ORIGINAL ARTICLE

Towards ecological flows: status of the benthic macroinvertebrate community during summer low-flow periods in a regulated lowland river

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ABSTRACT

Climate change along with the increasing exploitation of water resources exacerbates low-flow periods, causing detrimental effects on riverine communities. The main mitigation measure currently adopted to counteract hydrological alterations induced by off-stream diversion is the release of minimum flows (MFs), even if within the European Union Water Framework Directive an upgrade towards ecological flows is urgently required to achieve good ecological status (GES). In this study, we investigated the temporal evolution of the benthic macroinvertebrate community in an Italian regulated lowland river (Ticino River) to clarify the ecological effects of summer low flows, and we evaluated the current MFs in the perspective of meeting GES standard. Biomonitoring was carried out for four consecutive years (2019-2022), in a river site immediately below a large off-stream diversion. The four study years were characterized by different streamflow patterns, thus allowing us to compare the temporal trajectories of the community under different flow conditions. Moreover, the interruption of the low-flow periods due to overflow spilled by the upstream dam gave us the opportunity to assess the effects of experimental flow peaks. Contrary to the expectation, the macroinvertebrate assemblage kept almost unvaried across the years, showing great resistance and resilience to hydrological changes. Even in extraordinarily dry 2022, the community composition varied only slightly, with a reduction of mayflies and an increase of mollusks. However, a deterioration of the ecological status below GES standard was recorded that summer, indicating the need for an upgrading of the current MFs. This upgrade would include experimental flow peaks in critical periods, which act as intermediate disturbances, enhancing community richness, diversity, and overall quality, as well as compliance with a threshold of an index specifically developed for the hydrological pressure.

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INTRODUCTION

"How much water does a river need?" is a key question in ecohydraulics (Richter *et al.*, 1997), and the issue is still under debate (Poff and Matthews, 2013; Acreman, 2016; Palmer and Ruhi, 2019; Tickner *et al.*, 2020). Comprehensive answers to the previous question are becoming even more urgent in recent years, due to the magnifying effects of climate change. In fact, modification of temperatures, precipitations, and flow regimes induced by climate change can affect both water availability and freshwater demand for human activities, amplifying the pressure of off-stream diversion on the freshwater environment (Pletterbauer *et al.*, 2018; Pham *et al.*, 2019). Low-flow periods, which naturally characterize flow regimes, can be exacerbated by both water exploitation for multiple anthropic uses and global warming (Amraoui *et al.*, 2019; Tramblay *et al.*, 2020). Exacerbated low flows, in terms of magnitude, duration, or frequency, and the environmental alterations associated with them, can induce several detrimental effects on riverine communities (Bunn and Arthington, 2002; Poff and Zimmerman, 2010; Piano *et al.*, 2019a; Doretto *et al.*, 2020). For instance, the loss of wetted riverbed reduces the living space of the organisms and increases their density, while the reduction of habitat diversity reduces food sources and refugees, resulting in increased predation and competition, favoring generalist species (Dewson *et al.*, 2007; Piano *et al.*, 2019b). Moreover, the possible increase in pollution and thermal stress associated with flow reduction can also benefit more tolerant species, thus involving further changes in the composition of the riverine communities (Salmaso *et al.*, 2017, 2018).

The implementation of protective measures capable to mitigate hydrological alterations in regulated rivers is underdeveloped in many regions of the world, frequently limited to the release of minimum flows (MFs), mostly estimated through a simple hydrological approach (Quadroni *et al.*, 2017; Salmaso *et al.*, 2018). River protection requires instead the restoration of a range of flows strictly related to ecological outcomes (Poff, 2018), the so-called environmental flows, *i.e.*, the quantity, timing, duration, frequency, and quality of water flows required to sustain freshwater, estuarine and near-shore ecosystems as well as the human livelihoods and well-being (Tharme, 2000). For instance, the preservation of seasonal streamflow variation, in addition to MF release, allows maintaining benthic macroinvertebrate communities close to natural conditions (Quadroni *et al.*, 2021).

The concept of environmental flow is now worldwide recognized also by water protection policies. Within the European Union Water Framework Directive (WFD, 2000/60/EC), methods for the determination of the environmental flow are moving from the simple hydrological approach towards establishing a linkage between the hydrological regime and the good ecological status (GES) of the water bodies. Hence, ecological flow (e-flow) is defined as "a hydrological regime consistent with the achievement of the environmental objectives of the WFD in natural surface water bodies" (WFD CIS, 2015). Apart from the achievement of GES, the environmental objectives refer to the non-deterioration of the existing status and the compliance with standards and objectives for protected areas (*e.g.*, Natura 2000 sites within Birds and Habitats Directives).

Each member state of the European Union is required to implement and integrate into the River Basin Management Plans a methodology for the determination of e-flows, ensuring that rivers can achieve and maintain the GES. In Italy, a national decree (DD 30/STA) was approved in 2017 to update and provide homogeneous and scientifically advanced methodologies for the e-flow definition. However, to date, the general methodology adopted by the Italian River Basin Authorities is still based on the mentioned hydrological approach, mostly considering the percentage of mean annual flow, corrected through several coefficients related to different environmental aspects (Moccia *et al.*, 2020).

In a recent study (Salmaso *et al.*, 2021), we investigated the upstream section of an Italian lowland river (Ticino River) affected by substantial off-stream diversion, highlighting a significant increase in the duration of the low-flow periods and a concurrent reduction of summer flows over the last decades. We also detected a significant positive relationship between the magnitude of low flows and the diversity of the benthic macroinvertebrate community and between the duration of the low-flow periods and the total density of the community in summer.

