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- Tierno de Figueroa et al. 2009.
- Usseglio-Polatera et al. 2000.

Impacts of a micro-sewage effluent on the biota of a small Apennine creek

Tiziano Bo and Stefano Fenoglio*

*Department of Life and Environment Science, University of Piemonte Orientale, Di.S.A.V.,
Via T. Michel 11, 15121 Alessandria, Italy*

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Organic pollution of domestic origin represents the most important cause of water quality deterioration in rural and mountainous areas of the northern Apennines. In this study, the ecological consequences of a small sewage dump in the Caramagna Creek (northwestern Italy) were analyzed. The addition of organic matter and nutrients led to a dramatic change in the taxonomic richness and density of the macrobenthic community. Also functional, biological, and ecological composition of the invertebrate assemblages changed downstream of the effluent. Interestingly, benthic chlorophyll *a* showed only a weak increase in the downstream section, despite the increased levels of nutrients. This work emphasizes the importance of better management of sewage treatment also in remote areas.

Keywords: ■■■

Introduction

Organic pollution represents one of the most common causes of degradation of water quality in stream ecosystems (Paul and Meyer 2001). This kind of pollution is usually categorized as derived from point sources (Goudie 2006). Industrial and farm effluents, and urban run-off are surely important in this context, but sewage pollutants of domestic origin represent the greatest source of organic materials discharged into fresh waters (Mason 2002). In much of the developed world, the greatest part of the population is served by public sewers, and approximately the 80% of sewage receives at least secondary treatment, but the release of crude sewage into watercourses still remains a great ecological problem. Sewage from villages and towns is usually treated and then sent into river systems of medium-high order, but this practice is not so diffuse when we consider wastewaters from isolated houses and small housing assemblages. The impacts of these small point sources attain special relevance in circum-Mediterranean ecosystems, where water-level is scarce and temperature is elevated (Ortiz et al. 2005; López-Rodríguez et al. 2009).

The northern Apennines area, situated between the Alps and the Mediterranean, represents an important biodiversity hotspot, with peculiar climatic, geomorphologic, and biologic characteristics. This area has a low human population density; the economy is generally based on non-intensive agricultural and silvicultural practices,

*Corresponding author. Email: fenoglio@unipmn.it

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while medium and large industrial plants are lacking. Northern Apennine rivers are characterized by the presence of discharge peaks produced by autumn–winter rains, after which flows decline to baseflow in early summer. The hydrographic network of the area, because of the complex morphology of the Apennine mountains, is characterized by the presence of a few medium-sized rivers but a myriad of small to very small streams and creeks. These lotic systems host rich and diversified biological communities (Bo et al. 2009, 2010), with many rare and interesting taxa (Tierno de Figueroa et al. 2009; Fenoglio et al. 2010a). In the recent years, these environments have been increasingly threatened by human activities. Recent studies have emphasized the role of morphological alterations in damaging lotic systems of the northern Apennines (Hering et al. 2001), but little information is available about the effects of sewage micro-effluents on small Apennine creeks. The aim of this study was to analyze the effects of a micro-point source of organic pollution on the biota of a small Apennine creek, investigating the impacts of the effluent on the macroinvertebrate community structure and composition and on the benthic chlorophyll *a* abundance.

Methods and materials

This study was conducted in the Caramagna Creek, a small tributary of the Bormida River, northwestern Italy (44°36' N–8°32' E; 280 m a.s.l.). Dense woodlands with small scattered urban areas cover the entire catchment. Riparian vegetation is abundant and mainly composed by *Alnus glutinosa*, *Carpinus betulus*, and *Robinia pseudoacacia*, and the stream flows through a narrow, sinuous channel, characterized by a moderate slope. Riverbed width is approximately 2.0–2.5 m, and in this area substrate showed the following particle composition: 10% sand, 30% gravel, 50% pebbles, and 10% boulders. Moreover, there is a natural series of riffles alternating with shallow pools, with a generally moderate current velocity. At the study site, no morphological alterations are present, but the creek receives an effluent sewer from a small cluster of houses. To evaluate the impact of this point source of pollution, the creek was divided into two parts, and samplings were performed at one reach located 50 m upstream and one 50 m downstream of the sewer pipe. Main chemical and microbiological parameters were measured in six occasions (Table 1). Main physicochemical parameters were measured in the field

Table 1. Main chemical-microbiological parameters upstream and downstream of the sewage outfall in Caramagna Creek (mean ± SD).

