How hydromorphological constraints and regulated flows govern macroinvertebrate communities along an entire lowland river?

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ABSTRACT

Macroinvertebrates' response to hydromorphological alterations and regulated flows along lowland rivers is still poorly known despite ecohydrology's fundamental role in river science. Along the Oglio River (Northern Italy), several water abstractions and dams break it into segments with varying hydraulic and morphological properties. Three types of *a priori* different environments were identified (dammed, downstream and free flowing sections) and macroinvertebrate communities were sampled from each zone. This study aimed: I) to investigate patterns of macroinvertebrate communities along a regulated lowland river by testing the *a priori* zones; II) to find macroinvertebrate taxa that served as indicators of the various hydrological conditions and III) to verify hydromorphological control over ecological macroinvertebrate traits resulting in different trait values in each identified zone. Macroinvertebrate community was characterised in a total of 63 stations by means of two distinct[sf1] quantitative approaches, each exploring a surface of 0.5 m^2. The lowest richness values were found in dammed sites that tended toward lentic conditions. Ecnomidae (dammed zones), Limoniidae (downstream zones) and Heptageniidae (free flowing section) were identified as the best indicators of varying hydrological conditions. As suggested by the results of 4th Corner Method environmental constraints define communities with different ecological traits. These results highlight hydromorphological control over macroinvertebrate community structure and reflect how regulated flows affect the Oglio River in terms of biodiversity, indicator taxa and ecological traits. The authors wish to stress the importance of considering the ecological effects of dams and impoundments on river systems in upstream areas as well as downstream.

INTRODUCTION

Rivers and streams are among the most vulnerable and simultaneously exploited ecological systems on our planet (Allan and Castillo 2007). Humans have broadly altered river systems' hydrology through impoundments and diversions to meet their water, energy, and transport needs. In particular, dam construction has increased exponentially in recent decades, especially during the period 1960-1990 (Rosenberg *et al.*, 2000). Rivers and streams are shaped by their hydrology, which sets bottom features, the timing of flooding, transport of solids and dissolved materials, metabolic rates and biological communities (Allan and Castillo 2007). Any alterations in hydrology, such as those resulting from dams, have consequences for a number of lotic ecosystem properties. This latter issue seems obvious, but the implications of hydrological regime, river continuity and morphological conditions (together termed hydromorphology) for river and stream management were scarcely considered for a long time. Currently, there is a need to understand the ecological effects of a wide range of changes in physical habitat, as rivers are increasingly exploited, regulated or otherwise modified through flood-defence engineering, impoundments, restoration, climate change and the spread of alien species (Vaughan *et al.*, 2009). The need for studies linking hydromorphology and ecological response is a priority for river research and management that requires clearly stated hypotheses and adequate sampling programmes that are able to develop robust flow alteration-ecological response relationship (Vaughan *et al.*, 2009; Poff and Zimmerman 2010).

Hydromorphological elements and their assessment in lotic ecosystems were introduced recently in European legislation, with the Water Framework Directive (European Commission 2000) as a supporting tool for the comprehension of biological and chemical features. Unaltered hydromorphology is generally coupled with an elevated ecological status, and vice-versa (European Commission 2000).

Dam construction leads to a variety of demonstrated effects in stream hydraulics and properties, like the alteration of sediment transport (Ward and Stanford 1983, 1987; Syvitski *et al.*, 2005), inundation of terrestrial systems (Nilsson and Berggren 2000), fragmentation of riparian plant distribution (Jansson *et al.*, 2000), enhancement of greenhouse gas emissions (St Louis *et al.*, 2000), changes in thermal regimes and water chemical composition (Armitage 1984; Olden and Naiman 2010; Lessard and Hayes 2003) as well as a possible regime shift, from net heterotrophy to net autotrophy (Pinardi *et al.*, 2011). Furthermore, aquatic biodiversity seems to respond to hydraulic disturbance by changing community structures and resistance to invasion of exotic species (Stanford *et al.*, 1996; Bunn and Arthington 2002; Poff *et al.*, 2007). Different studies (Copp 1990; Irz *et al.*, 2006) reported a transition from lotic to lentic fish communities in dammed sites as well as increased in exotic and lake-adapted taxa (Pringle *et al.*, 2000). However, there are few similar studies focussing [sf2]on other aquatic taxa. The way the whole macroinvertebrate community respond to hydromorphological alterations and regulated flows in the long term, large-scale and lowland rivers has been poorly explored. Many researchers have investigated the ecohydrological changes that occur below a single dam, but few studies have examined changes in macroinvertebrate communities encompassing an entire river unit (Heppner and Loague 2008; Zolezzi *et al.*, 2011). Hydraulic stream conditions and other hydrologic factors, including a combination of current velocity, depth, surface slope and substrate roughness, instead seem to be important factors for invertebrate zonation patterns (Rempel *et al.*, 2000, Brooks *et al.*, 2005, Kennen *et al.*, 2010) and benthic ecology (Carling 1992). Nevertheless, the consequences of streams management and invertebrate hydraulic preferences are generally better known in small streams than in medium or large rivers (Mérigoux *et al.*, 2009), which is probably due to the complexity of these systems (Sparks 1995).

The effects of dams and barriers on macroinvertebrate communities are important because of the role that macroinvertebrates play in the functions and dynamics of stream ecosystems (Merritt *et al.*, 1984; Merritt and Lawson 1992). Dam use and the intensive exploitation of rivers, particularly in northern Italy, dates back to 1900. They were historically designed with little consideration for ecological effects such as migration pathways, minimum flow releases or hydropeaking problems. Here, as in many Mediterranean countries, rivers were converted into discontinuous systems with alternating or adjacent segments characterised by varying hydrologic conditions.

In this study three types of environments were identified *a priori* along the course of the regulated lowland Oglio River: a lentic stretch (dammed: upstream of the dams or weirs), a stream-like (downstream: downstream dams or water abstraction infrastructures) and a river-like section (free flowing) and macroinvertebrate communities were studied in each part. The main hypothesis is that macroinvertebrate community patterns are clearly addressed by different flow conditions affecting taxa richness, indicator families and ecological traits.

