Effects of high altitude reservoirs on the structure and function of lotic ecosystems: a case study in Italy

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Abstract

Alpine and pre-alpine lotic ecosystems are often remote and not affected by humans, which makes them some of the world's most pristine ecosystems. However, their status is often altered by the presence of reservoirs that are built to fulfil agricultural needs and hydroelectric demands. These reservoirs also disrupt stream continuity and alter the magnitude, timing, and frequency of natural

42 flows. The present work assessed how high altitude reservoirs affect the riverine ecosystems focusing

43 on: i) the macroinvertebrate communities; ii) the breakdown of organic matter; iii) the thermal regime.

44 Stretches altered by high altitude reservoirs had the best conditions for most macroinvertebrate

45 families due to a more stable flow conditions. The breakdown rate of coarse particulate organic

46 matter was not affected by high altitude reservoirs but its availability was higher in altered compared

47 to pristine stretches. The presence of hydroelectric power plants modified the stream thermal regime.

48 Reservoirs mitigate the atmospheric influence on stream water temperature while run of the river

49 plants strengthen it in the diverted stretches. Where both these alterations were present the thermal

50 regime of the stream was more similar to the natural ones compared to stretches subjected to only one

51 kind of alteration. This research showed how river impoundment alters the structure of

52 macroinvertebrate communities and the function of the downstream lotic ecosystems and can provide

53 the basis to correctly guide management strategies for lotic ecosystems affected by hydrological

54 alterations.

55 **Introduction**

56   Alpine and pre-alpine lotic ecosystems are often remote and not affected by human presence

57 and activities, which makes them some of the world's most pristine ecosystems (Füreder et al., 2002;

58 Hotaling et al., 2017). Moreover, due to significant habitat isolation and environmental heterogeneity,

59 they show high levels of biodiversity and some of the species living there are endemic and have thus

60 high naturalistic value (Muhlfeld et al., 2011; Jordan et al., 2016). The communities inhabiting alpine

61 ecosystems are confined to high elevation sites due to temperature requirements. However, global

62 warming is pushing them upslope toward mountaintops and shrinking their habitat (McGregor et al.,

63 1995; Brown et al., 2007). Considering their importance and vulnerability, studying and protecting

64 alpine lotic ecosystems is a priority.

65   The first reservoirs were built on alpine streams in the 19th century in order to fulfill

66 agricultural needs and hydroelectricity demand. At that time, the attention was mainly focused on

67 social-economic consequences and on the potential dangers to humans, while the environmental

impacts were mainly unknown and thus ignored. Nowadays it is well known that reservoirs disrupt the river continuity and produce hydrological alterations which can be defined as any anthropogenic disruption in the magnitude, timing, and frequency of natural flows (Zolezzi et al., 2009; Bocchiola, 2014). A common downstream effect is that the flood peak, and hence the frequency of overbank flooding, is reduced and sometimes displaced in time (Petts, 1984). Upstream, sediment accumulates in the slow moving water of the reservoir, while the water released downstream has low concentrations of suspended solids (Anselmetti et al., 2007). This modification of the sediment cycling in the river leads to major erosion downstream. Williams & Wolman (1984) concluded that 21 rivers in North America showed rapid riverbed erosion after reservoir construction. Reservoirs act as lake and the stored water can be affected by stratification in Summer/Winter and destratification in Spring/Autumn. For that reason, the temperature of water released from the reservoir into the river depends on stratification/destratification (Dickson et al., 2012) and on the level of the discharge. As an example Wiejaczka et al. (2018) found that the presence of the Czorsztyn-Sromowce Wyżne reservoir complex (Poland) decreased river water temperature in Summer, Winter and Spring (by 6.9, 0.7 and 7.9°C, respectively), whereas in Autumn it had an opposite effect, raising it by 7.9°C.

Reservoirs modify structural and functional characteristics of riverine ecosystems by altering the rate of degradation/transport of organic matter and the drift of organisms (Martínez et al., 2013). According to the Serial Discontinuity Concept (SDC, Ward & Stanford, 1995; Stanford & Ward, 2001), dams result in upstream-downstream shifts in biotic and abiotic patterns and processes; the direction and extent of the displacement depend on the variable of interest and are a function of dam position along the river continuum. The CPOM (Coarse Particulate Organic Matter) to FPOM (Fine Particulate Organic Matter) ratio declines naturally as the detritus is transported downstream (Fenoglio et al., 2015), but reservoirs greatly depress the ratio of coarse particulate to fine particulate organic matter (CPOM/FPOM) below the impoundment because the in-stream transport of detritus is blocked as highlighted by the SDC(Ward & Stanford, 1995). However, the response of organic matter to impoundment is variable in the literature: a study concerning the Colorado River showed a

94   suppression of CPOM below the impoundment with recovery beginning at 3 km (Voelz & Ward,

95   1991). In contrast, the highest value of CPOM was found at the first site below the Dam in the

96   Canning River (Australia), while it decreased sharply 5 km downstream (Storey et al., 1991).

97   Moreover, CPOM was reduced with the increase of fine sediment in the river and this also affected

98   the abundance of invertebrate shredders (Doretto et al., 2016). The CPOM/FPOM ratio is highly

99   influenced by water flow: while FPOM is transported downstream regardless of flood magnitude,

100   CPOM is more influenced by floods. The transport of CPOM downstream increases with increasing

101   flow because the greater water strength drags branches trapped between rocks and removed from the

102   trees.

103   The effect of impoundment on macroinvertebrate abundance and diversity is variable in the

104   literature. Principe (2010) found that macroinvertebrate richness and diversity increased in an

105   Argentine mountain river downstream from the dams, but there were no differences in

106   macroinvertebrate density. In contrast, Martínez et al. (2013) found that the average number of

107   macroinvertebrate taxa per sample was higher in upstream sites compared to downstream sites in five

108   low-order streams in Northern Spain. The taxa richness of shredders, collector-gatherers and scrapers

109   was lower in the streams downflow the dams than in the upflow streams. The Shannon diversity of

110   the entire macroinvertebrate assemblage, EPT (Ephemeroptera, Plecoptera, Trichoptera) richness and

111   total macroinvertebrate density were all lower downstream (Martínez et al., 2013). These differences

112   were likely related to the fact that freshwater systems were affected by multiple stressors and

113   macroinvertebrates might be affected differently by these stressors. Ward (1995) reported that river

114   regulation could reduce the biodiversity level due to the reduced transport of organic matter. Other

115   studies showed that filter-feeders, such as Simuliidae (Diptera), and collector-gatherers were often

116   abundant near dams with constant baseflow conditions where the availability of FPOM was larger

117   (Jones, 2013). Due to the variability in the response of freshwater systems to the same stressors in

118   different biogeographical areas, it is useful to develop specific studies for each area.

119   Alpine streams are characterized by low primary production because of the high water

120   velocity, low solar incidence, high bed instability, all of which limit the growth of phytoplankton,

121   macroalgae, bryophytes and angiosperms (Maiolini & Bruno, 2008; Bo et al., 2015). In these

122   conditions, the ecosystem is heterotrophic, and the food web is sustained by organic matter coming

123   from the riparian zone, such as leaf litter. Leaf litter breakdown, which involves the release of soluble

124   compounds the decomposition by microorganisms, and the feeding by benthic macroinvertebrates, is

125   a key process linking nutrient cycling, energy transfer and trophic interactions (McArthur & Barnes,

126   1988). For this reason, the measurement of leaf litter breakdown is a useful tool in alpine and pre-

127   alpine stream assessments as it links the characteristics of riparian vegetation with the activity of

128   microorganisms and invertebrates (Schmera et al., 2017). Moreover, leaf litter breakdown is affected

129   by natural and human-induced variations of a wide range of environmental factors. In the last twenty

130   years, many stream surveys (i.e. Danger & Robson, 2004; Graça et al., 2015) and experiments used

131   leaf breakdown rate as a direct measure of ecosystem functioning. Many of those studies focused on

132   the degradation of leaves abscised during the Autumn (McArthur & Barnes, 1988; Albariño &

133   Balseiro, 2002) using artificial leaf bags (Braioni et al., 2001). In Summer most of the leaves fallen

134   in the streams during Autumn have already been degraded (Slade & Riutta, 2012). However, some

135   leaf input occurs all year-round, in particular beech leaves are always abundant in the ephemeral

136   tributaries that are dry for most of the year. Leaves entering streams during Summer months, when

137   the allochthonous matter is scarce, may provide important energetic resources for lotic organisms.

138   Despite its importance, this source of organic matter is less studied than others (Maloney & Lamberti,

139   1995).

