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Effect of Multiple Stressors on Water Quality and Macroinvertebrate Assemblages in an Effluent-Dominated Stream

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Abstract Lura stream flows in the populated and industrialized conurbation North of Milan, Italy. The area suffers a sprawling urbanization which is leading to major alterations in water quality, hydrology and morphology of streams. These water bodies are known as *effluent-dominated streams*, because most of the baseflow is given by Wastewater Treatment Plant (WWTP) discharges. In this paper, a 5 year long assessment of Lura stream is presented and the collected data is discussed to understand overall ecological quality. Multivariate analysis carried out on macroinvertebrate assemblages and environmental variables suggests that invertebrate communities suffer severe alteration both upstream and downstream WWTP discharges. Results indicate that the high polluting loads coming from WWTP discharges affect seriously the stream water quality, but the most important cause of impairment are pulse perturbations related to the modified hydrology, causing droughts and flash floods, and to the spills of untreated sewage from overflows during rain events.

Keywords Benthic macroinvertebrates · Droughts · Floods · Pollution · Urban streams · Wastewater treatment plant effluents

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1 Introduction

The present research was carried out in order to provide the basis for studying and comparing possible recovery strategies for Lura stream, a small water body in Northern Italy. Lura can be defined as *effluent-dominated stream* (Schmidt 1993), a kind of watercourse greatly affected by the *urban stream syndrome* as described by Walsh et al. (2005). In such stream basins, land over-exploitation in urban areas stresses the flashiness of watercourses, decreasing their natural flows and leading to sudden floods and frequent droughts. Flows given by Wastewater Treatment Plant (WWTP) effluents become the major contribution to total baseflow.

Thus, this kind of streams presents a paradox (Boyle and Fraleigh 2003) in that the flow needed to support the development of biotic communities is essentially provided by the discharge of WWTP effluents, involving the input of residual but conspicuous polluting loads which can affect in other ways the communities themselves. In this kind of watersheds further problems are due to the point input of untreated sewage, the occurrence of flash floods and the consequent input of high polluting loads from the overflows of combined sewers during rain events, the alteration of riparian and riverbed morphology. Brooks et al. (2006) indicate various challenges in studying effluent-dominated streams: (1) absence of reference condition sites; (2) influence of site-specific conditions, which are difficult to point out, on water quality; (3) possible presence, as a worst-case scenario, of emerging contaminants; (4) difficult interpretation of data due to the influence of low flow and drought conditions on biological communities; (5) alteration of water quality given by effluents and stormwater; (6) conflict between the possible use of water (Smith 1993) and the integrity of stream ecosystem. The assessment of such streams is both difficult, due to variability and confounding factors (Nedeau et al. 2003), and important, due to the high number of people living in urban areas and asking for good environmental quality.

Therefore, an increasing number of studies is investigating these ecosystems, and particular attention is given to macroinvertebrate assemblage structures (Coimbra et al. 1996; Spänhoff et al. 2007) and their interaction with environmental variables such as physico-chemical (Daniel et al. 2002; Zeilhofer et al. 2006), hydrological (Nelson and Lieberman 2002; Wills et al. 2006) and morphological (Blakely et al. 2006; Kamp et al. 2007) indicators.

Thus, streams like Lura need a better comprehension of the synergistic effects of multiple anthropogenic stressors, and are a priority within the goals of assessment and restoration programs (Bernhardt and Palmer 2007).

This paper aims to assess the overall anthropogenic pressure of Lura stream basin, and to relate changes in biotic communities to alteration occurrence, basing on the results of a 5 year survey on water chemistry, environmental variables and macroinvertebrate assemblages. Lura stream is a valuable subject of study due to the uniformity of its small basin landscape, which makes stream morphology (width, depth, slope, reach order) quite homogeneous at watershed spatial scale. Thus, macroinvertebrate assemblages are less affected than normally by the high amount of variance due to substratum composition and hydraulic gradient (Gore 1978; Mérigoux and Dolédec 2004; Beauger et al. 2006) as well as to longitudinal ecological changes.

2 Materials and Methods

2.1 Study Area

Lura stream is 45 km long and passes through 17 municipalities in Lombardy (Italy), north of Milan, as shown in Fig. 1. Its catchment (about 130 km^2) is long and narrow, as is the typical case of lowland streams in this area. Lura receives water from superficial groundwater, wet meadows and small creeks, both on right and left side, and merges into the Olona river at Rho, close to Milan. Impairment is due to both the widespread urbanization, generating high polluting loads and catchment imperviousness, and to the presence of several industrial settlements. The three most critical aspects can be summarized (Canobbio and Mezzanotte 2003; Mezzanotte et al. 2005) in (1) the increasing water scarcity, determining frequent droughts in the upper part of the stream; (2) the occurrence of flash floods along the whole watercourse; (3) the loss of habitat suitability due to destruction of riparian vegetation and river bed morphological diversity; (4) the regular input of great polluting loads from WWTPs. Starting from the first