In order to contribute to the knowledge of hydromorphological constraints and regulated flows on macroinvertebrate communities, this study aims: I) to investigate patterns of macroinvertebrate communities along a lowland flow regulated river, testing *a priori* zonation; II) to find macroinvertebrate taxa that would serve as indicators of different hydrological conditions and III) to verify environmental and hydromorphological control over macroinvertebrate ecological traits resulting in different trait values in each zone.

METHODS

Study area

This study was carried out along the entire Oglio River (Lombardia, northern Italy), a manregulated watercourse of 154 km (Fig. 1). This river originates from an Alpine lake, the Lake Iseo (185 m a.s.l.), and flows into the Po River (16 m.a.s.l.). Water flow in the Oglio River is regulated by the Sarnico dam, at the southern extreme of Lake Iseo; regulation aims at the production of electricity and the maintenance of a water reserve in the upstream lake for irrigation purposes. The catchment of the Oglio River occupies an area of 3840 km^2, mostly exploited for agricultural activities (67%) and animal farming (about 600,000 cows and over 2,100,000 pigs); the human population comprises roughly 1,100,000 inhabitants.

The Oglio River suffers from various pressures. Briefly, intensive agriculture and farming have resulted in diffuse nitrogen pollution that affects surface and groundwater (Soana *et al*., 2011; Laini *et al*., 2011). The river itself has been heavily altered from its pristine status due to multiple agricultural and industrial water uses and from the construction of hydropower plants, low head dams and banks. Water diversions for irrigation date back to 1500 and are mainly located along the upper 29 km long reach; the sum of their water concession equals the average historical flow of the river (about 80 m^{3}s^{-1}), which means that the water flow could be entirely diverted. The realisation of hydropower plants is a more recent issue that dates back to 1950; six plants are operating at present, all located in the same upper reach mentioned above where the river is generally confined to a single channel, disconnected from its floodplain and with reduced sinuosity. Altered flow regime and damming are probably major causes of habitat heterogeneity loss. Hydraulic infrastructures result in variable riverbed widths (from <30 to about 100 m) and in variable water depths, from several meters upstream from the dams, to a few centimeters downstream from the water abstraction. The Oglio River has the typical features of a plain river, with gentle slopes and moderate water flow. The river substrate only partially varies along the longitudinal gradient from a typical gravel-dominated substrate to a fine sand-dominated substrate in the lowland areas. This is due to the presence of hydraulic infrastructures in the upper sections that affect the gravel substrate converting in silt and macrophyte-dominated substrate upstream from the dams.

Macroinvertebrate and environmental data

The macroinvertebrate community was sampled seasonally from July 2009 to May 2010 in a number of representative sites located along the Oglio River (Fig.1). Sampling sites, seasonally investigated, varied from a minimum of 15 to a maximum of 18. A few sites (mainly in dammed and free flowing zones) do not present a complete seasonal series due to vandalism or excess flow/floods. Sampling strategy reflected an *a priori* idea to split the water course into three environment types: i) dammed sites (basins upstream hydropower plants or low head dams); ii) sites immediately downstream to those described as dammed and iii) free flowing section in the lowland, meandering zone. Macrofauna community was characterised in a total of 63 stations by means of two distinct quantitative approaches, each exploring a surface of 0.5 m^2. At those sites belonging to dammed and free flowing zones artificial substrates were employed whilst at sites belonging to downstream zones a Surber net was used. At each station downstream hydropower plants or dams a total of 10 Surber units (1 surber unit = 0.05 m^2, with 500 µm mesh size net) were collected on each date by stirring and removing surface sediments and stones to remove any attached invertebrates. Explored areas within each station were proportional to the relative surface of all the microhabitats identified, according to Buffagni and Erba (2007).

Reliable and accurate collection of macroinvertebrates presents a certain degree of difficulty in deep sections of upstream dams and where flows are elevated. Here, the use of artificial substrates (Hester-Dendy modified e.g. Cairns and Dickson 1971; Battegazzore *et al.*, 1995) can represent a valid alternative to the Surber net (Solimini *et al.*, 2000; Buffagni *et al.*, 2007). Multiple-plate artificial substrates (hereinafter called AS) summing a total colonisable area of 0.5 m^2, were thus employed at dammed and free flowing stations. These samplers were anchored and suspended with ropes close to the bottom, as detailed in Buffagni *et al.* (2007). They were left *in situ* for 1 month to allow complete colonization and thereafter carefully retrieved. Each sampler was placed in a white plastic tray, and macroinvertebrates were removed with forceps from the plates and trapped sediment. The macroinvertebrates dislodged in the process of removing the AS from the river were collected immediately downstream with a 500-µm mesh net and added to the sample.

Macroinvertebrate samples and associated material, both from the Surber net or AS, were preserved in 70% ethanol and then examined under a stereoscope in the laboratory. All macroinvertebrate individuals were identified at family or genus level except for Hydracarina and Rissoidea gastropods. The sampling and processing effort at this taxonomic level allowed all groups from the invertebrate community to be investigated.

In all sampling dates and at all stations data on water flow, current velocity and depth were collected or provided by the Oglio Consortium (member of Alpine Lakes Controller Institutions, Civil Protection Department) (Table 1).

Data analyses

In this study two distinct quantitative approaches were used that contributed to the compilation of a large dataset. Different sampling methods can results in varying estimations of macrofauna abundance (Buffagni and Erba 2007), but they do not generally select among taxa so that presence/absence data are reliable with both approaches (Bo *et al.*, 2007). However the use of different sampling methods for different habitats is reported in many other studies (Gjerløv et al., 2003; Benstead et al., 2009). As a consequence, presence/absence information and not abundances were used in statistical tests. Furthermore, in order to avoid drawbacks due to the different taxonomic resolution, statistical analyses were generally performed by using a standardised taxonomic level (family data). Information about genus was included instead to improve ecological trait data analyses (see later).

The effects of seasonality and type of environments (predefined zones) on family richness were tested by using ANOVA analysis on the log transformed data.

