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Black flies (Diptera, Simuliidae) as ecological indicators of stream ecosystem health in an urbanizing area (Rome, Italy)

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Abstract
Introduction. The effectiveness of Simuliidae (Diptera, Nematocera) as indicators of stream health was tested in the urbanizing area of Rome, Central Italy.
Methods. 108 sampling sites were investigated in the spring to acquire data on environmental variables and black fly species.
Results and discussion. The comparison of sampling and literature data highlighted the disappearance of some species (*Simulium reptans*, *S. vernum*, *S. liriense*), possibly due to water pollution and land use changes. Correspondence analysis confirms altitude as the main factor influencing black fly species' distribution. However, water quality becomes predominant when considering exclusively plain sites: "ornatum" and "equinum" species groups show the greatest tolerance to chemical pollution. The high course of the Aniene river and its tributaries were sampled bimonthly, confirming the spatial-temporal black fly successions previously observed in central Apennines. The results also indicate that *S. ornatum* may supplant all other species in areas where the rural use is substituted by urban landscape.
Conclusion. In conclusion, black flies can be considered good environmental indicators to assess ecological health of both watercourses and surrounding landscapes.

Key words
- Simuliidae
- biological indicators
- watercourses
- human impact
- environmental health

INTRODUCTION

Black flies (Diptera, Simuliidae) are little insects well known worldwide because of their female adults' bite on humans and cattle [1, 2]. Pre-imago stages are extremely specialized, as strictly related to running waters, and can be found from snow melting brooks to large plane rivers [3]. In fact, larvae are passive filter feeders, predominantly fixed to smooth surfaces in typically lotic reaches of water courses. These insects have a key role in the ecology of water courses as important members in the food chain: they filter suspended particulate organic matter from water, making it available for a considerable number of other invertebrates, such as Thricoptera, which massively feed on them. Adult black flies are predominantly nectarivorous – males exclusively – but females need a blood meal in order to complete their egg development, and so they actively search for vertebrates to bite. While biting they inject anticoagulant saliva into the host animal, so that they can be vectors of etiologic agents such as viruses, nematodes and protozoa or they can be cause of allergic reactions to their saliva (Simuliotoxicosis). As a consequence, a large number of studies were carried out in order to detect veterinarian and medical consequences of their blood-sucking habits, such as dermal reactions [4], cattle murrains [5-7], or even transmission of serious human diseases (*e.g.* human river blindness [8]).

The interest of scientists for black flies also focused on the ecology of larvae. In fact, pre-imago stages generally have a well-defined altitudinal and seasonal distribution along unimpaired water courses and it is possible to identify different river zones, each characterized by distinctive black fly species or species-groups, on the basis of an ecological succession [9]. Actually, the distribution of black fly larvae strongly depends on the flow velocity and on the substratum, the latter represented by stones, rocks, vegetation or other submerged objects, provided that they are stable, smooth and free from mosses, algae and bacteria typical of eutrophic waters. Moreover, several studies demonstrated that the composition of

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black fly species assemblages in rivers is strongly dependent on some environmental variables, which are directly affected by human pressures on river ecosystems: loss of riparian vegetation [10], hydro-morphological modifications of the riverbed [11] and, most of all, chemical and physico-chemical impairment of water [12]. In fact, being filtering organisms and breathing dissolved oxygen, black fly larvae are generally disturbed by water pollution, even if some species take advantage from small increases of suspended organic matter, on which they can feed. However, when the organic matter concentration is too high, decomposer bacteria exhaust the dissolved oxygen, and also the most tolerant black fly species finally disappear [13].

During the last 60 years, black fly fauna of the province of Rome has been the subject of several occasional observations and only a few dedicated studies. In particular, the first information curiously come from the "Fauna of URSS" [14] regarding the identification of some black fly species collected around the city. In 1963, other records were reported from the countryside surrounding Rome (Albani hills, littoral areas; Rivosecchi, unpublished). A first detailed study on zonal and seasonal distribution of black flies in central Latium was carried out in the effluent river of the Lake Bracciano, soon followed by a study on the Mignone river, partly flowing in the province of Rome [15]. Finally, a study of black fly species of the metropolitan area of Rome was realized in 1996 [16] briefly presenting also a comparison with species from the countryside. However, no exhaustive study of the entire province has been performed until now, as well as in the rest of central Italy, despite the interest these dipterans excite both as possible medical-veterinarian problem and ecological indicators describing the effects on river ecosystems of the diffused urbanization affecting this territory.

