WFD ecological status indicator shows poor correlation with flow parameters in a large Alpine catchment

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36 **Summary**

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38 Since the implementation of the Water Framework Directive, the ecological status of
 39 European running waters has been evaluated using a set of harmonised ecological
 40 indicators that should guide conservation and restoration actions. Among these, the
 41 restoration of the natural flow regime (ecological flows) is considered indispensable
 42 for the achievement of the good ecological status, and yet the sensitivity of the
 43 current biological indicators to hydrologic parameters remains understudied. The
 44 Italian Star_ICMi well represents other similar WFD indicators; it is a
 45 macroinvertebrate-based multimetric index officially adopted to assess the ecological
 46 status of running waters at the national level. Recent legislation has also included the
 47 Star_ICMi as one of the indicators used to assess and prescribe ecological flows in
 48 river reaches regulated by water abstraction. However, the relationship between river
 49 hydrology and the Star_ICMi index is so far virtually unknown. Using data from the
 50 Trentino - Alto Adige Alpine region, we first assessed the relationship between the
 51 Star_ICMi and synthetic descriptors of the physico-chemical (LIMeco) and
 52 morphological (MQI) status of respectively 280 and 184 river reaches. Then, we
 53 examined the relation between the Star_ICMi and a set of ecologically-relevant
 54 hydrologic parameters derived from discharge time-series measured at 21
 55 hydrometric stations, representing both natural and regulated river reaches. Although
 56 the Star_ICMi showed significant and linear relationships with the physico-chemical
 57 character and, slightly, with the morphological quality of the reaches, its response to
 58 flow parameters appeared weak or non-existent when examined with linear models.
 59 Mixed quantile regressions allowed the identification of flow parameters that
 60 represented limiting factors for macroinvertebrate communities and the associated
 61 Star_ICMi scores. In particular, the index showed 'negative floors' where lower
 62 values were observed in reaches with large temporal variation in flow magnitude as
 63 well as frequent low and high flow events. The modelled quantiles also tracked the
 64 transition of the index from acceptable to unacceptable conditions.
 65 The results suggest that while the central tendency of the Star_ICMi index is not
 66 strongly influenced by river flow character, some key flow parameters represent
 67 limiting factors that allow the index to reach its lowest values, eventually 'pushing' the
 68 site towards unacceptable ecological conditions. The identification of limiting flow
 69 parameters can aid the setting of hydrologic thresholds over which ecological
 70 impairment is likely to occur. Overall, however, results imply caution is needed in
 71 using biological indicator like the Star_ICMi for the quantitative assessment and
 72 design of ecological flows.

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74 **Keywords:** Bioindicators; STAR_ICMi; Water Framework Directive; Ecological flows;
75 Quantile regression

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78 **1. Introduction**

79

80 The Water Framework Directive (WFD; European Commission, 2000) is the
81 principal legislative framework concerning the management and protection of
82 European waters. Through the definition of common approaches, the WFD
83 requires Member States to achieve ‘good ecological status’ objectives for
84 water bodies. Among the quality elements guiding the status classification of
85 streams and rivers, the hydrologic regime (the quantity and dynamics of river
86 flow, sensu Poff et al., 1997) is considered central in supporting the biological
87 elements and thus the achievement of good ecological status. Although not
88 explicitly mentioned in the WFD, the concept of ‘ecological flow’ (E-flow) is
89 increasingly considered and implemented in many river basin management
90 plans. Within the EU context, E-flows are defined as “an hydrologic regime
91 consistent with the achievement of the environmental objectives of the WFD
92 in natural surface water bodies”, and specific recommendations on the
93 definition of E-flows and their use in status assessment were also recently
94 provided (Guidance 31 by the European Commission; WFD CIS, 2015). In
95 particular, the Guidance 31 states that the “Ecological impacts of hydrological
96 alterations and their significance should be ultimately assessed with biological
97 indicators built on monitoring data that are specifically sensitive to
98 hydrological alterations”.

99 The use of biological indicators has a long tradition in freshwater ecology
100 where fish and macroinvertebrate based indices are widely used to define the
101 ecological integrity of waterbodies (e.g. De Pauw et al., 2006; Rosenberg and
102 Resh, 1993). In Europe, after the implementation of the WFD, there has been
103 substantial effort to harmonise the different eco-bio-indicators across EU
104 Countries (e.g for macroinvertebrates: Hering et al., 2004; Verdonschot and
105 Moog, 2006). These indicators are used to define the ecological status of
106 running waters and guide conservation and restoration effort.

