

## Change does not happen overnight: a case study on stream macroinvertebrates

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**Abstract** – The influence of day/night conditions on individual animal/plant species has been widely studied, but diel cycle studies of the entire stream macroinvertebrate community are extremely rare. This study explored potential dissimilarities between daytime and nighttime macroinvertebrate assemblages by extensive fieldwork conducted in the Lemme stream, a natural water course of NW Italy. Here numerous structural and functional metrics (richness, abundance, biomass, indicator taxa, composition, biomonitoring values and feeding groups) were evaluated at the family level. Small-scale environmental variables were investigated to understand possible differences between macroinvertebrate assemblages in the daytime/nighttime. After collecting and identifying 21 459 organisms of 50 taxa, Chironomidae (Diptera) was the most abundant under both day and night conditions. Our findings stressed that similar results and biological information on daytime/nighttime data were obtained. No marked differences could be related to various factors: heterotrophic condition of small-order streams, presence of aquatic predators under night and day conditions, absence of taxa with a specific phototaxis. Of all the environmental variables, velocity was always the most important in both situations, with some differences detected in the importance of the second variable (riverbed substrate diameter). This research, and future studies on different conditions and geographic areas, will contribute knowledge on stream macroinvertebrate diel activity, and provide useful information about efficient sampling strategies.

**Key-words:** aquatic ecology / diel cycle / lotic systems / aggregation / benthic invertebrates

**Résumé** – Le changement ne survient pas dans une nuit : étude sur la communauté de macro-invertébrés d'un cours d'eau. L'influence des conditions jour/nuit sur les espèces animales et végétales a été largement étudiée, tandis que les études à propos du cycle diurne de l'entier de la communauté des macro-invertébrés fluviales sont extrêmement rares. Dans le cadre de cette étude, les différences potentielles entre les assemblages de macro-invertébrés lors du jour et de la nuit ont été explorées grâce à un travail de terrain très appuyé. L'expérience a été conduite près du Lemme, un cours d'eau naturel situé dans le nord-ouest de l'Italie où des nombreux indicateurs structurels et fonctionnels (richesse, abondance, biomasse, taxons indicateurs, composition, valeurs de biosurveillance et groupes d'alimentation) ont été évalués au niveau Famille. Les variables environnementales à petite échelle ont été évaluées aussi dans le but de comprendre les différences possibles entre les assemblages de macro-invertébrés durant le jour et la nuit. Un total de 21 459 organismes appartenant à 50 taxons ont été collectés et identifiés, montrant que le Chironomidae (Diptera) est le plus présent le jour comme la nuit. Nos trouvailles nous ont montré que des résultats similaires ont été obtenus dans des conditions diurnes comme nocturnes. L'absence de différences marquées peut être due à plusieurs facteurs, parmi lesquels les conditions hétérotrophes des cours d'eau de petite portée, la présence de prédateurs aquatique le jour comme la nuit et l'absence de taxa avec phototaxie spécifique. En ce qui concerne les variables environnementales, la vitesse a été la plus importante dans les deux situations, alors que des différences ont été détectées avec le diamètre du substrat du lit du fleuve. Cette recherche, comme d'éventuels futures études portant sur différentes conditions et les zones géographiques, contribue à la connaissance de l'activité diurne de macro-invertébrés et peuvent fournir des informations utiles sur l'efficacité des stratégies d'échantillonnage.

**Mots-clés :** écologie aquatique / cycle diurne / systèmes lotiques / invertébrés benthique

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## 1 Introduction

Several studies have stressed the importance of light variation and the influence of day/night conditions on plants and animals by examining both physiological and ecological points of view (e.g., Gerrish *et al.*, 2009; Gaston *et al.*, 2013). For instance for terrestrial insects, their distribution or features can markedly differ from day to night as a survival strategy to avoid insectivorous birds (e.g., Guevara and Avilés, 2013). Unlike limnological research on lacustrine zooplankton distributions, which has long since recognized that species use different habitats in the daytime than they do at nighttime (e.g., Hutchinson, 1967; Dodson, 1990), very little is known about riverine communities. This is especially true for no-target taxa for conservation policies like invertebrates (e.g. Cardoso *et al.*, 2011).

