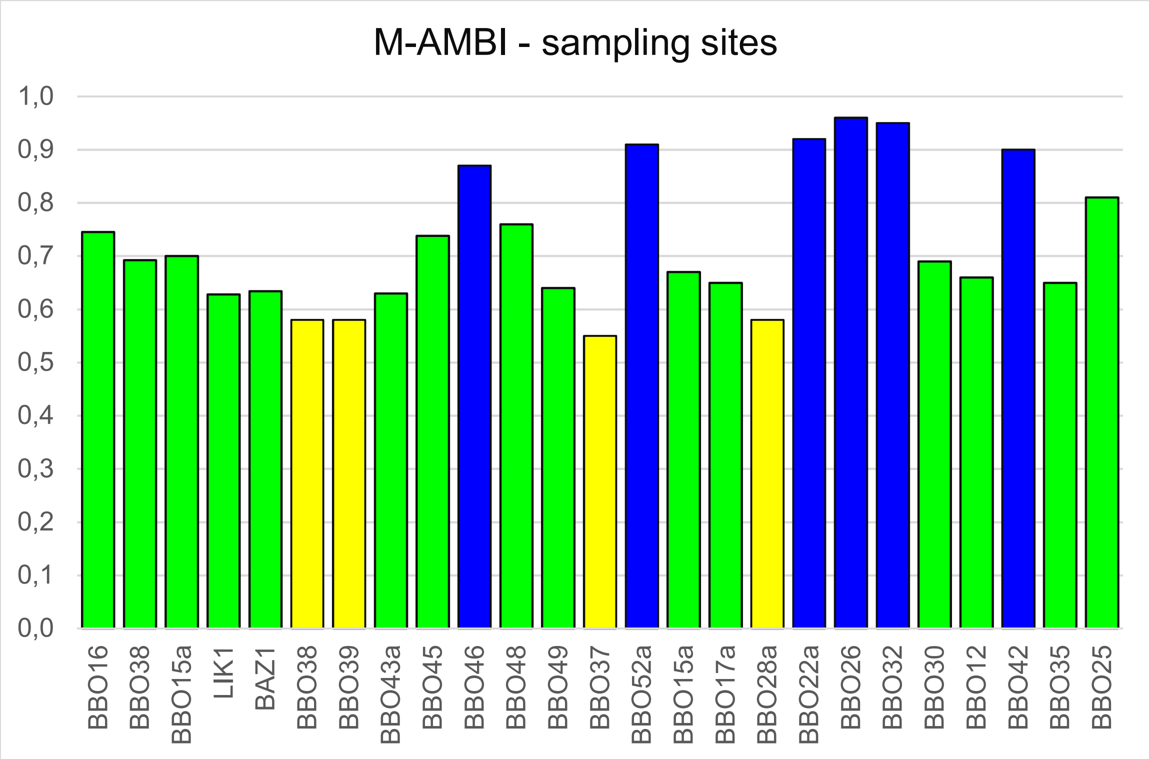


Figure 2.3.3. M-AMBI (2nd generation) for Coastal Water sampling sites (2012-2017) with indication of the Ecological Quality Status (EQS): blue (High), green (Good), yelow (Moderate).

Data selected for establishment of the 2nd generation NRC are compliant with WFD normative definition in terms of: 1) spatial coverage, 2) methodological approach and 3) requirement of minor impact from human activities. According to above-mentioned criteria we selected five NRS in coastal waters: WB O412-ZOI (BB-O46, BB-O52a); WB O422-SJI (BB-O26, BB-O32), WB O423-KVA (BB-O42).