107 However, although river organisms are clearly influenced by the hydrology,
 108 most present bioindicators were developed to emphasise organisms
 109 sensitivity to organic pollution and habitat degradation, and hence appear
 110 rather insensitive to hydrological alterations (Friberg, 2014; Poff and
 111 Zimmerman, 2010). Although some countries developed hydrologically-
 112 sensitive indicators based on flow preference of benthic invertebrates (UK:
 113 Extence et al., 1999; NZ: Greenwood et al., 2016), these are not yet
 114 implemented in the WFD. The implementation of evidence-based E-flows
 115 should be based on a sound understanding of the relation between river
 116 ecology (e.g. biodiversity) and flow characteristics (flow-ecology relationship:
 117 Rosenfeld, 2017; Stewart-Koster et al., 2014), ultimately requiring a
 118 fundamental association between water quantity and ecological quality. Yet,
 119 more effort has been dedicated internationally towards the definition and
 120 modelling of E-flows and water allocation for regulated rivers (e.g. residual
 121 flow) compared to the quantification of flow-ecology relationships (Davies et
 122 al., 2014; Tonkin et al., 2014). The natural flow paradigm is at the heart of the
 123 E-flow concept in that modified flow regimes should incorporate the natural
 124 variability in terms of flow magnitude, frequency, duration, timing and rate of
 125 change (Poff et al., 1997). Since the publication of the Nature Conservancy's
 126 Indicator of Hydrologic Alteration (IHA; Richter et al., 1997), parameters
 127 quantifying the different components of the flow regime have been widely
 128 used to characterise natural flow regimes and its alterations as well as to
 129 identify 'ecologically relevant' hydrological drivers (e.g. Worrall et al., 2014).
 130 Similarly, flow-ecology studies quantifying the influence of individual flow
 131 parameters on in-stream communities have been flourishing steadily in recent
 132 times (Tonkin et al., 2014); however, those that specifically assessed the
 133 response of multi-metric indicators such as those adopted by the WFD are
 134 scarce (Belmar et al., 2018; Monk et al., 2006; Nebra et al., 2014). This is
 135 surprising considering the emphasis given by the WFD on water abstraction,
 136 ranked as the second most common pressure on EU water bodies (WFD CIS,
 137 2015). Therefore, assessing how current WFD biological indicators respond to
 138 the different components of the flow regime is a prerequisite for managing E-
 139 flows and developing more specific indicators.

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141 Here we used a framework based on flow-ecology relationship to investigate
 142 the performance of a WFD bio-indicator to characterise hydrologic regimes
 143 and their alterations. As a case study, we used the macroinvertebrate-based
 144 Star_ICMi (Buffagni and Erba, 2007) officially adopted by the Italian legislation
 145 as the Biological Quality Element to guide the classification of running waters
 146 according to the WFD. The index is based on six normalized and weighted
 147 metrics also adopted by other EU Countries (Buffagni et al., 2006), and
 148 includes taxonomic richness and diversity, as well as taxa sensitivity to
 149 organic pollution. Alongside other purely hydrological and habitat-based eco-
 150 hydraulic indicators, the Star_ICMi also represents one of the methods
 151 adopted by the Italian law for the determination of E-flows in regulated rivers.
 152 However, since its official introduction in Italy, the few available studies have
 153 indicated a rather low sensitivity of the Star_ICMi to discharge alterations,
 154 especially where these are not coupled with a deterioration of water-quality,
 155 as it often occurs in Alpine and perialpine streams affected by hydropower
 156 regulation (Laini et al., 2018; Quadroni et al., 2017; Salmaso et al., 2018).
 157 Recently, some critical issues in using the STAR_ICMi to determine E-flows
 158 have been raised based mainly on the apparent lack of a direct relationship
 159 between the index and river discharge (Spitale and Bruno, 2018).
 160 Surprisingly, despite the specific requirements of the WFD, so far the
 161 relationship between the Star_ICMi and different flow parameters describing
 162 river discharge has been virtually unexplored in Italy (but see Laini et al.,
 163 2018). However, investigating how this ecological quality indicator responds to
 164 flow characteristics is indispensable to evaluate its use within the context of E-
 165 flows.
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 167 As a representative case study for the Italian Alpine area we analysed data
 168 from the Trentino-Alto Adige region where the main alterations of the natural
 169 flow regime are essentially related to hydropower schemes (Zolezzi et al.,
 170 2009). The study has two main objectives: first, we used the region-wide
 171 dataset to investigate the responses of the STAR_ICMi to the physico-
 172 chemical and morphological character of river reaches, as described by
 173 synthetic WFD quality elements. Second, by identifying a set of monitoring
 174 stations for which river discharge time-series were available, we quantified the

relationship between the Star_ICMi and a set of ecologically-relevant flow parameters, using both linear and quantile regressions. Because the latter analysis was based on limited data points, we did not attempt to disentangle and rank the individual effect of multiple environmental factors besides hydrologic regime (e.g. as in Booker et al., 2015). Instead, we appraised the extent to which other environmental covariates (i.e., anthropogenic stressors) might have influenced the observed flow-ecology relationship using the Procrustes analysis. Specifically, we tested if the correlation between hydrological parameters and macroinvertebrate communities increased with altitude where the influence of other anthropogenic stressors (e.g. nutrients, local land use) appeared to be weaker.