In this study, to clarify the ecological effects of summer low flows, we investigated the temporal evolution of the benthic macroinvertebrate community of the Ticino River by repeatedly sampling the assemblage during four consecutive years (2019-2022). In addition, we assessed interannual differences in the streamflow pattern and in the structure of the benthic macroinvertebrate community, and possible associations between them to critically analyze previous evidence (*i.e.*, the community is less diverse in summers characterized by a reduced magnitude of low flows, and the total density of macroinvertebrates is higher when low-flow periods last longer). Moreover, we evaluated if the current MF scheme could support achieving the GES by calculating the Italian index based on benthic macroinvertebrates, specifically developed to determine the ecological status of rivers, *i.e.*, the Standardization of River Classifications_Intercalibration Common Metric index (STAR_ICMi, Buffagni and Erba, 2007a). However, since the suitability of this index to highlight the ecological impacts induced by hydrological alterations has been already questioned (Larsen *et al.*, 2019), we also applied an index specifically developed for such purpose, *i.e.*, the Flow_T index, as required in the WFD CIS guidance document (WFD CIS, 2015). Flow_T is a macroinvertebrate trait-based index that can track the effect of flow variation, displaying consistent responses in different geographical regions across Europe (Laini *et al.*, 2022a). The information on flow velocity preferences derives indeed from a comprehensive trait dataset encompassing nearly 500 macroinvertebrate taxa at the European scale (Tachet *et al.*, 2010).

METHODS

Study area

The Ticino River is the main tributary of the Po River (northern Italy) in terms of mean annual flow (348 $m^3 s^{-1}$). It flows from the Swiss Alps to Lake Maggiore (the second largest Italian lake) for ~90 km and then from the lake to the Po River for ~110 km. Since 1943, the lake outflow is regulated by the Miorina Dam to optimize the water use in the downstream section of the river (Salmaso *et al.*, 2021; Espa *et al.*, 2022). Water releases through the dam depend on the water level of the lake, according to limits established by law. Discharge is controlled through movable gates that are fully opened during floods. About 7 km downstream, the largest off-stream diversion takes place at the Panperduto Dam, which has been active since 1884, and currently allows for a maximum off-stream diversion to the eastern side of the Ticino River of approximately 60% of the river mean annual discharge (*i.e.*, 170 m^3 s^{-1}), for irrigated agriculture and run-of-the-river hydropower (Fig. 1).

Mandatory monthly-modulated MFs are currently released below the Panperduto Dam. However, streamflow generally exceeds MFs during the periods of high-water availability, *i.e.*, mainly in spring, due to snowmelt, and in autumn, due to rainfall (Salmaso *et al.*, 2021; Espa *et al.*, 2022). Conversely, two periods of low flows usually occur in winter and in summer. In particularly dry years, the latter was more severe than the former, due to the co-occurrence of lower water availability and higher off-stream diversion for irrigation.

We selected a sampling site characterized by major hydrological alteration, located immediately below the Panperduto Dam (Fig. 1), where MF varies from 6% to 11% of the mean annual natural flow: 24 m^3 s^{-1} from January to May, 17 m^3 s^{-1} from June to August, and 31 m^3 s^{-1} from September to December. Here, river morphology is predominantly natural, with alternation of riffles, runs, and pools, and the substrate is mainly composed of cobbles (6-40 cm diameter).

Benthic macroinvertebrate sampling and analysis

Benthic macroinvertebrate sampling was carried out on two to six occasions per year (adopting a timespan of approximately two weeks between two consecutive sampling dates), according to the length of the summer low-flow period, from 2019 to 2022 (Fig. 2). On each sampling date, water temperature, dissolved oxygen concentration and saturation, pH, and electrical conductivity were measured *in situ* using the YSI Professional Plus portable multiparameter probe (YSI, Yellow Springs, USA). Benthic macroinvertebrates were collected with a Surber sampler of 0.05 m^2 area and 500 μm mesh, following a quantitative multi-habitat protocol (Buffagni and Erba, 2007b). The samples were preserved in alcohol and analyzed in the laboratory.

