

Taxonomic and functional responses of macroinvertebrate communities to dam construction in a non-wadeable river

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Abstract – Damming and impoundment are among the main flow-related alterations in rivers worldwide. However, compared to the highland river sections where the impacts associated with dams have been largely studied, still scarce evidence is available in scientific literature for large, lowland rivers. In this study, the ecological effects of a hydropower dam in the Po River (Italy) on the taxonomic and functional responses of benthic macroinvertebrates were examined by comparing one station upstream of the dam with one downstream station over a six-year period. Weak and not significant differences were observed for taxon richness, abundance and community composition. By contrast, community-level proportion of macroinvertebrates associated with water velocity increased during medium and high flow condition and decreased during low flow condition in downstream stations. Such differences were also mirrored by the Flow-T index, that is a biomonitoring index recently developed to assess hydraulic conditions in rivers. Results of this study support the adoption of stressor-specific metrics in river biomonitoring.

Keywords: Biomonitoring / river ecology / diversity / Community-Weighted Mean (CWM) / functional traits

1 Introduction

Natural flow regime (*i.e.* the natural variation of river discharge in terms of magnitude, frequency, duration, timing and flashiness) is widely considered the master variable that directly and indirectly affects the biological diversity and ecosystem processes of lotic ecosystems (Poff *et al.*, 1997). However, flow alterations are one of the most widely spread and massive impacts for rivers globally as a consequence of large and local scale pressures (López-Rodríguez *et al.*, 2019; Piano *et al.*, 2020). Climate change is altering the distribution patterns of precipitations in many areas of the world with an increase of extreme hydrological events, such as extended drying conditions and floods (Wu and Johnson, 2019). These altered hydrological conditions are often exacerbated at the local scale by massive water withdrawal, channelization, navigation and damming aimed at satisfying several human needs (Leitner *et al.*, 2021).

Among the above-mentioned alterations, damming represents one of the main and current causes of flow alterations in rivers, especially when associated with electricity production.

In the last decades, a proliferation of small to medium hydropower plants occurred in Europe, and especially in mountain areas, due to the urgent need for energy from renewable sources (Zarfl *et al.*, 2015). For instance, in a review on the natural integrity of Alpine rivers, Comiti (2012) highlighted that there are currently about 140 hydropower dams with height >15 m in the Italian Alps. Moreover, under the expected scenario of increased water shortage due to climate change dams and reservoirs are commonly seen as a useful solution to store water to be used during drying periods (Tonkin *et al.*, 2019).

Although the impacts of dams, including both the creation of the impounded section and sediment flushing operations, on the riverine biota have been already documented (Espa *et al.*, 2019), this scientific evidence is mainly focussed on mountain streams (Nukazawa *et al.*, 2020; Doretto *et al.*, 2021), while the impacts of dams in large, lowland rivers are still rarely investigated (Grzybkowska *et al.*, 2017; Horsak *et al.*, 2009). Beyond the greater occurrence of dams in mountain areas than lowland setting, this could be also explained by some specific features of large, lowland rivers (Leitner *et al.*, 2021). For instance, these lotic ecosystems pose several challenges with respect to the sampling of biological communities for

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Fig. 1. Pictures represent: (A) upstream station, (B) downstream station, (C) dam construction and (D) artificial substrates for macroinvertebrate sampling.

biomonitoring, especially when dealing with benthic organisms (*i.e.* macroinvertebrates). In large rivers, benthic macroinvertebrates are generally collected from the nearshore areas, when accessible, with evident limitations about their representativeness of the whole habitat. Another feature of large, lowland rivers is that they are usually affected by multiple stressors simultaneously because being usually located in the geographical areas most densely populated and where most anthropogenic activities occur. Beside local impacts, lowland sections of rivers generally suffer from the additive effects of impacts acting in the whole upstream river basin. Thus, it is difficult to discriminate the impacts attributable to the dams from other synergic and confounding factors and this aspect highlights the undeniable necessity for stressor-specific biomonitoring tools.