2. Methods

2.1 Study area

The Trentino-Alto Adige is a region in Northeast Italy with a surface of c. 13.000 Km² and a population of c. 900.000 inhabitants. The region mostly lays within the Alps with more than 75% of its territory above 1000 m of altitude. The Adige River and its tributaries form the largest river basin occupying about 80% of its territory. Minor river basins in the region included in the study were the Sarca, Brenta, Chiese and Vanoi. A total of 280 study reaches were included, which form the monitoring network of the Environmental Protection Agencies of the Provinces of Trento and Bolzano, across an altitudinal range of 175 - 1800 m a.s.l (Fig. 1).

Figure 1. Map of the main river networks in the Trentino-Alto Adige region in NE Italy and the analysed gauging stations (stars) and biological sampling stations (dots).

2.2. Data collection and computation of the WFD indicators

Data used in the present study come from the institutional monitoring programme of the Environmental Protection Agencies of the Provinces of Trento and Bolzano. Benthic macroinvertebrates were collected in 280 stream reaches between 2009 and 2014 (Fig. 1). Sampling followed the multi-habitat proportional technique according to the AQEM protocol (Hering et al., 2004), in which 10 replicate Suber samples (0.1m^2) were distributed along the reach proportionally to the different micro-habitat types present. Specimens were identified to genus and family levels as required for the calculation of the Star_ICMi. The index is computed combining sub-metrics related to the tolerance, richness and diversity of the different macroinvertebrate taxa observed (Appendix A in Supplementary Material).

In most of these biological sampling sites, data for the formulation of two additional WFD indicators were also gathered. To assess the physico-chemical quality element, we used the LIMeco index ("Livello di Inquinamento

218 dai Macrodescrittori per lo stato ecologico”), which scores river water quality
 219 in terms of dissolved oxygen and nutrient concentration (Azzellino et al.,
 220 2015), with data for 280 reaches. The morphological quality was assessed
 221 with the Morphological Quality Index (MQI; Rinaldi et al., 2013), with data
 222 available for a subset of 184 reaches. The MQI provides a score to the
 223 morphological quality of a river reach based on three main elements:
 224 geomorphological functionality (accounting for longitudinal and lateral
 225 continuity of river processes, channel patterns, river bed structure and
 226 substratum, riparian vegetation), degree of artificiality (e.g. presence of local
 227 and remote sources of hydro-morphological alterations, such as sediment
 228 mining, levees and embankments, artificial reservoirs), and observed recent
 229 channel adjustments.

230 Hydrological information was available from gauging stations located along
 231 the Adige River network (managed by the Ufficio Dighe for the Autonomous
 232 Province of Trento, and by the Ufficio Idrografico for the Autonomous
 233 Province of Bolzano), and we selected 21 gauged stations (Table 1) in
 234 proximity to biological sampling site (<5km stream distance, no influence of
 235 major tributaries) so as to pair hydrological and ecological data (Fig. 1).
 236 Overall discharge time-series differed in length among stations, but
 237 continuous flow records were available for all stations from 2007. This allowed
 238 us to associate 1-year antecedent flow series with each biological sample.

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Station code	River	Lat	Long	Flow regulation	Elevation (m a.s.l.)	Stream order	LIMeco	MQI	Mean annual discharge (m ³ /s)	Flow regime
N_Groe	Rio Gardena	5161409.89	702603.22	No	1190	3	0.96	NA	3.42	Pluvio-nival
N_Isa13	Isarco	5171220.03	699902.87	Reg	763	4	0.86	NA	63.36	Nivo-glacial
N_Vit15	Rio Funes	5168758.95	705884.66	No	1235	1	0.91	NA	0.90	Nivo-pluvial
O_Ahr2	Aurino	5202120.25	723398.50	No	1223	2	0.92	0.59	6.26	Nivo-glacial
O_Gad22	Gadera	5184328.69	719813.69	No	830	3	0.88	NA	8.95	Pluvio-nival
O_Gsi15	Rio Casies	5183969.79	739258.79	No	1191	2	NA	NA	2.55	Nivo-pluvial
O_Rei13	Rio Riva	5199898.44	725812.58	No	853	2	0.91	0.66	4.81	Nivo-pluvial
O_Svg9	Sant Vigilio	5177748.72	721932.96	No	1148	2	0.92	NA	2.00	Nivo-pluvial
PR000017	Leno	5082814.51	656927.22	Reg	175	2	0.88	0.24	4.86	Pluvio-nival
R14_Pas	Passirio	5179047.63	668502.74	No	490	3	0.79	0.56	11.76	Nivo-pluvial
R5_Pfe	Rio Plan	5183012.70	657564.60	No	1798	1	0.9	0.85	2.14	Nivo-pluvial
S_Egg11	Rio Ega	5151257.93	683769.13	Reg	305	3	0.82	NA	2.46	Pluvio-nival
S_Zwa15	Rio Nero	5134679.82	677086.61	No	280	2	0.74	NA	0.25	Pluvio-nival
SD000147	Adige	5083835.68	656296.52	Reg	228	5	0.74	0.55	217.18	Nivo-glacial
SD000149	Adige	5078284.74	655273.32	Reg	183	5	NA	NA	81.74	Nivo-glacial
SG000002	Adige	5104054.75	663619.22	Reg	289	5	0.76	0.52	201.23	Nivo-glacial
VP000004	Rabies	5140884.30	638493.02	No	1375	2	0.93	0.63	2.24	Nivo-pluvial
VP000026	Meledrio	5131002.37	644473.83	No	1022	2	0.92	0.74	1.58	Pluvio-nival
W_Fal22	Valsura	5165374.96	664469.30	Reg	299	2	0.89	NA	5.51	Pluvio-nival
Z_Ahr10	Aurino	5189045.77	723881.31	Reg	823	3	NA	NA	21.08	Nivo-glacial
Z_Rie8	Rienza	5188208.31	705884.66	Reg	730	4	NA	NA	46.96	Pluvio-nival