Most of the information about freshwater macroinvertebrate ecology has been collected during daytime sampling activities. Biomonitoring activities on aquatic ecosystems, which are probably the commonest way to obtain constant aquatic macroinvertebrate samples and data, originate from fieldwork done in the daytime. Any nocturnal activity is usually avoided for safety reasons, and also given the logistical constraints on researchers and technicians. Existing studies that are directly related to light and darkness dynamics in streams have generally investigated the relationship between macroinvertebrate drift and the predatory activity of fish (e.g., Roussel *et al.*, 1999; Davey *et al.*, 2011; Conallin *et al.*, 2012). In line with this, numerous macroinvertebrate taxa usually demonstrate a peak in drift activity related to darkness or nighttime conditions (e.g., Hansen and Closs, 2007).

Night is an important part of the diel cycle and several macroinvertebrates have demonstrated some form of nocturnal activity in different aquatic ecosystems (e.g. feeding or avoiding predation, Elliott 2000, 2002; Hampton and Duggan, 2003; Florencio *et al.*, 2011). In a North American stream, for example, it has been observed that several caddisfly larvae (Sericostomatidae) burrow during the day and resurface at night (Bergey and Resh, 1994).

Previous experimental approaches on day/night changes in aquatic macroinvertebrate assemblages have focused mainly on specific species, such as freshwater crustaceans (Johnson and Covich, 2000; Elliot 2005a), Ephemeroptera-Plecoptera-Trichoptera taxa (Elliott, 2000, 2002) or gastropods (Lombardo *et al.*, 2010). Likewise, marine and transitional (e.g., Guest *et al.*, 2003) or lentic and temporary ecosystems (Marklund *et al.*, 2001; Florencio *et al.*, 2011) have been previously investigated, but less is known about riverine ecosystems and the entire stream macroinvertebrate community (see Copp *et al.*, 2005, for an example in England).

This study evaluated and compared structural and functional metrics along the same river stretch, and different environmental variables were measured to: (i) provide a complete and quantitative assessment of day-to-night changes in the riverine macroinvertebrate community; (ii) assess the relevance of different environmental variables on macroinvertebrate compositions in different diel phases.

By addressing the first objective, we can gain an understanding of whether the biological and ecological information obtained during a specific period may be representative of the

general condition. By dealing with the second objective, we can investigate if different environmental variables are specifically associated with macroinvertebrate community compositions in the daytime and/or at nighttime.

After considering the nocturnal activities of some aquatic taxa and that some physico-chemical stream variables (e.g. water dissolved oxygen, pH) at night have been previously suggested as being capable of triggering nocturnal movements in lotic ecosystems (Wiley and Kohler, 1980; Brittain and Eikeland, 1988) we hypothesized that some discordances should be detected. We also expected to find main differences in the composition metrics values, such as aggregation. Similar results would demonstrate the consistency of the sampling data in any diel cycle stage.

Changes in macroinvertebrate distribution and diel variation are crucial for gaining a clearer understanding of population dynamics in aquatic ecosystems. Information on the diel activity patterns of macroinvertebrate larvae can enable us to understand the ecological role of particular taxa or groups, and may also prove useful when designing fieldwork studies and strategies. Throughout the article, we used the terms day and daytime to refer to daylight hours, and night and nighttime to refer to periods of darkness, which are in accordance with Helfman (1986).