The main aim of the present study is to test the effectiveness of the Simuliidae family as environmental indicators in streams of urbanizing areas, taking as a case study the territory of Central Italy surrounding the city of Rome. Therefore, in order to depict a comprehensive description of the present distribution of Simuliidae in the province of Rome and their relationships with environmental factors, a two year field research (2008-2009) was carried out in rivers and streams throughout this territory. Collected faunal data were used to perform a comparison with historical database, while the analysis of environmental variables associated to the sampling sites made it possible to detect how the impairment of the stream environments affects black fly assemblages' composition and distribution. Moreover, with a view to correlate seasonality and altitudinal features of the streams to black fly assemblages in natural conditions, a bimonthly sampling activity was carried out throughout a relatively unimpaired watercourse, from mountain springs to the Tevere valley.

MATERIALS AND METHODS

Study area

The Province of Rome (5353 km^2) is located in Central Italy *(Figure 1)* between the Apennine mountains and the Tyrrhenian sea, with an extremely high diversity from a geographical and ecological point of view, thanks to a complex system of geological, orographical, hydrographical and climatic factors [17]. Most of the cultivable land in this area is used for agriculture, principally for olive, vegetable and cereal farming, even if during the last 50 years a widespread process of urbanization has been deeply modifying the landscape. However, there are several patches of wood, most of which are protected in Regional parks or smaller reserves. The principal watercourses are the Tevere and the Aniene rivers, flowing almost perpendicularly respectively from the North and from the East. A considerable number of perennial streams cross the region, mostly tributaries of the two main rivers and a few flowing directly to the sea [18]. Samples were collected throughout the entire Province of Rome, considering perennial streams and the two principal rivers, for a total of 37 water-courses *(Table 1, Figure 1)*. 108 sampling sites were sampled in the spring during two years (2008-2009) to acquire data on land use, environmental variables and black fly species assemblages in rivers. The distribution of sites were quite homogenously arranged, but a higher number of sampling sites was located on urban streams in order to acquire more detailed information on the related pressure gradient. Sampling in the spring made it possible to detect the highest black fly species diversity during the year and at the same time mature larvae and pupae were easily collected. *Figure 1* shows the distribution of the sites in the study area; site code numbers were assigned in ascending order during the sampling campaigns, while the letters refer to the rivers. Most of the sampling sites (coded with capital letters in *Table 1*) are located in streams flowing in volcanic areas with siliceous substratum typical of central Latium, while a lower number of sites, in the Eastern piedmont zone of Apennines mountains, presents a calcareous substratum (coded in lower case in *Table 1*).

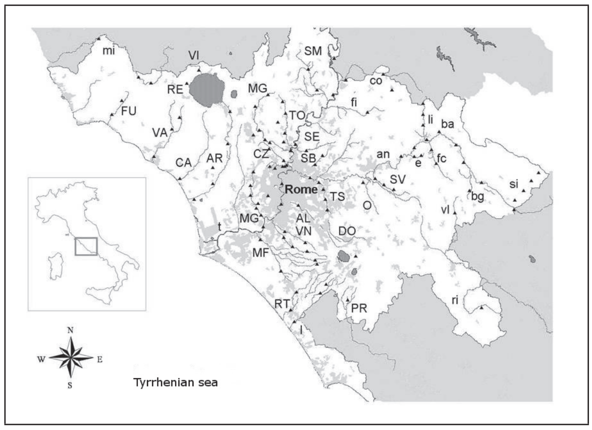

Figure 1
Study area and sampling sites: river codes are reported in *Table 1*; Province of Rome in white, with urbanized areas in light grey; inset: the location in Italy.