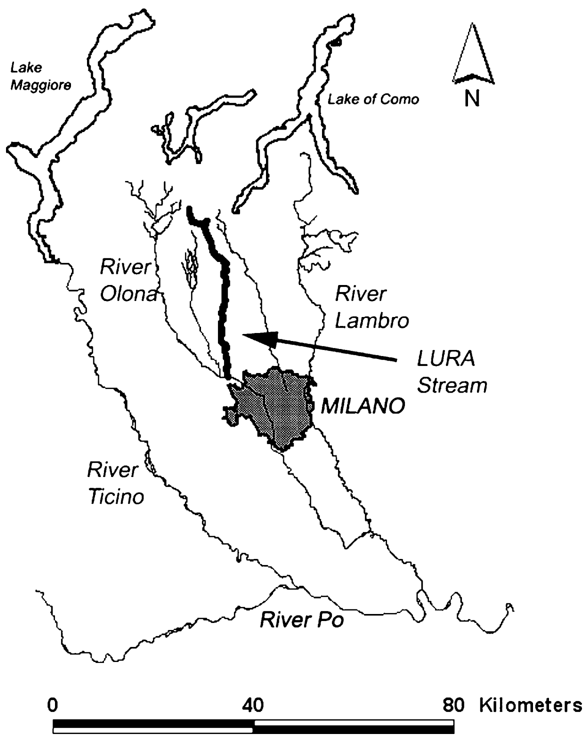

Fig. 1 Lura stream and the surrounding area

WWTP discharge, effluents constitute most, and sometimes the only, stream flow. Two of the existing WWTPs, Alto Lura and Lura Ambiente, about 150,000 Equivalent Inhabitants (EI) each, discharge directly into Lura, the other one (Livescia, about 40,000 EI) into a small tributary, called Livescia.

Thus, Lura stream can basically be divided in three sectors. The first one (about 7 km) can be considered as a "reference" sector, although already affected by anthropic presence. The surrounding area suffers from a sprawling urbanization, but natural base flow is still present for most of the year, though some droughts can occur in summer. Occasionally, some untreated sewage spills affect water quality.

The second sector, 7 km long, is subject to severe droughts, due both to changes in land use and to high river bed permeability. Water can be absent for many consecutive months, regardless of the season but with strict dependence from weather conditions.

The third sector, 31 km long, is effluent-dominated. The first WWTP (Alto Lura, AL-WWTP) is straightaway followed by the inflow of Livescia tributary (2nd WWTP). After the first WWTPs, the stream runs for 15 km in a wooden and farmed land, till the town of Saronno and the 3rd WWTP.

2.2 Data Collection

To study the hydrologic regime of the Lura stream, a detailed and physically based model has been built on a MIKE 11 platform (http://www.dhigroup.com/Software/WaterResources/MIKE11.aspx), which is one of the most reliable and commonly used tools for river simulation.

Rainfall data were collected continuously for two representative years of continuous rainfall data (2004 normal, 2005 dry) using three raingauges whose area of influence was estimated by the Thiessen method.

Basing on such data, hydrographs were made for four relevant stream sections (one in the first "reference" sector, one in the second, upstream of AL-WWTP, and two in the longer effluent-dominated one, just downstream and 8 km after the discharge of AL-WWTP), corresponding to the four sampling sites chosen for monitoring macroinvertebrate assemblages. Then, flow-duration curves have been plotted for each one of the four river sections, in order to assess the hydrological regime of the Lura stream in terms of number of days in the year during which the river flow rate has been equal or above a given threshold value.

Water quality has been studied for 5 years (2001–2006), by monthly sampling and physico-chemical analyses. Water samples were collected in 11 sites: one in the first sector, two in the second, eight in the third. Physico-chemical analyses included temperature, electric conductivity, dissolved oxygen (DO), pH, COD, total phosphorus (tot-P), total and ammonia nitrogen (tot-N and NH_4–N).

Macroinvertebrate assemblages were studied in four representative sites (as described above). Sampling campaigns have been carried out every 6 months, and every sampling campaign was done in very close days, with relatively homogeneous hydrological conditions. During each campaign, in each of the four sites six samples (covering 0.1 m^2) have been collected with a 500 μm mesh Surber sampler in different microhabitats, possibly along transects, thus trying to investigate the whole physical environment of the site. Invertebrates were stored in a final solution of 4% formaldehyde. In laboratory, macroinvertebrates were counted and taxonomically identified, generally to genus or family level. Invertebrates were identified using keys described in Sansoni (1992).

Extended Biotic Index (EBI—Woodiwiss 1978, adapted to Italian watercourses by Ghetti 1987), based upon DO sensitivity of taxa and their richness, was first determined, as well as Taxa Richness (*S*) and Shannon's Diversity Index (*D*). Environmental variables describing the macroinvertebrate habitats were estimated in field or in laboratory. Mean water depths and flow velocities for each site were recorded with replicate measurements at each sampling site.

Time from potential destructive flood and drought events was also recorded, and used to determine drought vicinance (quantified as the number of days from the last drought, if any, in 120 days), drought frequency (as the number of days of drought, if any, in 120 days) and flood vicinance (as the number of days from the last flood, if any, in 120 days). Finally, IFF, a river functionality index based upon RCE-2 (Petersen 1992), introduced in Italy by Siligardi and Maiolini (1993) and adopted by the Italian Environmental Protection Agency (ANPA 2000), was used to attribute a qualitative value to four environmental variables: riparian vegetation, bankside structure, riverbed morphology and general biota conditions. These values were recorded as a ratio between obtained values and IFF given optimum (maximum obtainable value) for each of the four variables.

2.3 Data Analysis

Macroinvertebrate assemblages and related environmental variable data sets were analyzed using CANOCO 4.0 (ter Braak and Smilauer 1998). Specific ordination techniques (Legendre and Legendre 1998) were used to examine patterns in the macroinvertebrate data (presence/absence of taxa) and to identify variables that were most closely associated to invertebrate distribution. Preliminary detrended correspondence analysis (DCA) on the taxa data revealed a gradient length <3 SD, indicating that most exhibited linear response (Fore et al. 1996) to environmental variations, thus justifying the use of linear multivariate analysis. Redundancy Analysis (RDA) was carried out with a forward selection of environmental variables. 999 Monte Carlo permutations were performed to determine which variables were significantly related ($P \leq 0.05$) on invertebrate distribution. The non significant variables were excluded from the analysis. Next, the Variance Inflation Factors (VIFs) of included variables were inspected. Variables with VIF>20, indicating strong multicollinearity, were excluded.