Here we analyse the taxonomic and functional response of benthic macroinvertebrates to a hydropower dam in the lowland part of the Po River (Italy). Differences in the diversity, abundance, taxonomic composition as well as biomonitoring indices were tested by comparing the macroinvertebrate communities from one station upstream of a recently constructed dam (*i.e.* impounded station) with one downstream station over a six-year period (*i.e.* before and after the dam creation). Because the selected stations are comparable in terms of land use and geomorphological conditions, we expected no significant variation in terms of richness, abundance and composition between the up- and downstream stations as a consequence of the dam creation. By contrast, we expected shifts in downstream station as compared to upstream

one in the functional traits profile of macroinvertebrate communities between stations, especially with respect to the traits associated with water velocity conditions. Finally, we expected differences between the two stations in the values of stressor-specific indices that incorporate such functional traits compared to generic biomonitoring indices that are based only on taxonomy and/or are not specifically designed for evaluating flow alterations.

2 Materials and methods

2.1 Area of study and data collection

The Po River originates from Monviso Mountain (Italian Cottian Alps – Northwestern Italy) wherein the “Parco del Monviso Natural Park” and is the longest river of Italy (651 km long; drainage area = 71,000 km²). The study was carried out in a lowland stretch of the Po River, near the Casale Monferrato village (45°08'03"N, 8°27'30"E, elevation: 115 m a.s.l.) where the watercourse is 200 m wide. From 2018 to 2020, a new large hydroelectric power plant was built with a maximum derived discharge >110,000 l/s. This infrastructure relies on a water inflatable rubber dam with a stable basement made of reinforced concrete (Fig. 1). Two sampling stations were selected along a river stretch of 1.5 km length: one upstream (hereafter U) and one downstream (hereafter D) of the dam (Fig. 1). Before the dam building medium river discharge was approximately of 500 m³/s and the upstream section was characterized by a multicourse channel, deep waters (>5 m)

and low flow velocity (medium < 1 m/sec), while the downstream section was similar to what we can observe today, with fast flow (medium > 1.5 m/sec), shallow waters (1.5 m) and an unicursal trend. Currently the upstream section is similar to a lentic habitat with deep waters (>6 m) and very low water velocity.

Artificial substrates (Fig. 1) were used to collect macro-invertebrates and consisted of eight layers of wood (every layer was 25 × 25 × 2 cm) separated by circular wooden spacers (diameter = 4 cm; height = 1 cm). In each station two artificial substrates were employed; in this way the total sampled area was equal to 1 m². These artificial substrates were tied to a rope anchored to the river bank and were left into the water for 60 ± 3 days to allow macroinvertebrate colonization over an adequate time period. On each removal date, artificial substrates were removed individually with special care being taken to minimize losses of organisms. Benthic invertebrates were removed from substrate and transferred in alcohol. In laboratory all macroinvertebrates were systematically identified to the family level, as required by the national biomonitoring program for rivers in Italy (Buffagni *et al.*, 2007), and counted. A total of seven monitoring dates were carried out, distributed in the following years: 2017 (2 dates ante-operam), 2019 (2 dates during the construction of the dam), 2021 and 2022 (3 dates post-operam). Overall, a total of 28 artificial substrates (7 dates × 2 stations × 2 substrates) were analysed.

On each removal date pH, water conductivity and dissolved oxygen were measured with a multiprobe (Hydrolab, mod. Quanta). In addition, 1 L water sample was collected in each sampling station and returned in laboratory where the following chemical parameters were measured by following standard procedures: nitrates concentration, total phosphorous concentration, COD, BOD5 and amount of *E. coli*.

2.2 Statistical analyses

Redundancy analysis (RDA) and PERMANOVA were performed to investigate variations in macroinvertebrate communities composition according to the physical and chemical variables and between stations over time. Macroinvertebrate communities were obtained by pooling together the data from the two artificial substrates. Macroinvertebrate abundances were Hellinger-transformed before performing RDA. A set of 8 physical and chemical variables were initially selected, including pH, oxygen concentration, total phosphorous, electrical conductivity, NO₃ concentration, BOD5, COD and the amount of *E. coli*. Stepwise forward selection was applied to retain only the most significant and non-collinear variables in RDA.