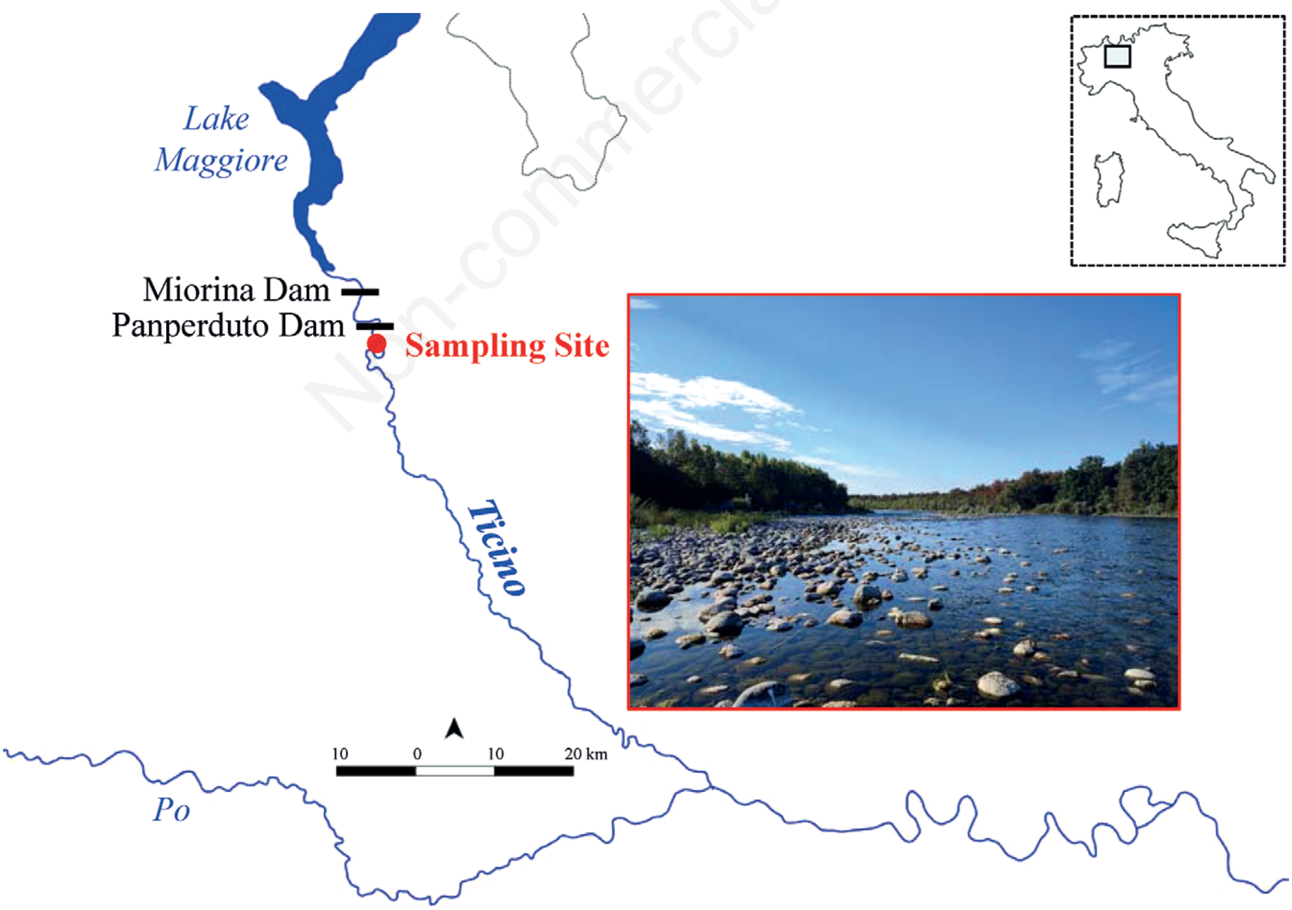

Fig. 1. Map of the study area in northern Italy and a photo of the Ticino sampling site in summer 2022.

Individuals belonging to Plecoptera, Ephemeroptera, Turbellaria, and Hirudinea were identified at the genus level and the others at the family level.

The structure of the benthic macroinvertebrate community was described through standard metrics: total density, the relative density of taxa belonging to Ephemeroptera, Plecoptera and Trichoptera (EPT), *i.e.*, the insect orders including species sensitive to environmental pressures, and the relative density of Chironomidae, *i.e.*, the family including species tolerant to environmental pressures. Moreover, the compositional dissimilarity between samples was quantified using the Bray-Curtis index. This index ranges from 0 (indicating complete similarity) to 1 (indicating complete dissimilarity). The Bray-Curtis distances were visually displayed in a two-dimensional ordination space using a non-metric multidimensional scaling (NMDS). One-way analysis of similarities (ANOSIM) was adopted to test the macroinvertebrate assemblage for significant differences between summers, and the similarity of percentages (SIMPER) was used to identify the taxa responsible for these differences.

The ecological status was determined through the STAR_ICMi (Buffagni and Erba, 2007a) that is ranked into five quality classes (bad, poor, moderate, good, and high), respectively bounded at 0.24, 0.48, 0.72, and 0.96. According to this method, the quality class assigned to a river reach depends on the annual mean of the seasonal values. However, in this study, only the temporal trend of this index during summer was investigated. The STAR_ICMi combines six sub-indices providing different information on the macroinvertebrate community: Average Score Per Taxon (ASPT), indicating tolerance; Log(Sel_EPTD+1), *i.e.*, the logarithm of the sum of the abundance of Heptageniidae, Ephemeridae, Leptophlebidae, Brachycentridae, Goeridae, Polycentropodidae, Limnephilidae, Odontoceridae, Dolichopodidae, Stratyomidae, Dixidae, Empididae, Athericidae and Nemouridae plus 1, indicating abundance and habitat; richness and diversity are instead represented by 1-GOLD, *i.e.*, 1 minus the relative abundance of Gastropoda, Oligochaeta and Diptera, and by the total number of families (N families), the number of EPT families (N EPT), and the Shannon-Wiener index.

Apart from the STAR_ICMi and its sub-indices, the FLOW_T index (Laini *et al.*, 2022a) was also calculated. Higher flow velocities typically result in communities characterized by higher scores of this index, 0 as the minimum and 1 as the maximum value.

Differences in the benthos indices between summers were analyzed by the Kruskal-Wallis test, followed by the Mann-Whitney test for pairwise comparisons.

The patterns of benthos indices were qualitatively compared to the local streamflow patterns to detect possible associations. The mean daily streamflow downstream from the Panperduto Dam from 2001 to 2022 was kindly provided by the water users association (Ticino Consortium) managing the mentioned hydraulic structures. Statistical analyses were performed using PAST 4.03 and R "biomonitoR" package (Laini *et al.*, 2022b).

RESULTS

Streamflow patterns

The four study summers were characterized by different streamflow patterns, thus supporting the comparison of the temporal trajectories of the benthic macroinvertebrate community under different flow conditions (Fig. 2).

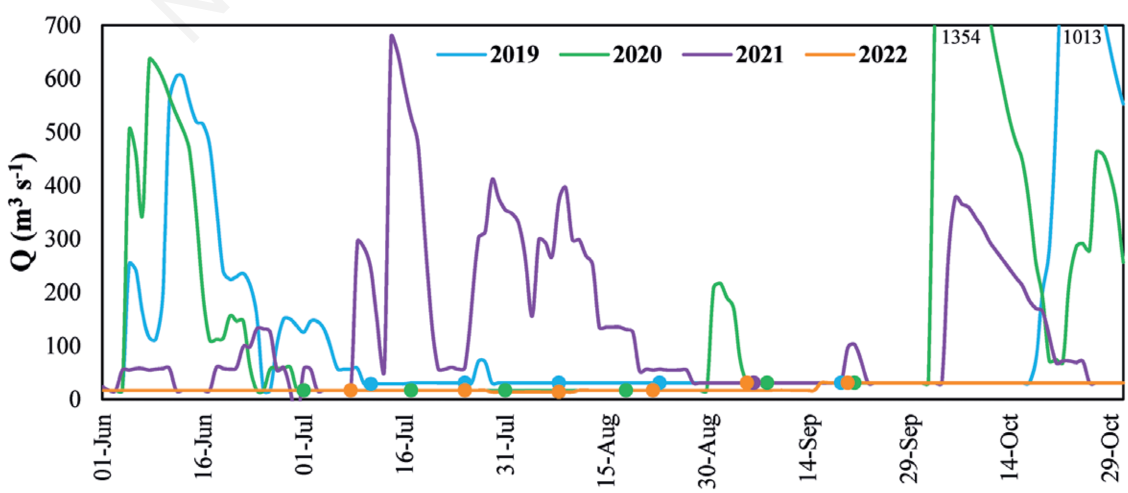

Fig. 2. Patterns of mean daily streamflow in the Ticino River downstream from the Panperduto Dam, from June to October 2019, 2020, 2021 and 2022. Benthic macroinvertebrates sampling dates are indicated by circles.