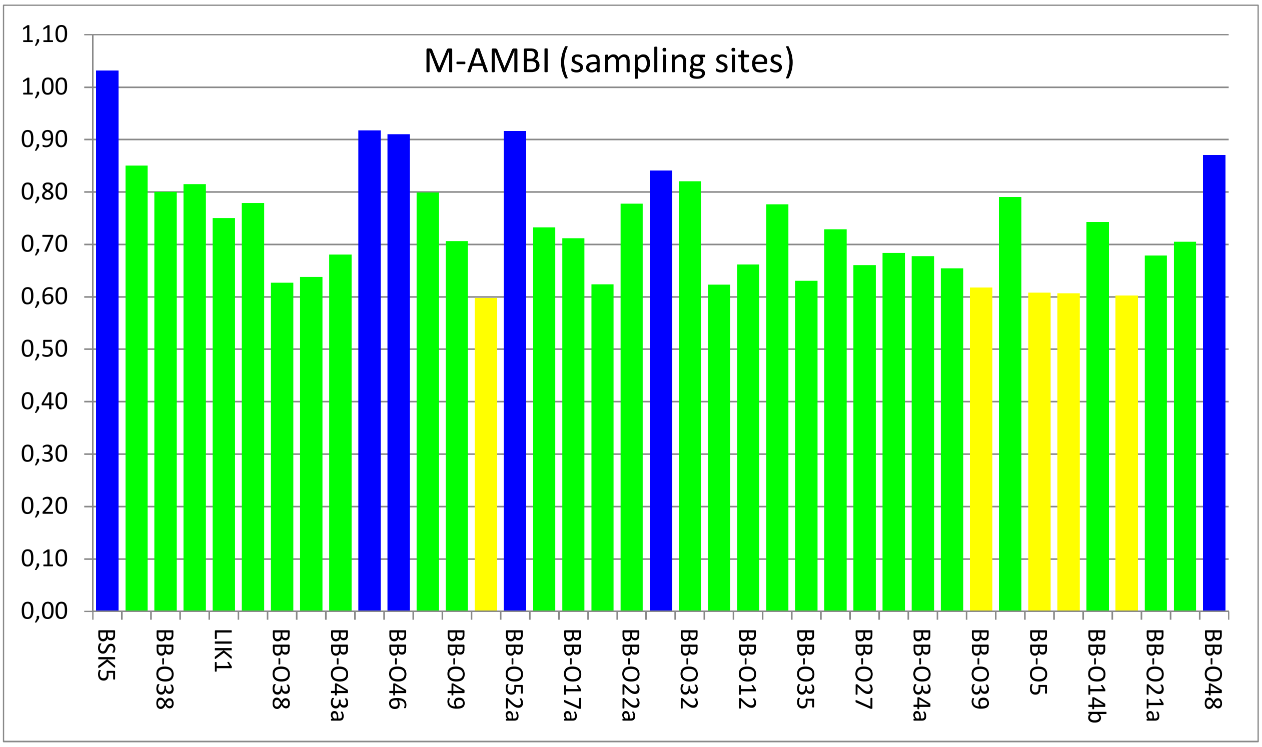
Up to now, we reported development of methodology based on 2012-2017 data set (100 grab samples collected during 25 sampling tours, at 23 sampling sites, within 17 Water bodies), using original Boundary Classes for EQR (REFCOND, 2003), and consecutive Reference Coditions describe above. Updated NRC was compliant to WFD normative definitions, as follows in Description of the biological communities in High and Good status.

However, the Republic of Croatia did not participate in IC exercises, due to late accession to the EU, so additional procedure was needed to harmonize National methodology within the MED-GIG. Since, Croatia and Slovenia are neighboring states that geographicaly share the eastern Adriatic coast, it can be assumed their coastal waters have the most similar ecological conditions and benthic communities. Therefore, and due to compliant method related to the analysis of taxonomic composition (see chapter 2.2.), we decided to harmonize methodology by acceptance of reference values and class boundaries for Slovenian coastal waters, developed during MED-GIG IC:

M-AMBI > 0.83, AMBI = 1.3, H '= 5.8, S = 110
that is, class boundaries
H / G = 0.83
G / M = 0.62

To achieve that goal we revised previous analyses, i.e. we calculated new M-AMBI (3rd generation) for updated Croatian data set (2012-2019), using Slovenian RC and class boundaries (BC) values, and the results are shown in the Figure 2.3.4. Two sites with the highest values of revised M-AMBI index (BB-BSK5 and BB-O52a) located in nearly totally pristine conditions, are selected as the reference sites.

Here, we report revised national results - and suggest updating of NRC in the Croatian part of the Adriatic Sea using data obtained during eight year WFD monitoring (2012-2019).



igure 2.3.4. Updated M-AMBI scores for Coastal Water sampling sites (2012-2019), following revision after Slovenian RC and BC.

2.4. NATIONAL BOUNDARY SETTING

Detailed description of methodology used to derive ecological class boundaries.

According to Water framework directive (WFD), the ecological status is preclassified into five classes (Bad, Poor, Moderate, Good, Very good/High), indicating differences in ecological status along a gradient of disturbance, based on BQE BI assessment. The initial boundaries between the ecological classes in the coastal waters of Croatia are those originally identified by REFCOND (2003) and applied by Muxika *et al.* (2007).