Statistical differences in the taxon richness, total abundance as well as the following biomonitoring indices: STAR_ICMi (Buffagni *et al.*, 2007) and Flow-T (Laini *et al.*, 2022) were tested using either paired *t*-tests or Wilcoxon signed-rank tests (see below). STAR_ICMi is the index adopted in Italy for the evaluation of the quality status of running waters according to the European Water Framework Directive 2000/60 (WFD). It is a multi-metric index calculated as the weighted sum of the following metrics: Average Score Per Taxon (ASPT); number of Ephemeroptera, Plecoptera and

Trichoptera families (EPT); total number of macroinvertebrate families; 1-GOLD (*i.e.* the relative abundance of Gastropoda, Oligochaeta and Diptera); Shannon index; logarithm of the abundance of selected Ephemeroptera, Plecoptera, Trichoptera and Diptera plus one (*i.e.* log (SEL_{EPTD} + 1)). Flow-T is calculated as the proportion, based on log-abundance data, of medium and fast classes over all classes for the trait “current velocity preferendum” described in Tachet *et al.* (2010). Artificial substrates are largely used as sampling technique for non-wadeable rivers as documented in previous studies and references (Blocksom and Flotemersch, 2005; Weigel and Dimick, 2011). Thus, we are confident that the taxonomic and functional metrics derived from the sampled community are methodologically reliable and suitable according to the sampling area and the aims of the study. Moreover, the adoption of artificial substrates for the calculation of the STAR_ICMi index is recommended by the Italian normative for non-wadable rivers (Buffagni *et al.*, 2007). Differences in the community and biomonitoring indices between the upstream and downstream stations were tested by means of paired *t*-test and Wilcoxon signed-rank test depending on the assumption of normality in the selected data. Paired *t*-test was used for biomonitoring indices, while Wilcoxon signed-rank test, that is the non-parametric equivalent of the paired *t*-test, was used for taxon richness and total abundance.

Finally, the functional response of the macroinvertebrate communities to the construction of dam was evaluated by calculating the community-level weighted means (CWM) of trait values (Laliberté *et al.*, 2014). To this end, 24 traits modalities (Tab. 1) belonging to four functional traits (*i.e.* respiration, locomotion and substrate relation, current velocity preferendum, and feeding habits) were selected (Tachet *et al.*, 2010). We choose these over other traits because they are generally considered to be sensitive to flow level alterations (Bonada *et al.*, 2007; Laini *et al.*, 2022). Wilcoxon signed-rank test was used for testing whether CWM for each trait modality varied between the upstream and downstream stations.

All analyses were run in the R statistical environment (R Core Team, 2022) by using the basic function and the following packages: biomonitor (Laini *et al.*, 2020), vegan (Oksanen *et al.*, 2015), FD (Laliberté *et al.*, 2014) and ggplot2 (Wickham *et al.*, 2016).