140   Aquatic ecosystems are vulnerable to climate change due to the close links among climate,

141   water availability, biological communities, and physical and chemical properties of stream water

142   (Null et al., 2013; Hotaling et al., 2017). A continuously heating atmosphere can absorb more water

143   vapor and can therefore offer a greater potential for heavy rainfall (Allan, 2012). Furthermore, due to

144   the increase of temperature, rainfall will occur in the form of rain rather than of snow, especially in

Spring and Autumn in the alpine and pre-alpine areas and in Summer at higher altitudes, so the frequency and intensity of medium and large flood events is expected to increase, as well as the occurring of floods in Spring and late Autumn (Viganò et al., 2015). At the same time, due to the increase of hot and dry Summer periods, low flow periods will occur more frequently, particularly at the end of Summer (Piano et al., 2019). In this scenario the increasing temperature in the atmosphere could lead to an increase in water temperature that could modify the ecological dynamics (Hette-Tronquart et al., 2013; Doretto et al., 2020) and increase the risks of pathogen transmission, especially among fish (Carraro et al., 2017). It is therefore important to assess the influence of high altitude reservoirs on the structure and function of alpine lotic ecosystems, in order to assess and disentangle the effects of different water management actions and climate change scenarios.

The present study used detailed monitoring to assess the influence of high altitude reservoirs on riverine ecosystems focusing on the alterations of: i) the macroinvertebrate communities; ii) the organic matter breakdown processes; iii) the thermal regime.

For the macroinvertebrate communities we tested the following hypotheses: (HM_1) there are structural differences among the communities inhabiting the pristine stretch and the stretch altered by high altitude dams while, downflow the confluence, the communities show an intermediate composition; (HM_2) there are families associated with the three considered stream stretches, representative of different alterations.

For the organic matter breakdown processes the tested hypotheses were: (HO_1) the Summer breakdown rates decrease with increasing percentage of catchment drained by high altitude reservoirs, due to the less intense mechanical degradation; (HO_2) coarse mesh leaf bags (accessible to invertebrates) show higher degradation rates than fine mesh bags (excluding invertebrates); (HO_3) both fallen and manually detached leaves are used as food sources for macroinvertebrates.

In the end, for the water thermal regime the tested hypotheses were: (HT_1) water temperature in the pristine stretch is higher than in the stretch altered by high altitude dams in Summer and lower in Winter while it is intermediate in the stretch downflow the confluence; (HT_2) in Summer daily

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water temperature variation in the pristine stretch is higher than in the altered stretch; (HT_3) daily water temperature variations below the confluence are bigger in the site affected by the run of the river power plant and such variations are similar to the ones observed in the pristine stretch.

Material and Methods

Study area

The study was carried out in the alpine valleys of Goglio stream in northern Italy (Figure 1). Sanguigno is the main left tributary of Goglio. The two streams were selected because they mainly differ for the presence of high altitude reservoirs. Goglio is characterized by the presence of five high altitude reservoirs that regulate the flow regime (sites G1 and G2, upper Goglio), while the flow regime of Sanguigno is considered pristine (sites S1 and S2). That's why in this study Sanguigno was used as the reference system. High-reservoirs are used only for hydro-electrical purposes, so they release only a minimum environmental flow into the Goglio stream. The flow discharge in Goglio is stable during the year, apart from the flood events caused by abundant precipitations. There are some small tributaries of Goglio that are not influenced by the high altitude reservoirs but all of them flow into Goglio downstream G1 site. Other anthropogenic activities in both watersheds are restricted to hikers and free-ranging livestock grazing, which makes them good reference systems. Downflow the confluence of Goglio and Sanguigno (sites G3, G4 and G5, lower Goglio) the anthropogenic activities in the watershed become more important with the presence of urban settlements and Run Of River hydroelectric power plants (ROR).
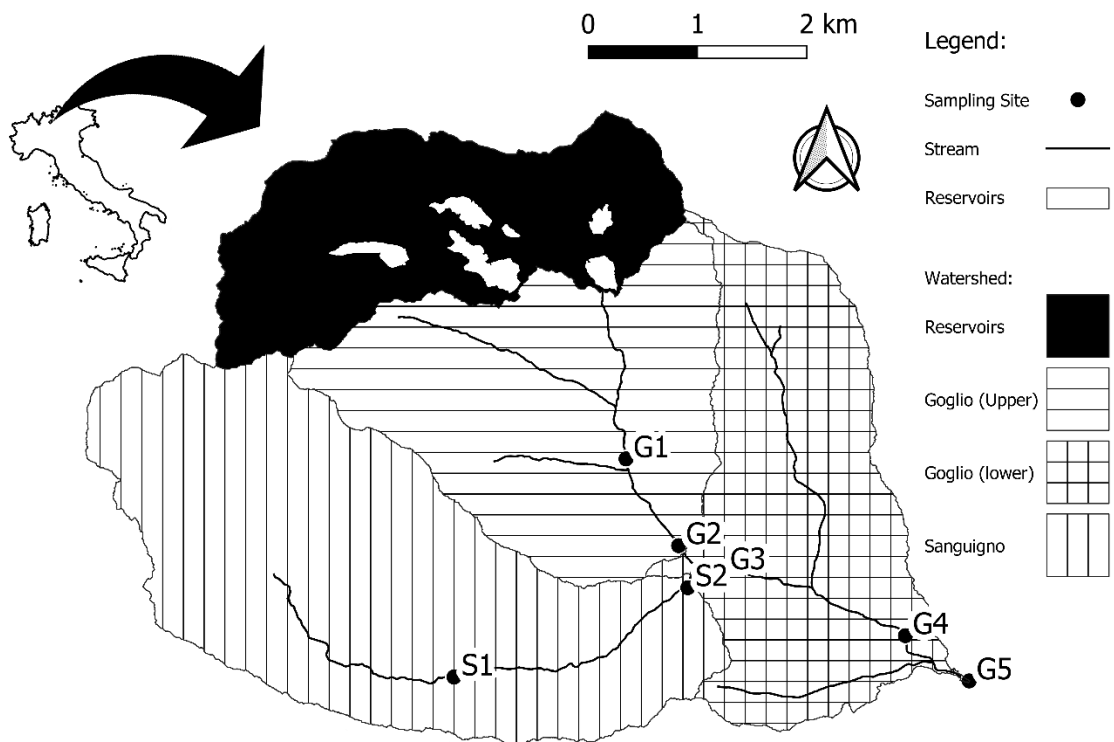
Fig. 1 Map of the study area with the location of sampling sites (G1, G2, G3, G4, G5, S1 and S2) and the representation of sub-watershed for Goglio (G) and Sanguigno (S) streams with the indication of the watershed area drained by the high altitude reservoirs.

All the studied sites are located below the tree line, with an elevation ranging between 600 and 1,400 m above sea level (a.s.l.). Tab.1 summarizes geographical information and watershed characteristics for each of them.

Tab. 1 Geographical information, main channel and watershed characteristics for each studied site. For each stream stretch, the main anthropic pressures are reported.

Stream stretch	Upper Goglio		Sanguigno		Lower Goglio		
Main impact sources	High altitude dams		Null		High altitude dams Run of river plant Urban settlements		
Site	G1	G2	S1	S2	G3	G4	G5
Altitude (m a.s.l.)	1,128	977	1,395	979	932	718	633
Distance from source (m)	1,540	2,620	4,290	6,970	3,140	5,020	5,850
Stream width (m)	6.3	7.1	7.2	2.4	4.6	6.7	16.7
Stream slope %	53.4	14.0	16.4	15.5	8.7	11.4	10.2
Watershed area (km^2)	8.1	13.6	7.7	11.5	25.4	31.0	32.2
Residual basin (%)	26.9	56.2	100.0	100.0	76.4	80.7	81.4

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Natural (%)	95.4	95.2	100.0	98.3	79.5	73.9	83.0
Agricultural (%)	4.5	4.7	0.0	0.7	16.0	22.6	9.1
Urban (%)	0.0	0.2	0.0	0.0	4.6	3.6	7.9

199   Sanguigno stream originates at 2,200 m a.s.l.. For a first stretch, it flows on a territory mainly

200   covered by natural meadows. Then it flows for a short stretch (2-3 km) on a flat plain with poor

201   riparian vegetation characterized by alders, pines and other firs. Lastly, due to the sudden increase of

202   the slope, it presents waterfalls and pools till the point of confluence with Goglio at an altitude of 940

203   meters after 7 kilometers. This portion of the territory is cover by a mixed forest (mostly beeches but

204   also alders, firs, birches and ash trees).

205   Goglio stream originates at 1,950 m a.s.l. from artificial reservoirs: Cernello, Aviasco,

206   Campelli, Sucotto and Nero. At that altitude, due to the high slope, the stream presents waterfalls and

207   pools and flows across a territory characterized by lithoid outcrops and permanent meadows. With

208   decreasing altitude, a mixed forest (mostly beeches but also alders, firs and ash trees) appears. Goglio

209   flows in the Serio River 3 kilometers after the confluence with Sanguigno. The substratum varies

210   from sand to bedrock including all the intermediate substrate classes without relevant differences

211   among stream reaches. Coarse classes are more represented while instream vegetation is made of

212   bryophytes.