## 2 Methods

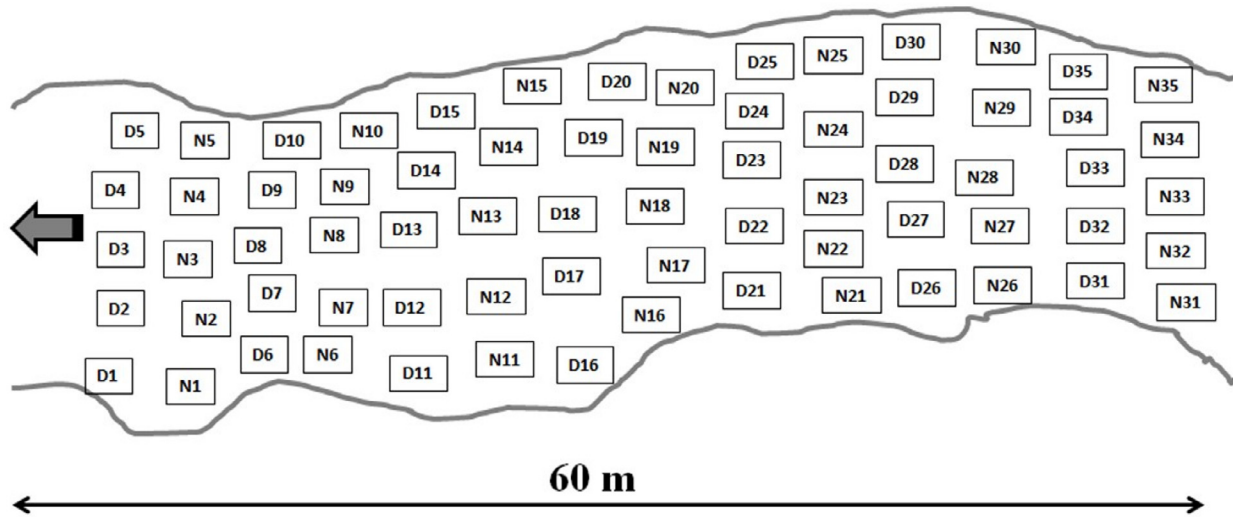
### 2.1 Study area

This study was performed in the Lemme stream, a typical small-sized (length 35 km) perennial Apennine stream tributary of the Orba stream (Po Basin, Piemonte Region, NW Italy). This kind of lotic ecosystem is widespread in continental and peninsular Italy as the Apennine Mountains extensively cover the country (Gumiero *et al.*, 2009). Dense woodlands with small scattered urban areas cover the entire catchment, where riparian deciduous vegetation is abundant. The sampling site (44° 36'49.71"N/8° 50'38.55"E, 342 m a.s.l.) is a representative 60-meter long stretch, with a mixture of riffles and runs, no deep pools, but with a moderate and constant slope. Riverbed width is approximately 4.0–5.0 m, and the substrate had the following particle composition: 10% sand, 30% gravel, 50% pebbles and 10% boulders (see Bo *et al.*, 2007 for further details).

As artificial lights, or extended and simulated periods of light and dark conditions, can apparently influence stream communities and associated terrestrial ecosystems (e.g., Fuller *et al.*, 1986; Perkin *et al.*, 2011; Meyer and Sullivan, 2013), a pristine stretch, located far from homes and villages, was considered to avoid any effects of anthropogenic factors on field data.

### 2.2 Biological and environmental data

Macroinvertebrates were collected with a 33 × 33 cm Surber sampler (255- $\mu$ m mesh) from December 13 to 20 in 2011. On a daily basis, five Surber samples were taken near midday and five near midnight to avoid dates with a full moon



**Fig. 1.** Example of sampling strategy (Surber distribution) in the studied Lemme stream reach (D1–D35: daytime Surbers, N1–N35; nighttime Surbers). The gray arrow indicates flow direction.

in order to maximize variation at natural light levels between night and day. Similar schedules have been considered in other studies (e.g., Elliot, 2002). Fieldwork was conducted at the end of fall, a period during which invertebrate abundance and diversity are generally higher in these ecosystems following natural leaf fall (Bo and Fenoglio, 2011). The Surber sampler is a quantitative approach (data refer to the sampled surface area), and is the most widely used technique in macroinvertebrate research and stream biomonitoring in Italy. Sampling units were selected to obtain a complete representation of all the microhabitat types along the stretch. However, as benthic taxa are not expected to recolonize disturbed sediments as quickly as other aquatic taxa do (e.g. fish), nighttime sampling was done approximately 0.5 m upstream from the corresponding daytime sampling point (following Copp *et al.*, 2005). By the time field activities had ended, 70 Surber units had been obtained along the studied stretch: 35 in the daytime (D) and 35 at nighttime (N) (Figure 1).