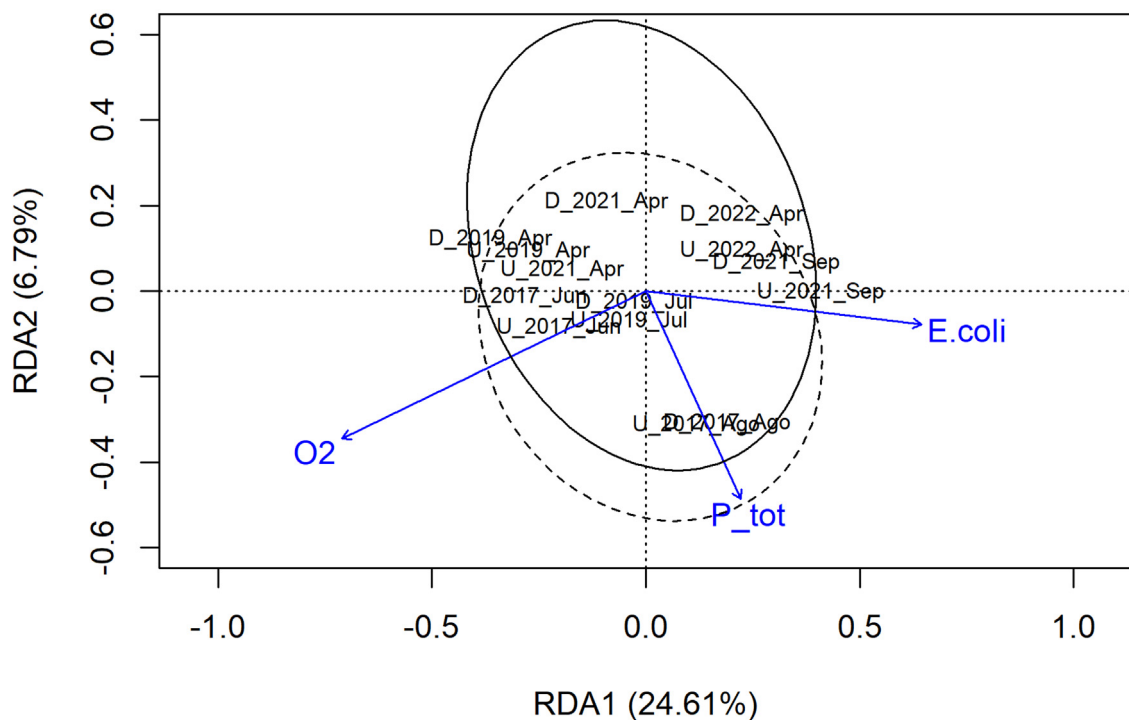
3 Results

A total of 10,782 macroinvertebrates were collected, belonging to 45 different families. Gammaridae, Bithyniidae, Chironomidae and Dugesidae were the most abundant taxa and accounted for 74.90% of the total macroinvertebrate community. The average number of taxa (±SD) per sampling occasion was 20 (±3.07), while the mean abundance (±SD) was 770 (±349.02) individuals per sampling occasion.

Based on the stepwise forward selection, only the variables concentration of oxygen, total phosphorous and *E. coli* were selected for the RDA analysis. Overall, these three variables significantly influenced the macroinvertebrate communities composition ($F_{3,10} = 3.55$; $p < 0.001$), accounting for 37.08% of the total variance. RDA1 explained 24.61% of the variance and was positively and negatively correlated with the amount of *E. coli* and oxygen concentration, respectively. By contrast,

Table 1. Community-level weighted means (CWM) of trait values for the two sampling stations based on the functional traits and modalities considered in this study. Significant ($p < 0.05$) values are in bold.