The quality of taxa inventory generated by seasonal sampling along the entire Oglio River and for the 3 groups of stations was checked using accumulation's curves. This approach is widely used to evaluate the representativeness of collected information (Soberón and Llorente 1993) and represents how the number of taxa within a geographical area varies as a function of the collection effort (Colwell and Coddington 1994). The slope of the curve decreases with sampling effort and reaches a hypothetical value of 0 when all taxa are detected. As the taxon richness is probably the main variable describing community diversity (Gaston 1996), accumulation's curves allow one to set reference terms for taxa richness given a fixed number of replicate samples. Different types of functions were fitted to family accumulation curves and the Weibull function provided the best match. The same function was demonstrated as a good compromise between the number of parameters to be fitted and also results in other studies on invertebrates (Jimenez-Valverde *et al.*, 2006; Tjørve 2003). The Weibull function was fitted to smoothed data and the asymptotic value (i.e., the taxa richness predicted for an ideally infinite sample size) was computed. The ratio of recorded to predicted richness (asymptotic score) was used as a proxy of representativeness of the database (in the three pre-defined zones and for the whole river).

A nonmetric multidimensional scaling (nMDS) analysis was performed to identify distribution patterns among the macroinvertebrate communities of the different sampled sites. NMDS is regarded as one of the most robust unconstrained ordination methods (Oksanen 2011) and is robust from deviation from multi-normality. Bray-Curtis distance was used as dissimilarity measure and stress was used to test the goodness of fit. The threshold above which the ordination was not considered reliable was set at 20%. Linear fittings were performed between the hydrological data (discharge, velocity and depth) and the output of nMDS ordination in order to identify environmental factors driving macroinvertebrate distribution. Analysis of similarities (ANOSIM) using Bray-Curtis distance was carried out to test whether there was a significant difference between the *a priori* proposed zones in terms of macroinvertebrate communities. This test was developed by Clark (1993) as a method for testing the significance of the groups that had been *a priori* defined. Prior to multivariate analysis, hydrological variables were transformed (log-transformation for quantitative variables) and standardised to improve linear relationships among variables, reduce distribution skewness and avoid distortions due to the effect of different transformations and magnitudes.

IndVal analysis was carried out to select the indicator family for each river zone (Dufrêne and Legendre 1997). This analysis evaluates the affinity of each taxon for one of the three environment types defined *a priori* (the Indicator Value: IV). Such an affinity is calculated on the basis of the frequency of each taxon in the identified groups. To take into account the unequal size of the sampling sites within each group the group-equalized IV was calculated according to De Cáceres and Legendre (2009). The significance of IV was tested using a Monte-Carlo test (999 runs) and Alpha level was set at 0.05. Taxa selected by IndVal should present environmental-specific ecological traits to allow their presence; the "4th Corner Method" (Legendre *et al.*, 1997) was used to check for differences in ecological traits between the different tested zones and flow conditions.

The matrix of ecological traits was built considering the traits and relative subgroups described by Usseglio-Polatera *et al*. (2000) and Tachet *et al*. (2002). The ecological characteristics used include the 7 traits related with hydrology and physical habitat, with a total of 37 possible modalities. The purpose of this method is to relate the ecological traits of the organisms to the habitat characteristics of the sites in which they live. The calculation is made possible by using traits, presence/absence (or abundance) and environmental matrices. Within the 5 models proposed by Dray & Legendre (2008) model number 2 "Environmental control over species assemblage" was chosen. In this model, the hypothesis is that taxa assemblages depend on the environmental feature characterising the sites where they were found. As shown by some authors (Bournaud *et al.*, 1996; Dolédec *et al.*, 1998) higher taxonomic levels can be suitable for an ecological study, so the first step was to select the families and genus collected in the Oglio River from the database. For the macroinvertebrate groups in which genus data were available, only the genus recovered in Oglio River were used. The second step was to calculate the relative frequency of each subgroup (i.e. lowlands, piedmont level or alpine level) belonging to a category (i.e. *Altitude*). The sum of the frequencies of the subgroup within a category is equal to 1.

All statistical analyses were performed using the statistical computing software R (R-Development core-team, 2010) with packages "Vegan" (Oksanen 2011), "ade4" (Chessel 2011) and "indicspecies" (De Cáceres 2011).

Results

Assessment of macroinvertebrate richness among different sampling methods

Results from ANOVA analysis (Table 2) suggested that the zone (p <0.01) and the season (p <0.05) were statistically significant variables affecting macroinvertebrate family richness along the Oglio River but not the interaction zone:season (p = 0.09). During the study period (1 year) about 40,000 organisms were identified to family or genus level and a total of 72 families were identified.

Focussing on the different sampling methods used: more than 75% of the recorded families were presented in Surber samples and also in AS samples. Concretely 62 families were detected using AS while 57 using Surber net and 47 were presented using both methods. The families recorded by exclusively a method were rare and were found in only few sites.

Assessment of macroinvertebrate inventory completeness and richness estimations along the three proposed zones

Downstream and free flowing zones hosted the richest sampling stations, with a total of 61 and 55 families recorded. On the other hand, stations within dammed zones exhibited the lowest richness, with 37 families recorded. Accumulation's curves showed the representative sampling effort for the 3 selected zones (Fig 2). This result suggested that the sampling effort accounted for at least 75% of the total families estimated for each zone (Table 3). Family richness seemed to increase slightly more rapidly in the downstream sites compared to the other zones. Moreover, dammed sites presented clearly lower family richness values compared to the other zones that appeared to be rather similar. Using data from all pooled sampling stations, the ratio between the recovered (72) and theoretical number of families predicted by accumulation's curves (78) equalled 92%.

Importance of hydromorphological environmental variables in determining macroinvertebrate communities

In the ordination space of the first 3 axes of non-multidimensional scaling the samples were arranged according to the *a priori* identified zones (Fig.3) and presented a stress value of 16%. Moreover, vector fitting among nMDS axes and hydromorphological parameters highlighted the importance of hydrological factors as drivers of the macroinvertebrate communities. The nMDS plot established three distinct groups that essentially consisted of the proposed *a priori* hypothesis. Considering axes 1 and 2, "dammed stations" appeared well-clustered on the right side of the plot while "downstream stations" were placed on the bottom and "free flowing section" essentially on the top left of the plot.

In detail (Fig. 3), downstream stations seemed to be characterised by reduced discharge and depth and partially by high velocity, while dammed ones were related to higher levels of depth. On the other hand, stations in the free flowing section were mainly characterised by high discharges and velocity. All variables presented a linear fitting statistically significant (p<0.01) between selected zones. Furthermore, the ANOSIM test showed there were significant differences (R = 0.522, p< 0.001) in macroinvertebrate assemblage composition among the three pre-defined zones.