After collecting macroinvertebrates, they were preserved in 96% ethanol and identified in the laboratory at the family level using the taxonomic keys proposed by Tachet *et al.* (2010). The use of higher taxonomic levels (e.g. family) has been widely utilized in previous studies on invertebrates because of the high correlation between family and species richness (Bournaud *et al.*, 1996; Baldi, 2003; Sánchez-Fernández *et al.*, 2006). Dolédec *et al.* (2000) evidenced minor differences in some functional traits, including dispersal, between macroinvertebrate taxonomic levels, and concluded that genus, and even the families level, can be suitable for describing many processes in macroinvertebrate communities. This can make measuring taxonomic richness and composition easier, which enables the study of the entire macroinvertebrate community. Moreover, macroinvertebrate family richness is generally considered one of the main biological metrics in aquatic ecosystem bioassessments (e.g. Birk *et al.*, 2012).

The richness (No. of taxa), abundance (No. of individuals), composition, level of aggregation (Morisita Index), inver-

tebrate biomass, biomonitoring results and indicator taxa were evaluated and compared. In order to complement our analysis and to consider functional information, each collected taxon was assigned to a functional feeding group (FFG: scrapers, shredders, gatherers, filterers and predators), as set out by Merritt and Cummins (1996). Each richness group value (No. individuals/group) was compared between D and N. Moreover, to obtain biomonitoring information, a test was run with the macroinvertebrate-based index STAR\_ICMi. This is the official multimetric index used in Italy for assessing water course quality status according to European legislation (for further details, see Buffagni *et al.*, 2006 and Buffagni and Erba, 2007). The index values were obtained and investigated for both the D and N periods.

To characterize the study areas and to assess the relevance of different factors, specific site variables (water velocity, depth, substrate size and coarse particulate organic matter (CPOM) amount) were measured at each sampling point (corresponding to each Surber) and were used in the multivariate analysis. Current velocity was measured with an Eijkelkamp 13.14 portable instrument, while the mean substrate diameter was calculated by weighing the mean diameter of all the mineral microhabitat sizes by the previously determined coverage percentage. The proportions of grain size classes on the surface area were visually estimated according to class as sand, gravel, microlithal, mesolithal, macrolithal and megalithal, as considered in the STAR\_ICMi protocol adopted in Italy (Buffagni and Erba, 2007).

To quantify CPOM, leaves and other vegetal detritus (diameter > 1 mm) were collected from each Surber sample. In the laboratory, this material was air-dried for 24 h, oven-dried (105 °C) for 24 h and weighed on an electronic balance (accuracy 0.01 g). The collected macroinvertebrate individuals were also oven-dried at 105 °C for 4 h, after which dry weight was recorded to obtain the macroinvertebrate biomass for each sample (accuracy 0.01 g).

### 2.3 Statistical analysis

To ensure that the results were consistent, the completeness of the entire taxa inventory generated was assessed using a nonparametric estimator (chao2, the “specpool” function of the R package “vegan”), as suggested by Walther and Moore (2005), which has already been used in macroinvertebrate community analyses (e.g., Martínez-Sanz *et al.*, 2010).

To examine the diel differences in different community descriptors (abundance, family richness, Shannon Index, biomonitoring results and functional role richness), *t*-tests on the transformed data (log or square root) were run. Homoscedasticity and normal data distribution were not constituted (not even with numerous transformations) for variables “No. of scrapers” and “biomass”. In these cases, analyses were performed by nonparametric Wilcoxon tests.

To investigate if the daytime and nighttime communities presented different aggregation levels, the Morisita Index of Intraspecific Aggregation (Morisita, 1959; Krebs, 1999) was resorted to. In order to assess the differences in the aggregation values between D and N, we examined the differences obtained with what would be expected by chance alone. To do this, we compared the difference in the aggregation values obtained in our samples (D value vs. N value) with the difference obtained from 9999 random draws of an equal number of sampling sites taken from the entire pool of sites to obtain a *p*-value ( $n = 70$ , including both daytime and nighttime).