Land use and water variables

Land use percentages for the upstream basins of each sampling site were obtained from the GIS CORINE

Table 1
List and codes of studied streams and rivers, and number of studied sites per watercourse (siliceous stream codes in capital letters)

Code	Stream name	Number of sites	Code	Stream name	Number of sites
AL	Almone	1	TS	Tor Sapienza	4
AR	Arrone	5	TO	Torraccia	4
CA	Caduta	1	VA	Vaccina	2
CR	Cremera	7	VN	Vallerano	5
CZ	Crescenza	7	VI	Vicarello	1
DO	Doganella	1	an	Aniene	8
FU	Fiume	2	ba	Bagnatore	1
I	Incastro	3	bg	Bagno	1
MG	Magliana	6	co	Corese	2
MF	Malafede	6	e	Empiglione	2
O	Osa	1	fc	Fiumicino	2
PR	Prefetti	1	fi	Fiora	1
RE	Renara	1	li	Licenza	4
RT	Rio Torto	2	mi	Mignone	3
SB	San Basilio	2	ri	Rio	1
SM	San Martino	2	si	Simbrivio	4
SV	San Vittorino	3	t	Tevere	8
SE	Settebagni	1	vl	Valle	1

Land Cover 2000 package, using a first clustering level of the land use categories: wood, pasture, agriculture and urban.

Water variables indicating agricultural and urban pollution were detected. Temperature, dissolved oxygen, pH, and electric conductivity were measured in the field with portable probes. Stream water samples were collected in sterile bottles following APAT protocol [19] and maintained at + 4 °C, while chemical analyses were carried out in the laboratory, within 12 hours after the sampling, in order to measure: nitrates (NO_3), phosphates (PO_4^{3-}), ammonium (NH_4^+), chemical oxygen demand (COD), and biological oxygen demand (BOD5). Spectrophotometrical analyses were performed following standard methods: ISO 7150-1 [20] for NH_4^+; ISO 7890-1[21] for NO_3^-; ISO 15705 [22] for COD; ISO 5815-1[23] for BOD5; and ISO 6878 [24] for PO_4^{3-}. Water *Escherichia coli* concentrations were detected within 24 h after the sampling, following the "membrane filtering" standard method reported in AWWA-APHA [25] and the results were expressed in CFU/100 mL (Colony Forming Units in 100 mL of water). *E. coli* concentration in river water is an indicator of domestic pollution.

Black fly sampling and identification

Black flies were predominantly collected in the springtime, when they quickly reach the last larval stages or the pupal stage and they can be easily identified. A longer sampling campaign was performed on the Simbrivio and Licenza streams, coded *si* and *li* in *Figure 1*, and on the high course of the Aniene river (coded *an*), that were sampled bimonthly for an entire year in order to correlate seasonality and altitudinal features of the streams to black fly assemblages. The choice of these watercourses lies on the fact that their watersheds still present limited human pressures and the possible impacts on biological communities can be more easily detected. Larvae and pupae were collected from different substrates (stones, plants, other smooth surfaces) in riffle meso-habitat, using entomological tweezers from pebbles and other removable surfaces, while a surber net was used for immobile surfaces. A 20 minute sampling effort was performed by a single operator in each site to standardize sampling. Such a collection method proved to be adequate to obtain representative samples of the local species occurrence (McCreadie *et al.*, 2004). Larvae and pupae were fixed in a 75% ethanol solution. Adults were obtained in the laboratory from mature pupae, which were sampled still attached to a removable substratum and kept in highly wet atmosphere until the emerging of the adults from their cocoon. Both preimaginal and adult stages were identified through morphological feature examination, with the help of specific keys for Italian Simuliidae fauna [26, 27] and an optical microscope.

Data analysis

In order to highlight which environmental variables best explain the distribution of black fly species and species-groups in the study area, a canonical correspondence analysis (CCA) was performed, so to obtain clusters of the different sampling sites based on the structure of black fly species assemblages, correlating them to the environmental variables (results available upon request). The statistical significance of a CCA ordination was assessed through Monte Carlo test. Before the analyses, environmental variable values were standardized following $X_{st} = (X_i - X_a)/SD$ (where X_{st} is the standardized value, X_i is the measured value, X_a is the average value and SD is the standard deviation [28]). The analyses were performed using PAST software [29].

RESULTS

Black fly community description

Black flies occurred at a total of 79 sampling sites (= 73% of all sites), while a total of 29 sites were completely lacking Simuliidae; they represent the heaviest degraded stream stretches in terms of land use and morphological and physical-chemical quality (*i.e.*, affected by strong urbanization or dramatic industrial pollution). A total of 2370 specimens were collected, belonging to 19 species representing about 26% of the total number of black fly species found in Italy (72 species, [30]). Three species belong to the genus *Prosimulium*, while all other species (16) belong to the genus *Simulium (Table 2)*. Additionally, several young and medium aged larvae of the genus *Simulium* were present in samples but could not be determined to species level, since diagnostic features such as the head's ventral cleft and the frontoclypeus' spots were still under development.