213   *Physical, chemical and GIS analyses*

214   In all the sampling sites, dissolved oxygen, electric conductivity (COND) and percent

215   saturation of dissolved oxygen (DO_PERC) were measured on field by a multiparametric probe

216   (HACH-Lange OD-30). Water velocity and water depth were measured by a HACH-FH950 portable

217   flowmeter. Water velocity was measured at 40% of the depth in order to obtain the mean velocity of

218   the water column in the sampling point. Cross sectional profiles were then used to calculate

219   discharges. Analyses of pH, Chemical Oxygen Demand (COD), total phosphorus (P_TOT), total

220   nitrogen (N_TOT) and ammonium (N_NH$_4$) in the water were carried out in laboratory according to

221   Standard Methods (APHA/AWWA/WEF, 2012).

222   QGIS 2.18.9 (QGIS Development Team, 2018) software was used to determine the relative

223   proportion of land use within the study area, while GRASS GIS 7.4.1 (GRASS Development Team,

224   2018) and the "watershed tool" were used to determine the watershed of each basin. Basin maps

225   were intersected with the DUSAF 4.0 map (land cover dataset updated at 2012) of the Lombardy

226   region.

227   In order to get a continuous measurement of water temperature, we placed data loggers

228   (iButton) at each site with a measurement range from -5 ° C to + 26 ° C and an accuracy of 0.125

229   °C, and loggers with a measurement range from -40 ° C to + 85 ° C and an accuracy of 0.0625 °C.

230   Sensors were set with 1-Wire software in order to get data every 10, 30 and 60 minutes. A one-minute

231   resolution dataset was obtained by the linear interpolation of temperature data within R software.

232   *Macroinvertebrate community assessment*

233   Macroinvertebrates were collected five times between Summer 2017 and Winter 2018 with a

234   Surber net (0.10 m^2, 500 µm mesh) by a standardized multi-habitat sampling procedure (Barbour et

235   al., 1999; AQEM Consortium, 2002; Hering et al., 2004). Ten replicated samples were collected from

236   different microhabitats according to their relative coverage in the examined site (only those with at

237   least 10% of coverage were considered) and then merged in the field and stored in 96% ethanol. In

238   the laboratory, taxa were identified at family level according to standard keys and the abundance of

239   individuals of each taxon was reported.

240   *Leaf bag experiments set-up*

241   Two separate experiments were carried out, one in Summer and one in Winter, in order to

242   evaluate if the degradation of CPOM during Summer was influenced by the presence of high altitude

243   dams and if the degradation rates of fallen and detached leaves during Winter were different. We used

244   fallen beech leaves to prepare leaf bags during the Summer experiment, while we used both fallen

245   and manually detached beech leaves during the Winter experiment. A total of 126 artificial leaf bags

246   were placed in the study sites in Summer and 24 leaf bags in Winter. The main focus of the Summer

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experiment was to assess the CPOM degradation along a gradient of alteration due to high altitude reservoirs (residual basin of the sampling sites) while the Winter experiment aimed to compare the degradation rate of fallen and manually detached leaves that are more commonly used in leaf bag studies (Boulton & Boon, 1991). The leaf bags used in the Summer experiment were prepared by using fallen beech leaves (*Fagus sylvatica*) collected in the study area. Only intact leaves were used for both the experiments. Leaves were brought to the laboratory shortly after collection and were dried at room temperature for 30 days. Five replicates were also dried for 24 h at 105 °C to obtain standardized moisture contents (Cabrini et al., 2013). Afterwards, we used the weight reduction obtained for leaves dried at 105 °C to calculate the dry weight of all the prepared leaf bags.

Leaves were placed in two kinds of synthetic net bags 20×20 cm: one with 0.5 mm and the other with 5 mm mesh sizes (Nanda et al., 2009; Wang et al., 2010). Each net bag was filled with about 3 g of leaves and identified by a unique number. In July we placed in the riverbeds a total of 126 leaf bags following this scheme: in all the sites we identified three habitats (riffle, pool and glide) and in each of them we placed 3 pairs of leaf bags (3 with coarse mesh + 3 with fine mesh) for a total of 18 bags per site (Table 2). For the Winter experiment we placed the leaf bags only in two sites: the first was a pristine one, while the second one was the most impacted by the high altitude reservoir (S2 and G1 respectively). In both sites, we placed in each habitat (riffle, pool and glide) one pair of bags made with fallen leaves (1 with coarse mesh + 1 with fine mesh) and one pair made with manually detached leaves (1 with coarse mesh + 1 with fine mesh) for a total of 12 bags per site (Table 2).

Tab. 2 Schematic representation of leaf bags positioning in the two experiments.

SUMMER - 7 SITES (Fallen leaves)		
Riffle 1 coarse + 1 fine	1 coarse + 1 fine	1 coarse + 1 fine
Pool 1 coarse + 1 fine	1 coarse + 1 fine	1 coarse + 1 fine
Glide 1 coarse + 1 fine	1 coarse + 1 fine	1 coarse + 1 fine
WINTER - 2 SITES		
Riffle 1 coarse + 1 fine (Fallen leaves)		1 coarse + 1 fine (Manually detached leaves)
Pool 1 coarse + 1 fine (Fallen leaves)		1 coarse + 1 fine (Manually detached leaves)
Glide 1 coarse + 1 fine (Fallen leaves)		1 coarse + 1 fine (Manually detached leaves)

268   All bags were tied with plastic strips to metal rods that were knocked into the sediment. Five

269   bags were brought to the laboratory and used to quantify the mass of leaves lost during

270   transport/manipulation operations. The Summer leaf bags were collected after 40, 62 and 98 days.

271   In each collection, six leaf bags were removed from each site, one pair for each habitat (riffle, pool

272   and glide), and shortly after transferred to the laboratory. All the Winter bags were collected after

273   140 days. In the laboratory, the leaves were washed and then dried for 24 h at 105 °C in order to

274   determine the remaining mass (Spänhoff et al., 2007). The mass loss was calculated by the

275   difference between the initial and the final dry mass of leaves and reported as % of initial mass.

276   *Data analyses*

277   Principal Component Analysis (PCA) was used to evaluate the correlations among physical,

278   chemical and geographical variables in all the samples of the dataset (Hotelling, 1933). PCA was

279   conducted using the function *prcomp* from the "factoextra" package (Kassambara & Mundt, 2017).

280   Non-Metric Multidimensional Scaling (NMDS), a gradient analysis approach based on a

281   distance or dissimilarity matrix, was used to visualize differences in the taxonomic structure of the

282   macroinvertebrate community among the studied stream stretches (Upper Goglio, Lower Goglio,

283   Sanguigno) defined *a priori* (Clarke & Ainsworth, 1993) based on anthropogenic impacts. NMDS

284   is an iterative procedure including several steps, using the function *metaMDS* from the "vegan"

285   package (Oksanen et al., 2017) in R project software (R Core Team, 2019). It is based on Bray-

286   Curtis dissimilarity distance evaluated using raw macroinvertebrate abundances, a non-Euclidean

287   distance used to quantify the compositional dissimilarity between two different samples.

288   Differences in the composition of communities among stream stretches were quantitatively

289   explored, as well as temporal controls, testing also the additive effects of "sampling period" within

290   a permutational multivariate analysis of variance (PERMANOVA) via the *adonis* function within

291   the "vegan" package. To determine the most sensitive taxa to the different groups of impact,

292   similarity percentages (SIMPER) analysis was implemented using the stream stretch as a primary

293 factor and the *simper* function. Its significance was tested using 999 permutations within the

294 "vegan" software package and abundance data were $\log_{10}(x+1)$ transformed.

295   As many of the leaf bags used for the Summer experiment were not recovered during the three

296 surveys (31% after 40 days, 45% after 62 days and 60% after 90 days) because they had drifted

297 downstream, a binary variable with value 0 or 1 for non-recovered and recovered leaf bags,

298 respectively, was associated to the data to reflect the probability of recovery. The probability of

299 recovery of the leaf bags were examined using "generalized linear mixed-effect model" (GLMM)

300 modelled with a Binomial distribution, which was performed by the *glmer* function in the "lme4"

301 package (Bates et al., 2015). The mesh size of each bag, the stay-in-place time and the percent residual

302 basin were used as fixed effects while sites and habitats within sites were used as random effects on

303 intercept accounting for any lack of spatial independence between samples. The *dispersion_glmer*

304 function within the "blmeco" package (Korner-Nievergelt et al., 2015) was used to ensure that

305 GLMM was not under- or overdispersed. To validate the assumptions of GLMM, simulated residuals

306 were plotted using the *simulateResiduals* function in the "DHARMa" package (Hartig, 2019).

307 Remaining masses from both experiments were tested using linear mixed-effect models (LMM) by

308 the *lmer* function in the "lme4" package. The percentage of remaining mass was square-root

309 transformed to normalize residuals and equalize variances. Mesh size, stay-in-place time of and

310 percent residual basin were used as fixed effects while sites and habitats within sites were used as

311 random effects on intercept for the Summer experiment. For the Winter experiment only two sites

312 were monitored, representative of Sanguigno and upper Goglio. The sites, the mesh size and the leaf

313 type were considered as fixed effects while habitats were used as random effects on intercept.