Table 1. Main characteristics of the hydrometric gauging stations used to derive flow parameters from discharge time-series.

2.3 Data analyses

Across the 280 monitoring sites, macroinvertebrate sampling occurred multiple times between 2009 and 2014 (3-10 times per site). We therefore calculated the mean Star_ICMi value to characterise the biological quality of each site. Similarly, multiple values of the LIMeco and MQI indices were averaged per site. For the first goal (relation among WFD quality indicators), we used ordinary least square regressions to relate the Star_ICMi index with the LIMeco and MQI indices.

For our second goal (flow-ecology relationship), we used the available discharge time-series to derive 21 flow parameters (Table 2) based on daily flow values (normalised relative to annual means). Following previous studies (Belmar et al., 2018; Worrall et al., 2014) two temporal scales were considered: 1-year and 60-days preceding the collection of benthic macroinvertebrates, thus representing the influence of both long and short-term antecedent hydrologic conditions. Flow parameters were derived following Indicator of Hydrologic Alteration approach (IHA; Richter et al., 1997) using the 'IHA' implementation in R (R Core Team, 2017). The flow

parameters represented ecologically relevant hydrologic characteristics regarding magnitude (e.g. 1-7-90 days maximum and minimum flow), frequency and duration (e.g. number and duration of low and high pulses), rate of change and variation (e.g. rise and fall rates, CV). No automatic selection of flow parameters or synthesis was performed (e.g. PCA reduction), so as to avoid the exclusion of relevant parameters, and to facilitate the interpretation of results (e.g. Schneider and Petrin, 2017). In addition, the overall number of parameters included was small (compared to most publication where >100 metrics are used) and represented arguably the minimum set of ecologically relevant flow characteristics. Parameters related to the timing of flow events were not calculated, because the computation would require longer (multiple-years) flow time-series, and because macroinvertebrate collection was conducted over different months of the year. Star_ICMi values from repeated observations in time (multiple biological samples per site) were not averaged in this case, but were all included for a total of 80 samples, each paired with 1-year hydrological information. This allowed us to increase statistical power and aid the visual interpretation of complex relationships. The longitudinal structure of the data was accounted for by including 'site' as random factor in linear mixed-models relating the Star_ICMi to flow parameters, using the nlme package in R (Pinheiro et al., 2018). The proportion of variance explained by the fixed factors (i.e. flow parameters) was expressed as marginal R^2 using the r.squaredGLMM function in the MuMin package (Bartoń, 2018).

Flow class	Flow parameter	Description
Magnitude	d1_Day_Min	Minimum flow, 1 day mean
	d3_Day_Min	Minimum flow, 3 days mean
	d7_Day_Min	Minimum flow, 7 days mean
	d30_Day_Min	Minimum flow, 30 days mean
	d90_Day_Min	Minimum flow, 90 days mean
	d1_Day_Max	Maximum flow, 1 day mean
	d3_Day_Max	Maximum flow, 3 days mean
	d7_Day_Max	Maximum flow, 7 days mean
	d30_Day_Max	Maximum flow, 30 days mean
	d90_Day_Max	Maximum flow, 90 days mean
	Base_index	7 days minimum / mean flow
Frequency and duration	Low_pulse_number	Number of flow events below 25th percentile
	High_pulse_number	Number of flow events above 75th percentile
	Low_pulse_length	Number of days below 25th percentile
	High_pulse_length	Number of days above 75th percentile
Rate of change and variation	Rise_rate	Median of all positive differences between consecutive values
	Fall_rate	Median of all negative differences between consecutive values
	Reversals	Number of times flow period switches from rising to falling and vice-versa
	max_min	Maximum flow - minimum flow
	CV	Coefficient of variation (SD/mean)
	CVmean30d	CV, mean over 30 days period

Table 2 - Flow parameters derived from daily flow-series included in the analyses

The 21 gauged reaches represented rivers with natural flow regime as well as reaches regulated by upstream hydropower schemes. These were all included in the analyses, because i) we were interested in assessing the relation between the Star_ICMi and specific flow parameters rather than quantifying differences among river reaches and ii) we wanted to include the full range of flow parameters expected in the region. Nonetheless, for aiding visual interpretation, regulated and non-regulated reaches were differently identified in the plots.