A nonmetric multidimensional scaling (NMDS) analysis was performed to visualize possible dissimilarities or patterns between the macroinvertebrate communities from the different sampling periods (D or N) using the entire dataset (No. sites = 70). Bray-Curtis distance was used as a dissimilarity measure, and stress was employed to test goodness of fit. Linear fittings, using the “envfit” function (vegan package, Oksanen *et al.*, 2013), were performed between the environmental variables and the output of each NMDS ordination (D and N, previously performed) to identify the environmental factors that drove the composition of macroinvertebrate communities at nighttime and in the daytime. The significance of the fitted vectors was assessed by a permutation procedure (9999 permutations). A Permanova analysis (Anderson, 2001) was carried out using Bray-Curtis distance to test whether there was a significant difference between the *a priori* proposed division (D and N) in terms of macroinvertebrate communities.

The daytime STAR\_ICMi value was obtained by considering the median value after 1000 randomizations of 10 Surber data (among 35 daytime Surbers). At the same time, the 2.5 and 97.5 percentiles were calculated to obtain the confidence interval (p.c.i.). The same procedure was followed using the nighttime Surber data to obtain a nighttime median STAR\_ICMi value and its p.c.i..

Finally, an indicator value analysis (Dufrêne and Legendre, 1997) was carried out using the indicpecies package (De Cáceres and Legendre, 2009) to select the indicator family for each sampling period (D and N). This analysis evaluates the affinity of each taxon for each *a priori*-defined group and provides an indicator value (herein called IV). All the analyses were performed with the R 3.0.1 software of the R statistical environment (R Development Core Team, 2013).

## 3 Results

### 3.1 Environmental characteristics and macroinvertebrates community

The current velocity, depth, CPOM and mean diameter of the substrate of the studied stretch were  $0.13 \pm 0.2 \text{ m}\cdot\text{s}^{-1}$ ,  $18.23 \pm 9.6 \text{ cm}$ ,  $2.32 \pm 2.9 \text{ g}$  and  $9.89 \pm 6.9 \text{ cm}$ , respectively (mean  $\pm$  SD). No differences were observed between the D and N measurements ( $p > 0.05$ , nonparametric Wilcoxon test).

We collected and identified 21 459 organisms of 50 macroinvertebrate higher taxa (49 families, plus Hydrachnidia, No. sites = 70). The most abundant taxon was Chironomidae (Diptera), followed by Taeniopterygidae (Plecoptera), Simuliidae (Diptera), Hydropsychidae (Trichoptera) and Baetidae (Ephemeroptera). Each one had more than 500 individuals in all (Table 1). The nonparametric estimator of species richness (chao2) suggested that at least 90.9% (50 of 55) of the total number of expected taxa were recorded in our pool of sampling units. The results showed that the data sets compiled for the Lemme stream stretch (as a whole) could be considered realistically complete if compared to the values proposed by other authors (e.g., Jiménez-Valverde and Hortal, 2003; Sánchez-Fernández *et al.*, 2008).

### 3.2 Comparing day and night communities

No differences were found between the daytime and nighttime assemblages for any studied community descriptor (*t*-test results, Table 2), or for the invertebrate biomass or the number of scrapers present (Wilcoxon test  $p = 0.112$  and  $p = 0.833$ , respectively). Forty-two taxa were obtained in the daytime and 46 at nighttime. The mean number of taxa in each Surber was strikingly similar under both conditions (mean  $\pm$  SD =  $11 \pm 3$  in the daytime;  $11 \pm 4$  at nighttime). In both cases Chironomidae (Diptera) and Taeniopterygidae (Plecoptera) were the most abundant taxa. The biomass mean values for each Surber were  $0.177 \text{ g} \pm 0.08$  and  $0.142 \text{ g} \pm 0.10$  for D and N, respectively. According to the intraspecific aggregation values obtained by the Morisita index, the daytime community gave a higher value (1.604) compared with the nighttime community (1.502). However, the difference (0.102) did not significantly differ from those obtained by chance after randomization (permutations test,  $p > 0.05$ ).