Parameters	Upstream	Downstream
Conductivity (μS/cm)	469.0 ± 41.8	510.8 ± 37.1
DO (mg/L)	11.30 ± 0.99	9.04 ± 0.69
T (°C)	8.30 ± 5.28	8.53 ± 5.84
Total P (mg/L)	0.05 ± 0.00	0.82 ± 0.68
COD (mg/L)	7.51 ± 1.40	24.0 ± 30.7
NH ₄ ⁺ (mg/L)	0.07 ± 0.07	2.88 ± 3.84
NO ₃ ⁻ (mg/L)	0.71 ± 0.34	0.69 ± 0.40
pH	7.91 ± 0.19	6.78 ± 2.85
Anionic tensioactives (mg/L)	0.05 ± 0.00	0.06 ± 0.01
<i>E. coli</i> (cfu/mL)	0.00 ± 0.00	1983.3 ± 3504.5

70 with Eijkelkamp 13.14 and 18.28 portable instruments. Water samples were also
collected from the sub-surface in acid-washed polythene bottles or sterile glass
bottles. In laboratory, some other chemical and bacteriological properties of the
water were assessed by using A.P.A.T. - I.R.S.A. (2003) methods (total P = M.598,
COD = M.014, NH_4^+ = M.589, NO_3^- = M.020, anionic tensioactives = M.268, and
75 *Escherichia coli* = M.001).

Macroinvertebrate community composition and structure were evaluated using
a $20 \times 20 \text{ cm}^2$ Surber sampler (255 μm mesh). Samples were collected at each station
monthly from January 2005 to March 2006. In the laboratory, all organisms were
counted and identified to the genus level, except for Annelida and early instars of
80 some Trichoptera and Diptera, which were identified to the family level. Each taxon
was also assigned to one of the following functional feeding groups: scrapers (Sc),
shredders (Sh), collector-gatherers (Cg), filterers (F), or predators (P) according to
Merritt and Cummins (1996). Moreover, a classification of taxa into seven biological
and seven ecological groups was conducted according to the Usseglio-Polatera et al.
85 (2000) species traits approach. In the same period, benthic chlorophyll *a* was assessed
on six occasions by positioning 84 ceramic tiles in the stream reach. Tiles were left in
place for 1 month and then brushed to collect attached material. We determined
chlorophyll *a* concentrations by spectrophotometry following extraction in 90%
acetone according to Steinman and Lamberti (1996). We used the Mann–Whitney
90 U-test to evaluate differences in biological measures between the upstream and the
downstream sites.

Statistical analyses were performed with Systat 8.0 (Wilkinson 2000).

Results and discussion

Totally, we collected 156 samples and identified 13,981 organisms belonging to 92
95 taxa in the section upstream of the sewage outfall and 48,549 organisms belonging to

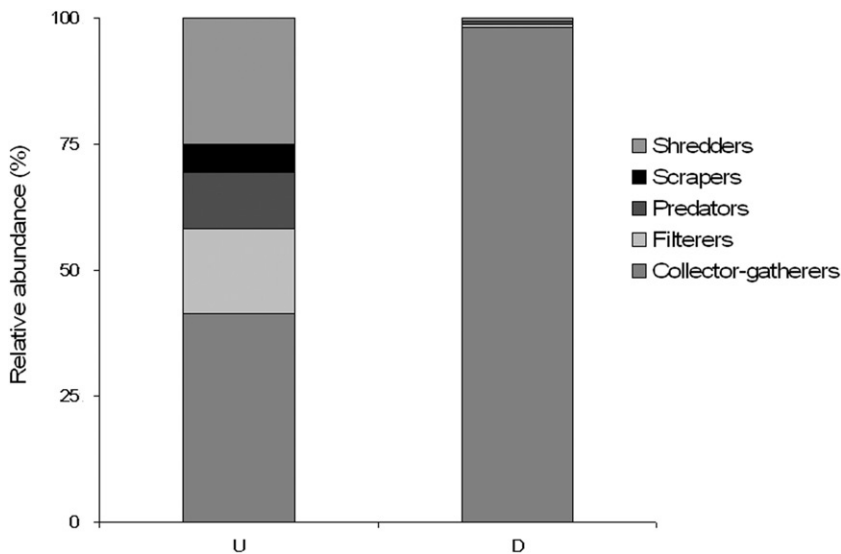


Figure 1. Relative abundance of functional feeding groups upstream (U) and downstream (D) of the sewage outfall in Caramagna Creek.