3 Results and Discussion

3.1 Hydrological Parameters

On the whole, the MIKE 11 model confirmed the differences among sites and the partition of stream in three sectors presenting different hydrological conditions, as shown in Fig. 2.

Comparing the 2004 and 2005 curves, the ephemeral regime of the stream, due to the lack of groundwater

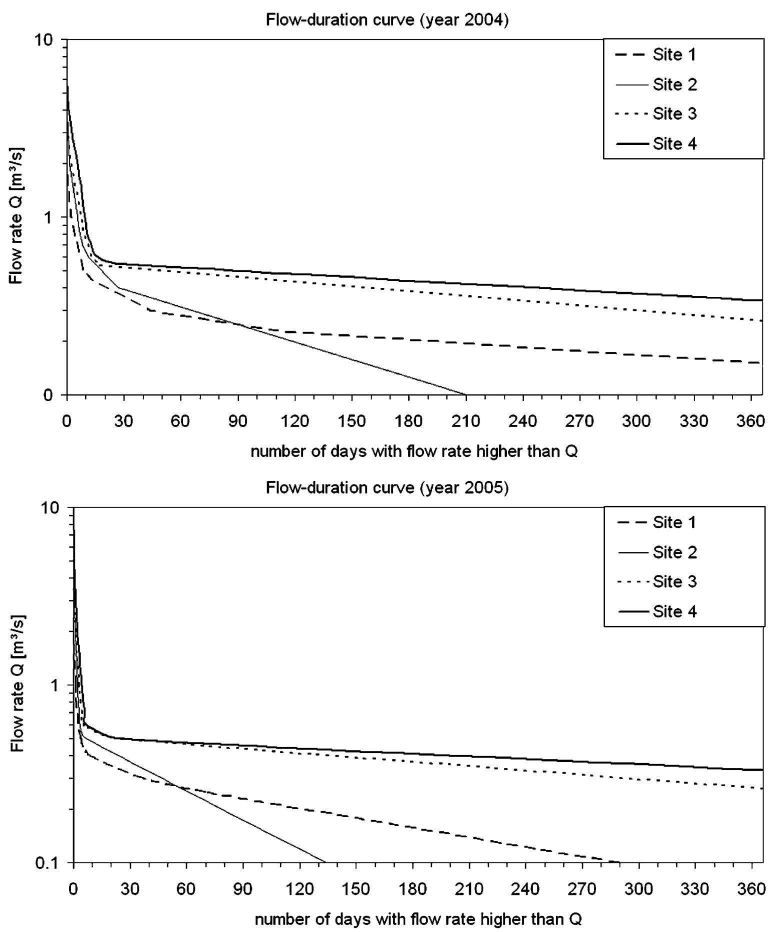

Fig. 2 Flow-duration curves for four Lura sections in years 2004 and 2005

contribution to the river flow, can be observed from the trends in Sites 1 and 2. In 2005 (representative of scarce rainfall years, rain/year=544 mm), water was completely absent for 75 and 230 days in Sites 1 and 2 respectively. In 2004, when rainfall was higher (rain/year=839 mm), a baseflow was always present in Site 1 and in 210 days in Site 2. The difference between the two sites is chiefly related to the high permeability of the riverbed in site 2 and to the consequent water loss.

In sites 3 and 4 flow, in dry periods, is made essentially of the effluents discharged by WWTPs, while the direct rainfall-runoff raises the stream flow for no more than one day after the event. Site 3 receives the effluent from AL-WWTP while site 4 receives also the input from Livescia (the most important tributary), whose flow, in dry periods, is completely made of the effluent from Livescia WWTP.

The flashiness of floods is clearly shown in Fig. 2 by the short duration of higher flows. In agreement with the greater amount of rain fallen in 2004, peak flows were higher than in 2005, furtherly confirming the strict dependence of flow from meteorological events.

3.2 Physico-chemical Parameters

Trends of physical and chemical factors in low flow (site 2 dry), moderate flow (site 2 with a base flow) and flood peak conditions are shown in Fig. 3. In low and moderate flow conditions, the most noticeable event was always the input of the effluent from AL-WWTP (site 3), which strongly affected the values of all parameters.

Water temperature was usually higher after WWTP discharges (indicated by arrows in Fig. 2) and decreased downstream, due to exchange with air temperature, especially in low flow conditions. When a base flow was present upstream the AL-WWTP discharge, temperature peaks were controlled by the immediate dilution. With higher flows (flood condition) temperature was almost homogeneous in the entire stream, due to the very little contribution of WWTP effluents to the total streamflow.

Though the stream receives WWTP effluents which commonly have low DO saturation, drops in DO saturation were not significant as expected. This is due to the fact that AL-WWTP effluent presents high DO saturation itself, mainly because of the use of ozone as the main disinfectant. Thus, mean DO saturation was never below 80%, even downflow the effluent discharges. Drops in DO saturation were almost cancelled with the presence of significative base flow. During floods, starting from site 2 the action of various sewage overflows, pouring in high loads of ammonium nitrogen and organic matter, caused oxygen saturation to decrease.