314   The *dredge* function within the "MuMIn" package (Bartoń, 2019) was then used to derive the

315 optimal set of fixed effects tested within each LMM and GLMM. This function fits different models

316 comprising all the combinations of fixed effects and ranks them by the Akaike Information Criterion

317 corrected for small sample size (AICc). The most parsimonious model within 2 AICc units of the

318 model exhibiting the lowest AICc value was selected as the "optimal" model. The explanatory power

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319   of the statistical models was derived from marginal pseudo r-squared values (r^2m; see Nakagawa &

320   Schielzeth, 2013), which quantify the variance explained by the fixed effects and were obtained using

321   the *rsquared.glmm* function in MuMIn. The significance of each optimal model was obtained via

322   likelihood ratio tests (White et al., 2018).

323   Finally, we used analysis of variance (ANOVA) to test for differences in mean daily water

324   temperatures and daily temperature variations among the stream stretches considering the Summer

325   (June, July and August) and Winter (December, January and February) months. One-way ANOVA

326   with Tukey's test for pairwise comparisons was also used to analyze the differences among stream

327   stretches and seasons. We selected one site for each stream stretch for temperature comparisons,

328   specifically, G2 as representative of Upper Goglio, S2 as representative of Sanguigno, G3 as

329   representative of Lower Goglio without the effect of ROR plant (G3 is located only 200 meters

330   downstream the confluence between Upper Goglio and Sanguigno and the water diversion) and G4

331   as representative of Lower Goglio subjected to the effect of ROR plant (G4 is located almost 2

332   kilometers downstream G3).

333   All statistical analyses were performed using R project software (R Core Team, 2019).

334   **Results**

335   *Environmental variables*

336   The results of PCA are shown in Figure 2 for variables related to physical and chemical water

337   characteristics and land use (n=35). The total variance explained by the first two axes was 49.6%:

338   34.8% by the first principal component and 14.8% by the second one. The first principal component

339   was positively correlated with watershed area, urban and agriculture coverage and water total nitrogen

340   and negatively with natural land use and elevation. The second principal component was positively

341   correlated with water conductivity and COD and negatively with percent residual basin, water

342   temperature, flow and oxygen saturation. The plots of the two first principal components show high

343 orthogonality between the effects of high altitude reservoirs, represented by the residual basin (2nd

344 PC) and the effects of watershed area and land use modifications (1st PC).

345 All the studied sites were characterized by a level of oxygenation close to saturation (97.8 ±

346 5.5 Mean ± SD) in all seasons. Nutrient concentrations were low (< 0.7 mg/L) in all Sanguigno and

347 Goglio sites; the only parameter that showed a clear spatial pattern was the total nitrogen whose

348 concentration increased progressively up to a maximum (1.1 ± 0.2 mg/L) in G4, then decreased

349 considerably (0.6 ± 0.2 mg/L). The influence of high altitude reservoirs on Goglio basin was already

350 reduced in G2. In fact, only 43.8% of the basin area is drained by the reservoirs. In G3 the influence

351 of the reservoirs became marginal (23.6%).
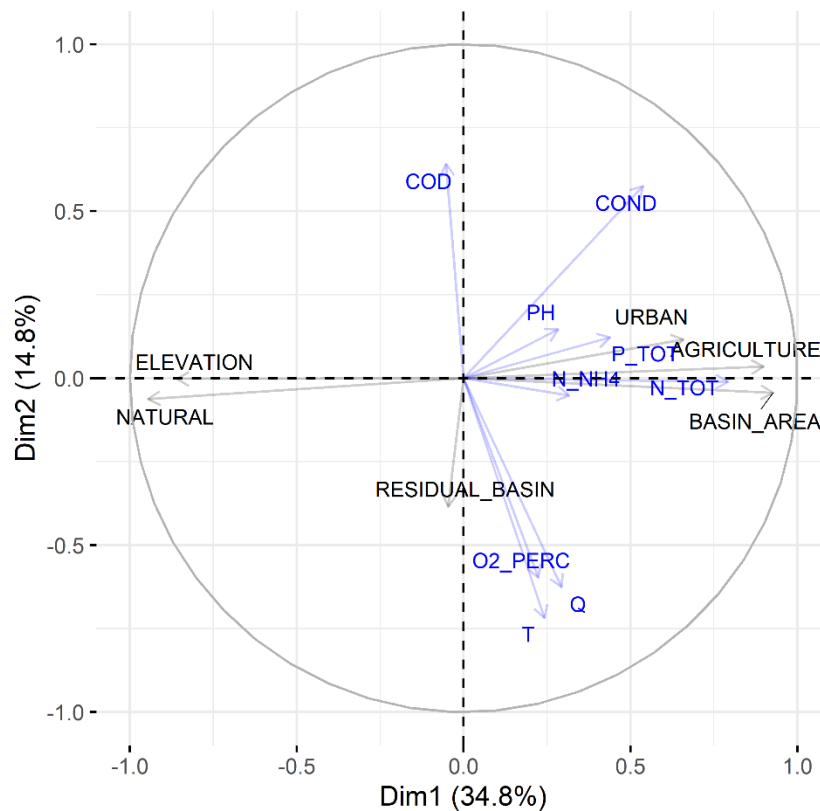
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353 **Fig. 2** Principal Component Analysis plot of the watershed characteristics (black text) of the seven

354 sampling sites and of the water chemical characteristics (blue text) during the five samplings. Vector length

355 and direction is proportional to its relationship with each axis.

356 *Similarity among macroinvertebrate communities*

357   The NMDS plot of macroinvertebrate communities (Figure 3) shows that the community

358   inhabiting upper Goglio clustered separately from the communities inhabiting Sanguigno and lower

359   Goglio supporting our HM_1 hypothesis. Such results were confirmed by PERMANOVA that

360   highlighted significant differences among the communities of the three stream stretches ($F = 1.64$, *p-*

361   value $=0.018$). SIMPER analysis highlighted that the densities of some macroinvertebrate families

362   were significantly different in the three cases, and that differences between regulated (Upper Goglio)

363   and non-regulated (Sanguigno) systems (Table 3) were particularly sharp, confirming our HM_2

364   hypothesis. Eleven macroinvertebrate families, belonging to several taxonomic orders, displayed

365   greater affinities for the regulated sites in upper Goglio than for the pristine sites in Sanguigno while

366   no family showed the opposite pattern (Table 3). The comparison between upper Goglio and lower

367   Goglio showed that some families had greater affinity for upper Goglio, that is affected only by

368   hydrological alterations, but none of them had greater affinity for lower Goglio, that is affected by

369   both hydrological and chemical alterations. Five families showed significantly different distributions

370   between Sanguigno and lower Goglio: Perlidae and Heptageniidae were more abundant in Sanguigno

371   while Leuctridae, Limoniidae and Simuliidae had greater densities in lower Goglio.
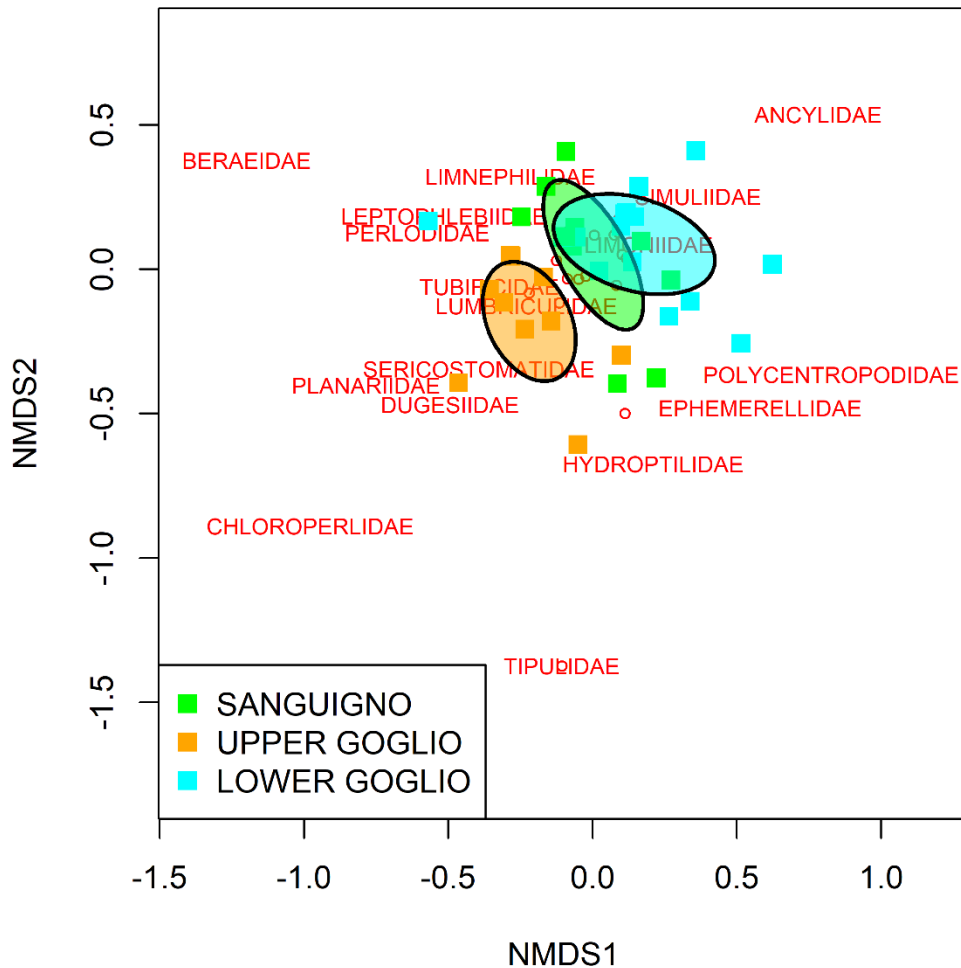
Fig. 3 Nonmetric multidimensional scaling (NMDS) ordination plot for aquatic macroinvertebrate communities where the a-priori identified stream stretches are colored. Green denotes the communities that belong to Sanguigno stream (n = 10), orange and cyan denote respectively the communities that belong to upper (n = 10) and lower stretches of Goglio stream (n = 15). Shaded ellipses represent the 95% confidence interval surrounding the centroid of each stream stretch in the ordination space. Each square represents the overall macroinvertebrate community at each sampling. Macroinvertebrate families are positioned in the ordination space with red uppercase labels, as weighted averages. 3D stress = 0.18.