In our analytical approach, we recognise that streamflow conditions are among the many factors that influence macroinvertebrate assemblages across the study sites. These include, for instance, water quality parameters and temperature, resource availability and riverbed morphology among others (Allan, 1995). However, streamflow characteristics in some reaches can represent a limiting factor for macroinvertebrates, where other stream features would allow different density or diversity to be observed. These limits can be considered as either 'ceilings' or 'floors' when the biological metric shows upper or lower limits as a function of a flow parameter, respectively. In these cases, the biological metric is unlikely to display a central response to flow parameters and ordinary regressions are not suited to quantify the limits (Konrad et al., 2008; Lancaster and Belyea, 2006). Conversely, quantile

313 regressions allow modelling the effect of a predictor variable over different
 314 quantiles of the dependent variable. In other words, the model fits the 'limiting
 315 response' of the y variable by identifying its conditional quantiles with respect
 316 to the predictor variable x . When quantifying the 80th percentile, for example,
 317 80% of the values of y are equal or less than the modelled function of x (Cade
 318 and Noon, 2003). In the present study, we assessed both the central and
 319 limiting response of the Star_ICMi index to the different flow parameters. We
 320 used linear mixed-models to account for repeated sampling within site using
 321 the 'nlme' package in R. For the quantile approach we employed a recently
 322 developed algorithm for linear quantile mixed-models implemented in the
 323 'lqmm' package in R (Geraci, 2014).

324
 325 Lastly, we used Procrustes analysis to appraise the extent to which other
 326 confounding factors may influence the flow-ecology relationship in the study
 327 area. Similarly to Mantel test, Procrustes analysis quantifies the association
 328 between multivariate data matrices, but it also provides a vector of residuals
 329 that represent the differences between homologous observations (i.e. sites,
 330 samples) across the two matrices (Lisboa et al., 2014). The residuals vector is
 331 a measure of the fit between the two matrices and can be used to further
 332 understand how the matrices are related. For example, the Procrustes
 333 residuals can be used to appraise whether another factor influenced the
 334 degree of matching between observations. Here we used Procrustes analysis
 335 to quantify the match between the matrix of macroinvertebrate communities
 336 (*samples x taxa*) and the matrix of flow parameters (*samples x parameters*).
 337 Then, we extracted the residuals vector and used it to investigate the
 338 influence of other environmental covariates. Specifically we used altitude
 339 (ranging 175 - 1800 m a.s.l.) as a proxy for many correlated factors and
 340 stressors such as temperature, land-use and water quality, and a linear
 341 mixed-models was used to relate the Procrustes residuals vector with altitude.
 342 Procrustes analysis requires the same dimensionality between multivariate
 343 matrices. Therefore, we first harmonised the dimensionality of each matrix
 344 using Principal Component Analysis (PCA) by keeping the first five
 345 components for each matrix. Macroinvertebrate densities were Hellinger-
 346 transformed prior to PCA (Lisboa et al., 2014).

3. Results

The Star_ICMi index showed linear and relatively strong ($R^2=0.36$, $P < 0.0001$; $n=280$) correlations with the LIMeco index and, to a lesser extent, with the MQI ($R^2=0.2$, $P < 0.0001$; $n=184$) (Fig. 2).

Only three of the flow parameters calculated from 1-year flow series were significantly (at $P < 0.05$) and negatively correlated with the Star_ICMi according to linear mixed models, namely CV_mean30d (marginal $R^2=0.3$), d1_Day_Max (marginal $R^2=0.1$) and max-min (marginal $R^2=0.1$) (Fig. S1 in SM; see description of the parameters in Table 1). No significant correlations were observed when flow parameters were derived from 60-days flow series preceding the macroinvertebrates collection (Fig.S2 in SM).

Figure 2- Regression of the Star_ICMi with LIMeco and MQI. Red lines = linear fit; grey areas = 95% CI. Background colours denote threshold between acceptable (green) and unacceptable (yellow) conditions (sensu WFD). Grey circles = reaches affected by hydropower upstream (HP); white circles = reaches not affected by hydropower (No)

Conversely, the use of quantile regressions allowed the identification of additional flow parameters that appeared to limit the scores of the Star_ICMi (Fig. 3). In particular, peak flows (d1_Day_Max), the number of low and high

pulses as well as parameters related to flow variation (CV, max_min) represented 'negative floors', which led the Star_ICMi below unacceptable conditions. Put in other words, lower values of the aforementioned flow parameters limited the minimum scores of the Star_ICMi. When flow parameters were derived from 60-days flow time-series, the influence of low and high flow pulse number remained significant, while the effect of hydrologic reversals became apparent, also in the form of a 'negative floor' (Fig. 4). That is, the minimum scores of the Star_ICMi were observed in reaches characterised by frequent hydrologic reversals. At this shorter time-scale, the effect of daily rate of change in flow also became apparent, with a 'negative floor' observed with Rise_rate (Fig.4).