As stated in the previous chapter, we accepted Slovenian methodology for the assessment of the Ecological Quality Status, using M-AMBI classification method, including Slovenian RC and BC values. Accordingly, ecological class boundaries were revised as follows: H/G = 0.83, G/M = 0.62, M/P = 0.41, P/B = 0.20.

M-AMBI intervals associated with each of five ecological class is presented in Table 2.4.1.

Table 2.4.1. EQS classification scheme with corresponding EQR intervals. Based on results obtained during eight year monitoring on total of 156 samples, taken at 39 sampling tours from 30 sampling sites (Fig. 2.4.1.).

ECOLOGICAL STATE CATEGORY (EQS)	M-AMBI EQR CLASS BOUNDARIES
HIGH / VERY GOOD	0.83-1.00
GOOD	0.62-0.82
MODERATE	0.41-0.61
POOR	0.20-0.40
BAD	0.00-0.20

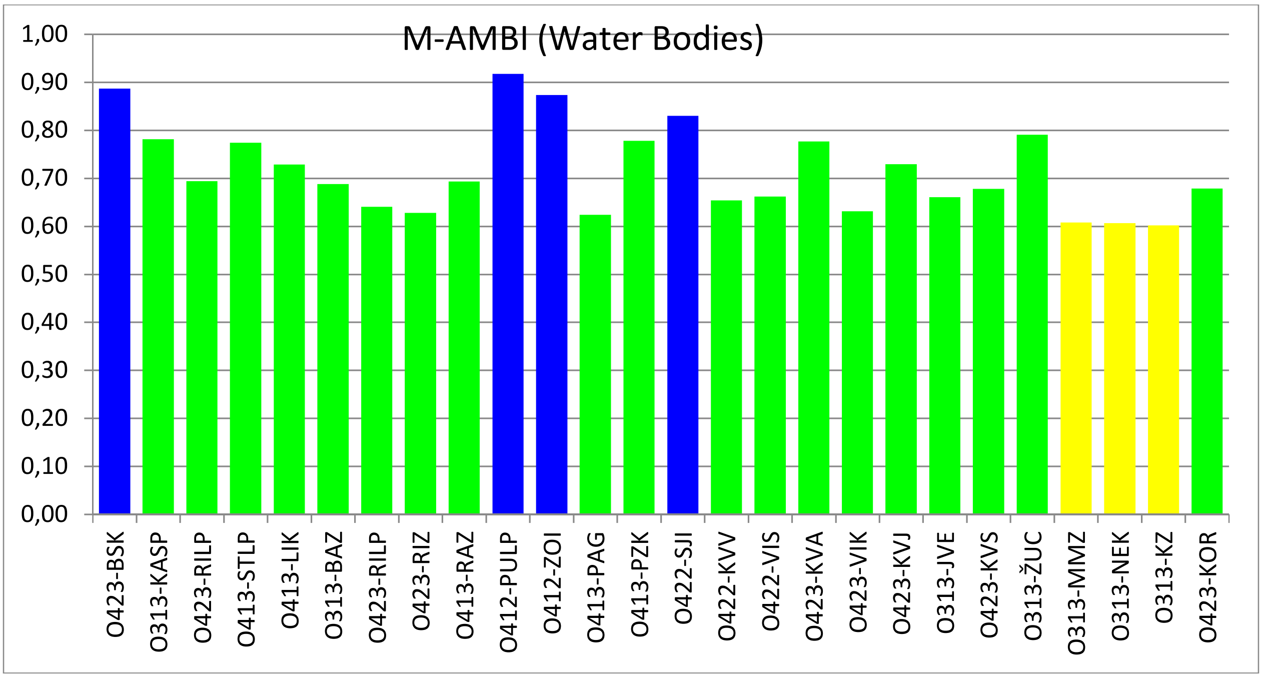


Figure 2.4.1. Type-specific water bodies in Croatian Coastal Waters (2012-2019). Ecological quality status (EQS) after acceptance of Slovenian M-AMBI classification method. Croatian CW dataset for National Boundary Setting related to 25 WB monitored during 2012-2019.

Results obtained during eight year monitoring were unequally distributed between High and Moderate classes, indicated High EQS for 3, Good EQS for 12 and Moderate EQS for 10 WB (Fig. 2.4.1.)