381 **Tab. 3** SIMPER analysis of univariate responses of macroinvertebrates to environmental alterations:

382 . = $p \leq 0.01$; * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$; NS = non-significant. Code for contrast are (UP)

383 Upper Goglio – High altitude dams, (SA) Sanguigno – No impact, (LG) Lower Goglio – High altitude

384 dams/Run of river plant/Urban settlements. Families that show significant differences for at least one

385 contrast are reported in bold. Average abundances for each sampled family are reported as $\log_{10}(x+1)$

386 transformed values.

| Family | Contrast | | | Average | Average | Average |
	UG-SA	UG-LG	LG-SA	UG	SA	LG
Chloroperlidae	*	NS	NS	0.14	0.00	0.00
Leuctridae	NS	NS	*	1.26	1.36	1.77
Perlidae	NS	NS	*	0.66	0.61	0.33
Perlodidae	NS	.	NS	0.46	0.16	0.14
Nemouridae	NS	NS	NS	1.83	1.19	1.29
Baetidae	NS	NS	NS	2.17	2.18	2.18
Ephemerellidae	NS	NS	NS	0.14	0.40	0.34
Heptageniidae	NS	NS	.	1.99	2.27	1.85
Leptophlebiidae	NS	NS	NS	0.34	0.15	0.11
Beraeidae	NS	NS	NS	0.00	0.00	0.05
Hydropsychidae	NS	NS	NS	1.61	1.26	1.23
Hydroptilidae	**	NS	NS	0.03	0.00	0.00
Limnephilidae	NS	NS	NS	0.37	0.45	0.35
Odontoceridae	*	NS	NS	0.03	0.00	0.00
Philipotamidae	NS	NS	NS	0.59	0.48	0.59
Polycentropodidae	NS	NS	NS	0.00	0.00	0.11
Rhyacophilidae	*	NS	NS	1.18	0.64	0.78
Sericostomatidae	***	**	NS	0.41	0.00	0.40
Elmidae	NS	NS	NS	1.81	1.25	1.30
Hydraenidae	NS	NS	NS	0.94	0.57	0.70
Athericidae	NS	*	NS	1.22	0.69	0.59
Blephariceridae	NS	NS	NS	0.24	0.28	0.28
Chironomidae	NS	NS	NS	1.43	1.04	1.53
Limoniidae	NS	NS	*	0.57	0.55	1.01
Psychodidae	NS	NS	NS	0.06	0.00	0.13
Simuliidae	NS	NS	*	0.58	0.44	1.22
Tipulidae	*	NS	NS	0.03	0.00	0.00
Scirtidae	**	*	NS	0.74	0.14	0.21
Ancylidae	NS	NS	NS	0.00	0.00	0.15
Planariidae	***	**	NS	0.58	0.00	0.00
Dugesiidae	***	NS	NS	0.03	0.00	0.00
Tubificidae	*	NS	NS	0.05	0.00	0.00
Lumbricidae	NS	NS	NS	0.22	0.14	0.12
Lumbriculidae	**	*	NS	0.78	0.04	0.31

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Leaf bags results

389   The percent residual basin was included in the optimal model developed for the probability of

390   recovery for the leaf bags of the Summer experiment (n = 123) as well as the mesh size and the stay-

391   in-place time (Table 4). The model partially supported our HO_1 hypothesis. The probability of

392   recovery decreased with increasing stay-in-place time and percent residual basin; moreover, coarse

393   mesh bags always showed a lower probability of recovery (Figure 4A) fully confirming our HO_2

394   hypothesis. The remaining mass in the leaf bags of the Summer experiment (n = 69) was significantly

395   associated with the stay-in-place time and the mesh size, always lower in coarse mesh bags than in

396   fine mesh ones, and decreased from a maximum of 96.9% in the first collection to a minimum of 5

397   % in the last one (Figure 4B). Those results suggest that our HO_1 hypothesis should be partially

398   revised: where the percent catchment area drained by high altitude reservoirs was larger the transport

399   of CPOM increased but the breakdown rate did not. The remaining mass in the leaf bags of the Winter

400   experiment (n = 24) was significantly associated with the leaf type ("Fallen" or "Manually detached")

401   and the mesh size. The remaining mass was lower for bags filled with falling leaves than for bags

402   filled with leaves collected from the litter; this effect was greater in the coarse mesh bags than in the

403   fine mesh ones (Figure 4C), confirming that the access of macroinvertebrate is a significant factor in

404   the degradation rates for the two type of leaves (HO_3).

405   **Tab. 4** GLMM and LMM outputs examining the response of the probability of recovery and the

406   percent remaining mass to the mesh size, the stay-in-place time and the residual basin for the summer

407   experiment. LMM outputs examining the response of remaining mass to the mesh size and the leaf type for

408   the winter experiment.

Experiment	Response	Fixed terms	X^2	p-value	r^2m
Summer	Probability of recovery (%)	Mesh + Time + Residual Basin	25.79	<0.001	0.31
	Remaining mass (%)	Mesh + Time	19.91	<0.001	0.19
Winter	Remaining mass (%)	Mesh + Leaf Type	20.81	<0.001	0.56
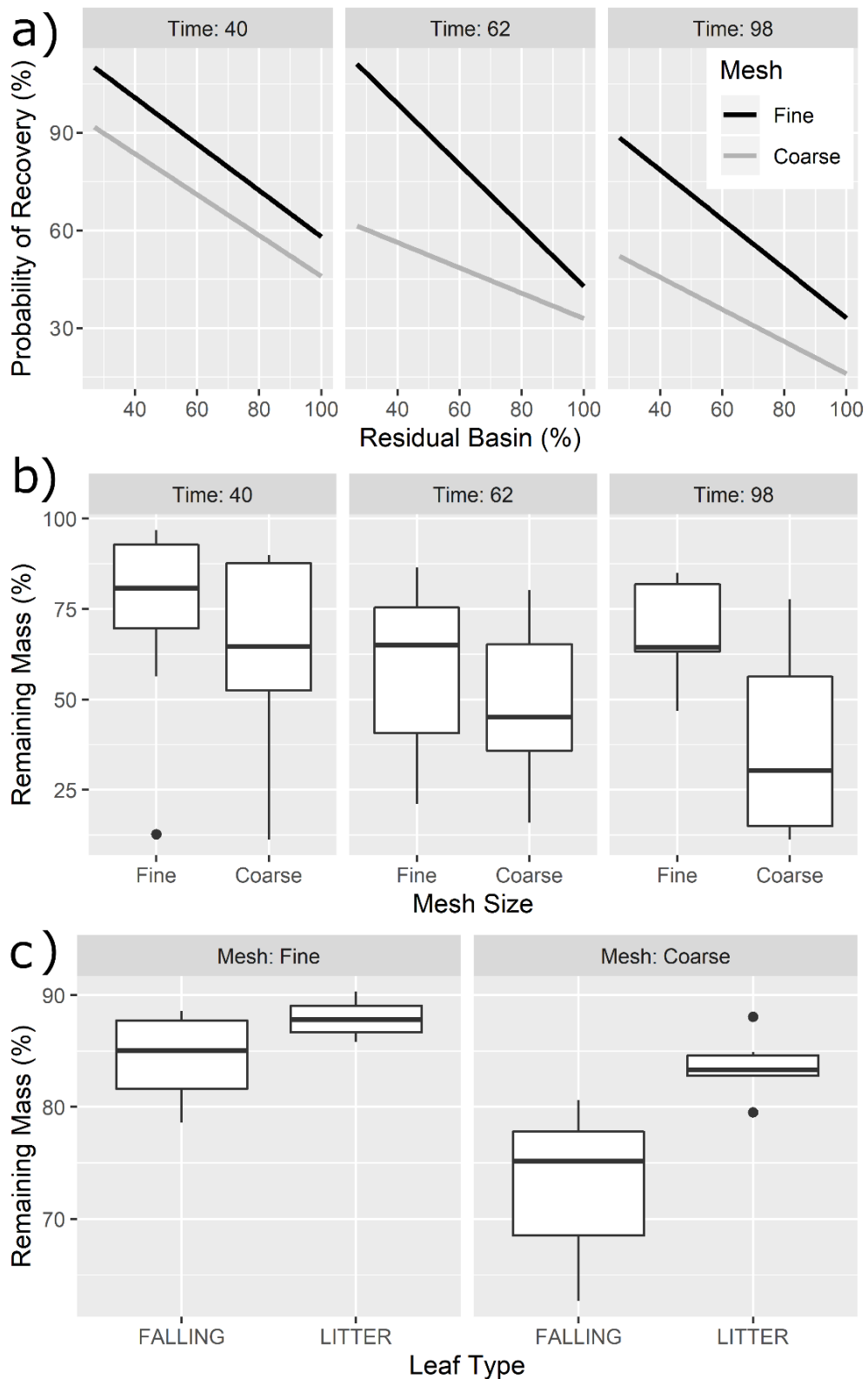
Fig. 4 Graphical representation of the results for the two leaf bags experiments. a) Probability of recovery for the leaf bags of the Summer experiment as a function of residual basin, mesh size and stay-in-place time (5 sites n = 126). b) Remaining mass for the leaf bags of the Summer experiment as a function of mesh size and stay-in-place time (5 sites, n = 69). c) Remaining mass for the leaf bags of the Winter