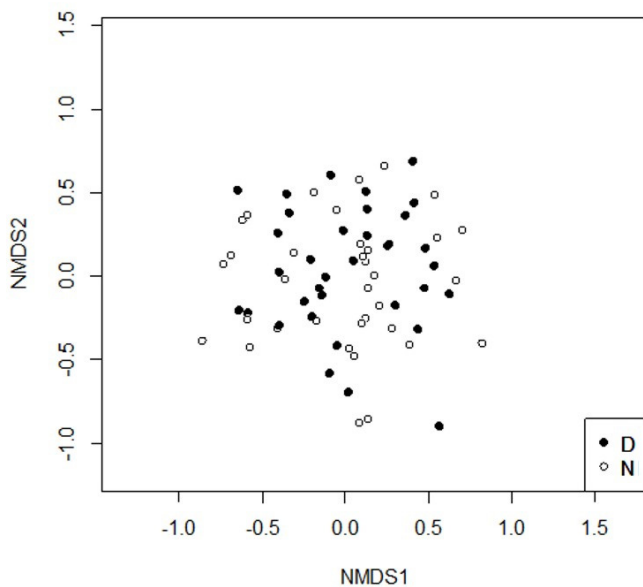
The NMDS ordinations of the macroinvertebrates communities (Figure 2, first two axes displayed) showed no clear clustered patterns, but the daytime and nighttime sites completely overlapped. The final stress value for the three-dimensional ordination was 0.19. According to the “envfit” analysis results, both communities (D and N) presented similar relationships to the environmental variables (Table 3). In each case, velocity was the most important variable to be related with community compositions in terms of explained data variance. However, the  $r^2$  value obtained for the daytime community was higher ( $r^2 = 0.58$  vs.  $r^2 = 0.46$ , Table 3). This variable was followed by “CPOM values” for the nighttime community and by the “mean substrate diameter” for the daytime community (both with  $p < 0.01$ , Table 3).

**Table 1.** Taxa list and number of individuals obtained in the Lemme stream (Order and Family, except Hydrachnidia). D = daytime; N = nighttime (35 Surber in both cases).

Order	Family	D abundance	N abundance	
<b>Plecoptera</b>	Capniidae	3	3	
	Chloroperlidae	1	0	
	Leuctridae	75	71	
	Nemouridae	31	14	
	Perlodidae	0	2	
	Taeniopterygidae	670	894	
<b>Ephemeroptera</b>	Baetidae	265	272	
	Caenidae	0	2	
	Ephemeridae	2	3	
	Heptageniidae	21	17	
	Leptophlebiidae	21	11	
<b>Trichoptera</b>	Beraeidae	2	2	
	Ecnomidae	0	1	
	Goeridae	1	0	
	Hydropsychidae	298	248	
	Hydroptilidae	2	2	
	Leptoceridae	6	12	
	Limnephilidae	2	4	
	Polycentropodidae	3	1	
	Psychomyiidae	13	9	
	Rhyacophilidae	96	113	
	Sericostomatidae	2	2	
	<b>Diptera</b>	Athericidae	4	0
Blephariceridae		0	6	
Chironomidae		9432	7610	
Empididae		9	8	
Limoniidae		62	51	
Psychodidae		4	3	
Simuliidae		144	577	
Stratiomyidae		3	1	
Tipulidae		3	4	
<b>Coleoptera</b>		Dryopidae	1	1
		Elmidae	36	39
	Gyrinidae	3	3	
	Hydraenidae	2	1	
	Scirtidae	1	0	
<b>Odonata</b>	Aeshnidae	5	6	
	Gomphidae	7	18	
<b>Pulmonata</b>	Ancylidae	1	3	
	Lymnaeidae	0	3	
	Physidae	1	1	
<b>Amphipoda</b>	Gammaridae	3	6	
<b>Isopoda</b>	Asellidae	0	2	
<b>Tubificida (Oligochaeta)</b>	Naididae	51	99	
<b>Opisthopora (Oligochaeta)</b>	Lumbricidae	6	6	
<b>Lumbriculida (Oligochaeta)</b>	Lumbriculidae	0	2	
<b>Arhynchobdellida</b>	Erpobdellidae	0	2	
<b>Tricladida</b>	Dugesidae	6	8	
<b>Gordea (Nematoda)</b>	Gordiidae	1	3	
<b>Trombidiformes</b>	Hydrachnidia	8	6	

**Table 2.** Results of the  $t$ -test between the daytime and nighttime data. Variables “Scrapers” and “Biomass” are not displayed as they were evaluated with Wilcoxon tests (see the values and the results in the main text).  $df$ : degrees of freedom for the  $t$ -statistic.