In low flow conditions, NH_4–N concentrations measured at Site 1, though considered as a reference site, were high (average>1 mg/l), probably because of uncontrolled inputs in rural areas. After AL-WWTP discharge (site 3), mean conductivity was about 1,500 μS/cm, COD 60 mg/l, total P 1.5 mg/l and total N 17 mg/l. With moderate flows trends were similar but concentrations were lower. However, some spills in urban areas originated other peaks. During floods, peaks in COD and total N and, especially, NH_4–N were already present in site 2 due to the already cited sewage overflows. At site 2, during floods, ammonia concentration raised to about 2 mg/l (as mean flood value), leading to estimate polluting load as very high, taking into account the high diluting flows (7.555 m^3/s in November 2004 sampling campaign, which is Q_1 flow for site 2 in Fig. 2).

3.3 Macroinvertebrate Assemblages

45 taxa were identified in the four monitored sites. First, Taxa Richness (*S*), Shannon's Diversity Index (*D*) and EBI were calculated in order to obtain a simple ranking tool. Results are reported in Table 1. Site 1, representing the first "reference" reach, is characterized by benthic invertebrate assemblages of higher quality. Both the highest Taxa Richness (from 10 to 16, mean S=12.3) and the most sensitive taxa were detected, including Plecoptera (Leuctridae and Nemouridae) and various Ephemeroptera. Stoneflies disappeared completely in the other sampling sites, while the only mayflies detected downstream were *Baetis* spp. In site 2 *Baetis* spp. and some Diptera (especially Chironomidae and Simulidae) were usually dominant, while in site 3 and 4 Trichoptera Hydropsichidae was the most noticeable taxon. *Baetis* almost disappeared in site 3 but was always found in site 4. In these two sites, Chironomidae were ubiquitous and extremely abundant. An occasional presence of Gastropoda, sometimes abundant, could be observed.

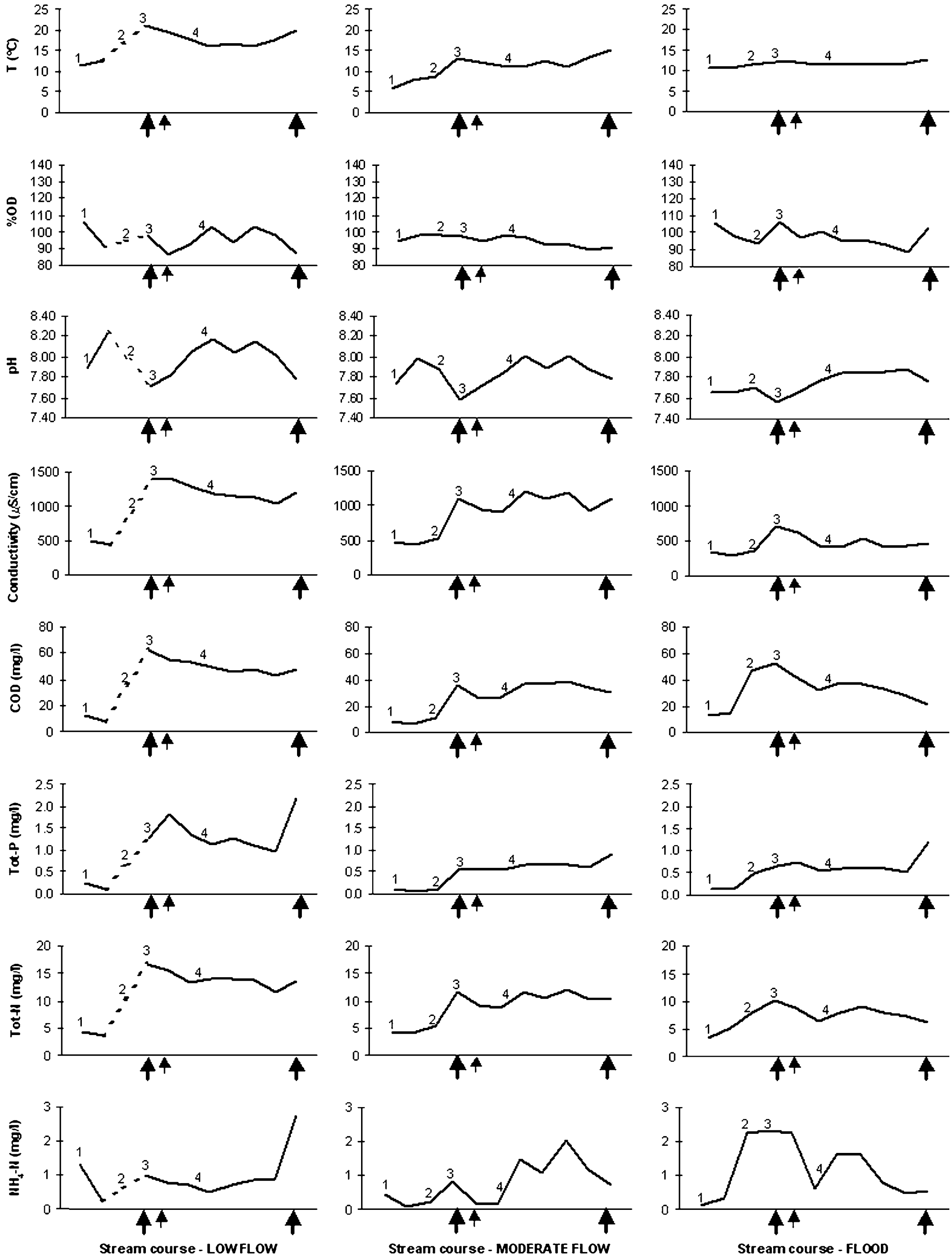

Fig. 3 Mean values of physico-chemical parameters in low flow (*column A*), moderate flow (*column B*) and flood (*column C*) conditions along the stream course during 2001–2006. *Numbers* indicate macroinvertebrate sampling sites, *arrows* indicate WWTP discharges