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414 experiment as a function of mesh size and leaf type (2 sites, n = 24). All the plots were created using the raw

415 data.

Analysis of water temperature

417 Water temperature was measured continuously in order to detect temperature changes at very

418 short time scale (<1 hour). As it can be seen in Figure 5, upper Goglio was colder in Summer and

419 warmer in Winter than Sanguigno. One-way ANOVA (p < 0.001) and Tukey's test (always p < 0.001)

420 show a significant difference in the mean daily temperature (letters **c** and **d** and **f** and **g** in Figure 6A)

421 between the two sites, both in Summer and Winter. Moreover lower Goglio had an intermediate mean

422 daily water temperature compared to upper Goglio and Sanguigno both in Summer and Winter as

423 highlighted by Tukey's comparisons (letters **a-b** compared with **c** and **d** for Summer and letter **e**

424 compared to **f** and **g** for Winter, Figure 6A) fully supporting our HT_1 hypothesis.

425 One-way ANOVA, (p < 0.001) and Tukey's test (p < 0.001) highlight a significant difference

426 in the daily temperature variation in Summer between Sanguigno and upper Goglio, with the first one

427 showing larger variations (letters **b** and **c** in Figure 6B) supporting our HT_2 hypothesis. No difference

428 in the daily temperature variation during Winter was observed among the three sites.

429 Daily water temperature variations during Summer months in lower Goglio were larger than

430 in the upper Goglio, especially in the site affected by the ROR power plant where the variations were

431 similar to the ones observed in Sanguigno. This supports our HT_3 hypothesis (p < 0.001, letters **a** and

432 **b** compared to **c**, Figure 6B).

433 The seasonal variability in Sanguigno and in lower Goglio was higher than in upper Goglio.

434 In Winter the average temperature was ≈0°C in Sanguigno and ≈2.6°C in lower Goglio while the

435 average temperature of upper Goglio was ≈4°C; in Summer the average temperature was ≈13°C in

436 Sanguigno and ≈12.6°C in lower Goglio while the average temperature of upper Goglio was ≈10.3°C.

437 Considering the extremes of the water temperature range the data show that during Winter Sanguigno

438 reached also negative values (-0.72 °C in site SAN1), lower Goglio got to 0.89 °C (in site G5) while

439 upper Goglio achieved only 1.25 °C (in site G1); in Summer Sanguigno reached 17.05 °C (in site

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440   SAN2), lower Goglio got to 15.09 °C (in site G4) while upper Goglio achieved only 12.30 °C (in site

441   G2). Obviously, the Spring and Autumn data of water temperature had intermediate values (either in

442   the average and in the extremes) compared to the rest of the year.
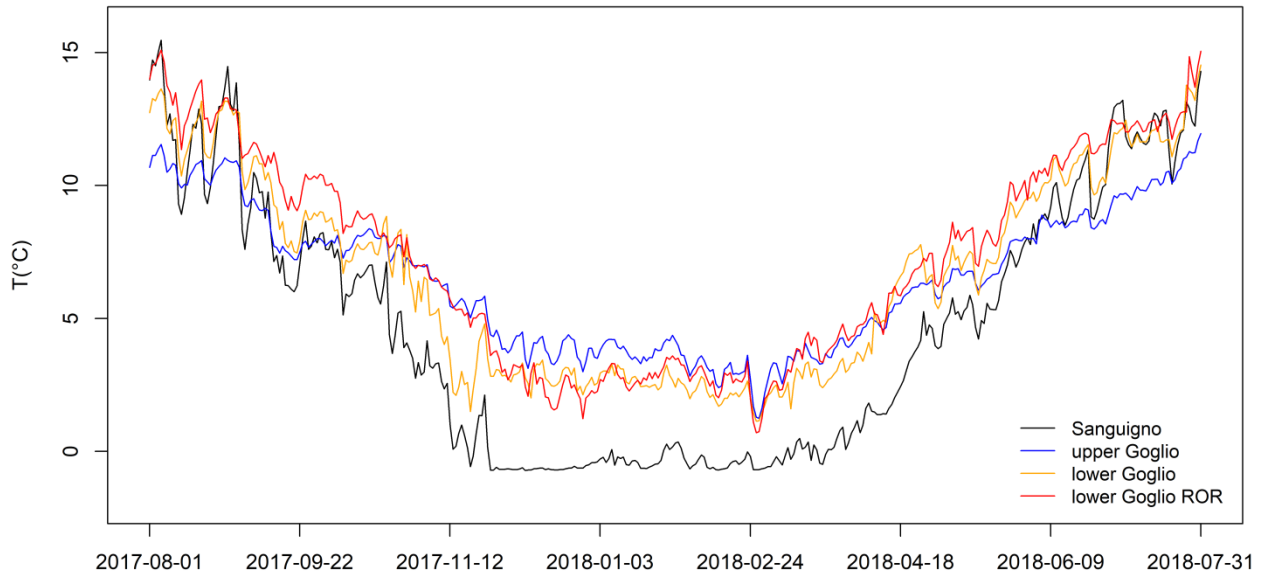
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444   **Fig. 5** Mean daily water temperature of Sanguigno (site SAN1 – pristine), upper Goglio (site G1 –

445   profoundly altered by high altitude dams), lower Goglio (site G3 – partially altered by high altitude dams)

446   and lower Goglio with ROR (site G4 – partially altered by high altitude dams and by a Run Of the River