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Table 2. Relative abundances (%) for macroinvertebrates collected upstream (U) and downstream (D) of the sewage outfall.

Taxon	U	D	Taxon	U	D
Plecoptera			<i>Calopteryx</i> sp.	0.036	0.004
<i>Capnia bifrons</i>	0.336	0.006	<i>Onychogomphus forcipatus</i>	0.129	0.002
<i>Leuctra</i> sp.	2.525	0.035	<i>Orthetrum</i> sp.	0.029	0.000
<i>Nemoura</i> sp.	1.280	0.189	Coleoptera		
<i>Protonemura</i> sp.	0.072	0.004	Haliplidae	0.007	0.000
<i>Isoperla</i> sp.	1.001	0.027	Dytiscidae		
<i>Brachyptera</i> sp.	13.140	0.272	<i>Deronectes delarouzei</i>	0.000	0.000
Ephemeroptera			<i>Deronectes moestus</i>	0.007	0.000
<i>Electrogena</i> sp.	0.143	0.000	<i>Hydroglyphus pusillus</i>	0.007	0.000
<i>Serratella ignita</i>	0.837	0.132	Unidentified larvae	0.043	0.004
<i>Epeorus sylvicola</i>	0.036	0.000	Hydrophilidae	0.014	0.000
<i>Siphonurus lacustris</i>	0.486	0.132	Dryopidae		
<i>Caenis</i> sp.	0.694	0.014	Unidentified larvae	0.014	0.000
<i>Baetis</i> sp.	14.700	0.692	<i>Helichus substriatus</i>	1.688	0.027
<i>Torleya major</i>	0.014	0.004	Hydraenidae	0.000	0.000
<i>Centropitulum luteolum</i>	1.016	0.000	<i>Hydraena similis</i>	0.000	0.002
<i>Ecdyonurus</i> sp.	2.775	0.066	<i>Hydraena solarii</i>	0.000	0.000
<i>Ephemera danica</i>	0.229	0.004	<i>Hydraena andreinii</i>	0.658	0.023
<i>Paraleptophlebia</i> sp.	0.315	0.000	<i>Hydraena assimilis</i>	0.093	0.000
<i>Habropleptoides</i> sp.	0.608	0.021	<i>Hydraena heterogina</i>	0.021	0.000
<i>Habroplebia</i> sp.	3.169	0.043	<i>Hydraena truncata</i>	0.086	0.000
Trichoptera			<i>Hydraena subimpressa</i>	0.286	0.000
Polycentropodidae	0.000	0.002	<i>Hydraena devillei</i>	0.021	0.000
Hydroptilidae	0.007	0.000	<i>Limnobia mucronatus</i>	0.007	0.000
Beraeidae	0.043	0.000	Gyrinidae	0.322	0.006
<i>Hydropsyche</i> sp.	1.395	0.031	Elmidae		
<i>Wormaldia</i> sp.	0.486	0.004	Unidentified larvae	0.479	0.016
<i>Philopotamus</i> sp.	0.229	0.002	Unidentified adults	0.615	0.014
Leptoceridae	0.501	0.000	<i>Exolus</i> sp.	0.021	0.000
Goeridae	0.043	0.000	Scirtidae	0.622	0.002