2.5. PRESSURES ADDRESSED

Pressures considered ascertaining the relationship between anthropogenic pressures and BQE BI were land usage (urbanization, industry, agriculture) and sea usage (mariculture, ports, wastewater discharge). The relationship between Ecological Quality Status based on benthic invertebrates and anthropogenic pressures were tested by integrative measure of antrophogenic pressure "z" that was used in previous RBMP to determine impact of pressures on water bodies of transitional and coastal water and calculate risks Since individual pressures are expressed in different units of measurement or as dimensionless numbers, i.e. indices or points, all values were standardized by being converted to z-scores (Kušpilić et al., 2016).

A z-score is a numerical measurement that describes a value's relationship to the mean of a group of values. Z-score is measured in terms of standard deviations from the mean. If a Z-score is 0, it indicates that the data point's score is identical to the mean score. The process of standardization is a basic mathematical method which is explained in numerous statistical handbooks and applied in scientific papers (Sauliere et al., 2019.; Šolić et al., 2022.-Figs 4.,5. and 7.).

Integrative measure of antrophogenic pressure “z” consists of eight different pressures as indicated in the table below:

individual pressure	**description**
annual loads (t/yr/km3) of selected pollutants from point sources	(BOD5, TN, TP, Cd, Cu, Pb, Hg, Ni, Zn, antracen, fluoranten) – *z* was calculated as average of all calculated z values
annual loads (t/yr/km3) of selected pollutants from inhabitants outside wastewater systems	(BOD5, TN, TP, Cd, Cu, Pb, Hg, Ni, Zn, antracen, fluoranten) – *z* was calculated as average of all calculated z values
LUSI-index values	
marine traffic intensity - the area of the water body to which the average number of vessels refers	"scores" were associated to particular WB given the average number of vessels per km2 and the percentage of WB area occupied by vessels through area correction factor; score 1 (<30 average vessels/km2), 2 (30-70 av. vessels/km2), 3 (71-140 average vessels/km2), 4 (>140 av. vessels/km2). Correction factor f=affected area (%)x0,01
annual loads of N and P (t/yr/km^3) from mariculture	(TN, TP)
assessed impact of invasive species on BQEs	WB was scored according to scores: 0 (no impact, invasive species not found in WB)-1 (no impact probable) - 2 (impact probable)-3 (impact detectable)
assessed impact from fishing	WB was scored according to the area the fishing gear can cover, times of workload, impact of the fishing gear on BQE in the WB, WB area under impact - average scores were calculated per fishing gear per water body.
hydromorphological pressures	WB was scored according to hydromorphological status, Hymo is H and G = 0, HYMO is less than G =1

“z” score was calculated for each coastal water body using the formula:

$z=(x-\mu)/\sigma$ where x=single measurement, μ=average value, σ=standard deviation

Positive z - scores indicate above average pressures to which the water body is exposed. Negative z – scores indicate below average pressures. $EQR_{M\text{-}AMBI}$ values were tested against pressure variable “z”. The linear regression analysis of $EQR_{M\text{-}AMBI}$ with integrative metrics of antrophogenic pressures (“z”), have shown good and significant negative relationship for EQR_{AMBI} (Figure 2.5.2.). Therefore, $EQR_{M\text{-}AMBI}$ can be considered as suitable metric and “z” as suitable indicator of antrophogenic pressure in Croatian coastal waters.