Figure 3- The Star_ICMi vs IHA metrics based on flow time-series from 1-year preceding the biological sampling. Blue line indicates significant mixed quantile regression at $q=0.2$. Significance levels are at $P<0.01$ for all parameters except for CV at $P=0.04$. Background colours denote threshold between acceptable (green) and unacceptable (yellow) conditions (sensu WFD). Grey circles = reaches affected by hydropower upstream (HP); white circles = reaches not affected by hydropower (No)

Figure 4- Star_ICMi vs IHA metrics based on flow series from 2 months preceding the biological sampling. Blue line indicates significant quantile regression at $q=0.2$. Significance levels are at $P<0.05$. Background color denotes threshold between acceptable (green) and unacceptable (yellow) conditions (sensu WFD). Grey circles = reaches affected by hydropower upstream (HP); white circles = reaches not affected by hydropower (No)

Our study reaches encompassed a wide altitudinal range, which potentially acted as a confounding factor in the analysis of flow-ecology relationships as altitude was related to changes in the main physico-chemical parameters and local land use across the study reaches (Fig. S3 in SM). In fact, the Star_ICMi index increased linearly with altitude (Fig 5A; mixed-model marginal $R^2=0.2$, $P=0.01$), and most of the regulated river reaches occurred at lower elevations. The residuals vector from the Procrustes analysis associating the matrices of flow parameters and macroinvertebrate communities declined with altitude (Fig. 5B; mixed-model marginal $R^2=0.15$, $P=0.04$), indicating a significant and negative effect of altitude. These results indicate that the match between the biota (taxonomic matrix) and the hydrological parameters was stronger in

higher-altitude locations, likely because the influence of confounding stressors was weaker.

Fig. 5 - Relation between the Star_ICMi (A) and Procrustes residual (B) with altitude. Legend as in Fig. 3 and 4.

4. Discussion

We used monitoring data from a large Alpine river network to assess the relationship between WFD quality elements, and the response of the official Italian macroinvertebrate-based indicator to measured hydrological parameters. The Star_ICMi showed a relatively strong relation with the physico-chemical character of the river reaches, as expressed by the LIMeco index. This was expected, as stream macroinvertebrates are known to be particularly sensitive to water quality parameters (e.g. Friberg et al., 2010; Guilpart et al., 2012). Moreover, the LIMeco index specifically reflects the concentration of organic pollutants (nitrates and phosphates), to which the Star_ICMi is designed to respond (Quadroni et al., 2017). Parallel findings were recently reported by Azzellino et al. (2015) for the nearby Lombardy region (northern Italy), where

442 water quality, as expressed by the LIMeco scores, explained c. 50% of the
 443 variation in the Star_ICMi across a range of river reaches with similar
 444 environmental settings as those studied here.

445 The influence of rivers' morphological features on benthic invertebrates, as
 446 expressed by the MQI, was also significant, but apparently weaker. The MQI
 447 reflects the integrity of both channel and riparian habitat that can influence in-
 448 stream organisms in multiple ways, for instance by providing refugia and
 449 resources (Matthaei et al., 2000; Naiman and Décamps, 1997). Hence, our
 450 results suggest that across the Trentino-Alto Adige region, physico-chemical
 451 water quality was the main determinant of macroinvertebrate community
 452 integrity as measured by the STAR_ICMi, which was only secondarily affected
 453 by riparian and in-stream morphological features. Other studies in Italy and
 454 elsewhere indicated that substratum and riparian characteristics mostly
 455 influenced the functional composition (e.g. feeding habits) of benthic
 456 invertebrates, rather than their taxonomic identity and diversity (e.g. Larsen
 457 and Ormerod, 2010; Manfrin et al., 2016). This could in part explain the lower
 458 sensitivity of the taxonomic-based Star_ICMi to the MQI.

459 Results from our second objective also indicated a rather poor sensitivity of
 460 the Star_ICMi index to hydrological parameters. Analyses of flow-ecology
 461 relationship were based on a reduced sample size, because we selected
 462 those reaches for which daily discharge time-series were available from
 463 adjacent gauging stations. Nonetheless, the reaches were distributed across
 464 the whole extent of the study area and included both natural and regulated
 465 rivers and likely represented the entire range of flow parameters observed in
 466 the region. These parameters were derived at two temporal scales (1-year
 467 and 60-days preceding biological sampling) and provided similar but not
 468 identical results. When assessing the central response (using linear mixed-
 469 models), only parameters derived at 1-year time scale appeared to
 470 significantly and negatively affect the biological indicator, and included the
 471 monthly coefficient of variation in flow and the overall range and maximum
 472 daily flow. These all indicate a generally negative effect of large daily flow
 473 variation on aquatic communities, as also reported elsewhere (e.g. Bruno et
 474 al., 2010; Konrad et al., 2008; McGarvey, 2014).