At site 1, EBI values were comprised between 7 and 8, corresponding to Class II–III (of V), while Shannon's Diversity Index (*D*) had a mean value of 1.62. In Site 2 water quality was still acceptable, but, as already stated, the watercourse suffered from important hydraulic fluctuations, with long droughts and sudden floods. Such situation led *S* to 6–11 (mean value 7.9) and EBI and *D* to lower values. At site 3

Table 1 Synthesis of macroinvertebrate sampling results and EBI classification

Site	N. sampling campaigns	Taxa richness (*S*)	abundance of individuals (n/0.1 m^2)	Shannon's Diversity Index (*D*)	EBI value
		Min–max (mean±SD)	Min–max (mean±SD)	Min–max (mean±SD)	Min–max (mean±SD)
1—Olgiate	10	10–16 (12.3±2.0)	194–580 (375±149)	1.49–1.88 (1.62±0.13)	7–8 (7.8±0.4)
2—Bulgaro	8	6–11 (7.9±1.9)	53–1,082 (516±296)	0.94–1.60 (1.30±0.28)	5–6 (5.4±0.5)
3—Guanzate	10	4–11 (7.8±2.4)	119–2,601 (1,092±697)	0.65–1.33 (1.04±0.22)	2–6 (5.1±1.3)
4—Lomazzo	10	6–10 (7.8±1.1)	100–844 (503±238)	0.85–1.80 (1.31±0.26)	5–6 (5.8±0.4)

the AL-WWTP effluent influence brought to highly variable EBI values (2 to 6, corresponding to Class III to V). Such variability, involving also *S* and *D* values, was mainly related to flow conditions. At site 4, better riverbed morphology and a residual self-purification ability of the stream (which can buffer pulse disturbances and polluting load peaks) led to a slightly better situation: EBI raised to 5–6, ranking site 4 in Class III–IV, while *D* was comprised between 0.85 and 1.80.

Mean values for environmental variables observed during macroinvertebrate sampling are presented in Table 2, as well as standard deviations, minimum and maximum values. Water depth was comparable between sites, and stream width increased with flow. Mean velocity values were similar in site 1, 3 and 4, but much lower in site 2. This variable was related to drought frequency and drought proximity. The values observed for physico-chemical parameters during macroinvertebrate sampling campaigns were comparable to the values measured over the whole period of monitoring of water quality (see Fig. 3). Site 1 had the best vegetation (with willows, alders and cottonwoods as valuable riparian elements within the black locust woods) and biota, because of normal periphytic cover and availability of coarse particulate organic matter (CPOM).

Higher values of rainfall were related to the proximity of flood events but lower values were not related to droughts, because droughts involved only site 2 (and partially site 1). Thus, collinearity between scarce rainfall and droughts could only be observed for site 2. Droughts were recorded also at site 1 but only before autumn sampling campaigns (years 2003 and 2005).

Redundancy Analysis was applied to macroinvertebrate community data and associated environmental variables as shown in Figs. 4 and 5. In Fig. 4, different colors indicate EBI classes for each site in each sampling campaign. Site 2 is represented by eight scores only, because in spring/summer 2003 and in autumn/winter 2005 (samples 2.04 and 2.09) droughts covered the whole period and sampling was not possible. Site scores are separated along the two axis that account for most of the variability based on benthic invertebrates assemblage structure. For this RDA, the first two axes showed 26.8% of macroinvertebrate variability and 51.5% of species–environment relation variability. The test of significance for both the first axis and overall RDA was highly significant (P=0.001).

Position of site scores indicates a clear division within the first axis among sites upstream and downstream the AL-WWTP in all seasons. Occasionally, sites downstream the effluent discharge (4.05; 3.03, 3.05, 3.06) were more similar to upstream site 2. This was directly related with floods event vicinity and, thus, with higher base flow in the upstream sites and higher diluting flow downstream. This, however, brought to the lowest EBI values (3.03 and 3.06) due to flood destructivity and sewage overflow spills. WWTP upstream sites (sites 1 and 2) are distinctly separated by vertical Axis 2, as site 2 is mainly affected by drought events.

Environmental variable vectors represent gradients through the community data, with the arrow pointing the area of higher value for each variable, but the gradient extends through the whole set. Axis 1 is positively related with droughts, pH and biota quality, and negatively related with temperature, ammonia nitrogen, conductivity and riverbed morphology (the latter presents low values along all the stream). These variables are also collinear with other, omitted chemical parameters and they are all related with the effluent dominated sector of the stream. Worse EBI results for sites downstream AL-WWTP discharge occur in two clusters near highest value of NH_4–N and

Table 2 Environmental variables at macroinvertebrate sampling sites during monitoring campaigns; mean values±SD (min.–max.)