Glossosomatidae	1.473	0.016	Hemiptera	0.007	0.000
Psychomyiidae	0.315	0.006	<i>Velia</i> sp.	0.007	0.000
Limnephilidae	1.209	0.008	<i>Hydrometra stagnorum</i>	0.000	0.000
<i>Rhyacophila</i> sp.	0.000	0.002	Gerridae	0.000	0.002
<i>Hypporhyacophila</i> sp.	0.036	0.002	<i>Micronecta</i> sp.	2.589	0.004
<i>Anabolia lombarda</i>	0.057	0.000	Hirudinea		
<i>Potamophylax cingulatus</i>	0.050	0.000	<i>Dina</i> sp.	0.007	0.000
<i>Odontocerum albicorne</i>	0.336	0.000	<i>Helobdella stagnalis</i>	0.007	0.000
<i>Sericostoma</i> sp.	0.136	0.000	Annelida		
Diptera			<i>Eiseniella tetradra</i>	2.296	0.035
Culicidae	0.007	0.000	Lumbricidae	0.858	0.014
<i>Anopheles</i> sp.	0.007	0.000	Lumbriculidae	0.479	0.091
<i>Atherix</i> sp.	0.136	0.002	Naididae	1.802	79.730
Chironomidae	11.780	16.980	Tubificidae	0.093	0.187
Ceratopogonidae	0.966	0.181	Tricladida		
<i>Dixa</i> sp.	0.043	0.000	<i>Dugesia</i> sp.	5.443	0.051
Tabanidae	0.143	0.000	Bivalvia		
<i>Tipula</i> sp.	0.944	0.045	<i>Pisidium</i> sp.	0.029	0.000
Stratiomyidae	0.107	0.004	Gastropoda		
Limoniidae	0.272	0.004	<i>Ancylus fluviatilis</i>	0.007	0.000
Simuliidae	14.650	0.661	<i>Lymnaea peregra</i>	0.100	0.004
Empididae	0.043	0.006	Arachnida		
Psychodidae	0.107	0.004	Hydracarina	1.874	0.037
Sciomyzidae	0.000	0.002	Nematomorpha		
Rhagionidae	0.007	0.000	<i>Gordius</i> sp.	0.043	0.000
Megaloptera			Nematoda		
<i>Sialis fuliginosa</i>	0.007	0.000	Unidentified species	0.072	0.117
Odonata			Mermithidae	0.021	0.019
<i>Cordulegaster boltoni</i>	0.093	0.000	Hymenoptera		
<i>Boyeria irene</i>	0.021	0.000	<i>Agriotypus armatus</i> (in Goeridae)	0.014	0.000

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56 taxa in the downstream section of Caramagna Creek. The upstream and downstream reaches were different from each other in many biological aspects.

The upstream section of Caramagna Creek is a typical Apennine low-order watercourse. Richness and density of the macroinvertebrate community were high and in the range reported for other streams in this area (Fenoglio et al. 2005, 2010b). However, taxonomic richness was significantly ($p < 0.001$) different between upstream ($N = 17.7 \pm 5.76$ SD) and downstream ($N = 8.70 \pm 6.18$ SD) sites. Likewise, organism density was significantly ($p < 0.001$) different, being 128.2 ± 106.6 SD organisms/m² upstream but 1032.9 ± 1495.6 SD organisms/m² downstream.

Furthermore, in the upstream section, the functional composition of the benthic biocenosis seemed well structured. Collector-gatherers comprised the most abundant functional feeding group, followed by shredders, filterers, predators, and scrapers. Downstream of the sewage effluent, the functional composition was completely altered, and collector-gatherers represented the dominant and almost exclusive group. Considering the relative importance of the functional feeding groups in the upstream and downstream communities, significant ($p < 0.001$) differences were also present for all functional groups except the collector-gatherers (Figure 1). Ephemeroptera/Plecoptera/Trichoptera taxa were present, with 34 taxa (representing 49.7% of the total number of organisms) in the upstream reach and 24 taxa (1.7% of the total number) in the downstream reach (Table 2).

Considering the relative importance of the biological traits group in the two communities, significant ($p < 0.001$) differences were evident in all trait groups except group 'b' (Figure 2). The most represented biological trait group was the 'e' group (small- or medium-sized organisms, uni- or plurivoltine, with aquatic respiration, crawlers), followed by the 'f' group (medium-sized or large monovoltine organisms, with aquatic respiration, crawlers), while the 'b' group was the rarest (medium or large crawlers or burrowers, mostly ovoviviparous).

Considering the relative importance of the ecological traits groups, significant ($p < 0.001$) differences were also apparent between the two communities for all groups except the 'E' group (i.e., eurythermic or thermophilous mesosaprobic, living in lentic riverine microhabitats) (Figure 3). The most important group was the 'B' group (organisms adapted to rhithronic and oligotrophic environments with coarse substrate), followed by the 'C' group (those living in rhithronic or epipotamic oligotrophic environments with slow-medium current velocities). The 'F' and 'D' groups (organisms avoiding the main channel and living in semi-lentic habitats, oligo- or mesotrophic) were also abundant.

Downstream of the sewage outfall the biological community changes dramatically. Density of invertebrates increased eightfold, while the taxonomic richness of the community collapsed, with the complete loss of most taxa. Oligochaeta Naididae and Diptera Chironomidae represented, respectively, 79.7% and 17.0% of the total invertebrates collected in the downstream samples. These groups are usually well known for their tolerance to organic pollution and, as collector-gatherers, are advantaged by the increased presence of fine particulate organic matter downstream. Naididae are multivoltine, burrowers/interstitial, microphagous deposit-feeders (biological trait group 'F'), while Chironomidae are small/medium-sized organisms, plurivoltine, and short-lived crawlers with varied feeding habits (group 'E').