In 2019, the summer low-flow period started on July 10 and ended on October 18, with a total duration of 100 days; the flow was constant at 31 $m^3 s^{-1}$except for two consecutive days (July 27 and 28) when a flow increase up to 70 $m^3 s^{-1}$ (*i.e.*, a value approximately double than that recorded in the previous days) was recorded. In July and August, flow values were higher than the MF prescribed for these months, due to a larger water availability.

In 2020, the summer low-flow period started ten days before that in 2019 (on June 30), and ended two weeks before (on October 3), lasting 95 days. Low flow corresponded to the MF during the whole period, *i.e.*, 17 $m^3 s^{-1}$until the end of August and 31 $m^3 s^{-1}$ later, except for five consecutive days (from August 31 to September 4), when a flow increase up to 217 $m^3 s^{-1}$ (170 $m^3 s^{-1}$ on average, *i.e.*, a value approximately 10 times larger than the MF) occurred.

Both in 2019 and in 2020, the interruption of the prolonged low-flow periods due to overflow spilled by the upstream dam gave us the opportunity to assess the effects of experimental flow peaks.

Summer 2021 was characterized by the absence of a prolonged low-flow period: in fact, MF values were recorded only six days at the beginning of July (from 3 to 8) and 38 days from August 28 to October 4, also interrupted by three consecutive days (September 20-22) of higher flows (maximum value 103 $m^3 s^{-1}$, mean value 90 $m^3 s^{-1}$).

2022 was an extraordinarily dry year, characterized by the absence of the typical high-flow period in spring: low flows started in the autumn of the previous year (November 23, 2021) and continued until the end of the study period. Due to this extreme drought, the MF threshold established for July and August was not complied during 13 days from July 29 to August 10, 2022.

During the 90 days before the first macroinvertebrate sampling, the hydrological conditions were different between the four investigated years (Tab. 1). Mean values decreased from 2019 to 2022, while median values were equal to MF from 2020 to 2022 and higher in 2019. Peak values were higher in 2019 and 2020.

In 2022 flow variation was lower than that in previous years: MFs were always recorded except for one day, when the flow value was 29 $m^3 s^{-1}$. The number of MF days was instead lower in 2019, *i.e.*, approximately one-third of the considered timespan, and increased up to approximately half in 2020 and 2021.

Water physico-chemical parameters

The values of water physico-chemical parameters measured on each sampling occasion were in ranges suitable for most aquatic organisms inhabiting lowland rivers in the study area (Salmaso *et al.*, 2017): temperature 20.3-28.6°C (mean 24°C), pH 7.26-9.44 (mean 8.59), electrical conductivity 137-385 µS cm^{-1} (mean 193 µS cm^{-1}), dissolved oxygen concentration 6.8-10.5 mg L^{-1} (mean 8.5 mg L^{-1}) and saturation 85-126% (mean 101%). Maximum values of temperature and electrical conductivity were recorded in 2022.

Patterns of the benthic macroinvertebrate community

The total density of benthic macroinvertebrates differed between the four summers: higher values were recorded in summer 2020 (mean 6008 ind. m^{-2}) and 2021 (mean 5976 ind. m^{-2}) than in summer 2019 (mean 1418 ind. m^{-2}) and 2022 (mean 2038 ind. m^{-2}) (Kruskal-Wallis and Mann-Whitney test, $p<0.05$, 2021 excluded). In 2020, a relevant density reduction occurred after the previously mentioned flow peak that interrupted the low-flow period (Fig. 3).

The benthic macroinvertebrate community was always dominated by the three insect orders Ephemeroptera, Trichoptera and Diptera. However, the relative density of EPT had a different pattern between years. In 2019 and 2022 it decreased in August and then increased in September, while in 2020 a decreasing trend during all the monitored period was recorded, probably due to the flow peak detected at the beginning of September. The mean value of % EPT was similar in 2019 (73%), 2021 (78%),

Tab. 1. Basic statistics of the mean daily streamflow recorded downstream from the Panperduto Dam during the 90 days before the first macroinvertebrate sampling for each year, from 2019 to 2022. The number of days when the streamflow equaled the minimum flow (MF) value was also considered.

Statistics	2019	2020	2021	2022
Mean ($m^3 s^{-1}$)	155	135	58	21
Median ($m^3 s^{-1}$)	69	24	24	24
Standard deviation ($m^3 s^{-1}$)	183	176	73	4
Minimum ($m^3 s^{-1}$)	17	17	17	17
Maximum ($m^3 s^{-1}$)	677	636	391	29
Coefficient of variation (%)	118	131	125	17
Number of MF days	32	52	54	89

MF, minimum flow.