Environmental variables		1—Olgiate	2—Bulgaro	3—Guanzate	4—Lomazzo
Depth (mean)	cm	13±2 (10–18)	20±4 (15–26)	18±3 (15–23)	21±2 (18–26)
Width	m	3.1±0.1 (2.9–3.3)	3.9±0.3 (3.5–4.4)	5.5	6.3±0.1 (6.3–6.7)
Flow speed (mean)	m/s	0.34±0.05 (0.27–0.42)	0.15±0.04 (0.09–0.21)	0.39±0.05 (0.34–0.51)	0.32±0.05 (0.28–0.45)
T	°C	8.2±5.8 (1.5–16.5)	12.8±2.8 (9.4–17.5)	17.5±6.0 (11.2–26)	14.0±5.8 (5.8–22.1)
DO	%	98.1±10.6 (82.1–118.5)	105.2±36.8 (61.0–186.1)	98.0±15.9 (73.8–124.1)	97.4±10.9 (81.7–114.0)
pH	pH u.	7.81±0.29 (7.27–8.21)	8.04±0.31 (7.56–8.56)	7.47±0.27 (6.93–7.8)	7.86±0.29(7.18–8.22)
Conductivity	μS/cm	441±175 (254–815)	419±56 (309–506)	1320±506 (418–1876)	1341±461 (398–1764)
COD	mg/l	9±3 (5–17)	19±19 (5–60)	53±17 (35–83)	52±12 (31–72)
P-tot	mg/l	0.174±0.108 (0.047–0.370)	0.217±0.187 (0.056–0.632)	1.359±0.682 (0.325–2.618)	1.567±0.858 (0.537–2.782)
N-tot	mg/l	5.017±1.640 (2.740–8.590)	4.599±2.295 (2.300–9.170)	17.449±5.626 (6.950–24.750)	16.420±5.630 (5.830–23.735)
NH_4–N	mg/l	0.632±0.835 (0.082–2.580)	0.592±1.021 (0.000–2.465)	2.966±4.901 (0.029–15.950)	1.213±2.446 (0.045–7.950)
Riparian vegetation condition	IFF value/IFF optimum	0.65	0.37	0.53	0.42
Riparian structure condition	IFF value/IFF optimum	0.55	0.13	0.56	0.67
River bed condition	IFF value/IFF optimum	0.41	0.42	0.44	0.55
Biota condition	IFF value/IFF optimum	0.53	0.22	0.18	0.20
Rain	mm (120 days)	265±124 (64–420)	269±135 (80–517)	283±133 (129–568)	278±110 (167–567)
Droughts (vicinance)	1–(days from dry/120 days)	0.152±0.251 (0.000–0.625)	0.590±0.407 (0.000–1.000)	0.000	0.000
Droughts (frequency)	days of dry/120 days	0.171±0.299 (0.000–0.750)	0.498±0.398 (0.000–1.000)	0.000	0.000
Floods (vicinance)	1–(days from event/120 days)	0.352±0.330 (0.000–0.833)	0.190±0.297 (0.000–0.833)	0.413±0.380 (0.000–0.900)	0.335±0.341 (0.000–0.778)

floods. Site 2 scores are related with both droughts and (along Axis 2) floods, and are defined by low EBI values. On the other hand, site 1 scores are the best of the entire set and are only slightly related with droughts, but strongly related with good riparian vegetation and biological quality.

Taxa distribution is represented in Fig. 5. Environmental variables were omitted from graph to make it clearer, but their gradients are reported in Fig. 4. The greatest diversity is positively related with Axis 1 and negatively related with Axis 2, and is thus in the lower-right quadrant where site 1 scores are clustered (presenting negative relationship with both pollution on axis 1 and droughts on axis 2). All sensitive taxa are here, including all stoneflies and mayflies. Some taxa presented positive relationship with droughts, particularly Crustacea (both Gammaridae and Asellidae) and some Gastropoda (*Ancylus*). Caddisflies of Rhyacophilidae family were found only in one sample at site 2, thus being allocated in the same area. Some taxa were extremely tolerant about pollution, preferring sites 3 and 4 which, on the other hand, ensure to invertebrate communities a constant base flow. Such characteristics were preferred by Odonata, some Diptera, Gastropoda (*Lymnaea* and *Physa*) and Trichoptera Hydropsichidae. Both Oligochaeta Tubificidae and Diptera Chironomidae were completely ubiquitous and are centered in the origin of the graph.