475

476 It is important to highlight how focusing on the central response of
 477 macroinvertebrate metrics can provide only limited insight into the effects of
 478 streamflow. Lotic invertebrates are influenced by a wide range of abiotic and
 479 biotic factors and are unlikely to display a central or linear response to
 480 streamflow parameters (Konrad et al., 2008; Rosenfeld, 2017). In these
 481 cases, the use of quantile regressions allow the identification of those factors
 482 that appear to locally limit the maximum or minimum values of the response
 483 variable. Previous studies with benthic invertebrates have shown the validity
 484 of this approach for the identification of the environmental constraints on local
 485 community density and richness (Fornaroli et al., 2015; Lancaster and Belyea,
 486 2006). Here, we used a novel approach based on mixed quantile models and
 487 were able to identify some key flow parameters that appeared to determine
 488 the lower limits of the Star_ICMi. Interestingly, the significant quantiles all took
 489 the form of 'negative floors', whereas no significant 'ceilings' were observed.
 490 Negative floors imply that the lower limits of the biological indicator declined
 491 with increasing values of the flow parameters. This resulted in the modelled
 492 quantiles to apparently 'track' the transition of the ecosystem into
 493 unacceptable conditions. Viewed in terms of ecological constraints, these
 494 negative floors suggest that the biological integrity was maintained within
 495 acceptable conditions (sensu WFD) by lower values of the flow parameters,
 496 which evidently represented favourable hydrologic conditions. As the value of
 497 the flow parameters increased, the hydrologic conditions deteriorated thus
 498 leading some sites to drop to a lower quality status. The identified limiting
 499 parameters were mostly related to flow variation and the frequency of flow
 500 events. Specifically, we found that high peak flows, frequent low and high
 501 pulses and large variations in daily flow (as CV) apparently acted as stressors
 502 for macroinvertebrates leading to a marked decline in the Star_ICMi at some
 503 sites. Similar patterns were observed by Konrad et al. (2008) in 111 stream
 504 sites in the western U.S.A., where invertebrate abundance and the proportion
 505 of intolerant taxa showed quantile relations in the form of negative floors with
 506 parameters describing discharge variation.

507 Our results also parallel those Worrall et al. (2014) in showing that not only
 508 the long-term flow regime, but also short-term antecedent flow conditions can
 509 influence benthic communities. Our modelling procedure identified additional

510 flow parameters as limiting factors when derived from 60-days preceding
 511 macroinvertebrate collection. In this case, the lower values of the Star_ICMi
 512 were limited by large daily rise rates in flow and the number of hydrologic
 513 reversals, which are also parameters quantifying hydrologic variations.
 514 It should be noted that most reaches characterised by higher values of the
 515 limiting flow parameters (e.g. yearly CV, frequent high and low pulses,
 516 reversals) were located downstream of hydropower plants, albeit at different
 517 distances. Hydropower operations in the region are known to affect the
 518 natural flow variability by often increasing the frequency and amplitude of flow
 519 oscillations and sharp transitions (Zolezzi et al., 2009). Therefore, the
 520 identified limiting flow parameters were likely outside their natural range of
 521 variability, and thus represented stressing factors for the communities.

522
 523 The large scatter or variance in the relation between the Star_ICMi and flow
 524 parameters clearly indicates the influence of additional limiting factors (e.g.
 525 water quality and altitude, as seen here). Disentangling the different source of
 526 variation in these cases can be challenging as these can include both natural
 527 and anthropogenic factors as well as biotic and abiotic processes. In these
 528 cases, the use of quantile modelling has offered clear advantages (Fornaroli
 529 et al., 2015; Konrad et al., 2008), as also observed in the present study. We
 530 specifically attempted to quantify the influence of other covariates on the
 531 observed flow-ecology relationship using the multivariate Procrustes analysis
 532 and the associated residuals vector. We used Procrustes to first associate the
 533 matrices of flow parameters and macroinvertebrate communities (sites x taxa
 534 densities). Then, we derived the vector of residuals that quantified the
 535 mismatch between homologous observations (sites) in the multivariate space
 536 defined by the two matrices (Lisboa et al., 2014). This residuals vector
 537 showed a significant and negative correlation with altitude. This means not
 538 only that altitude acted as an important covariate, but also that the match
 539 between the biota and the hydrology was stronger in upland reaches
 540 compared to lowlands. Upland reaches were likely less influenced by potential
 541 confounding factors related to human activity, including nutrient inputs and
 542 land use conversion, and the influence of streamflow characteristics on local
 543 communities was evidently stronger.

This contingency has wider implications for the development of general flow-ecology relationships in the area and potentially across Europe, where analogous biological indicators are adopted in line with the WFD requirements (Buffagni et al., 2006), and further emphasises the need for research and management that acknowledges the complexity of multiple stressors acting on river ecosystems (Ormerod et al., 2010).

5. Conclusions

In this study, we used data from a large Alpine river network to assess the relation among different WFD quality elements and to contribute to the development of evidence-based ecological flows. This was also motivated by the warning from the European commission (WFD CIS, 2015) stating that “in cases where hydrological alterations are likely to prevent the achievement of environmental objectives, the assessment of the gap between the current flow regime and the ecological flow is a critical step to inform the design of the programme of measures”.