Among the species-groups, "ornatum" group (*S. intermedium* and *S. ornatum* s.l.) presented the highest frequency (81% of all sampled sites), followed by the "equinum" group (*S. pseudequinum*, *S. equinum* and *S. lineatum*) found in 39% of sites, the "aureum" group (*S. velutinum* and *S. angustipes*) in 35%, the "variegatum" group (*S. argyreatum*, *S. variegatum* and *S. monticola*) in 22% and finally the "hirtipes" group (*P. hirtipes*, *P. rufipes* and *P. latimucro*) in 8% of sites. However, when dealing with larvae of species included in these groups, it is not always possible to arrive to the species level identification due to the shape similarity of their head's ventral clefts, even if available cocoon-emerging adults were identified in the laboratory to confirm determination, whenever available.

The largely most common species was *S. ornatum* (complex), collected in the 80% of the total black fly hosting sites, and diffused from the sea level up to 800 m *(Table 3)*. 2 species were collected in nearly 20% of the total sites: *S. velutinum* and *S. pseudequinum*, respectively belonging to "aureum" and "equinum" species-groups. While *S. velutinum* presented an altitudinal diffusion similar to *S. ornatum*, *S. pseudequinum* resulted limited in plain stream sites. All other species had an occurrence between 5% and 20%, with the exception of 4 species that showed only a punctual distribution: *S. erythrocephalum*, *S. brevidens*, *S. lundstromi* and *P. latimucro*.

Table 2
Comparison between literature (A, see text for details) and present study (B) lists of black fly species in the province of Rome. Codes are given for species collected in this study

Species	List A	List B	Code
Prosimulium (Prosimulium) hirtipes (Fries, 1824)	+	+	*PPh*
Prosimulium (P.) rufipes (Meigen, 1830)	-	+	*PPr*
Prosimulium (P.) latimucro (Enderlein, 1925)	-	+	*PPl*
Prosimulium (P.) tomoswaryi (Enderlein, 1921)	+	-	
Simulium (Eusimulium) velutinum (Santos Abreu, 1922)	+	+	*SEv*
Simulium (E.) angustipes Edwards, 1915	+	+	*SEa*
Simulium (E.) petricolum (Rivosecchi, 1963)	+	-	
Simulium (Nevermannia) angustitarse (Lundstrom, 1911)	+	+	*SNa*
Simulium (N.) cryophilum (Rubzov, 1959)	+	+	*SNc*
Simulium (N.) lundstromi (Enderlein, 1921)	+	+	*SNl*
Simulium (N.) vernum Macquart, 1826	+	+	*SNv*
Simulium (N.) marsicanum (Rivosecchi, 1962)	+	-	
Simulium (N.) brevidens (Rubzov, 1956)	+	+	*SNb*
Simulium (Wilhelmia) equinum (Linnaeus, 1758)	+	+	*SWe*
Simulium (W.) lineatum (Meigen, 1804)	+	+	*SWl*
Simulium (W.) pseudequinum Séguy, 1921	+	+	*SWp*
Simulium (W.) balcanicum (Enderlein, 1924)	+	-	
Simulium (Simulium) variegatum Meigen, 1818	+	+	*SSv*
Simulium (S.) argyreatum Meigen 1838	+	+	*SSa*
Simulium (S.) monticola Friederichs, 1920	+	+	*SSm*
Simulium (S.) ornatum complex (Meigen, 1818)	+	+	*SSo*
Simulium (S.) intermedium Roubaud, 1906	+	+	*SSi*
Simulium (S.) hispaniola Grenier & Bertrand, 1954	+	-	
Simulium (S.) rivosecchii Rubzov, 1954	+	-	
Simulium (S.) reptans (Linnaeus, 1758)	+	-	
Simulium (S.) liriense Rivosecchi, 1961	+	-	
Simulium (S.) bezzii (Corti, 1914)	+	-	
Simulium (Boophthora) erythrocephalum (De Geer, 1776)	+	+	*SBe*
Simulium (Obuchovia) brevifile Rubzov, 1956	+	-	