447   power plant) in the period 01st August 2017 - 31st July 2018.
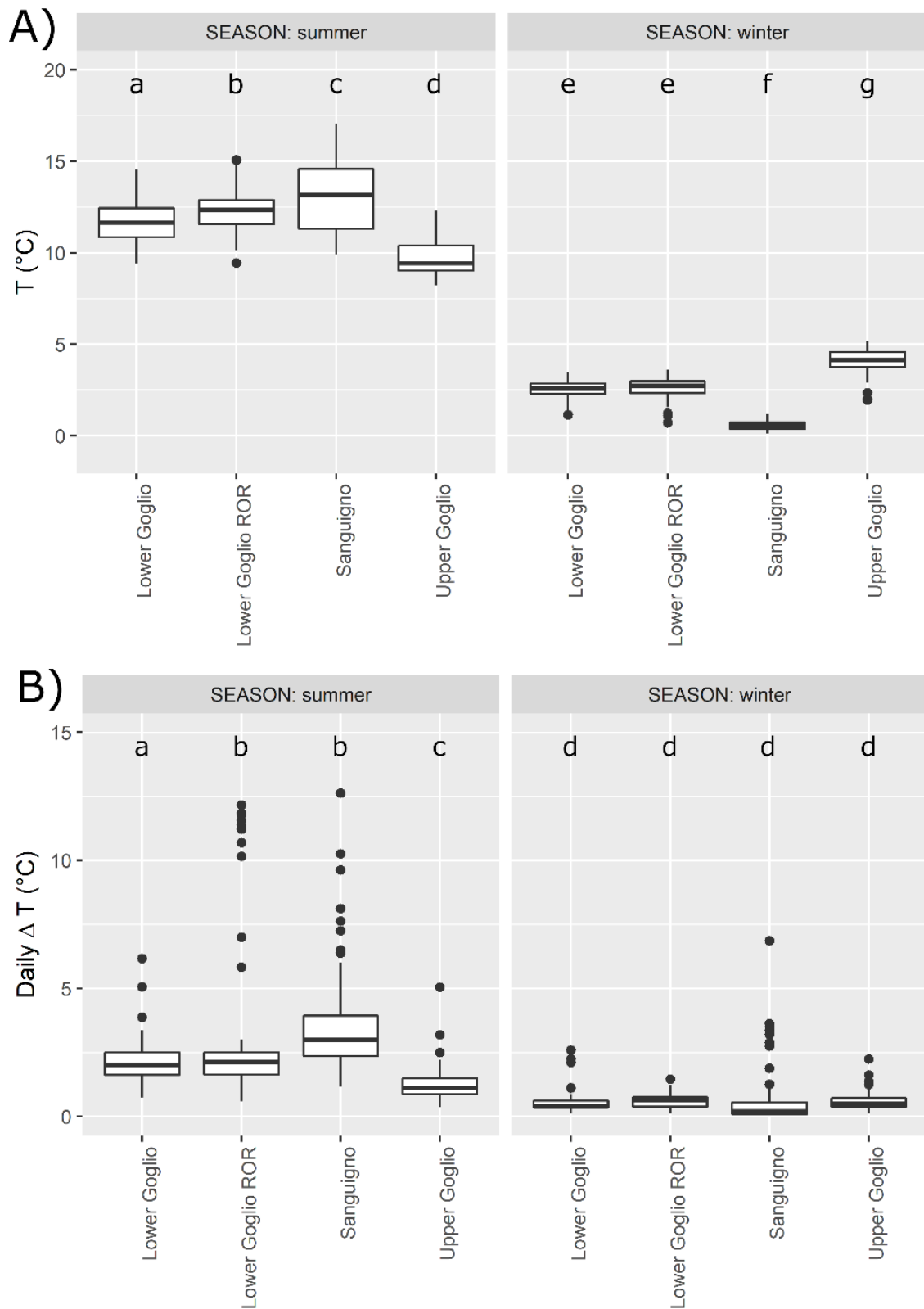
Fig. 6 Box plot presentation of mean daily temperatures (A) and daily temperature variations (B) grouped by stream stretch (Lower Goglio, Lower Goglio ROR, Sanguigno and Upper Goglio) and seasons (Summer and Winter). Different lowercase letters indicate significant differences among categories (Tukey's multiple-comparison test, $p < 0.001$).

Discussion

Environmental variables

The results of physical and chemical analyses show that the studied streams were characterized by a level of oxygenation close to saturation and by low concentrations of nutrients both in Winter and in Summer. The increase of the concentrations of total nitrogen and other parameters (P_TOT and N_NH$_4$) between G3 and G4 was due to the urban settlement of Valgoglio, while the decrease of nutrient concentrations in G5 was due to the dilution caused by the input of water coming from the high altitude reservoirs, which passes through the Aviasco Hydroelectric plant and is finally released by the ROR hydroelectric plant located upstream G5. The increase of total nitrogen and water flow between S1 and S2 was due to natural inputs. There is no evidence of the effects of the high altitude reservoirs on water chemistry and this is related to the very high location of the dams within the river network (Stanford & Ward, 2001) but also to the absence of other anthropogenic impact except for the urban settlement downflow the dam and the main tributary.

Macroinvertebrate community

The analysis of macroinvertebrate samples shows that the presence of high altitude reservoirs changed the structure of the macroinvertebrate community. Due to the decrease of the influence of the high altitude reservoirs, and to the confluence of Sanguigno, the communities inhabiting lower Goglio showed a high similarity with the one inhabiting Sanguigno, fully supporting our HM$_1$ hypothesis and in agreement with the predictions from the Serial Discontinuity Concept (Stanford & Ward, 2001). In fact, only the densities of five families differed significantly between them. An interesting finding that emerged from the survey is that 11 macroinvertebrate families, spanning across several taxonomic orders, displayed greater affinities for the regulated sites in upper Goglio than for the pristine sites in Sanguigno while no family showed the opposite pattern. Below high altitude reservoirs the flow regime was more stable and the flood events dampened, creating more favorable conditions for the establishment of some families that were not present in the pristine sites.

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478   The effects of stream regulation on mayflies is well documented in the literature (Brittain &

479   Saltveit, 1989; Mantel et al., 2010). Mantel et al. (2010) found an increase in some taxa (Baetidae,

480   Caenidae) and a decrease in others (Teloganodidae and Heptageniidae) below dams. Similar changes

481   were observed by Brittain & Saltveit (1989) who suggested that dietary and habitat were the reasons

482   for the observed patterns, as the increase of certain baetids below dams could be linked to greater

483   algal growth (as a food source and predation refuge), while the density of heptageniids that prefer

484   clean rocks for attachment might decrease below dams. Heptageniid abundances in our study

485   decreased in lower Goglio but not in upper Goglio compared to Sanguigno. This suggests that their

486   abundance was driven by pollution loads rather than by alterations of the flow and temperature

487   regimes. This result is consistent with their BMWP (Biological Monitoring Working Party, Armitage

488   et al., 1983) score (10) that classifies them as indicators of good water quality but seems to be less

489   congruent with the LIFE (Lotic-invertebrate Index for Flow Evaluation, Extence et al., 1999) score

490   (I: rapids) as Heptageniidae are also abundant in stretches with altered flow regimes.

491   Armitage (1978) found that Plecoptera, Trichoptera and Coleoptera were poorly represented

492   and occurred at low mean densities in a site situated just below the Cow Green dam (River Tees,

493   Upper Teesdale, England) while a few hundred meters below Coleoptera (Elmidae) were more

494   abundant. In our study, the response of Plecoptera was not uniform. Perlidae showed the same pattern

495   as Heptagenidaee, while Chloroperlidae were significantly more abundant in upper Goglio than in

496   Sanguigno, indicating their preference for a more stable hydrological regime even if they had a high

497   LIFE score (I: rapids). Moreover, many species of Plecoptera are cold-stenothermal (Fochetti, 2020)

498   and our results on water temperature clearly show that upper Goglio is, on average, colder than

499   Sanguigno suggesting that Plecoptera abundance was probably affected by the joint effect of

500   hydrological conditions and thermal regime. Leuctridae were significantly more abundant in lower

501   Goglio than in Sanguigno, indicating that their abundance was positively affected by moderate

502   anthropogenic impacts and water pollution. In contrast to Armitage's (1978) and Ward's (1995)

503   results that showed the negative effects of dams on both organic transport and biodiversity, we found

504   that some Trichoptera families (Hydroptilidae, Odontoceridae, Rhyacophilidae and

505   Sericostomatidae) were significantly more abundant in upper Goglio than in Sanguigno; the high

506   abundance of those families in hydrologically altered stretches had been already reported (Cortes et

507   al., 1998) and may be linked to the synergistic effect of larger food supply and more stable flow, as

508   suggested by Boon (1987). This hypothesis is corroborated by our leaf bags experiment: in upper

509   Goglio leaf bags were not transported by water flow, thus representing an important source of food

510   for shredders such as those trichopteran families. Coleoptera (Scirtidae) were more abundant in upper

511   Goglio than in Sanguigno and lower Goglio, suggesting their preference for a more stable

512   hydrological regime, in agreement with their LIFE score (IV: slow and standing waters) and the

513   results of Armitage (1978).

514   The increase of more ubiquitous macroinvertebrate orders including Diptera or Oligochaeta

515   are often reported downstream from impoundments (Ogbeibu & Oribhabor, 2002; Phillips et al.,

516   2016; Krajenbrink et al., 2019) and this is in agreement with our results as many families belonging

517   to those orders are significantly more abundant in upper Goglio than in Sanguigno. Conserving

518   natural landscapes is important not only to preserve species but also to have reference systems that

519   can be studied to understand the pristine state and functioning of ecosystems and correctly evaluate

520   the effect of alteration on other systems (Grill et al., 2019; Milner et al., 2019). This survey shows

521   that conditions in the upper Goglio supported the most diverse macroinvertebrate community

522   compared to other sites, in spite of being profoundly altered by high altitude reservoirs. Our analysis

523   did not identify any macroinvertebrate family impacted by high altitude dams due to the coarse

524   systematic resolution (i.e. family level): sub-family level identification of organisms may produce

525   different results in terms of impacts on community and specific genus/species responses (Hotaling

526   et al., 2017). Some authors reported that in high alpine streams the anthropogenic flow regulation

527   tended to ameliorate stream conditions resulting in higher diversity but this did not favour certain

528   species that are specialist in the harsh natural conditions (Füreder et al., 2000). Although further

529   work is required to address the impact of taxonomic resolution, our results highlights how important

530   is the definition of the reference condition (i.e. expected community composition in pristine sites

531   such as S1 and S2) for bioassessment (Wilding et al., 2018; Fornaroli et al., 2019) and how

532   biomonitoring indices must account for the deviation from the reference condition both as increases

533   or decreases in diversity and abundance of organisms.