Our results suggest that existing macroinvertebrate-based biological indicators, like the Star_ICMi used as a case study and prescribed by the Italian national legislation, may mostly reflect local physico-chemical water quality and to a lesser extent the morphological integrity of the reaches, as expressed here by the LIMeco and MQI descriptors, respectively. This result was expected given previous observations and the known sensitivity of the index to organic pollution (Azzellino et al., 2015; Quadroni et al., 2017). As such, the Star_ICMi showed rather poor correlations with flow parameters when examined in its central response. Nonetheless, quantile modelling allowed the identification of key flow parameters that limited the minimum scores of the index (i.e. ‘negative floors’), and apparently tracked the transition of the ecosystem into unacceptable conditions. The identification of these flow limits can aid the implementation of E-flows by allowing managers to compare local conditions with the given limits and set hydrologic thresholds over which ecological impairment is likely to occur. In the study area, most of the negative limits identified were related to the magnitude and frequency of flow variations, which were likely altered by upstream hydropower operations. However, as also emphasised by Konrad et al. (2008), these limits show that

the biological response to local hydrologic characteristics is contingent upon a range of local and regional factors including both natural (e.g. altitude) and anthropogenic ones (water quality), as demonstrated here by the Procrustes analysis. This has clear implications for both fundamental flow-ecology research and for water management, because the response of biological communities and associated indicators to flow regulation cannot be predicted without detailed information on the wider environmental setting of a river reach.

Although some important limiting hydrologic parameters were identified, results from the present study imply caution is needed in using the current WFD biological indicators based on analogous principles to the one adopted in Italy, especially to guide the management of ecological flows. Further research is needed to better quantify flow-ecology relationships and develop hydrology-sensitive indicators. Similar efforts were pursued by other countries where empirical waterflow preferences of benthic invertebrates were synthesised into a river flow index (e.g. LIFE index; Extence et al., 1999). However, the validity of the LIFE index in other environmental settings needs to be tested (Dunbar et al., 2010) and, more generally, the index is designed to reflect changes in flow velocity and might correlate poorly with other flow parameters likely affected by river regulation (i.e. frequency and magnitude of variation). Ideally, effort and resources should be directed to the development of ecological indicators targeting specific flow characteristics that are most likely altered by river regulation and water uses.

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804 SUPPLEMENTARY MATERIAL

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806 Appendix A

807 Description of the STAR_ICMi index (adapted from [http://www.life-inhabit.it/cnr-](http://www.life-inhabit.it/cnr-irsa-activities/en/cnr-irsa-activities-related-inhabit/ecological-status/staricmi)
808 [irsa-activities/en/cnr-irsa-activities-related-inhabit/ecological-status/staricmi](http://www.life-inhabit.it/cnr-irsa-activities-related-inhabit/ecological-status/staricmi),

809 Buffagni et al., 2005, 2007, 2008).

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811 The STAR_ICMi is a multimetric index which includes six metrics:

- 812 1. Average Score Per Taxon (Armitage et al., 1983);
- 813 2. Log10(sel_EPTD+1): Log10 (sum of Heptageniidae, Ephemeridae,
814 Leptophlebiidae, Brachycentridae, Goeridae, Polycentropodidae,
815 Limnephilidae, Odontoceridae, Dolichopodidae, Stratyomidae, Dixidae,
816 Empididae, Athericidae & Nemouridae;
- 817 3. 1-GOLD 1:1 - (relative abundance of Gastropoda, Oligochaeta and Diptera);
- 818 4. Number of EPT families;
- 819 5. Total number of families
- 820 6. Shannon-Weiner diversity index.

821 The calculation of the STAR_ICMi is performed in 4 steps:

- 822 1. calculation of the raw value for each of the 6 metrics;
- 823 2. calculation of the Ecological Quality Ratio for each of the 6 metrics by
824 dividing the observed value (i.e. obtained for the considered samples) by the
825 median value of the metric calculated from the reference river type;
- 826 3. calculation of the weighted average of the EQR using a specifically assigned
827 weight for each metric (0.333, 0.266, 0.067, 0.167, 0.083, 0.083, respectively
828 for metrics 1-6);
- 829 4. normalization of the obtained value by dividing the value of the considered
830 sample by the STAR_ICMi expected in reference samples.

831 Values of STAR_ICMi vary between 0 and +1, and different intervals correspond to
832 the five quality classes defined by the Water Framework Directive: high, good,
833 moderate, bad, poor.

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839 SUPPLEMENTARY FIGURES

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843 **Supplementary Figure S1** - Correlation coefficients between the Star_ICMi and flow

844 parameters calculated from 1-year flow-series preceding the biological sampling.

845 Only three flow parameters showed significant correlation (red stars) based on linear

846 mixed models.

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849 **Supplementary Figure S2** - Correlation coefficients between the Star_ICMi and flow

850 parameters calculated from 60-days flow-series preceding the biological sampling.

851 No significant correlation was observed based on mixed models.

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Supplementary Figure S3 - Relationship between altitude and main physico-chemical parameters and local land use across the study reaches used in the flow-ecology analysis. Grey circles = reaches affected by hydropower upstream (HP); white circles = reaches not affected by hydropower (No)