Potential indicator taxa and ecological trait analysis

IndVal analysis identified indicator taxa for the three environment types proposed (Table 4). Five families were significant indicators for dammed sites: Ecnomidae, Coenagrionidae, Viviparidae, Lymnaeidae and Limnephilidae. Some authors (Bonada *et al*., 2008, following Dufrêne and Legendre, 1997) considered an IV > 25 as key value to consider adequate an indicator taxa, so the first two presented an important IV value and great significance level (p<0.001). Stations included in the downstream zones presented a heterogeneous list composed by fourteen indicator families: Limoniidae, Psychomyidae, Lumbricidae, Baetidae, Neritidae and Rhyacophilidae with the best significance level (p<0.001). These stations presented a heterogeneous clustering of taxa, with different ecological characteristics and varying taxonomic positions. Finally, seven families were good indicators for the free flowing section with Heptageniidae (essentially genus *Heptagenia*) with the highest IV followed by Calopterygidae (genus *Calopteryx*), Gammaridae, Platycnemidae, Hydropsychidae, Gomphidae and lastly Tubificidae.

Results from the "4th Corner Method" showed distinct patterns of ecological traits in the three different *a priori* hypothesised zones: dammed, downstream and free flowing stretch (Table 5). When focussing upon ecological traits like *transversal distribution*, *longitudinal distribution* or *current velocity* it is very interesting to note that the three pre-defined zones presented macroinvertebrate communities with different ecological traits. In these cases, dammed and downstream zones presented almost always opposite and complementary values. In particular, analysing *transversal distribution* in dammed zones presented a macroinvertebrate community with negative correlation with habitats like river channel and a strong and positive relation with habitats like ponds and pools (0.25; p<0.01) and also with lakes (0.07; p<0.01). On the other hand, macroinvertebrate communities inhabiting downstream zones presented a negative relationship with habitats like lakes (-0.09; p<0.01) and ponds (-0.11; p<0.05) and positive value with banks habitats (0.12; p<0.01). In free flowing section significant and negative relationships were obtained with ponds (-0.10, p<0.05) and temporary waters (-0.09; p<0.01). Focussing on *longitudinal distribution* dammed zones presented negative relationship with crenon and epirhithron zones (-0.11 and –0.21; p<0.01) and positive relationship with metapotamon habitats (0.17; p<0.01). Again, downstream zones presented opposite values compared with dammed stations (except for estuary value) with positive relationships with crenon and epirhithron areas (0.09 and 0.12; p<0.01) and negative relationships with epipotamon (-0.08; p<0.01) and metapotamon (-0.08; p<0.05). In this ecological trait free flowing sites presented complex results with positive relationships with metarhithron zones (0.11; p<0.01) and negative with estuary (-0.12; p<0.01) and metapotamon (-0.06; p<0.05).

Also considering *altitude* trait, macroinvertebrate communities inhabiting dammed and downstream zones presented opposite signs between lowlands, piedmont and alpine levels.

Observing *substrate preference* dammed zones were essentially related to microphytes and macrophytes (0.14 and 0.12; $p<0.01$), while downstream zones with flags, twigs and roots (0.07 and 0.09; $p<0.01$) and silt (-0.09; $p<0.01$). The free flowing section was positively related to silt, sand and gravel (0.14; 0.12; 0.09 with $p<0.01$). Furthermore, considering *current velocity*, dammed zones presented negative and significant relationships with medium and fast velocity (-0.18 and –0.24; $p<0.01$) while the others presented a positive relation, although less significant. Analysing the *trophic status*, it was interesting to note that dammed zones were positively related with eutrophic conditions (0.13; $p<0.01$) while the other ones presented opposite results with negative relationship with eutrophic conditions. The *temperature* trait seemed important because dammed zones presented a negative and significant relationship with psychrophilic, i.e. cold-stenothermal organism (-0.099; $p<0.01$) and a positive relationship with eurythermic conditions (0.06; $p<0.01$), while other zones did not present significant values.

Discussion

Species level resolution is preferable in ecohydrological researches when it is available (Monk *et al.* 2012). However, the present study considered the taxonomic level used as adequate in order to characterise the ecological traits of most groups with respect to riverine hydrology and a good compromise between classification effort and gathered information (Marchant *et al.*, 1995; Bournaud *et al.*, 1996; Dolédec *et al.*, 1998). Also, macroinvertebrate family richness generally presents a high correlation with species richness in Mediterranean areas (Sánchez-Fernández *et al.*, 2006) as well as in boreal systems (Heino and Soininen, 2007) which seems to suggest how species-level assemblage patterns could be reproduced by using genus- and family-level data. Furthermore, recently Belmar *et al.* (2012) focussed on hydrological variables found a relatively strong relationship between community composition and flow regimes at different taxonomic levels, from species to family level.

In this study, the decision to work on the presence/absence of families and not on abundance is justified first by the necessity of using two distinct sampling techniques which were demonstrated to recover the same group of organisms. Additionally, outputs from the NMDS analysis were likely to provide a similar qualitative data ordination when performed on abundance (after data transformations as Wisconsin double standardization) and on presence/absence.

At the scale of the entire river, the seasonal samplings and the number of stations investigated were adequate in order to provide a reliable inventory of the macroinvertebrate community. In fact, according to the outputs of the accumulation’s curves, more than 80% of the total families were censused, and only a few families were missing in order to reach the asymptotic theoretical richness value. Focussing on each environment type, about 78% of the expected families were found in dammed stations, and it is likely that sampling efforts could have been slightly improved in order to reach higher completeness values (Jiménez-Valverde and Hortal 2003; Sánchez-Fernandez *et al.*, 2008). Highly representative family inventories were instead realised for downstream and free flowing stations.

The representativeness of the macroinvertebrate community for the entire river and for the three groups of *a priori* selected reaches is an important requirement for the analyses performed and guarantees the robustness of the main outputs. Results from the present study clearly suggest that hydrological parameters and regulated flows play a key role in structuring macroinvertebrate communities in a regulated lowland river. This outcome has a high degree of novelty as, to current knowledge, similar results focussing on large scale and entire river units, specifically on macroinvertebrate communities and ecological traits are very scarce in the literature, at least in ecohydrology research and similar geographic areas.