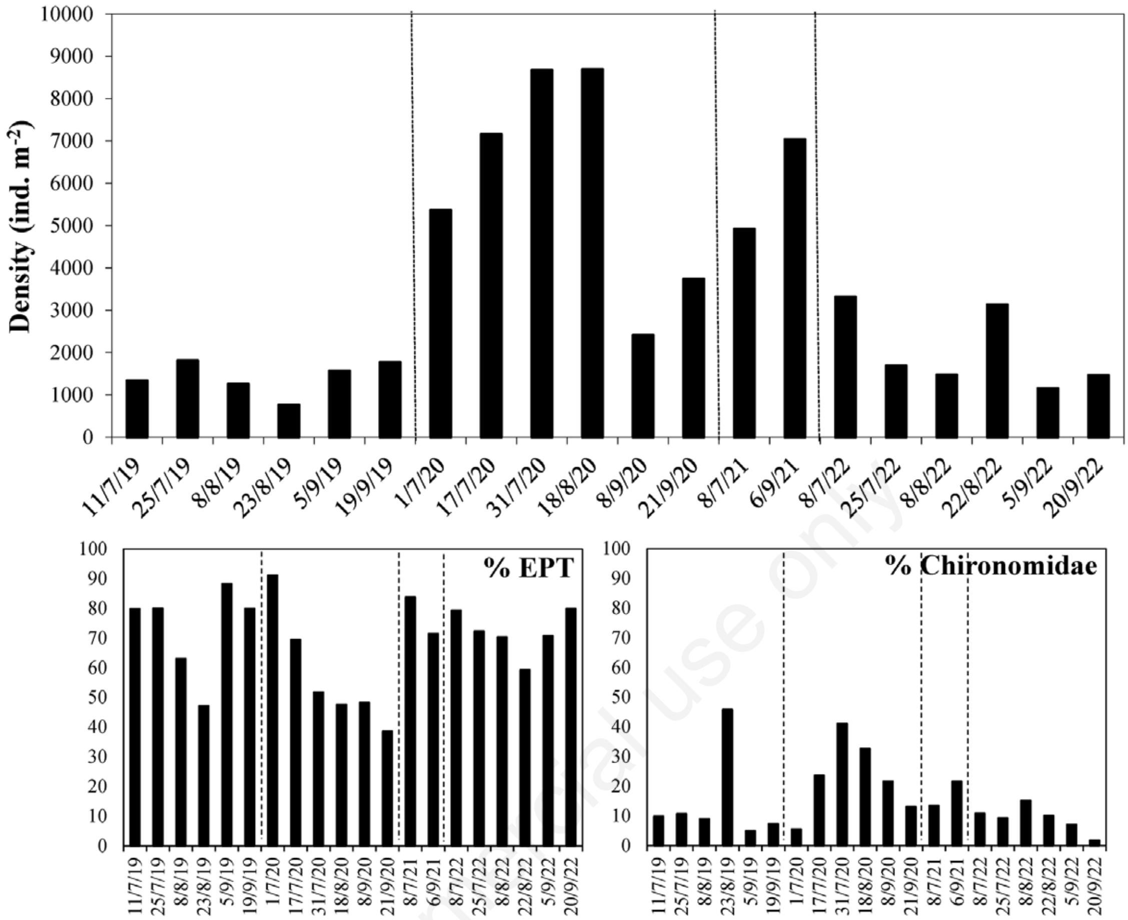

Fig. 3. Total density of benthic macroinvertebrates and relative density of individuals belonging to EPT orders (% EPT) and Chironomidae family in summer 2019, 2020, 2021 and 2022.

and 2022 (72%) while lower in 2020 (58%). The patterns of the relative density of Chironomidae were instead similar in 2020 and 2022, but with higher values in the first year (23%) than in the latter (9%). In 2019 a peak of this metric was recorded at the end of August, and the average value (15%) was similar to that recorded in 2021 (18%) (Fig. 3). However, no significant differences in both % EPT and % Chironomidae were detected between years (Kruskal-Wallis and Mann-Whitney test, $p<0.05$, 2021 excluded).

The whole taxonomic composition of the benthic macroinvertebrate community was similar in 2019 and 2022, but significantly different in 2020 (ANOSIM, $R = 0.41$, $p=0.0005$). Although 2021 could not be statistically tested due to the low number of samples (only two), in the NMDS the assemblage was located within the range of 2020 samples and distant from 2019 and 2022 samples (Fig. 4). The major differences between years were ascribed to differences in the density of the most abundant taxa, *i.e.*, Hydropsychidae, *Baetis*, Chironomidae, and Simuliidae. Most taxa displayed the highest mean densities in 2020 and

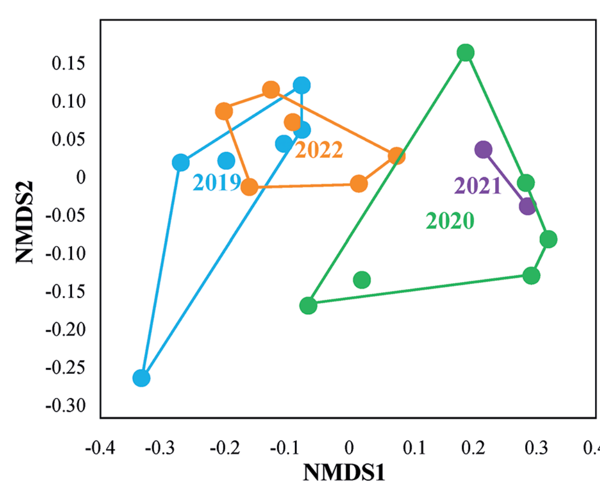

Fig. 4. Non-metric multidimensional scaling (NMDS, stress= 0.10) showing the distance (Bray-Curtis index) between samples of benthic macroinvertebrates collected in summer 2019, 2020, 2021 and 2022.

2021, except for the mollusks belonging to Neritidae and Dreissenidae families, more abundant in 2022. Between 2019 and 2022, higher mean densities of most taxa were recorded in 2022, except for some Ephemeroptera genera, mainly *Serratella* (Tab. 2). Individuals belonging to this genus were detected only in the first sampling of 2022 (8/7/2022), and even *Ecdyonurus* was absent in the last two sampling occasions.

In Fig. 5, the temporal patterns of the STAR_ICMi and its six sub-indices were reported. The STAR_ICMi values mainly corresponded to a good ecological status (GES), except for some sampling occasions in 2019 and 2022. In 2022, an almost decreasing trend was recorded with a minimum value in the last sampling date. Both in 2019 and in 2020 the maximum value was recorded after the flow peak that interrupted the low-flow period. Considering the mean value over the summer, the ecological status was moderate in 2022 (0.68), good in 2020 (0.86) and 2021 (0.78), and at the border between these two quality classes in 2019 (0.72). Among the sub-indices composing the STAR_ICMi, the ASPT was the less variable, with mean values ranging from 5.6 to 6. A decreasing trend of the Log(Sel_EPTD+1) was recorded both in 2020 and 2022, with lower values in the latter year. The mean value was indeed 0.95 in 2022 and 1.93 in 2020. In the other two years averages were 1.21 and 1.62 in 2019 and 2021, respectively. In all summers, the 1-GOLD decreased until the end of August and then increased in September. The mean values were similar (0.77-0.80) except for 2020, when the average was lower (0.68).