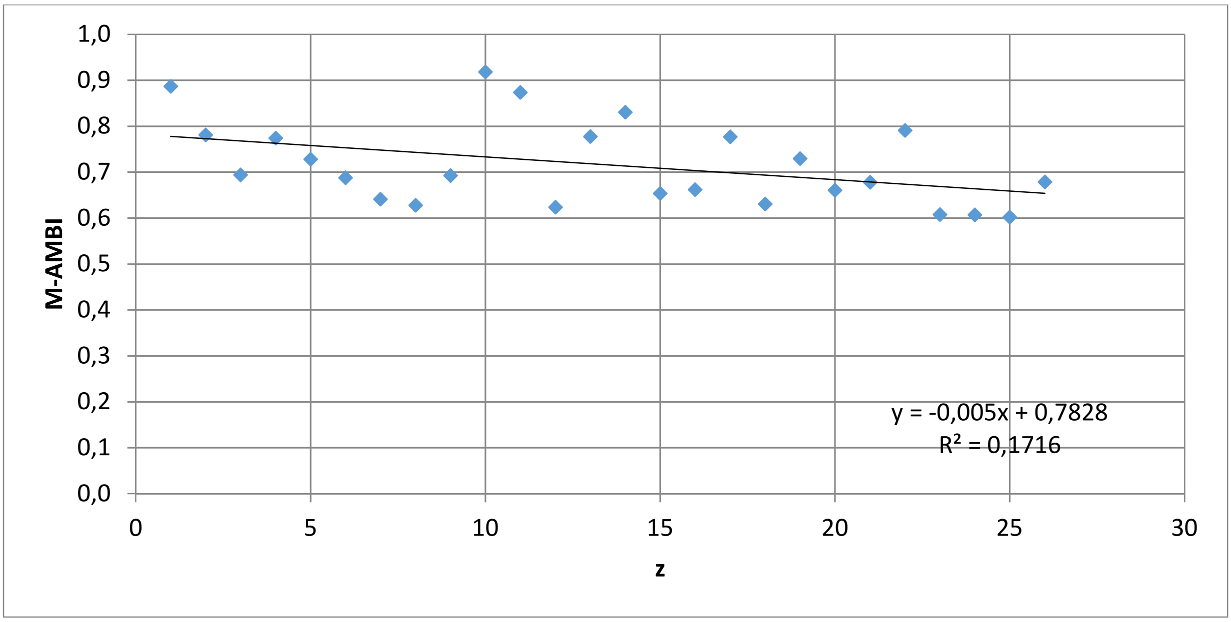


FIGURE 2.5.2. Linear regression between M-AMBI and integrative measure of anthropogenic pressure ("z"), n=25, df=23. Statistically significant negative correlation (N=25, df=23, R^2=0.1716, R=0.4142, $p<0.05$) is established.

3. WFD COMPLIANCE CHECKING

The first step in the Intercalibration process requires the checking of national methods considering the following WFD compliance criteria.

Table 2. List of the WFD compliance criteria and the WFD compliance checking process and results

Compliance criteria	Compliance checking
Ecological status is classified by one of **five classes** (high, good, moderate, poor and bad).	Yes
High, good and moderate ecological status are set in line with the WFD's **normative definitions** (**Boundary setting procedure**)	Yes
All relevant parameters indicative of the biological quality element are covered (see Table 1 in the IC Guidance). A **combination rule** to combine parameter assessment into BQE assessment has to be defined. If parameters are missing, Member States need to demonstrate that the method is sufficiently indicative of the status of the QE as a whole	Yes
Assessment is adapted to **intercalibration common types** that are defined in line with the typological requirements of the Annex II WFD and approved by WG ECOSTAT	Yes
The water body is assessed against **type-specific near-natural reference conditions**	Yes
Assessment results are expressed as **EQRs**	Yes
Sampling procedure allows for **representative** information about water body quality/ecological status **in space and time**	Yes

All data relevant for assessing the biological **parameters** specified in the WFD's normative definitions are covered by the **sampling procedure**	Yes
Selected taxonomic level achieves adequate **confidence and precision** in classification	Yes

4. IC FEASIBILITY CHECKING

The Republic of Croatia joined to EU on July 2013. At that time, the intercalibration process among MS involved in MED-GIG for CW was already completed. However, Croatia share the same assessment method with Slovenia and derived same class boundaries and reference conditions, that successfully applied and intercalibrated method in MED-GIG IC process. Although there were differences in terms of taxonomic identification level between Slovenia and Croatia, validation test provided by linear regression analysis of two corresponding Slovenian datasets has confirnmed compliance of methodology on the issue (see chapter 2.2.). The next step of adjustment and calibration of Croatian results according to the Slovenian methodology was: 1) acceptance of Slovenian benchmarks and ecological class boundaries, 2) application of adopted RC and BC values to the national data set, and 3) calculation of new EQR values for assessment of Ecological Quality Status in Coastal Waters, compliant to Slovenian methodology.

4.1. TYPOLOGY

Does the national method address the same common type(s) as other methods in the Intercalibration group? Provide evaluation if IC feasibility regarding common IC types.
Typologies are not relevant in the MEDGIG ecosystem as it was concluded in MEDGIG CW benthic invertebrate working group, and therefore they are not relevant in MEDGIG IC procedure for macro-invertebrates element (EC 2013b).

4.2. PRESSURES ADDRESSED

Does the national method address the same pressure(s) as other methods in the Intercalibration group? Provide evaluation if IC feasibility regarding pressures addressed.

4.3. ASSESSMENT CONCEPT

Does the national method follow the same assessment concept as other methods in the Intercalibration group? Provide evaluation if IC feasibility regarding assessment concept of the intercalibrated methods.