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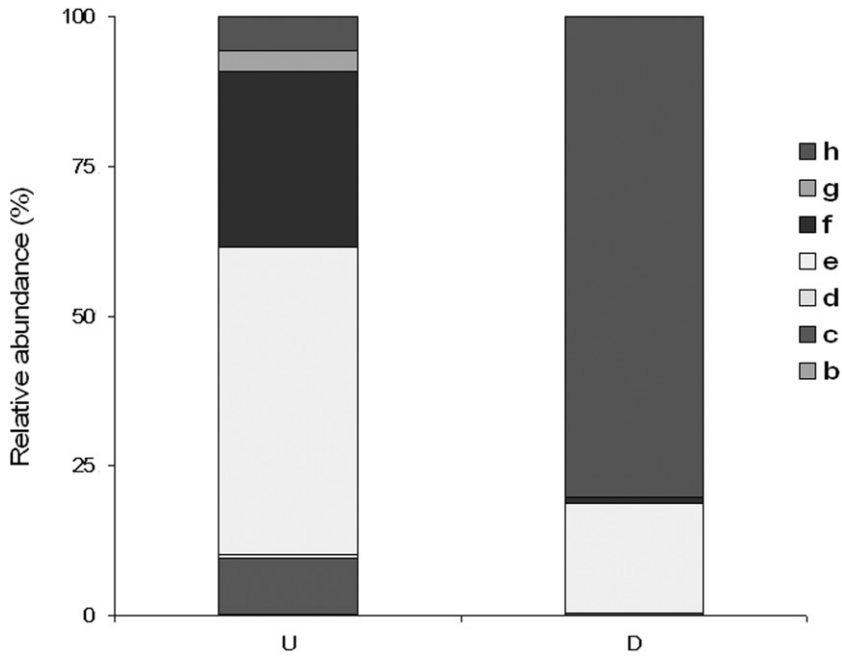


Figure 2. Relative abundance of biological traits groups (Usseglio-Polatera et al. 2000) in the functional feeding group presence in the upstream (U) and downstream (D) reaches of Caramagna Creek.

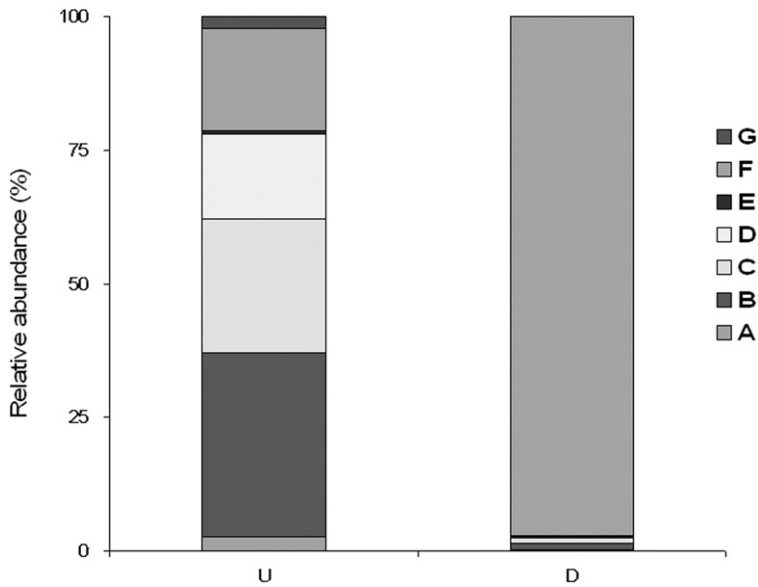


Figure 3. Relative abundance of ecological traits groups (Usseglio-Polatera et al. 2000) in the functional feeding group presence in the upstream (U) and downstream (D) reaches of Caramagna Creek.

Ecologically, both Naididae and Chironomidae are organisms adapted to the life
145 in slow flowing or semi-lentic, mesosaprobic environments with fine and organic
substrata (ecological group 'F').

The increased concentrations of nutrients downstream versus upstream would
suggest an increase in the instream autotrophic component. However, although the
amount of chlorophyll *a* found on artificial substrates was slightly higher
150 downstream of the sewage outlet (0.44 ± 0.50 SD $\mu\text{g}/\text{cm}^2$) than upstream
(0.32 ± 0.19 SD $\mu\text{g}/\text{cm}^2$), the difference was not significant.