The sequence of hydropower plants, low head dams and water abstraction infrastructures has created a discontinuum of hydrological conditions in the Oglio River with alternating lentic-like and strictly lotic-like reaches near upstream and downstream infrastructures. As a consequence, macroinvertebrate communities do not present an upstream-downstream gradient along the rivercourse, as predicted by river continuum theories (Vannote *et al*., 1980). Rather, the presented results clearly describe identifiable and alternating lentic and lotic communities along the rivercourse. The results of NMDS analysis match the proposed *a priori* grouping of the investigated stations according to three distinct hydrological features. Differences in terms of taxonomic composition among the proposed zones were also reflected in the ANOSIM test while environmental types (predefined zones) and the season seems to be important factors affecting macroinvertebrate richness values.

These different zones will be discussed separately later. The macroinvertebrate community structure is probably shaped by factors such as the substrate, vegetation and chemical gradients at the microscale (i.e. dissolved oxygen availability in porewaters), that are directly related with local hydrology. Furthermore, hydrological change and interaction with substrate may affect the availability of potential microhabitats to some species while increasing habitat availability for others (Statzner *et al*., 1988). Gore et al. (2001) stressed that aquatic organisms are probably restricted to those combinations of velocity, depth, and substrate that allow morphological and behavioural resistance to flow to be exceeded by energetic gains and predicted an increasing emphasis on incorporating hydraulic variables as a part of bioassessment. A dynamic and natural hydrological connectivity among waterbodies, in terms of space and time, has been proven to drive patterns of macroinvertebrate biodiversity and ecosystem functions in different floodplain rivers (Amoros and Bornette 2002; Leigh and Sheldon 2009).

Dammed stations

This group included sites characterised by features typical of shallow lakes of a few meters depth, no apparent water velocity, soft substrate and dense macrophyte stands. Here, hydrology and depth were the main drivers for the ordination of data (cluster on the right of the nMDS plot). Indicator taxa like *Ecnomus tenellus* (Ecnomidae) or Coenagrionidae, Viviparidae and Lymnaeidae were in agreement with this output, with absence of water current and high depth as selecting factors for the taxa colonizing dammed stations. Ecological trait analysis added further evidence in this respect, as recovered macroinvertebrate communities are generally related to lentic habitats like ponds or lakes with null current velocity and macro and microphytes substrate. Among macrophytes, *Vallisneria spiralis* was abundant in all dammed stations. Despite the fact that they were located in the upper zone of the Oglio River these sites did not present invertebrate communities typical of rhithron zones. This is in part due to the natural conditions of the lowland river, but considering the hydromorphologic variable values, it seems to be clear that regulated flows and dams act as alterations within the natural river continuum.

Here, the Oglio River is also often disconnected from its floodplain, although the ecological importance of this area as part of a river ecosystem has been recognised (Burt *et al.*, 2008; Burt *et al.*, 2010).

Stations upstream from hydropower plants and low head dams had poorer measured and estimated biodiversity, probably due to net habitat loss during the shift from a lotic to an artificial lentic system (Bonada *et al.*, 2005; Ribera 2008) that included reduced sinuosity and the loss of meandering zones loss (Garcia *et al.*, 2012). Aquatic environments such as rivers display large habitat heterogeneity, including pool-riffle sequences (Vannote *et al.*, 1980; Allan and Castillo 2007) as well as a number of different micro-habitats at reach scale (Cogerino *et al.*, 1995; Allan *et al.*, 1997; Boyero 2003). The habitat heterogeneity of lotic ecosystems may allow the presence of a higher number of taxa in comparison to ponds or lakes, although under natural conditions, these environments generally contribute to the presence of rare and unique species (Williams *et al.*, 2003). Furthermore, dammed stations, essentially in the upper zone of Oglio River, presented macroinvertebrate communities negatively related with psychrophilic conditions, which seems to emphasise the importance of thermal regimes (Olden and Naiman 2010) in environmental flows assessments.

Downstream stations

Current velocity, reduced depth and type of substrate (mainly flags or mesolithal) suggested that stations downstream from the dams or water abstraction structures had those features that characterise pristine, rhithral and stream-like environments. This is another artificial condition which is a consequence of a sudden decrease in water flow for multiple water uses. The reversal of lentic-like features and the re-establishment of lotic characteristics were described in the Serial Discontinuity Concept (SDC) (Ward and Stanford 1983) and in other studies (Odum 1997). The SDC viewed dams as clear discontinuities within the river continuum and proposed that rivers have a tendency to reset ecological conditions toward unregulated or natural conditions as distance downstream from the point of regulation increases (Stanford and Ward 2001).

In downstream stations, selected indicator families like Psychomyidae, Ephemerellidae (*Ephemerella*) or Rhyacophilidae were typical of rhithral ecosystems, while other families provided multiple and often unclear information with respect to environmental features. For example, the presence of Neritidae (*Theodoxus*) and other Mollusca could be an indicator of an hyporhithral or potamal environment, while that of *Dreissena polymorpha* does not, and its presence is probably due to a drift effect from dammed upstream coupled with the high dispersive capacity of this invasive species.

The ecological traits analysis, and in particular the traits *transversal distribution*, *longitudinal distribution*, *current velocity* and *altitude* suggested negative relationships between macroinvertebrate communities of downstream stations with lakes, potamal zones and null velocity and positive relation with alpine level altitude, fast velocity and rhithron zones, features that are generally typical of stream-like environments with limited water discharge.

Free flowing stations

These stations characterised a lowland, ~100 km long free-flowing river course which was devoid of infrastructures that created longitudinal discontinuities of relevant water flow variations. Flows and water velocity were constant or tended to increase and the upstream-downstream variations of chemical and biological features probably followed the predictions of the Vannote *et al.* (1980) conceptual model.