The number of all families (N families) and of the families belonging to EPT (N EPT) had a similar pattern within the same summer. They were almost stable in 2019, with an increasing trend in 2020 and a decreasing trend, although with some oscillations, in 2022. The mean values of both indices were highest (26 and 11, respectively) in 2020, while the minimum mean value was recorded in 2019 for N families (19) and in 2022 for N EPT (8). The maximum values of both indices were recorded after the flow peaks interrupting the low-flow period, both in 2019 and in 2020. The Shannon-Wiener index as well had its maximums after these high-flow events. However, the maximum value recorded in 2019 was also observed in 2022, when no flow peaks took place. As for the other indices, except for 1-GOLD, the maximum mean value was recorded in 2020 (1.86).

In summary, STAR_ICMi, Log(SelEPTD+1), N families and N EPT were significantly higher in 2020 than in 2019 and 2022 (Kruskal-Wallis and Mann-Whitney test, $p<0.05$), while the other indices did not differ significantly between these three years. Moreover, the lowest values of N EPT and Log(Sel_EPTD+1) detected in 2022 seemed responsible for the decrease of the STAR_ICMi.

The FLOW_T index did not vary markedly, both within and between the four summers (Fig. 6). Mean values ranged from 0.52 to 0.60. However, it was significantly higher in 2019 than in 2020 and 2022 (Kruskal-Wallis and Mann-Whitney test, $p<0.05$). Although not statistically tested, the two values of the Flow_T index recorded in 2021 were similar to those in 2019.

Tab. 2. Results of SIMPER analysis with values of average dissimilarity (AD) in the composition of benthic macroinvertebrate community between the four summers (2019-2022), and the relative contribution (C %) of the main taxa. For each taxon, the mean density in the four periods was reported.

	19 *vs* 20	19 *vs* 21	19 *vs* 22	20 *vs* 21	20 *vs* 22	21 *vs* 22				
	AD 63.4	AD 65.7	AD 39.7	AD 40.7	AD 56.2	AD 57.8	Mean density (ind. 0.5 m^{-2})			
Taxon	C %	C %	C %	C %	C %	C %	2019	2020	2021	2022
Hydropsychidae	25.8	50.0	37.5	29.8	23.2	45.4	349	979	1560	531
Chironomidae	25.1	18.1	9.5	21.7	26.3	18.9	83	783	545	96
Baetis	14.2	14.6	10.5	13.0	15.0	15.2	101	419	441	112
Simuliidae	8.5	3.9	9.6	8.5	9.2	4.7	55	203	103	80
Dugesia	8.1	0.29	4.2	7.3	7.2	1.5	8	165	1	34
Serratella	3.7	1.7	5.3	3.3	3.5	1.6	36	84	37	3
Caenis	2.8	0.52	0.7	2.3	3.0	0.58	5	70	14	1
Ecdyonurus	2.0	0.36	0.8	1.8	2.2	0.46	6	57	14	5
Hydroptilidae	1.8	1.56	2.4	2.5	1.8	1.7	8	47	43	14
Rhyacophilidae	1.7	2.61	2.2	1.8	1.4	2.4	18	55	74	26
Leuctra	0.95	3.4	3.6	2.7	0.7	2.6	11	32	98	33
Neritidae	0.26	0.10	3.4	0.2	0.87	0.89	1	5	3	22
Physidae	0.02	0.43	0.15	0.4	0.04	0.45	0	0	9	1
Dreissenidae	0.02	0	3.1	0.02	0.97	0.89	0	0	0	20