4.4. CONCLUSION ON THE INTERCALIBRATION FEASIBILITY.

The Croatian classification method for benthic invertebrates in coastal waters is harmonized with the Slovenian methodology including: 1) validation of the taxonomic identification method, 2) acceptance of Slovenian RC and BC values, and 3) their application to the Croatian data set.
Fitting the Croatian classification method with the results of the completed intercalibration of the MED GIG was completed by testing obtained EQR values against general type pressure. Linear regression analysis found out statistically significant negative relationship between M-AMBI and integrative measure of anthropogenic pressure, suggesting suitability of the Croatian classification method and intercalibration feasibility.

5. DESCRIPTION OF THE BIOLOGICAL COMMUNITIES

DESCRIPT AT HIGH STATUS BQI BENTHIC INVERTEBRATES COMPRISES OFTEN HIGH NUMBER OF SPECIESI ON OF THE BIOLOGICAL COMMUNITIES AT HIGH STATUS

At High status M-AMBI for BQE Benthic Invertebrates ≥0.83, ranged between 0.84 and 1.03 (median 1.03). It was reported from seven sampling sites (BB-BSK5, BB-O16, BB-O26, BB-O45, BB-O46, BB-O48 and BB-O52a), and established in three Water Bodies: O423-BSK, O412-PULP, O422-SJI and O412-ZOI.
A disturbance-sensitive taxa of the type-specific communities associated with undisturbed conditions (e.g. *Aponuphis bilineata, Euclymene lumbricoides, Myriochele heeri, Poecilochaetus fauchaldi, Timoclea ovata, Turitella turbona* etc.) are present and rather abundant in communities composition. Relative abundances of EG assessed by AMBI indicate normal or slightly impoverished community and unpolluted site. Distribution of species within five EG was: EGI=38.5, EGII=35.1, EGIII=20.6, EGIV and EGV=6.3.

At High Ecological Quality Status AMBI ranged between 0.77 and 2.03 (median 1.37), Shannon-Wiener diversity index (H') between 4.87 and 5.83 (median 5.36), and species richness (S) between 60 and 166 (median 82.50) species per site. Both, number of species and species diversity are rather high.

OF THE BIOLOGICAL COMMUNITIES AT GOOD STATUS

At Good status M-AMBI for BQE Benthic Invertebrates ≥0.82, ranged between 0.62 and 0.82 (median 0.71). It was established at 28 sampling sites belonging to in 22 Water Bodies.

Most of the sensitive taxa of the type-specific communities are present. Relative abundances of EG assessed by AMBI indicate slightly unbalanced community and slightly polluted site. At Good status, percentage contribution of Ecological Groups is similar to that in High status communities EGI=28.8, EGII=43.2, EGIII=17.8, EGIV and EGV=10.3.), but in this EQS category indiferent species are mainly most abundant than sensitive one and percentage of opportunistic species is higher, as well. Many sensitive and indifferent species that characterize inflarittoral/circalittoral communities (*Antalis dentalis, A. novemcostata, Hyala vitraea, Nucula nitidosa, Mangelia paciniana, Marphysa bellii, M. kinbergi, Labioleanira yhleni, Laevicardium crissum, Lumbrineris latreilli, Maldane glebifex, Pista lornensis, Poecilochaetus fauchaldi, Tellina donacina, Terebellides stroemi, Turitella communis* etc.) are present and abundant in communities composition.
At Good Ecological Quality Status AMBI ranged between 0.97and 258(median 1.68), Shannon-Wiener diversity index (H') between 3.49 and 5.23 (median 4.09), and species richness (S) between 18 and 99 (median 28) species per site.

DESCRIPTION OF THE BIOLOGICAL COMMUNITIES AT MODERATE STATUS

At Moderate status M-AMBI for BQE Benthic Invertebrates ≥0.41, ranged between 0.60 and 0.62 (median 0.61). It was established at 5 sampling sites belonging to in 5 Water Bodies.
At Moderate Ecological Quality Status AMBI ranged between 0.42 and 2.41 (median 1.98), Shannon-Wiener diversity index (H') between 1.87 and 3.62 (median 3.41), and species richness (S) between 19 and 38 (median 22) species per site. In addition to comparatively low species richness and diversity, communities at Moderate EQS, characterize with enlarged contribution of oportunitic species (22,8%). At our sites, increased contribution of opportunistic species is related to increasing abundance of EGIV bivalves *Clausinella fasciata* and *Corbula gibba*.

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