Trait	Trait modality	CWM Downstream station (mean $\pm$ SD)	CWM Upstream station (mean $\pm$ SD)	<i>p</i> -value
Respiration	Tegument	0.448 $\pm$ 0.026	0.390 $\pm$ 0.074	0.047
	Gill	0.554 $\pm$ 0.029	0.597 $\pm$ 0.070	0.078
	Plastron	0.003 $\pm$ 0.003	0.005 $\pm$ 0.007	0.219
	Spiracle	0.004 $\pm$ 0.004	0.008 $\pm$ 0.011	0.375
Locomotion and substrate relation	Flier	0.001 $\pm$ 0.001	0.002 $\pm$ 0.003	0.687
	Surface swimmer	0.014 $\pm$ 0.018	0.024 $\pm$ 0.021	0.078
	Water swimmer	0.154 $\pm$ 0.082	0.181 $\pm$ 0.098	0.219
	Crawler	0.562 $\pm$ 0.107	0.597 $\pm$ 0.089	0.078
	Burrower	0.127 $\pm$ 0.029	0.111 $\pm$ 0.029	0.156
	Interstitial	0.063 $\pm$ 0.037	0.043 $\pm$ 0.018	0.109
	Temporary attached	0.074 $\pm$ 0.033	0.040 $\pm$ 0.024	0.031
	Permanent attached	0.001 $\pm$ 0.001	0.0005 $\pm$ 0.0005	0.078
Current velocity (preferendum)	Null	0.307 $\pm$ 0.112	0.303 $\pm$ 0.143	0.998
	Slow	0.450 $\pm$ 0.078	0.534 $\pm$ 0.098	0.016
	Medium	0.143 $\pm$ 0.037	0.095 $\pm$ 0.039	0.031
	Fast	0.099 $\pm$ 0.036	0.067 $\pm$ 0.038	0.031
Feeding habits	Absorber	0.002 $\pm$ 0.001	0.003 $\pm$ 0.003	0.999
	Deposit-feeder	0.119 $\pm$ 0.062	0.076 $\pm$ 0.035	0.031
	Shredder	0.265 $\pm$ 0.135	0.349 $\pm$ 0.179	0.078
	Scraper	0.232 $\pm$ 0.083	0.215 $\pm$ 0.084	0.297
	Filter-feeder	0.212 $\pm$ 0.089	0.197 $\pm$ 0.094	0.296
	Piercer	0.004 $\pm$ 0.004	0.011 $\pm$ 0.013	0.375
	Predator	0.147 $\pm$ 0.056	0.136 $\pm$ 0.070	0.375
	Parasite	0.017 $\pm$ 0.008	0.013 $\pm$ 0.008	0.015

**Fig. 2.** RDA ordination plot of macroinvertebrate community. Arrows indicate gradients in the three selected variables after stepwise forward selection (*i.e.* concentration of oxygen = O₂, concentration of total phosphorous = P_{tot}, and amount of *E. coli* = E.coli). Labels indicate the macroinvertebrate community composition for each station (D = downstream; U = upstream) on each sampling occasion (year_month). Ellipses represent standard errors around the centroids for the downstream station (solid line) and upstream station (dashed line).