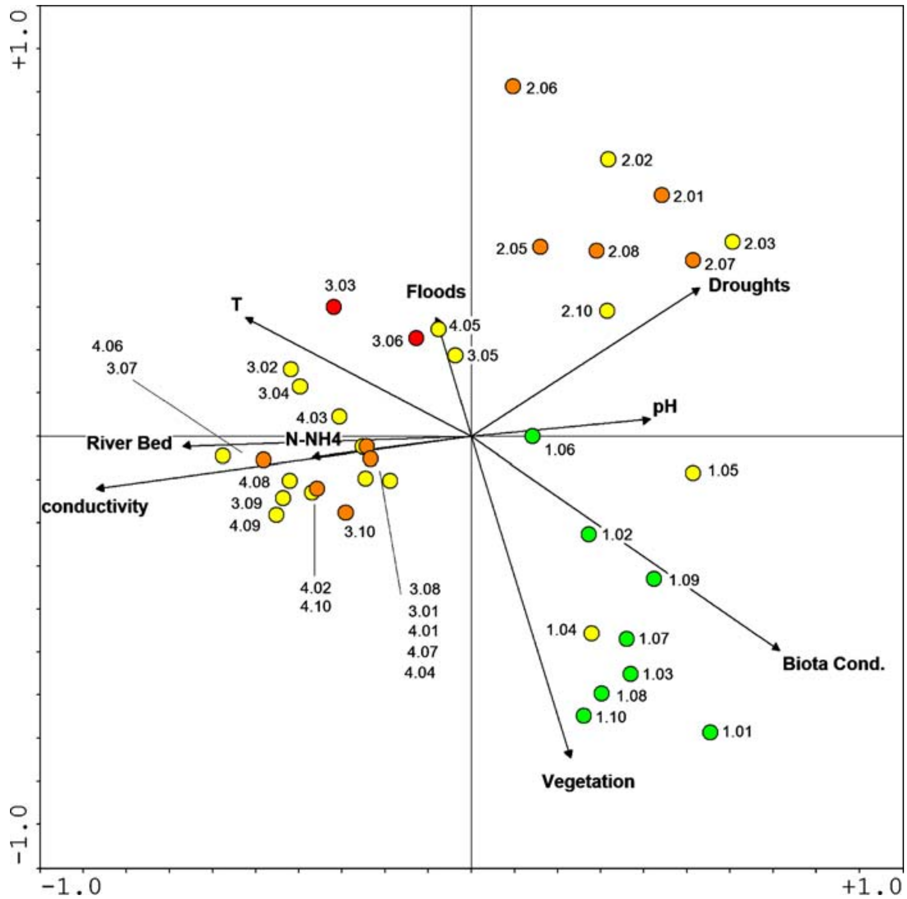

Fig. 4 RDA biplot graph indicating relationships between environmental variables (*arrows*) and macroinvertebrate communities sampled in the four sites. *Labels* show also a number indicating the period of sampling, every 6 months from fall/winter 2001 (0.01) to spring/summer 2006 (0.10). Site scores are indicated with different colors showing their EBI value (*red*: 2–3; *orange*: 4–5; *yellow*: 6–7; *green*: 8)

4 Conclusions

According to the results of physico-chemical monitoring, the most important factor affecting water quality of Lura stream is Alto Lura WWTP discharge. Effluents input continuously a too high polluting load (especially organic matter and nutrients) which cannot be diluted enough by the scarce or void baseflow which is usually present. Hydrologic regime, which is heavily affected by the basin intense urbanization, has a key role in influencing WWTP effluent effects on stream water quality. Collected data shows that very different physico-chemical conditions occur when the three hydrologic regimes (low flow, moderate flow and flood) alternates. The best conditions, with low pollutant concentrations, were generally observed during moderate flow periods, due to the stream diluting capability. However, hydrological parameters analysis shows that such periods are short, especially in years presenting scarce rainfall. Low flow is a more standard condition in effluent-dominated streams, usually influenced by their basin land use and, thus, presenting ephemeral regime. Flash floods are another hydrological distinctiveness of the stream, and even if they present a low frequency, as shown by hydrological parameters analysis, they strongly influence the stream overall ecological quality. During floods, spills of untreated wastewater occur along the stream, balancing the very high diluting capability of such high flows with greater polluting loads. The main consequence is that some peaks in pollutant concentrations (especially ammonia nitrogen and organic matter) can be found, affecting both the stream sector upstream WWTP discharges and the effluent-dominated one. Moreover, this kind of perturbation occur when the mechanical action of floods already affects biotic communities.

Thus, the altered hydrologic cycle and the consequent alternance of drought and flood events have the greatest impact on the ecology of Lura stream. This happens both upstream the WWTP discharges, where water scarcity originates destructive droughts

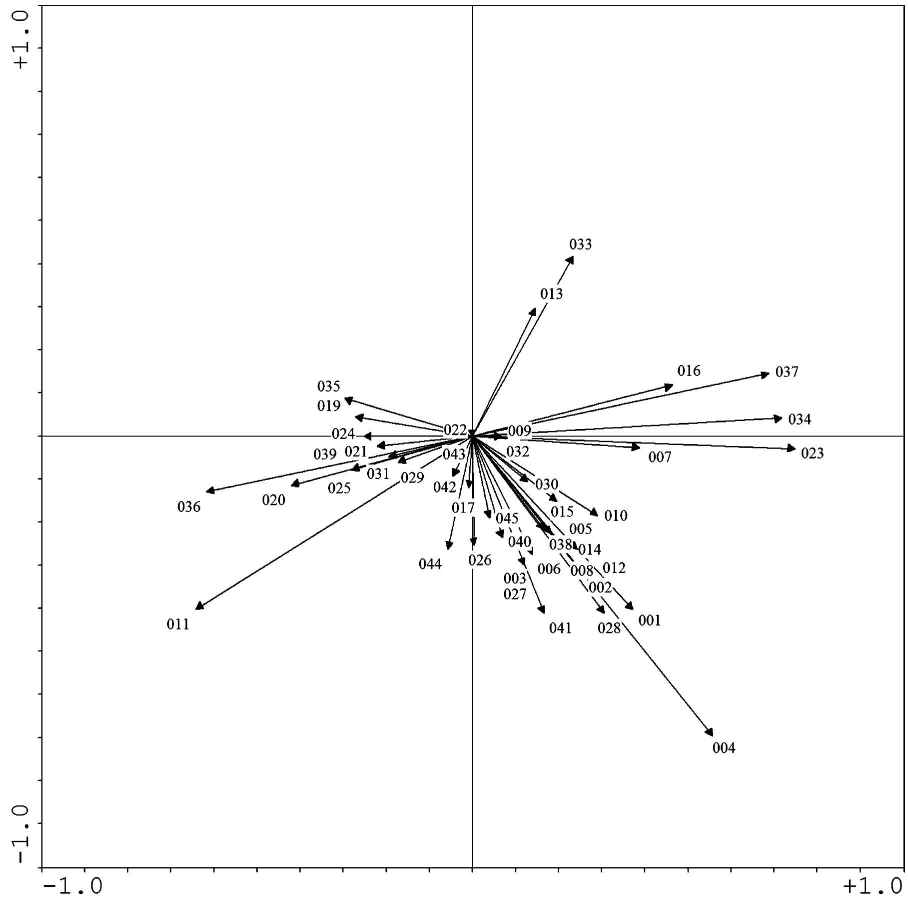

Fig. 5 Scatter diagram of macroinvertebrate taxa found in Lura Stream. Scores are related to environmental variables and sites as shown in Fig. 4. Legend:

n.	*Taxa*	*n.*	*Taxa*	*n.*	*Taxa*	*n.*	*Taxa*	*n.*	*Taxa*
001	*Leuctra*	010	*Paraleptophlebia*	019	*Onychogompus*	028	Dixidae	037	*Ancylus*
002	*Anphinemura*	011	Hydropsichidae	020	*Orthetrum*	029	Rhagionidae	038	*Planorbis*
003	*Nemoura*	012	Limnaephilidae	021	*Crocothemis*	030	Athericidae	039	*Haemopis*
004	*Ecdyonurus*	013	Rhyacophilidae	022	Chironomidae	031	Anthomyidae	040	*Dina*
005	*Rithrogena*	014	Philopotamidae	023	Simuliidae	032	*Nepa*	041	Naididae
006	*Habrophlebia*	015	Goeridae	024	Limonidae	033	Gammaridae	042	Lumbricidae
007	*Baetis*	016	Dytiscidae	025	Tabanidae	034	Asellidae	043	Tubificidae
008	*Procleon*	017	Elminthidae	026	Tipulidae	035	*Lymnaea*	044	Lumbiculidae
009	*Habroleptoides*	018	Hydraenidae	027	Psychodidae	036	*Physa*	045	Mermithidae

preventing a balanced development and survival of invertebrate assemblages (as described by Lake 2003), and after them, where the effect of floods, taking place frequently, becomes dominant (Scrimgeour and Winterbourn 1989). As expected (Boyle and Fraleigh 2003), variation in macroinvertebrate assemblages is associated with various classes of variables. Multivariate analysis divides macroinvertebrate communities upstream and downstream WWTP discharges along Axis 1, as shown also in Couceiro et al. (2007), but the worst EBI and Shannon's *D* Index values, indicating the poorer biotic communities, are confined in clusters influenced by the simultaneous presence of the worst water quality (Mattei et al. 2006), given by ammonia nitrogen spills, and of recurring floods. Thus, while the effluent-dominated section of the stream obviously presents a worse macroinvertebrate assemblage structure if compared with the reference site (lower Taxa Richness, Shannon's *D* Index and EBI values), the worst impacts affecting the stream ecology are not given by WWTP treated effluents, but are caused by untreated discharges (mainly due to sewer overflows), according to literature (Rueda et al. 2002; Gücker et al. 2006), and altered hydrology. Samples related to such extreme conditions show not only a worse quality in taxa sensitivity (no Plecoptera, only tolerant Ephemeroptera and Tricoptera), typical for all the samples in the effluent-dominated portion of the stream, but also a significantly lower taxa richness, caused by the absence of even some tolerant taxa, and density. The relationship between macroinvertebrate taxa and environmental variables given by multivariate analysis shows that there are very few taxa positively related with the presence of hydrological perturbations (both floods and droughts), while there are various taxa (mainly tolerant ones) that show a positive relationship with pollution indicators of the effluent-dominated sector of the stream. Tolerant macroinvertebrates can use the stream environment downstream the WWTP discharges as a stable living habitat (due to the continuous presence of water), but communities are greatly affected by episodic perturbation such as droughts, floods and spills of untreated wastewater. Moreover, the overall quality of the stream morphology is low and this causes an increase in all the other alteration effects, due to low habitat availability and absence of refugia for biotic communities (Boulton 2003).

On the basis of the disturbance characteristics and the way they occur in the stream, perturbations affecting Lura can be divided in two kinds, that can be called *press* and *pulse* (Bender et al. 1984). Actually, *press* impacts (WWTP discharges) showed a more evident effect on water quality but *pulse* ones (droughts, floods, spills) presented worse effects on biota, due to the low resistance of the stream ecosystem. Paradoxically, WWTP discharges grant a stable baseflow to biotic communities and, thus, macroinvertebrate assemblages in the effluent-dominated sector, when no other perturbation occur, show high densities of individuals and intermediate quality. Such quality is significantly lower if compared with reference site but much better than the quality of assemblages affected by *pulse* perturbations, both upstream and downstream WWTP discharges. In site 2, where water quality is generally good but *pulse* perturbances occur, values of Taxa Richness, Shannon's *D* Index and EBI can drop far below the mean values of the effluent-dominated sites, if perturbations like droughts, floods and/or sewage overflows take place. On the other hand, in the effluent-dominated sector of the stream, the worst ecological quality of the whole stream is found when *pulse* perturbances (floods, untreated wastewater discharges) sum their effects to the continuous *press* disturbance given by WWTP discharges.

In effluent-dominated streams like Lura, WWTP effluents can be perceived as the greater impact. It is obvious that, if compared with available reference sites, the water quality and biotic communities of effluent-dominated sites result greatly altered. However, it must be considered that the same basin features (especially sprawling urbanization) that normally lead to a conspicuous amount of effluent discharges, originate other kinds of perturbations, both *press* (such as reduced baseflow and altered morphology) and *pulse* (droughts, flash floods and spills of untreated wastewater) that are both difficult to assess and greatly impacting. *Pulse* perturbations do not allow the ecosystem to regulate itself to the new conditions, while *press* perturbations concur in making the ecosystem resistance and resilience weak. Thus, all the mentioned alterations have great influence and involve themselves reciprocally. Environmental studying and restoration planning in effluent-dominated streams should not be limited to water quality, but should consider, in example, measures for increasing habitat quality, providing acceptable flow conditions (Brunke et al. 2001) and improving morphology.

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