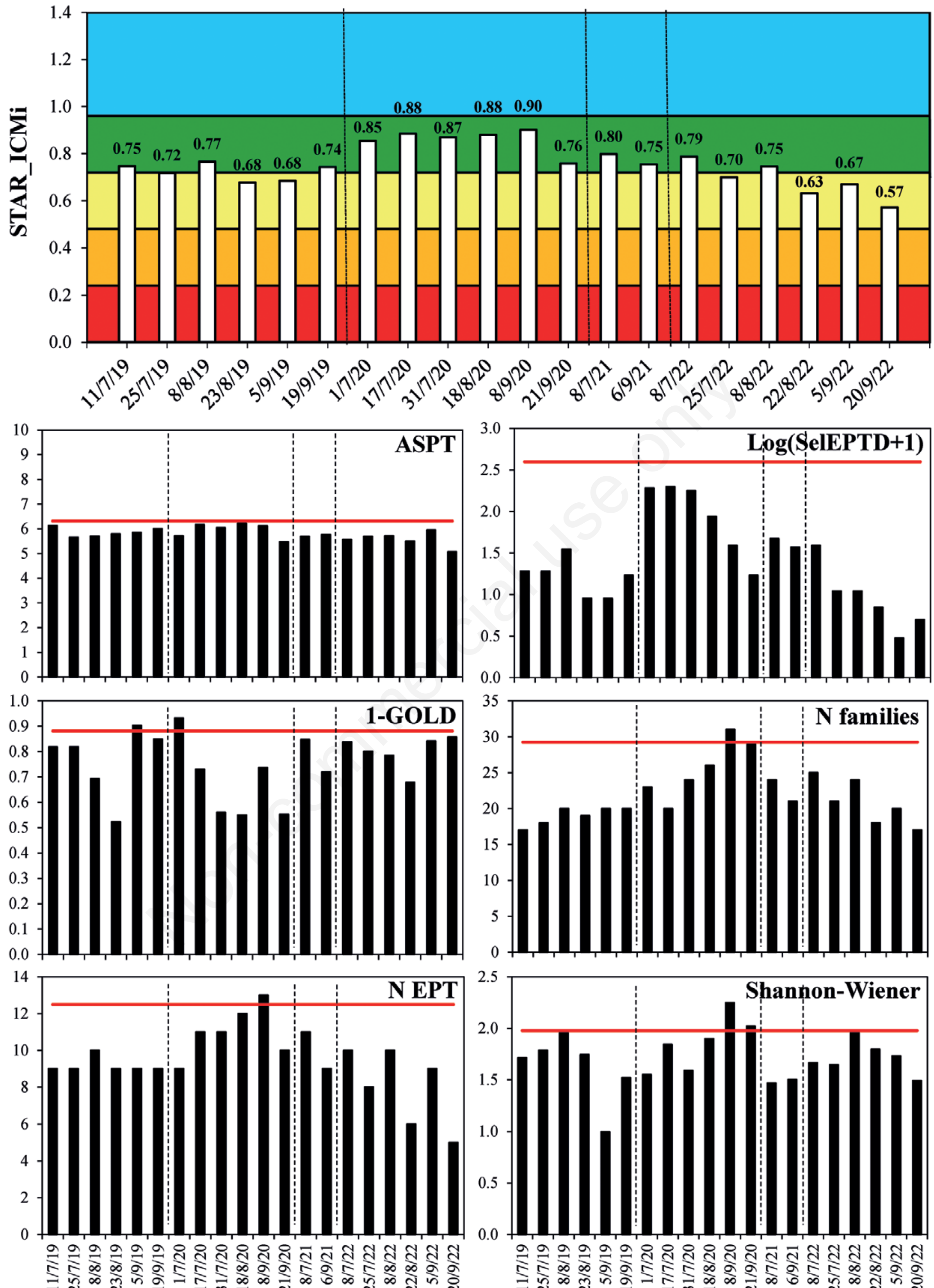

Fig. 5. Temporal patterns of the STAR_ICMi (quality classes range from high - light blue - to bad - red) and its six sub-indices (red line represents the reference value) in summer 2019, 2020, 2021 and 2022.

DISCUSSION

The flow regime is a major determinant of riverine communities, directly and indirectly influencing biodiversity and ecosystem processes of lotic ecosystems (Poff *et al.*, 1997; Guareschi *et al.*, 2012; Rolls *et al.*, 2018). As well known, the number of free-flowing rivers is decreasing worldwide (Grill *et al.*, 2019), with dams and related water management schemes representing the main cause of flow alterations in rivers (Ogbeibu and Oribhabor, 2002; White *et al.*, 2017). However, dam impact on aquatic biota can vary from moderate to severe also depending on the local geo-morphological setting (Leitner *et al.*, 2021), and, particularly in recent years, on the climate-related hydrological changes (Wu and Johnson, 2019).

The relevant differences in the streamflow patterns observed between the four investigated years in the Ticino River provided us with a unique opportunity to assess the ecological impact of hydrological alterations on the benthic macroinvertebrate community of a regulated large lowland river. Among the four years, 2022 was extraordinarily dry, with both reduced low-flow values, even lower than the established MF threshold, and increased duration of the low-flow period, with no interruptions during the monitored time span. We thus expected a noticeable change in the macroinvertebrate community in that year.

Contrary to expectations, the macroinvertebrate assemblage resulted almost unvaried across years, showing substantial resistance and resilience to hydrological changes. In particular, no significant differences were detected between the assemblage collected in 2022 and that sampled in 2019, despite the marked difference in the streamflow patterns not only during the summer, but also in the 90 days before the first macroinvertebrate sampling. We could only detect lower densities of some Ephemeroptera genera, probably due to an early adult emergence caused by the higher temperatures recorded in 2022 (Shipley *et al.*, 2022). Moreover, this year, differently from the other ones, we noticed a higher density of mollusks belonging to Neritidae and Dreissenidae families, probably advantaged by the constant dry conditions, making the river environment less lotic. This change in the community composition arises a further important issue associated with the spread of invasive alien species such as *Dreissena polymorpha* (Pyšek *et al.*, 2020).

The high resistance/resilience of the benthic macroinvertebrate community detected in this study can be related to the river size (*i.e.*, in smaller rivers where relevant flow reduction causes a strong decrease of microhabitat availability, the effects can be different and the resilience lower; Piano *et al.*, 2019a, 2019b; Doretto *et al.*, 2020) and to the general characteristics of the assemblage, already shaped to face significant hydrological alterations. In fact, as typical in regulated rivers (Quadroni *et al.*, 2017, 2021), the dominant taxa are opportunistic and characterized by traits enabling them to face disturbed conditions, *i.e.*, rapid population turnover, tendency to drift, high mobility, multivoltinism, poorly synchronized emergence.

In addition to the four dominant taxa, the Ticino community was generally composed of other 15-20 families, of which about ten belong to EPT orders. Its ecological status defined through the STAR_ICMi was good or mostly good from 2019 to 2021, while it was mostly below the GES standard in 2022. In this latter year, the lowest value of the STAR_ICMi and of its two sub-indices that indicate the sensitivity of the community to

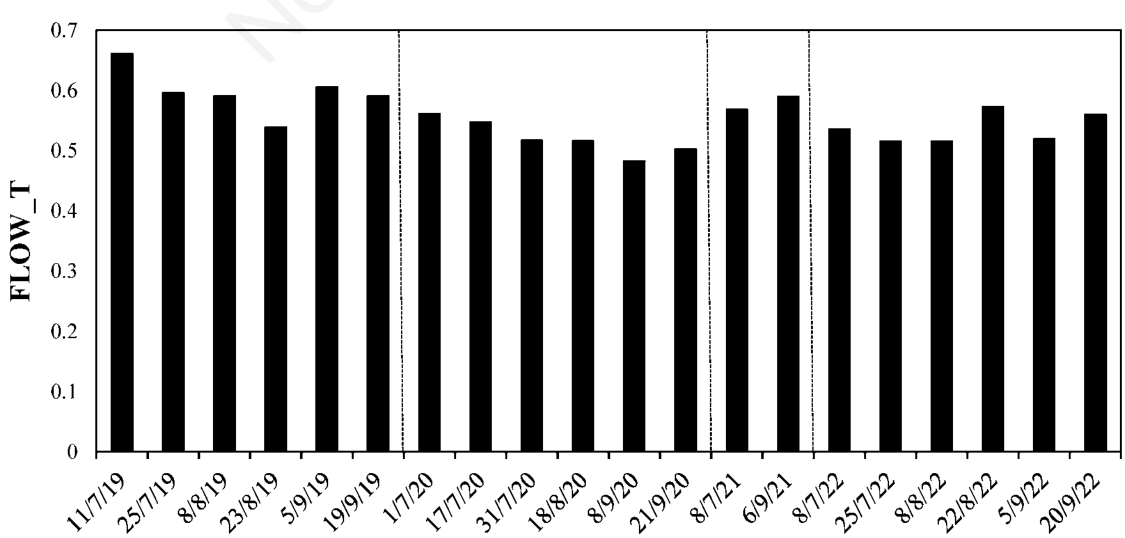

Fig. 6. Temporal pattern of the FLOW_T index in summer 2019, 2020, 2021 and 2022.

environmental pressures (Log(SelEPTD+1) and N EPT), was recorded. This result shows that the low flows recorded in this period cannot be considered "ecological".