Black flies and environmental quality

The result of CCA is presented in *Figure 2*, plot 1, where axes 1 and 2 together account for 31.57% of the cumulative variance (similar values are often obtained in ecological studies [31]). The execution of the Monte Carlo test (999 permutation) highlights the statistical significance of the scatter for both axes 1 and 2 ($p < 0.01$). In the resulting plot, sites (dots) and species (asterisks) are clustered in two groups, denoted with the letters A and B in the picture, whose separation is supported by some physico-chemical and land use parameters that present the highest correlations with the two axes (correlation > 0.30). Group A contains all sites characterized by high O_2 saturation in water, high wood land use percentages in the upstream basin and elevated altitude. On the contrary, sites included in group B present elevated concentration of nitrates,

Table 3
Altitude ranges of black fly species distribution (0-20 m: sea level; 21-200 m: plane; 201-400 m: low hill; 401-600 m: high hill; 601-800 m: low mountain; 801-1300 m: mountain)

	Altitude (metres a.s.l.)					
Species	0-20	21-200	201-400	401-600	601-800	801-1300
P. hirtipes	-	-	-	+	+	-
P. rufipes	-	-	-	-	-	+
P. latimucro	-	-	-	-	-	+
S. ornatum	+	+	+	+	+	-
S. velutinum	+	+	+	-	-	-
S. angustipes	-	+	-	+	+	-
gr. aureum	+	+	+	+	+	-
S. angustitarse	-	+	+	-	-	-
S. equinum	+	+	+	-	-	-
S. lineatum	+	+	+	-	-	-
S. pseudequinum	+	+	-	-	-	-
gr. equinum	+	+	+	-	-	-
S. argyreatum	-	-	+	+	+	-
S. variegatum	-	+	+	+	+	-
S. monticola	-	+	+	+	-	+
gr. variegatum	-	+	+	+	+	+
S. cryophilum	-	+	+	+	-	-
S. lundstromi	-	+	-	-	-	-
S. brevidens	-	-	-	+	+	-
S. erythrocephalum	+	-	-	-	-	-

phosphates and chemical oxygen demand and their basin's land cover is dominated by urban and agricultural use. The position of species in the graph explains how they influenced the distribution of sites between group A and B: species of "hirtipes" and "variegatum" species-groups characterize sites included in group A, while species of the species-groups "ornatum", "equinum" and "aureum" result typical of sites belonging to group B.

As a whole, the distribution of the species in the sites results to be strongly influenced by the altitude, which presents the highest correlation with the CCA first axis. In *Table 3*, it is reported the distribution of species in 6 altitudinal ranges, on the basis of the data collected in this study. Species represented by single specimens were not considered for the possible occurrence of drift events. The meaning of these ranges is partly explained through the distinction among black fly species on the basis of their reproductive behavior and water feature preferences. Some species are typical of mountain habitat and cold waters, such as those of the *Prosimulium* genus, which reproduce once during the year (univoltine species). Others, such as "equinum" group species, *S. erytrocephalum* and *S. angustipes*, prefer slower and warmer waters, and are typically collected in plane rivers. The third group, finally, includes all species that reproduce several times during the year (polivoltine species): as the spring progresses, "variegatum" species reproduce in higher and higher areas, until they overlap the areas previously occupied by *Prosimulium*. However, as a consequence of this pattern, it is possible that some species showed different altitudinal ranges due to the fact that the sampling campaign lasted during all spring and the first part of summer, with most sites only sampled once, so that each site might have presented a slightly different black fly fauna if sampled in an earlier or later month.

In order to clarify how the environmental variables influence black fly species distribution in areas with higher human presence (*i.e.* plane land of the study area), the analysis was repeated including only the sites located under 300 m a.s.l. (*Figure 2*, plot 2). In the graph, axes 1 and 2 together account for the 38% of the total variance. Monte Carlo test highlights the statistical significance of the first two axes ($p < 0.02$). *S. ornatum* results the most frequent species in urban areas with high water pollution (group C in the figure). However, it is present also in not urban and less impaired situations, where it coexists with *S. lineatum* ("equinum" group), which also shows quite a high tolerance to water pollution. Sites in the best chemical and land use conditions (grouped in A) host black fly assemblages constituted by a large number of species: *S. cryophilum* (SNc), *S. monticola* (SSm), *S. angustitarse* (SNa), *S. velutinum* (SEv), *S. argyreatum* (SSa), *S. angustipes* (SEa) and *S. pseudequinum* (SWp). Nevertheless, the three species of the "equinum" group (SWe, SWp and SWl) show a good tolerance to water pollution (sites of the group B).