Due to its length, this reach included a number of different habitats whose features could partially overlap those characterising downstream stations (i.e. the substrate, at its beginning) as well as those characterizing dammed stations (i.e. water depth, toward its end). Such heterogeneity is reflected by the results of the IndVal and Ecological traits analyses. In fact, selected macroinvetebrate indicators taxa of free flowing stations like Gammaridae, *Heptagenia* or different taxa of Odonata are essentially related with lowland rivers. However, results from the ecological trait analysis, and in particular those related to the trait *longitudinal distribution*, suggested a rhithral more than potamal macrofauna community. In terms of altitude traits, this section presented a macroinvertebrate fauna more related with a piedmont level community than with a lowland level. The use of different analysis (IndVal and traits analysis) can improve the quality of the results bringing additional ecohydrological information. This section that would be expected to present macroinvertebrate communities closely related with a potamon condition, really presented heterogeneous communities that may be partially associated with an alteration of the rhithron-potamon boundary. The topic regarding a possible shift in the rhithron-potamon boundary was in part stressed by Stanford *et al.* (1996) who suggested that in rivers that are free flowing for long distances downstream from large dams, the position of the rhithron-potamon transition could be predicted from the operational mode of the dams relative to the influence of tributaries. Furthermore this topic is quite specific and necessarily requires supplementary researches.

Final considerations

The relationship between habitat alteration and river ecology is finally receiving increasing attention (Vaughan *et al.*, 2009; Poff and Zimmerman 2010) and specific macroinvertebrate index or invertebrate preferences research, related to flow alteration and hydroecology topics, have been recently developed (Extence *et al.*, 1999; Mérigoux *et al.*, 2009; Armanini *et al.*, 2011a, Armanini *et al.*, 2011b). However, biomonitoring activities by environmental agencies and scientific interest focus widely on the impact of dams and hydroelectric plants on downstream sections (Ligon *et al.*, 1995; Power *et al.*, 1996; Galbraith and Vaughn 2011) while less attention to the macroinvertebrate communities is generally devoted for upstream, dammed stations. Here, drastic changes in macroinvertebrate communities can occur, as demonstrated by the present study in terms of indicator taxa and selection of different macroinvertebrate ecological traits. The authors suggest that monitoring activities should also prioritise those zones where human intervention has created river reaches with lentic features. Pringle (1997), focussing on fish communities, had already stressed the importance of considering the upstream as well as downstream effects of dams and impoundments because disturbances can also be transmitted upstream.

Results from the present study also suggest altered macrofauna communities in zones located upstream and downstream from barriers or dams. For example, abundant densities of exotic invertebrates like *Dreissena polymorpha*, *Corbicula* or *Orconectes limosus* characterising dammed and downstream stations, are likely a consequence of flow alteration (Bunn and Arthington 2002) coupled with other anthropogenic causes. Upstream, invertebrate communities suffer stagnation and habitat loss, resulting in biodiversity loss (Stanford *et al.*, 1996) as more exigent, strictly lotic taxa such as most EPT taxa (Ephemeroptera, Plecoptera, Trichoptera) cannot cope with such conditions. Downstream, macroinvertebrate communities suffer highly artificial variable flows, resulting in habitat instability that promotes the presence of communities with numerous indicator taxa.

Results from the IndVal analyses may represent an effective monitoring tool when the effects of river flow regulations or the realisation of infrastructures must be evaluated. Particularly informative, with this respect, are those macroinvertebrate taxa characterised by high indicator values.

Ecohydrological research and sustainable water flow management should be central in the present and near future in order to achieve the quality targets set by the Water Framework Directive (Acreman and Ferguson 2010; Boon *et al.*, 2010) as well as for modified waterbodies such as the Oglio River. This is particularly important also because hydroclimatic models predict that European rivers will collectively show reduced discharge and seasonally would have lower summer flow (Arnell 1999). Moreover, flow management may even be relatively ineffective in restoration solutions or environmental conservation when provided in the absence of pollution abatement, riparian management and habitat restoration (Arthington *et al*., 2010).

Renöfalt *et al.* (2010) have suggested prioritising among different restoration actions, starting with projects that have positive effects on the largest areas or on projects and actions that can serve as learning experiences through scientific experimentation and testing. In this perspective, the investigated area from a human-dominated landscape should be exploited as useful test case (Jackson *et al.*, 2009) for the sustainable management of environmental flow and restoration of floodplains in other similarly altered areas.

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Table 1. Environmental and hydrological variables measured and used in the analysis.

Variables	Dammed stations	Downstream stations	Free flowing stations
Discharge (m^3 s^{-1})	42.88 ± 27.72	21.23 ± 14.33	86.23 ± 54.53
Depth (m)	3.10 ± 0.75	1.13 ± 0.49	1.96 ± 0.84
Velocity (m s^{-1})	0.23 ± 0.22	0.51 ± 0.32	0.95 ± 0.31

Table 2. Summaries of ANOVA used to assess the effects of Zone, Season and the interaction on macroinvertebrate richness. df: degrees of freedom. *** *p*<0.001; ** *p*<0.01; * *p*<0.05

Variables	**df**	**Mean Sq**	**F-value.**	***p*-value**
Zone	2	0.7403	6.206	0.00387 **
Season	3	0.3712	3.112	0.03425*
Zone: Season	6	0.2283	1.914	0.09634

Table 3. Number of stations sampled (Stations), number of observed (S obs) and estimated families (S exp) for each Oglio zone (obtained by Accumulation's Curves). For each zone the completeness degree (% Compl) is also displayed.

	Stations	**S exp**	**S obs**	**% Compl**
Dammed stations	14	47	37	78
Downstream stations	24	69	61	89
Free flowing stations	25	65	55	84
Oglio River (total)	63	78	72	92

Table 4. Results of INDVAL analysis for each zone. Indicator Value and significant *p*-value are displayed.

Dammed stations

Order	**Family**	**I.V.**	***p*-value**
TRI	Ecnomidae	64.3	0.001
ODO	Coenagrionidae	38.6	0.001
ARC	Viviparidae	25.5	0.004
PUL	Lymnaeidae	25.0	0.024
TRI	Limnephilidae	14.3	0.039

Downstrem stations

Order	**Family**	**I.V.**	***p*-value**
DIT	Limoniidae	54.8	0.001
TRI	Psychomyidae	53.7	0.001
OPI	Lumbricidae	53.3	0.001
EFE	Baetidae	46.0	0.001
NER	Neritidae	42.0	0.001
EFE	Ephemerellidae	40.7	0.002
VEN	Dreissenidae	40.2	0.011
TRI	Rhyacophilidae	39.9	0.001
TRI	Lepidostomatidae	37.5	0.003
DIT	Empididae	33.6	0.005
ARH	Erpobdellidae	30.6	0.045
HEM	Naucoridae	25.5	0.005
VEN	Corbiculidae	24.1	0.044
DIT	Tipulidae	17.4	0.04