Both in 2019 and in 2020, maximum values of richness (both total and EPT), diversity and quality were recorded after the flow increase that interrupted the low-flow period. Therefore, these events acted as intermediate disturbances reducing total density, limiting the dominance of taxa, and thus rebalancing the community. This agrees with evidence from previous studies (Fornaroli *et al.*, 2019; Robinson, 2012; Salmaso *et al.*, 2021), highlighting the significant control exerted by high flows on benthic assemblages of regulated rivers.

The index especially sensitive to hydrological alterations, Flow_T, was less variable than expected. The poor variability of this index across different hydrological summers was probably due to the already-mentioned stability of the Ticino community composition at the monitoring site. However, it indicated a better condition in 2019 and 2021 than in 2020 and 2022. Compared to 2020 and 2022, 2019 was characterized by the highest flows during springtime, and by higher low-flow values during the whole summer. In contrast, 2021 was characterized by large high flows during most of the summer.

Obviously, hydrology is not the only factor shaping benthic macroinvertebrate communities, particularly in large lowland rivers, where multiple pressures usually co-exist (Laini *et al.*, 2019; Racchetti *et al.*, 2019; Dolédec *et al.*, 2021; Leitner *et al.*, 2021). The results of the spot measurements of the main water physico-chemical parameters during benthos sampling occasions did not show worrying values, even if during the 2022 prolonged low-flow period particularly high values of temperature and conductivity (*i.e.*, an indirect measure of pollution) were recorded. Exacerbated low flows could thus have also induced thermal stress and eutrophication phenomena in this period. Moreover, events of nutrient and organic pollution cannot be excluded over the entire study period, for instance concurrently with the peak of the relative density of Chironomidae detected in 2019. In our previous work in the same area (Salmaso *et al.*, 2021), we found that the relatively high nitrate-nitrogen concentrations detected in the summer period decreased the relative abundance of EPT, favoring at the same time Chironomidae, *i.e.*, a family including species characterized by relatively high tolerance to pollution (Salmaso *et al.*, 2018).

In general, communities below dams are both resilient and resistant, are dominated by generalist taxa, and river regulation associated with increased eutrophication and other environmental stresses, particularly at low flows, may induce a food web simplification (Dolédec *et al.*, 2021). The presence of the Panperduto Dam since 1884 and of the upstream Miorina Dam since 1943 has likely shaped the benthic macroinvertebrate assemblage of the Ticino River, which is able to face different hydrological conditions, also extreme conditions. Exceptional dry years such as 2022 already occurred in the past, for instance in 2003 and 2005 (Fig. 7). However, they were followed by wetter years, so it is difficult conjecturing the possible effects of low flow persistence over the next years.

CONCLUSIONS

In our opinion, the results of this study can support the development of improved ecological criteria and the related upgrading of the current environmental-flow scheme, still based on the MF concept alone.

Overall, the MFs currently adopted to mitigate the hydrological alteration of the Ticino River proved to be

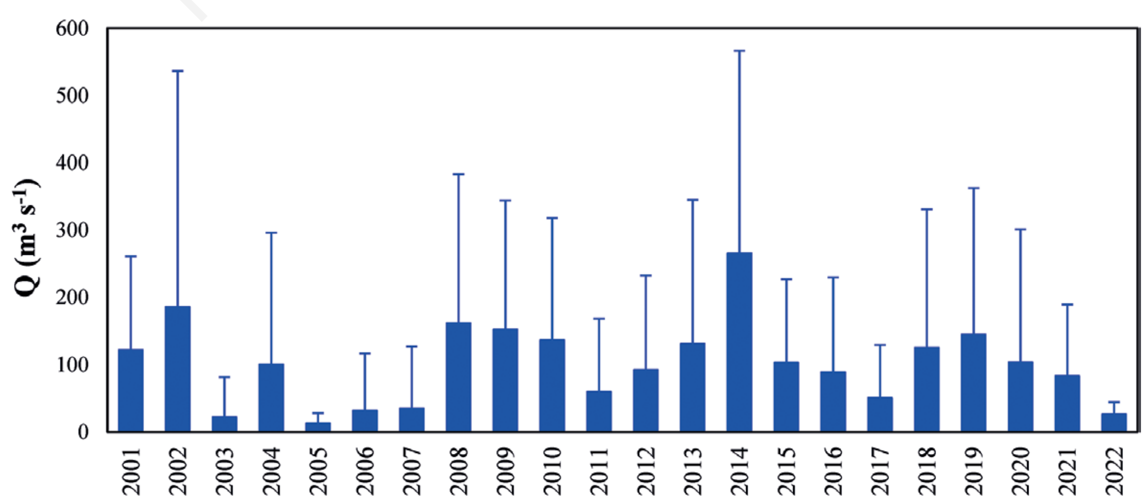

Fig. 7. Mean annual flow plus standard deviation in the Ticino River below the Panperduto Dam from 2001 to 2022.

adequate to maintain the benthic macroinvertebrate community in the study reach. However, prolonged periods of low flows such as that occurred in 2022 can induce the decline of the ecological status below the WFD threshold (GES). In these cases, streamflow increases temporarily interrupting low-flow periods could determine increases of richness, diversity, and overall quality of the riverine ecosystem. Though water allocation during droughts is especially challenging, water resource managers would consider this environmental need together with those of the other river stakeholders. This is particularly relevant in the next future, as low-flow periods are predicted to increase in magnitude, duration, and frequency as a consequence of climate change. Moreover, the eco-compatibility of a regulated flow regime could be assessed by considering other environmental objectives besides the GES standard, for example, as suggested in the WFD CIS guidance document (WFD CIS, 2015), the compliance with thresholds of indexes specifically developed to detect the hydrological pressure, such as the Flow_T index. However, further research is necessary to support this proposal. A successful e-flow policy could be achieved only if limitations in institutional capacity, scientific knowledge and monitoring resources will be overcome (Le Quesne *et al.*, 2010).

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