Variables	$df$	$t$	$p$ -value
Abundances	68	-0.4269	0.6708
Families' Richness	68	-0.0329	0.9739
Shannon Index	68	0.0838	0.9335
Predators (No. ind)	67	0.9308	0.3553
Collectors (No. ind)	67	-0.9911	0.3252
Shredders (No. ind)	67	0.6572	0.5133
Filter feeders (No. ind)	68	0.0326	0.9741



**Fig. 2.** NMDS plot where the *a priori* identified sites are colored. Black denotes the sites that belong to the daytime sampling ( $n = 35$ ), while white indicates the sites that belong to the nighttime sampling ( $n = 35$ ). 3D stress = 0.19

The Permanova test showed no significant differences ( $F$ -value = 0.50,  $r^2 = 0.01$ ,  $p > 0.05$ ) in the macroinvertebrate assemblage composition between the two pre-defined groups (D and N).

The median STAR\_ICMi values were 0.960 and 0.985 for the D and the N period, respectively. In both cases, ecological status always varied between the Good and High classes, with a prevalence of the latter and their p.c.i. (0.866–1.059 and 0.855–1.085, respectively) almost completely overlapping. Finally, the indicator value analysis did not identify strong groups of indicator taxa for the two community conditions under study (D and N). Only oligochaetes, which belong to the family Naididae, presented a high (IV = 0.49) and significant indicator value for the nighttime community ( $p = 0.036$ ). No indicator taxa were obtained for the daytime community, and all the other families displayed low values with no significant relationships found in both cases.

## 4 Discussion

This study analyzed macroinvertebrate communities from daytime and nighttime samplings. No relevant differences were found when taxonomic, functional and biological information was considered. In this freshwater ecosystem (Northern Apennine stream), the information obtained at the family level (even for biomonitoring purposes) during a period (daytime or nighttime) was representative of the entire diel cycle and macroinvertebrate community.

When focusing on an intermittent small stream in Australia, Dell *et al.* (2015) recently obtained similar results, and detected no functional differences between the diurnal and nocturnal invertebrate communities in a specific experimental pool bed. Similarly, few or no diel differences in invertebrate densities were found in a British river (Copp *et al.*, 2005).

Our findings contrast with our initial prediction. We suggest three potential reasons for the lack of major differences between the day and night data: (i) the heterotrophic condition of small-order streams (*e.g.*, mid-mountain Apennine streams); (ii) presence of predators under both night and day conditions; (iii) absence of taxa with a specific phototaxis (*e.g.* positive phototaxis: movement toward light). The influence of these possibilities, either alone or combined, could be responsible for the results we obtained. Regarding the first reason, it is important to stress that allochthonous material (such as riparian leaves and wood) is a crucial energy source in these freshwater ecosystems (Allan and Castillo, 2007), and the source and availability of these components is largely independent of the light patterns that reach the aquatic system. A second reason could be the presence of different predators in the daytime and at nighttime, which could lead to similar predation pressure on macroinvertebrates. The confirmed presence of diurnal fish predators, such as brown trout (*Salmo trutta*, Linnaeus, 1758) and chub (*Squalius cephalus*, Linnaeus, 1758), could be balanced by the presence of nocturnal white-clawed crayfish (*Austropotamobius pallipes* complex), with abundant populations in the study area and in this watercourse (Zaccara *et al.*, 2004; Nardi *et al.*, 2005). Normally this crayfish has been considered an opportunistic omnivorous animal, but the importance of macroinvertebrates in its diet has been well documented in such ecosystems, and even in different life stages (Scalici and Gibertini, 2007). The third and last suggestion could be connected with the indicator values analysis results. In our case, the only nighttime indicator taxa were the oligochaetes of the family Naididae. This finding seems to indicate that taxa with a specific phototaxis were lacking. The wider distribution and greater abundance of these aquatic worms at nighttime has already been, be it indirectly, reported by Elliott (2005b), whose study analyzed gut content and the diet of a predaceous caddisfly. This could be related to the nocturnal habits of oligochaetes as a group. However, additional studies are essential before being able to recommend their use as indicator taxa.