Free flowing stations

Order	**Family**	**I.V.**	***p*-value**
EFE	Heptageniidae	74.3	0.001
ODO	Calopterygidae	49.0	0.001
ANP	Gammaridae	48.0	0.004
ODO	Platycnemidae	44.4	0.001
TRI	Hydropsychidae	43.8	0.035
ODO	Gomphidae	42.9	0.001
TUB	Tubificidae	23.2	0.033

Table 5. Summaries of ecological traits results following the traits description of Usseglio-Polatera et al. (2000). The results from the global test (F) and their significance p obtained by permutations in the "4th Corner Method" are presented (Legendre *et al.*, 1997). For dammed, downstream and free flowing sites, the r-values from the correlation traits-habitat matrix are given. The significance of r-value was also tested by permutations (999 runs). All p-values include Holm correction. *** $p<0.001$; ** $p<0.01$; * $p<0.05$

Ecological Traits	**F-test**	***p*-value**		**Dammed**		**Downstream**		**Free flowing**	
					r-value		**r-value**		**r-value**
Transversal distribution									
river_channel	13.705	0.001	***	-0.159	0.003	0.054	0.034	0.072	0.016
banks_	15.167	0.001	***	-0.160	0.003	0.116	0.003	0.008	0.408
ponds_pools	36.554	0.001	***	0.255	0.003	-0.107	0.014	-0.095	0.014
marshes_peat_bogs	1.284	0.126		0.026	0.282	0.027	0.282	-0.049	0.084
temporary_waters	4.066	0.001	***	0.032	0.098	0.060	0.016	-0.088	0.003
lakes	4.702	0.001	***	0.071	0.004	-0.087	0.003	0.034	0.113
groundwaters	2.813	0.001	***	0.016	0.208	-0.071	0.003	0.061	0.003
Longitudinal distribution									
crenon	8.075	0.001	***	-0.113	0.003	0.093	0.003	-0.005	0.417
epirhithron	24.772	0.001	***	-0.210	0.003	0.120	0.003	0.045	0.106
metarhithron	21.290	0.001	***	-0.191	0.003	0.038	0.120	0.114	0.003
hyporhithron	6.407	0.001	***	-0.110	0.003	0.051	0.032	0.035	0.068
epipotamon	4.446	0.009	**	0.073	0.014	-0.082	0.009	0.026	0.194
metapotamon	15.750	0.001	***	0.171	0.003	-0.080	0.022	-0.055	0.034
estuary	8.242	0.001	***	0.040	0.037	0.088	0.003	-0.124	0.003
outside_river_system	17.688	0.001	***	0.175	0.003	-0.118	0.003	-0.019	0.293
Altitude									
lowlands	9.678	0.001	***	0.135	0.003	-0.056	0.052	-0.050	0.052
piedmont_level	10.184	0.001	***	-0.135	0.003	0.033	0.129	0.075	0.008
alpine_level	5.319	0.002	**	-0.091	0.003	0.077	0.004	-0.006	0.413
Substrate (preferendum)									
flags	3.083	0.005	**	-0.001	0.487	0.069	0.004	-0.071	0.003
gravel	7.247	0.001	***	-0.099	0.003	-0.013	0.324	0.093	0.003
sand	12.812	0.001	***	-0.137	0.003	-0.005	0.451	0.115	0.003
silt	10.609	0.001	***	-0.064	0.004	-0.085	0.004	0.140	0.003
macrophytes	8.486	0.001	***	0.120	0.003	-0.019	0.230	-0.078	0.006
microphytes	10.592	0.001	***	0.141	0.003	-0.060	0.044	-0.052	0.044
twigs_roots	4.278	0.001	***	-0.054	0.010	0.088	0.003	-0.049	0.011
organic_detritus	0.774	0.164		0.032	0.153	-0.033	0.153	0.009	0.325
mud	2.024	0.059	.	0.027	0.151	-0.062	0.018	0.043	0.134
Current velocity (preferendum)									
null	31.875	0.001	***	0.239	0.003	-0.087	0.013	-0.103	0.008
slow	6.559	0.001	***	0.107	0.003	-0.020	0.200	-0.066	0.004
medium	35.694	0.001	***	-0.246	0.003	0.056	0.068	0.140	0.003
fast	19.390	0.001	***	-0.189	0.003	0.087	0.008	0.062	0.022
Trophic status (preferendum)									
oligotrophic	9.936	0.001	***	-0.133	0.003	0.086	0.006	0.018	0.266

mesotrophic	3.372	0.008	**	0.050	0.058	-0.078	0.009	0.041	0.059
eutrophic	9.031	0.001	***	0.130	0.003	-0.047	0.041	-0.056	0.024
Temperature									
psychrophilic	5.234	0.001	***	-0.099	0.003	0.043	0.062	0.036	0.066
thermophilic	0.409	0.411		0.024	0.333	0.003	0.446	-0.022	0.333
eurythermic	2.153	0.032	*	0.063	0.006	-0.037	0.126	-0.013	0.318