534   *Leaf bags experiment*

535   Statistical analysis for the Summer leaf bags experiments shows that the probability of

536   recovery (%) of leaf bags is significantly influenced by Mesh size, Time and Residual Basin. High

537   altitude reservoirs partially drain the basin and reduce the water inputs to the Goglio stream.

538   Consequently, the magnitude of flood events was reduced and lead to reduced transport of organic

539   matter (i.e. higher probability of recover). Conversely, such differences in flood magnitude did not

540   seem to alter the degradation processes of CPOM, as also observed by Casas et al. (2000) in a

541   Mediterranean stream, giving no support for our HO_1 hypothesis.

542   As the residual basin increases in lower Goglio, the effect of reservoirs on flood events

543   becomes marginal and the flow regime was regulated mainly by the inputs from the tributaries. In

544   these conditions, the magnitude of flood events increased, and this was reflected in a decrease in the

545   probability of recovery of leaf bags, which were more easily removed from the riverbed. This

546   phenomenon was more evident for coarse bags, probably because debris carried by the floods could

547   easily anchor to the meshes and increase the strength exerted by the flowing water. This result

548   suggests that natural leaf packs can follow similar dynamics (Braioni et al., 2001), with comparable

549   breakdown rates but with higher CPOM availability in altered stretches than in pristine one (Martínez

550   et al., 2013), especially during Summer when high flow events are more frequent. The probability of

551   recovery was also reduced, especially for coarse mesh bags, by the time spent in water, mostly due

552   to the higher number of high flow events occurred since the positioning of leaf bags.

553   The remaining mass (%) of leaf bags was significantly influenced by Mesh size and Time.

554   Coarse mesh leaf bags showed a lower percentage of residual mass than fine mesh leaf bags because

555   leaves go through the mesh more easily after being smashed by mechanical degradation; furthermore,

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they give access to larger invertebrates and are potentially subject to higher biological degradation (Graça et al., 2001; Slade & Riutta, 2012). As the time of exposure of bags to mechanical and biological degradation increases, the % of remaining mass decreases.

During the Summer experiment different high flow events occurred, while low flow conditions lasted for the whole duration of the Winter experiment. The results of the Winter experiment highlight that the remaining mass (%) was significantly affected by the type of leaf: leaf bags filled with falling leaves showed a lower remaining mass than those filled with leaves collected from the litter as previously highlighted by (Gessner & Chauvet, 2002). Probably the retention of labile carbon and nutrients in fresh leaf litter facilitated their utilization by leaf-associated micro-organisms and invertebrates, and this raised the importance of biotic processes with respect to physical processes such as leaching (Gessner, 1991). Moreover, as for the Summer experiment, coarse mesh bags showed higher degradation rate and this was particularly evident for bags filled with falling leaves. Those results confirm that both fallen and manually detached leaves are a food source for macroinvertebrates and highlight that CPOM availability is controlled also by the flow regime and not only by its seasonal availability.

Water temperature:

The different thermal regime of the stream stretches is mostly due to the hydrologic regulation, as Goglio is regulated by high altitude reservoirs while Sanguigno is not. In Summer thermal stratification occurs in reservoirs: in the deeper layers the water is colder and denser than in the surface ones which are continuously heated by the solar radiation. That's why in upper Goglio, which is fed on water released from the lower layers of reservoirs, water was colder than in Sanguigno due to the reservoir release. This supports the HT_1 hypothesis and is in line with other studies (Webb & Nobilis, 1995; Toffolon et al., 2010). An opposite situation (the reservoir discharge increases water temperature downstream) would occur if the release of water from the reservoirs would take place from the surface or where riverine water is cooler due to strong snow meltwater inputs, as observed by Dickson et al. (2012). In Winter, as the lake surface was entirely frozen, the temperature at the

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582 bottom was close to 4° C so the water temperature downstream was closely linked to the temperature

583 of the hypolimnetic layer. The reservoir release increased the downstream water temperature as

584 described in Céréghino et al. (2002) and Carolli et al. (2008). Reservoirs regulation caused an increase

585 in water temperature, comparable in amplitude to the increase observed by Zolezzi et al. (2011) in

586 the Noce river basin and by Dickson et al. (2012) in the Eisboden catchment (that is ≈3°C above a

587 lower base temperature). In Sanguigno valley, the Winter water temperature dropped even below zero

588 confirming the results of Malard et al. (2006) and Tockner et al. (2010). In the absence of Winter

589 snow cover, water temperature records became very similar to air temperature also due to the fact

590 that the flow is naturally at minimum during Winter months (Jansson et al., 2003).

591   The water temperature in lower Goglio (G3) was lower than in upper Goglio (G1) in Winter

592 and higher in Summer. Those differences were due to the mixing of waters from upper Goglio and

593 Sanguigno, but also to the contribution of the water swirled by the hydropower station and released

594 upstream G3 station. The specific contribution of the swirled water was not determined because the

595 flow data were not available. The Summer cooling downstream the hydropower release was

596 consistent with the one measured in the Ticino river, downstream from the Biasca hydropower release

597 (Frutiger, 2004), even if the river size and the discharge flow are very different. Considering the water

598 temperature measured in G4 and G5 the previous elements seem not sufficient to explain a thermal

599 regime much more similar to the one of Sanguigno compared to upper Goglio, otherwise lower

600 Goglio (G3) and lower Goglio ROR (G4) would be not so different (our HT_3 hypothesis), as it has

601 proved by the Tuckey's test. The key driver in this case is represented by the ROR hydropower plants

602 presence. Water temperature depends mostly by the air temperature, but water has a strong thermal

603 inertia due to his high thermal capacity, therefore larger volume and fast flows reduce the effects of

604 the heat exchanges between water and air. ROR diversions, embezzling water from the stream, reduce

605 the total amount of water and decrease the flow speed so the dependence of water temperature on air

606 dynamics turns out to be strengthened (Brown et al., 2006). For this reason the daily variability in

607 Sanguigno was not statistically different from the variability in lower Goglio subjected to ROR (G4)

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608 but proved to be higher than in the other stretches during summer (HT$_2$). However, the drivers that

609 control the thermal regime of rivers regulated from ROR power plants need to be more deeply studied

610 and quantified.

611 **Conclusion**

612 The presence of hydroelectric power plants (high altitude reservoirs or ROR plants) modifies

613 the stream ecosystem with regards to all the aspects investigated in this study: macroinvertebrate

614 community composition, organic matter breakdown and thermal regime.

615 Macroinvertebrate communities that inhabit pristine sites are generally less diverse than in

616 other sites and more specialized for highly rheophilic environment due to the strong influence of high

617 flow events. In our case study the stretch subjected to the effect of high altitude dam showed the best

618 conditions for most of the macroinvertebrate families due to the abundance of food (especially CPOM

619 and dead wood) and the reduced stress due to high flow events. We did not identify any family that

620 could be considered representative of undisturbed conditions. The observed lack of representative

621 taxa for undisturbed sites could also be due to the coarse taxonomic resolution (i.e. family level) and

622 sub-family level identification might have produced different specific responses. This would

623 highlight the importance of systematic resolution and the need to develop community-wise metrics

624 that can properly assess this kind of alterations.

625 Macroinvertebrates communities in the stretch subjected to both hydrological and chemical

626 alterations were characterized by the abundance of families which can tolerate disturbed conditions

627 such as Leuctridae, Limoniidae and Simuliidae pointing out that, as often reported in literature,

628 alterations due to anthropogenic polluting loads are easier to identify than alterations due to

629 hydrological alterations.

630 Organic matter availability is positively affected by high altitude dams. In pristine sites leaf

631 bags were often removed from the riverbed causing a lower availability of this food source for the

632 macroinvertebrate community. Conversely, the breakdown processes seemed to be only slightly

633 altered by the presence of the high altitude reservoir as mesh sizes and residence time were the only

two factors having a significant effect on breakdown rates. Moreover, our results highlight that the Summer input of CPOM to low-order stream can be an important food source, comparable to the Winter input of recently fallen leaves. This can be of great importance in pristine sites were the effects of high flow events shorten the residence time of organic matter.

Thermal regime is profoundly altered by high altitude dams and less influenced by meteorological conditions. The ecological consequences of thermal alterations need to be specifically investigated, especially with mesocosm experiments or ideal case studies that allow to disentangle the effect of thermal and flow regime on biological populations. Those alterations make the stream stretches less subject to the effect of climate change and especially of heat waves that are becoming more and more frequent and intense in the alpine and pre-alpine environments. Reservoirs mitigate the atmospheric influence on stream water temperature while run of the river plants strengthen it in the diverted stretches. Where both these alterations were present the thermal regime of the stream was more similar to the natural ones compared to stretches subjected to only one kind of alteration and profoundly driven by meteorological conditions.

This research has provided elements for a better understanding of the impact of river impoundments on stream ecosystem structure and functioning. Such elements can be of great use in planning management strategies to protect the environmental quality of watercourses affected by the presence of hydroelectric plants, with particular reference to the growing importance of climate change.

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