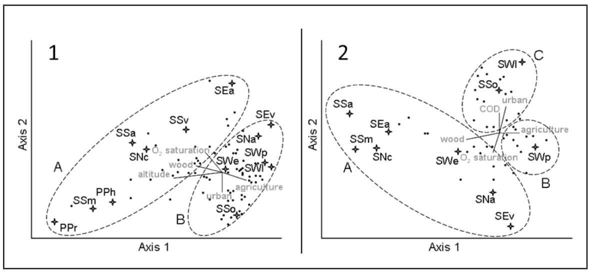

Figure 2
Canonical correspondence analysis (CCA) on environmental variables and black fly species abundances in studied sites (sites represented with dots; species codes in *Table 2*; Plot 1: group A contains mountain sites; group B contains hill and lowland sites; axis 1 + axis 2: 31.57% total variance explained; Plot 2: group A: best conditions; group B: intermediate conditions; group C: worst conditions; axis 1 + axis 2: 38% total variance explained).

Altitudinal and seasonal changes in black fly assemblages and water pollution

The zonal and seasonal distribution of black flies was studied in some water courses that still present fair conservation status. In the highest sites of both the tributary streams (Simbrivio and Licenza), black flies assemblages are dominated by species belonging to *Prosimulium* genus during winter and early spring. During the same period, all sites located downstream are dominated by species of the group "variegatum" (*S. variegatum*, *S. monticola* and *S. argyreatum*). As the spring season proceeds, at first the number of species collected in each site increases, then the species of the group "variegatum" displaces all others. At the end of summer and in early autumn the situation of the two streams diverges. In fact, while all the sites of the Simbrivio stream maintain a dominance of "variegatum" group species, in the sites of the Licenza stream there is an outbreak of *S. ornatum*, that temporary displaces all other species in sites located downstream urbanized areas and presenting significantly more polluted waters. The only exception is the highest site of the Licenza stream, located upstream any pollution sources and where *S. argyreatum* and *S. monticola* still characterize the black fly assemblage, together with "aureum" group species (*S. velutinum* and *S. angustipes*). However, when winter returns, *S. ornatum* disappears while *Prosimulium* and "variegatum" species recover the dominance respectively in mountain and medium course sites. A similar seasonal pattern was observed for the sites of the Aniene river, where "variegatum" group species are present all the year long together with *S. ornatum*, the latter becoming predominant in downstream sites during the second half of the year.

DISCUSSION

The results of this study are consistent with those of several studies regarding the use of black flies in the assessment of environmental quality of streams and rivers [10, 11, 32]. In fact, beside the relative simplicity in sampling both larvae and pupae, leading a sessile life, these insects are quite abundant and ubiquitous. Actually, their absence was detected only in strongly altered water courses of the studied area, where industrial or mining waste had generated conditions (foams, suspended dusts) almost unbearable for any macroinvertebrate species. Moreover, the taxonomy of Simuliidae has been deeply studied due to the medical and veterinarian implications, and, among Diptera, the species of this family are the most frequently used as biological indicators, together with Chironomidae [33].

In consideration of these insects' value as biological indicators, a comparison between black fly data collected in the present study and those reported in literature can help picturing how the urbanization of the Roman area is influencing stream ecosystems. Actually, comparing species lists themselves is a significant indicator of the ongoing environmental changes. Literature reports 27 black fly species collected in previous studies in the detected area [26], 17 of which were found again in the present study, while 2 more species were found which had never been collected before in the area (*P. rufipes* and *P. latimucro*) [26]. On the other hand, 10 species reported in literature were not collected during this study and different reasons may be found to explain it. A first group of species (*S. rivosecchii* and *S. balcanicum*) has been probably erroneously reported in a publication on the fauna of the Soviet Union [14] and never found again. A second group was found in extremely localized mountain sampling sites (*S. marsicanum*, *S. petricolum* and *S. hispaniola*) and their collection would require a specific research that is beyond the ecological scope of this study. A third group of species (*S. liriense*, *S. reptans* and *S. pontinum*) was surely present in the area when reported, but probably disappeared due to the impairment of the streams where they were detected [34]. The last group only numbers *S. vernum*, whose disappearance proves to be closely related to the urbanization of the area. In fact, this species was reported in small streams surrounded by woods of poplars (*Populus nigra*) in the Albani hills (site DO in *Figure 1*), where the adult females ambushed horses and cows from the trees [26]. The recent low urbanization of the area preserved the chemical status of the water, so that other black fly species were still found (*S. angustipes* and *S. ornatum*), but, nevertheless, it marked the disappearance of the poplar wood, with the consequence that no *S. vernum* was collected during the present study.