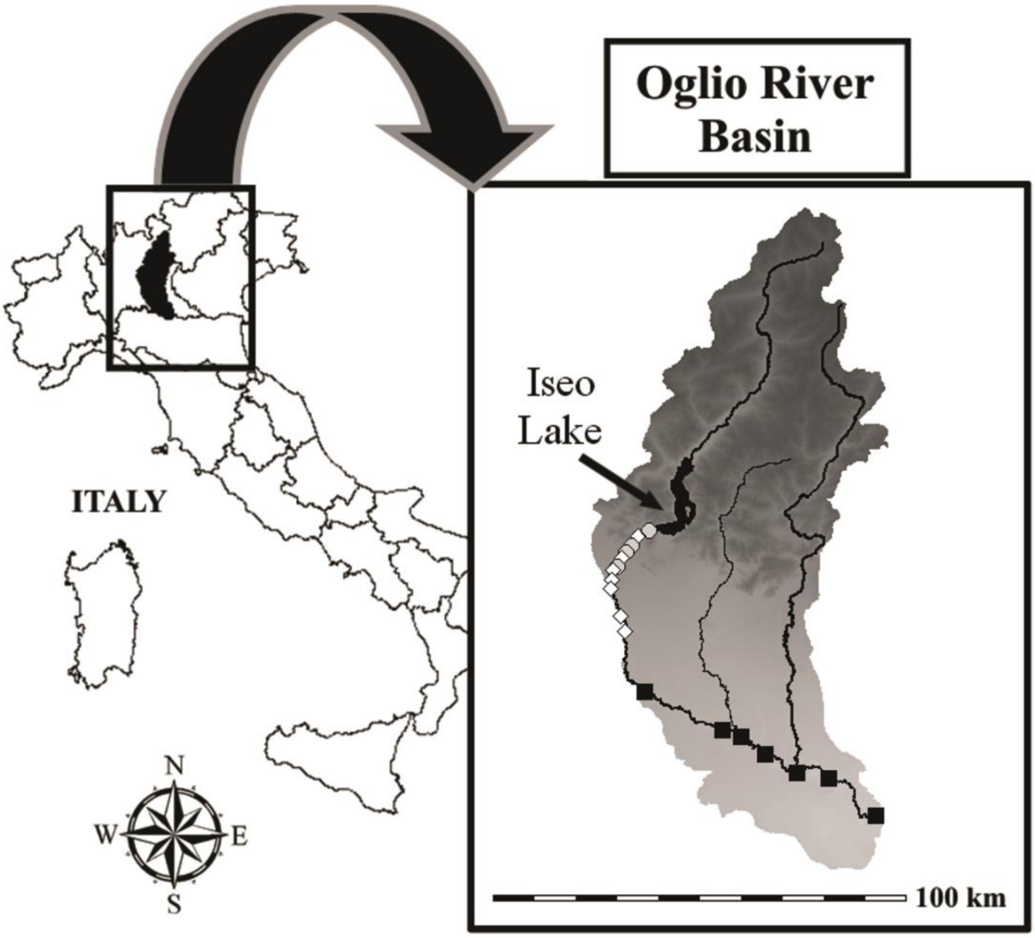

Fig 1. Map of Oglio basin (Northern Italy) and sampling sites along the river. In grey circles the dammed sites, in white downstream sites and in black squares the sites belonging to the free flowing section.

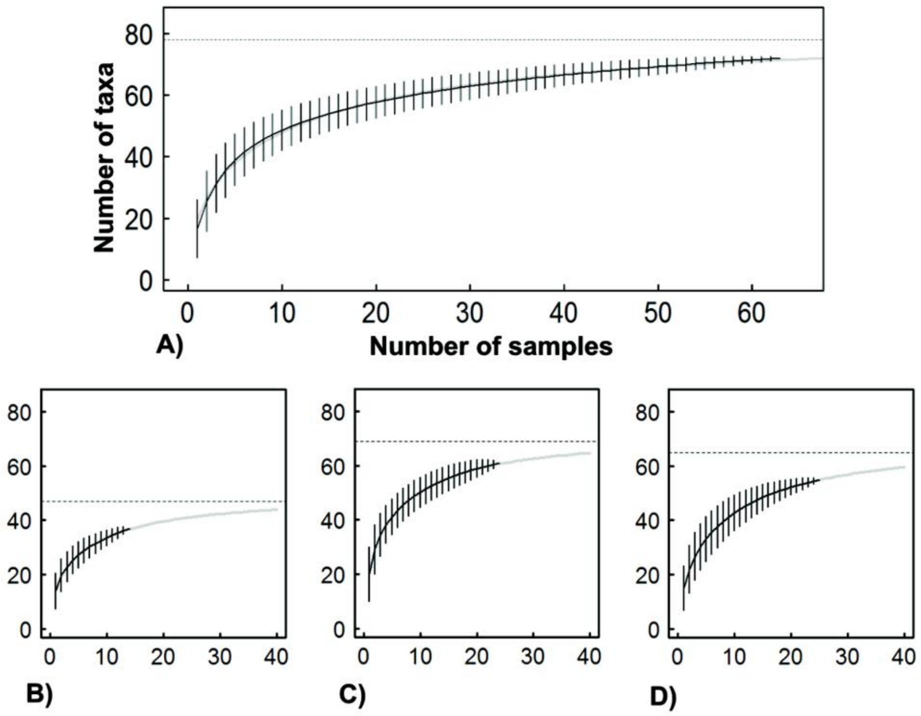

Fig 2. Accumulation's Curves in the Oglio River (A). The three zones separately are also displayed: Dammed stations (B), Downstream stations (C) and free flowing section (D). Expected asymptote is always displayed. The numbers of samples are always displayed on the x-axis while the number of taxa on the y-axis.

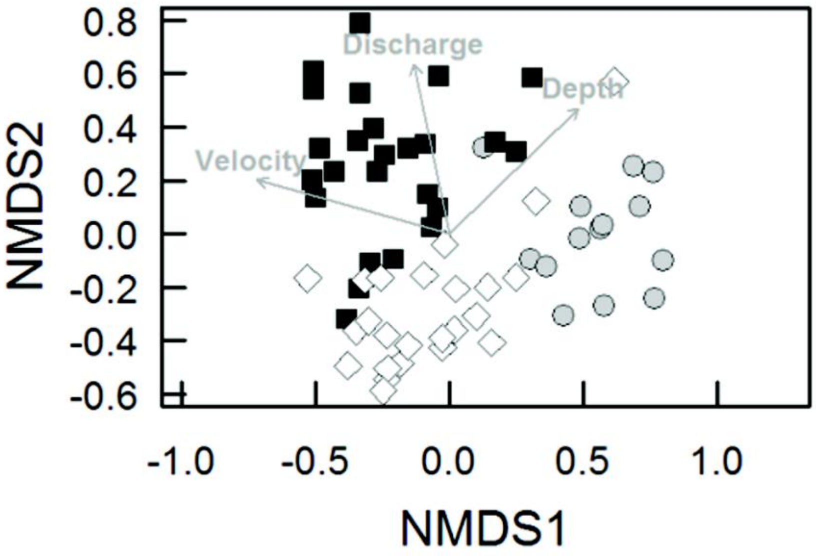

Fig 3. NMDS plot and the *a priori* identified zones coloured. In black color sites belonging to the free flowing section, in grey dammed sites and in white downstream sites (stress=0.16). Hydromorphological variables marked are also displayed.

[sf3]