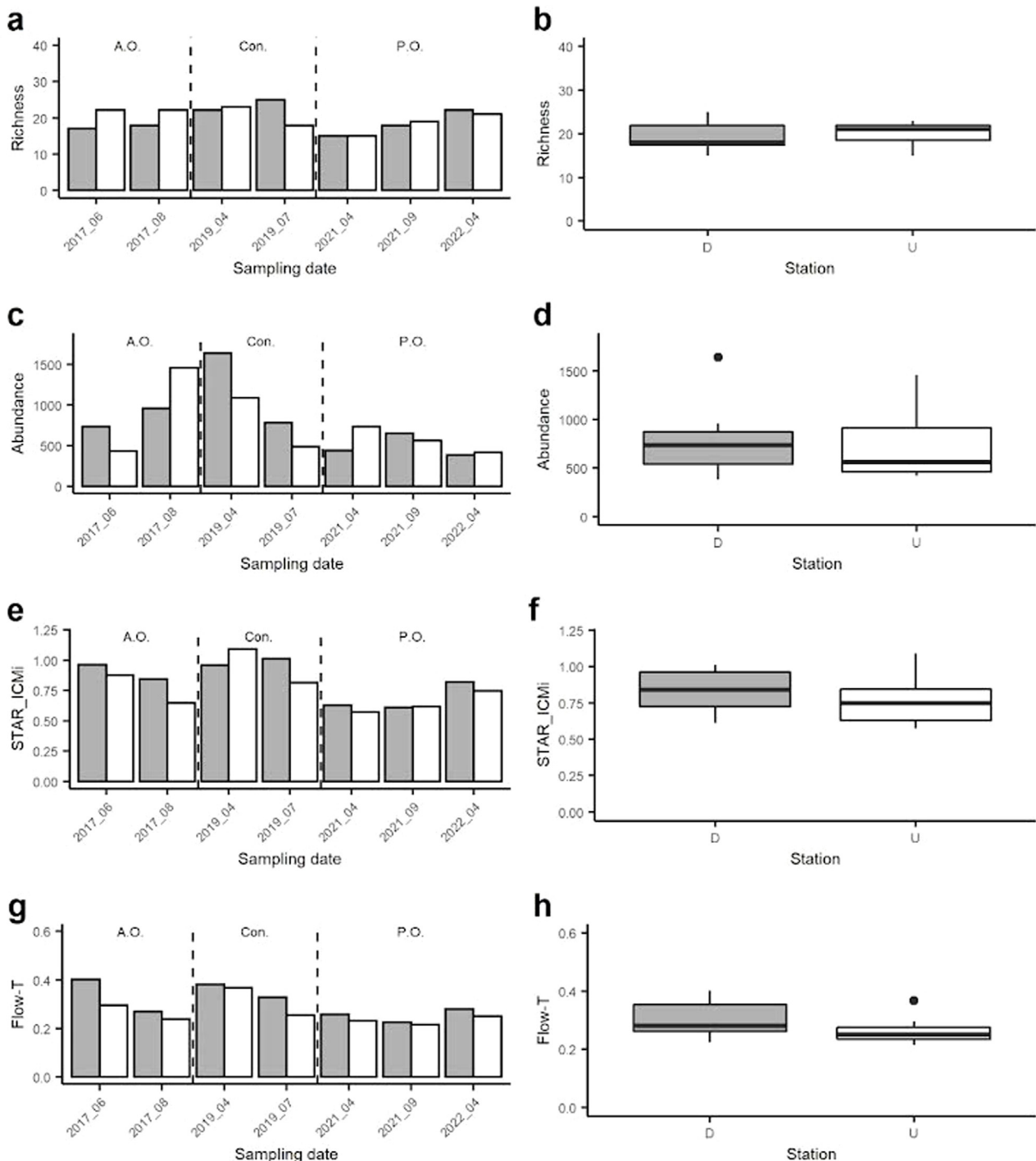


Fig. 3. Bars and boxplots illustrate the variation in: (a, b) taxon richness, (c, d) total abundance, (e, f) STAR_ICMi index and (g, h) abundance-based Flow-T index over time and between the downstream (D; gray) and upstream (U; white) sampling stations. In boxplots: horizontal black line=median; lower and upper box edge=first and third quartiles, respectively; vertical lines indicate ± 1.5 interquartile distance.

RDA2 explained 6.79% of variance in the macroinvertebrate communities composition and was negatively correlated with the increase in the concentration of total phosphorous (Fig. 2). However, PERMANOVA showed no significant differences in communities composition between the two sampling stations ($F_{1,10} = 0.568$; $p = 0.749$).

Negligible variations in taxon richness were observed over time (Fig. 3a) with no statistical differences between sampling stations ($V = 13$, $p = 0.67$; Fig. 3b). By contrast, total number of macroinvertebrates varied over time with lower abundances recorded after the dam construction (Fig. 3c). However, the total number of macroinvertebrates did not vary statistically between stations ($V = 10$, $p = 0.578$; Fig. 3d). When looking at

the biomonitoring indices, STAR_ICMi index slightly decreased over time and was, on average, higher in the downstream station compared to the upstream one, but these differences were not statistically significant ($t=-1.55$, $p=0.171$; Figs. 3e and 3f). The Flow-T index was, on average, lower after dam construction compared to the previous sampling occasions (Fig. 3g). Moreover, Flow-T index was significantly higher in the downstream station (including before dam construction) compared to the upstream one ($t=-3.026$, $p=0.023$; Fig. 3h).

Community-level weighted means (CWM) of trait values analysis showed significant results for seven out of the 24 trait modalities examined in this study (Tab. 1). Among the modalities related to respiration only the trait modality tegument significantly varied between the sampling stations, with higher CWM values found in the downstream station. Similarly, macroinvertebrates associated with permanent attached habits had significant higher CWM values in the downstream section than upstream of the dam. When looking at the preferences for water velocity, 3 out of 4 trait modalities significantly varied between stations (Tab. 1). CWM values for the modalities medium and fast were, on average, higher in the downstream station, while an opposite result was found for the community-level proportion of macroinvertebrates associated with slow water velocity.