When considering the second aim of this research work, we found very few differences when studying small-scale environmental variables. Velocity was the most important variable to be related with the macroinvertebrate composition in both cases. This variable has already been demonstrated as one of the most important factors of macroinvertebrate community

**Table 3.** Correlations of environmental variables with the NMDS ordinations of macroinvertebrates (D and N separately) and the significance of the correlation based on the envfit function (9999 permutations). The goodness-of-fit statistic is the squared correlation coefficient ( $r^2$ ) (mean\_diam = mean riverbed substrate diameter).

Variables	Daytime (D)			Nighttime (N)		
	$r^2$	$p$ -value		$r^2$	$p$ -value	
Velocity	0.578	0.0001	***	0.457	0.0002	***
Depth	0.023	0.6831		0.128	0.1129	
CPOM	0.194	0.0305	*	0.289	0.0048	**
mean_diam	0.297	0.0033	**	0.075	0.2859	

structures in different streams and regions (Boyero and Bailey, 2001; Brooks *et al.*, 2005; Guareschi *et al.*, 2014). The importance of the CPOM resource for stream macroinvertebrate distribution has been previously stressed in similar Apennine ecosystems, particularly in relation to shredders distribution (Fenoglio *et al.*, 2005).

In our fieldwork study, the only difference found in terms of community composition and environmental variables was represented by the “mean riverbed substrate diameter” variable, which was relevant when considering the daytime data. The dependence of community structure on riverbed typology is a matter of constant debate (*e.g.*, Culp *et al.*, 1983; Barnes *et al.*, 2013; Laini *et al.*, 2014), although features like size, constitution or complexity have often been shown to influence macroinvertebrate diversity, structure or preferences in different streams and regions (*e.g.*, Gayraud and Philippe, 2001; Graça *et al.*, 2004; Barnes *et al.*, 2013; Fu *et al.*, 2015).

#### 4.1 Final remarks and future research lines

In this kind of research, it is important to stress that some specific results may be affected by the sampling methods employed. For instance in lentic ecosystems, Florencio *et al.* (2011) suggested that dip-netting was especially appropriate for sampling macroinvertebrates in different microhabitats, whereas fyke nets were a better option for capturing nocturnal and fast-swimming invertebrates. Similar problems may affect lotic ecosystems, where the use of a Surber sampler alone might not be capable of collecting fast-swimming invertebrates like some Coleoptera (*e.g.*, family Gyrinidae), or skaters like aquatic Hemiptera (*e.g.*, Gerridae). We opted for the Surber sampler for both conditions because obtaining quantitative and comparable data was considered the most important factor and was in line with our study aims.

Nevertheless, more specific findings could probably be obtained by complementary methods to evaluate specific movements (*e.g.*, traps or artificial substrates in different directions, Bruno *et al.*, 2012) and to better comprehend the importance of each small scale variable. Further research is also required on the possibility of intra-stretch changes in macroinvertebrates distribution at nighttime by separately analyzing different habitats (*e.g.*: surface/bottom, sand/pebbles, sediment/macrophytes). For instance, by focusing on macrophyte patches (*Ceratophyllum demersum*) in an Argentinian stream, Ferreiro (2014) found a higher abundance value for invertebrates (planktonic and benthonic organisms) at nighttime, and suggested quite an abrupt change once the sun sets.

Our results potentially represent a reference and natural condition in Apennine streams as to day-night changes at the family level, and may provide useful information about sampling strategies. As behavioral adaptations may be species-specific, it would be interesting to compare these results by studying the entire macroinvertebrate community at a higher taxonomic resolution (*e.g.* species), despite this entailing having to make considerable efforts in terms of resources.

Coupling experimental approaches (laboratory) with fieldwork observations in different geographic areas is recommended. Complementary studies in urban streams and on the effects of light pollution are also advisable (Meyer and Sullivan, 2013), and even by considering different wavelengths (*e.g.*, Barmuta *et al.*, 2001) would be extremely interesting to better understand diel cycles in stream macroinvertebrates and their relationship with artificial pollution.

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#### Conflict of interest

The authors have declared no conflict of interest.

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