The CCA analysis of black fly assemblages in relation to environmental variables also gave evidences of the strong impact of urbanization on river communities. In fact, while site altitude proved to be the most important factor in black fly assemblage composition when all sampled sites were analyzed (confirming the upstream-downstream ecological succession already described by previous studies [4, 6, 12, 35]), trophic status and domestic pollution of water resulted the most important variables when analyzing exclusively plane stream communities. In particular, the presence of *S. ornatum,* as a dominant species in the assemblages or even the only one present, seems to point out the sites in the most impaired ecological conditions. Also the species of the group "equinum" proved to be quite tolerant to water pollution when the surrounding landscape maintains a good level of natural or agricultural use, where these dipterans can still find cattle to bite and ambushing sites. Similar results had been found in a previous study carried out by Rivosecchi [33] dealing with the landscape and the attacks of black flies to humans and cattle. In particular, the disappearance of livestock farming due to the urbanization of landscape seems to favor *S. ornatum* over "equinum" species. In fact, these species, although resulting as tolerant as *S. ornatum* to water pollution, present a higher sensibility to landscape changes, since they cannot split their breeding habits from cattle to other vertebrates occurring in urbanized areas, such birds, rats or even humans. In summary, the modification process of the black fly fauna along a gradient of urbanization can be outlined with the initial disappearance of naturally present species, firstly substituted by the assemblage of "equinum" and "ornatum" species and finally by the "ornatum" group alone, which takes competitive advantage from the rural to urban land use change.

The two-month sampling of the sites located in the medium-high course of the Aniene river and its main tributaries made it possible to confirm the observations on the ecological succession of black fly species in Apennine mountains in particular [13] and in European mountains in general [36, 37]. *Prosimulium* species characterize mountain sites of the Licenza and Simbrivio streams. *S. variegatum*, *S. monticola* and *S. argyreatum* dominate the medium high courses of the streams and the Aniene river, with other species representing a small fraction of the black fly assemblages. Finally, low course of the Licenza stream and the Aniene river already present species typical of plane site, such as "equinum" and "aureum" group species. The lower plane course of the Aniene river, highly polluted and made turbid by mining sediments, was not investigated. The variations to this scheme, such as the reported outbreaks of *Simulium ornatum* in sites located downstream of any organic pollution source, can be useful indicators of river impairment due to human activities. The discussed outcomes partially disagree with the observations carried out in Central Europe by Illesova *et al*. [36], according with which *S. ornatum* would be more easily collected in mesotrophic waters in association with *S. variegatum*, while *S. lineatum* and *S. erythocephalum* should be considered typical of more severely eutrophic waters. In particular, the data collected in Central Italy give the "ornatum" species a wider ecological value and, in agreement with previous studies [10, 38], depict this group as the one containing the European black fly species most tolerant also to water pollution.

CONCLUSIONS

The bio-indicator value of black flies is generally based on the ecological features of larvae, strictly related to stream chemistry and hydrology. The results of this study are consistent with the ecological succession of black fly species at larval stage, but also the peculiar feeding habits of the adults, strongly dependent on landscape arrangement, results to be closely related to the black fly species distribution. In fact, alterations of land use affect two principal necessities of black fly adults: the presence of ambushing sites and the availability of vertebrates to bite. Ambushing sites, such as wet ground, grass, trees or hedges are extremely important for female black flies, that are used to resting in these areas while waiting for a vertebrate to bite [3]. Moreover, the presence of specific animals, necessary for black flies that feed on their blood, is strongly related to land cover, since different vertebrate species may be found only in some environmental contexts (*e.g.*, ungulates and birds in woods, cattle in rural areas, rats and birds in urban context). Therefore, black flies can be not only considered as status indicators for watercourse health but, as suggested in previous studies [39-41], they may be also considered as landscape indicators for the assessment of the environment surrounding streams and rivers.

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Conflict of interest statement

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