The impact of small-not-regulated sewage sources on high quality aquatic
environments is important. The protection of lotic environments should not be
entrusted only to an efficient management of large sewage treatment plants, but it
155 must assume an accurate control of the many, small organic waste dumps that are
present also in isolated and rural areas.

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References

- A.P.A.T. - I.R.S.A. 2003. Metodi analitici per le acque. Manuale e linee guida n. 29. IGER,
Roma. p. 1149.
- 165 Bo T, Fenoglio S, López-Rodríguez MJ, Tierno de Figueroa JM. 2009. Phenology of adult
Stoneflies (Plecoptera) of the Curone stream (northern Apennines, Italy). *Journal of
Freshwater Ecology*. 24:279–283. 6
- Bo T, López-Rodríguez MJ, Fenoglio S, Cammarata M, Tierno de Figueroa JM. 2010.
Feeding habits of *Padogobius bonelli* (Osteichthyes, Gobiidae) in the Curone creek
(northwest Italy): territoriality influences diet? *Journal of Freshwater Ecology*.
170 25:367–371. 6
- Borchardt MA. 1996. Nutrients. In: Stevenson J, Bothwell ML, Lowe RL, editors. *Algal
ecology: freshwater benthic ecosystems*. New York, NY: Academic Press. p. 184–228. 5
- Fenoglio S, Bo T, Agosta P, Malacarne G. 2005. Temporal and spatial patterns of coarse
particulate organic matter and macroinvertebrate distribution in a low-order Apennine
175 stream. *Journal of Freshwater Ecology*. 20:539–547. 6
- Fenoglio S, Bo T, Cammarata M, Malacarne G, Del Frate G. 2010b. Contribution of macro-
and micro-consumers to the decomposition of fish carcasses in low-order streams: an
experimental study. *Hydrobiologia*. 637:219–228. 6
- 180 Fenoglio S, Bo T, Lopez-Rodriguez MJ, Tierno de Figueroa JM. 2010a. Life cycle and
nymphal feeding of *Besdokus ravizzarum* (Plecoptera: Perlodidae), a threatened stonefly.
Insect Science. 17:149–153. 6
- Goudie AS. 2006. *The human impact on the natural environment: past, present and
future*. 6th ed. Oxford: Blackwell. p. 357.
- 185 Hering D, Buffagni A, Moog O, Sandin L, Sommerhäuser M, Stubauer I, Feld C, Johnson R,
Pinto P, Skoulikidis N, et al. 2001. The development of a system to assess the ecological
quality of streams based on macroinvertebrates – design of the sampling programme
within the AQEM Project. *International Review of Hydrobiology*. 88:345–361. 6

- 190 López-Rodríguez MJ, Tierno de Figueroa JM, Fenoglio S, Bo T, Alba-Tercedor J. 2009. Life strategies of 3 Perlodidae species (Plecoptera) in a Mediterranean seasonal stream in southern Europe. *Journal of the North American Benthological Society*. 8:611–625. **6**
- Mason C. 2002. *Biology of freshwater pollution*. 4th ed. Edinburgh: Pearson Ed. p. 388.
- Merritt RW, Cummins KW. 1996. *An introduction to the aquatic insects of North America*. Dubuque, IA: Kendall/Hunt. p. 863.
- 195 Ortiz JD, Martí E, Puig MA. 2005. Recovery of the macroinvertebrate community below a wastewater treatment plant input in a Mediterranean stream. *Hydrobiologia*. 545:289–302. **6**
- Paul MJ, Meyer JL. 2001. Streams in the urban landscape. *Annual Review of Ecology and Systematics*. 32(1):333–365.
- 200 Steinmann AD, Lamberti GA. 1996. Biomass and pigments of benthic algae. In: Hauer FR, Lamberti G, editors. *Methods in stream ecology*. New York, NY: Academic Press. p. 295–313.
- Tierno de Figueroa JM, Bo T, López-Rodríguez MJ, Fenoglio S. 2009. Life cycle of three stonefly species (Plecoptera) from an Apenninic stream (Italy) with the description of the nymph of *Nemoura hesperiae*. *Annales de la Société Entomologique de France*. 45:339–343. **6**
- 205 Usseglio-Polatera P, Bournaud M, Richoux P, Tachet H. 2000. Biological and ecological traits of benthic fresh-water macroinvertebrates: relationships and definition of groups with similar traits. *Freshwater Biology*. 43:175–205. **6**
- 210 Wilkinson L. 2000. SYSTAT vers. 8.0. Evanston, Chicago, IL, USA.