4 Discussion

Damming and impoundment are widely recognized as flow-related alterations in rivers (Ogbeibu and Oribhabor, 2002; White *et al.*, 2017), causing modest to severe impairments on the aquatic biota depending on the local geomorphological setting. In fact, previous research dealing with these alterations focused mostly on mountain areas, while their effects in large, lowland rivers remains underestimated.

In a large-scale study Leitner *et al.* (2021) evaluated the correlation between ten candidate macroinvertebrate community metrics and eight different pressures, including impounding and damming, in large rivers across Europe and by controlling also for river types. Authors found that for the Continental Lowland type, damming was the pressure that played the heaviest impact, but they also found opposite trends in some selected candidate metrics for the pressures impoundment and damming depending on the river type. For the impoundment, authors concluded that site-specific hydro-morphological conditions can affect in different manner the abundance of macroinvertebrates according to the local pool of taxa, leading to varying responses in terms of biotic and biomonitoring indices. By contrast, for the pressure caused by dams, authors stated that methodological constraints associated with macroinvertebrate sampling in large rivers may be inadequate for the bioassessment of certain pressures (Leitner *et al.*, 2021). Overall, this emphasizes the importance of adopting stressor-specific approaches.

To our knowledge, our study represents one of the few attempts, at least in Italy, aimed at monitoring the flow-related impacts associated with a dam in a section of a large, lowland river by using benthic macroinvertebrates. Multivariate analysis showed weak and negligible variations in the taxonomic composition of macroinvertebrate communities

throughout the study, with changes varying over time (*i.e.* year or season) rather than between locations (*i.e.* upstream and downstream of the dam). Furthermore, no significant differences in the total taxon richness and total abundance were found between the upstream and downstream stations. Similarly, STAR_ICMi index was slightly higher (*i.e.* indicating better ecological conditions) in the downstream station compared to the upstream, impounded station. However, these differences were not statistically significant and this finding was expected insofar as STAR_ICMi is a generic biomonitoring index that poorly performs in relation to specific pressures, as demonstrated in previous studies (Doretto *et al.*, 2019; Larsen *et al.*, 2019).

When looking at the performance of the Flow-T index, a recently developed multimetric index designed for assessing flow conditions (Laini *et al.*, 2022), index values were significantly higher (*i.e.* indicating more reophilous conditions) in the downstream station than in the upstream impounded station and, on average, decreased over time, especially after the dam construction. To our knowledge, this study represents one of the first applications of the Flow-T index and findings indicate that, among the community metrics and biomonitoring indices here considered, this index was the only one able to detect the changes in the hydraulic conditions between the two stations as a consequence of the dam creation. These findings are also corroborated by those obtained from the species traits analysis. The choice of incorporating the abundances of taxa in the functional analysis (*i.e.* in the form of community-level weighted means (CWM) of trait values) was successful in detecting differences associate with the hydraulic conditions up- and downstream of the dam, especially in relation to the functional trait “water velocity preferendum”.

One of the main advantages of using functional traits in community ecology is that they allow to better relate the effects of the environmental conditions on the biota, based on the assumption that environment filters species according to their ecological and biological traits (*i.e.* species sorting scenario). In the last decades the use of functional traits or groups in river ecology has increased and has been invoked as a solution to make river biomonitoring even more specific and effective (Bonada *et al.*, 2006; Merritt *et al.*, 2017). This study also partly supports the idea that the Flow-T index could be a candidate biomonitoring tool for assessing the flow-related alterations, including impoundment and damming, in large, lowland rivers.

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Author contributions

Tiziano Bo: conceptualization, methodology, writing – original draft, writing – review & editing. Alberto Doretto: formal analysis, writing – original draft, writing – review & editing. Anna Marino: formal analysis, writing – original draft, writing – review & editing. Alex Laini: formal analysis, writing – original draft, writing – review & editing.

Alessandro Candiotto: conceptualization, methodology, writing – review & editing.

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