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ORIGINAL ARTICLE

Settling distances of benthic invertebrates in a sediment mobilization simulation in semi-natural flumes

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ABSTRACT

*Drift time and distance depend on the ability of the drifting invertebrates to alter their body posture or by swimming, and these behaviours may change according to the local hydraulic environment, resulting in different distances travelled before exiting the drift. Such drift and settlement-mediated invertebrate movement determine dispersion processes and ultimately generates distribution patterns within streams. We conducted an experiment in an open-air, artificial flume system directly fed by an Alpine stream, where we disturbed the sediment in the flumes, inducing catastrophic drift in the benthic community, and then assessed the settlement distances of benthic invertebrates. For each flume, we collected drift samples by disturbing the substrate at 1.5 m intervals, at increasing distance from the downstream end, for a total of 7 disturbances and a maximum settling distance of 10 m in each flume, with five replicates (*i.e.*, five flumes) for each disturbance. The disturbances induced a massive catastrophic drift in Ephemeroptera, Plecoptera and Trichoptera, always higher than the behaviourally-occurring basedrift. The Settling Index calculated over the total drift collected at each distance increased with increasing distance, and after 10 m, 90% of the drifting animals had settled. Evenness and taxa richness progressively decrease with increasing settling distance. All drifting taxa were represented mainly by young instars. We used the drift collected at 1 m from the disturbance to standardize the remaining samples, based on the assumption that 1 m is not a distance long enough to allow animals to settle at that water velocity. We calculated the percentage of possible drifters which settled by computing a Settling Index for each taxon. The drifting taxa listed by decreasing Settling Index scores were* Epeorus *sp.,* Rhithrogena semicolorata*,* Isoperla *spp.,* Sericostoma *spp.,* Ecdyonurus *spp.,* Nemoura *spp.,* Leuctra *spp.,* Baetis *spp.,* Hydropsyche *spp.,* Rhyacophila *spp. We have shown, in accordance with numerous other studies, that entrained EPT nymphs travel only short distances before returning to the substratum, and that the actual distance travelled while drifting and the total time spent in drift varies between species. The results of this study can provide suggestions to assess taxon-specific availability to colonization which generates distribution patterns within streams and, on a smaller scale (*i.e.*, flume simulations), our results can be extrapolated to other studies conducted in artificial flumes, or to support evidences from field studies.*

Key words: Invertebrate drift; downstream dispersion; EPT; larval settlement.

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INTRODUCTION

Drift regulates densities, dispersal, and life cycles of benthic invertebrates in streams (Brittain and Eikeland, 1988; Céréghino and Lavandier, 1998; Maier, 2001; Mochizuki *et al*., 2006). In temperate streams, invertebrate drift is represented mainly by larval stages of Ephemeroptera, Diptera Simuliidae and Chironomidae, Plecoptera and Trichoptera, which are the most common benthic taxa in such habitats (Brittain and Eikeland, 1988; Hieber *et al*., 2003). Catastrophic drift (*sensu* CULP *et al*., 1985) is usually associated with flood conditions, during which the substrate is physically disturbed. Drift time and distance depend on the ability of the drifting invertebrates to alter their body posture or swimming, and these behaviors may change according to the local hydraulic environment. Similarly, the hydraulic conditions and the near-bed flows may initially influence the ability of organisms to settle by reducing access to particular areas of the streambed (Fonseca and Hart, 2001; Oldmeadow *et al*., 2009 and references therein). Thus, distances travelled before exiting the drift are determined by the physical constraints imposed by the hydraulic environment on the morphological and mechanical swimming abilities of organisms, and the decisions individuals make regarding whether to terminate or prolong drift (Oldmeadow *et al*., 2009). In the end, invertebrate movement, mediated by drift travelling distances and settling abilities, generates distribution patterns within streams (Oldmeadow *et al*., 2009).

Today, experimental flumes of different typology have become a common tool to study the responses of biological communities to physical, chemical, hydrological manipulations, using behavioral and catastrophic drift as the measured response and based on the hypothesis that any invertebrate showing an immediate avoidance or displacement reaction would rapidly drift (Holomuzki and Biggs, 2000; Imbert and Perry, 2000; Suren and Jowett, 2001; Mochizuki *et al*., 2006; Carolli *et al*., 2012; Fenoglio *et al*., 2013; Bruno *et al*., 2013, 2016). Although drift catches in these simulations were often high, it is difficult to assess the significance of these responses, since if a high proportion of the benthos enters the drift for a short period of time, the drifting invertebrates may have originated from the area immediately downstream from the disturbance and could have returned rapidly to the benthos and thus not have been caught. Alternatively, if a low proportion of the benthos enters the drift, and the drifting invertebrates do not return rapidly to the streambed, the animals caught in the drift sampler may have drifted over a long distance and originated from an extensive area downstream from the disturbance point. This issue cannot be resolved until more is known about the distance travelled and/or the time spent in the drift by the different taxa present in the flumes, as already noted by Elliott (2002).

In this study we describe the results of a manipulative experiment where we disturbed the sediment in a set of artificial flumes inducing catastrophic drift in the benthic community, and then assessed the settlement distances of benthic invertebrates, particularly EPT. We examined the invertebrates drift distances and the rate at which they return to the substrate, based on the assumption that the differences in drift abundance/composition recorded between the samples collected at a given distance x from the disturbance and those collected at the upstream distance x+1 are presumably due to those taxa/individuals which settle on the substrate between x and x+1. The main questions investigated in our research were the following: i) does the community settle along a distance gradient, or after a certain drifting distance threshold?; ii) which are the drifting and settling tendencies for the EPT taxa present in benthic communities?; iii) when collecting drift after a certain distance from disturbance, which is the minimum distance threshold after which a significant number of individuals/taxa have settled?

The general contribution of our simulations to the understanding of invertebrate drift is to help assessing the relative importance of potential larval supply (*i.e*., drifting invertebrates) and post-settlement persistence in generating distribution and abundance patterns in streams after catastrophic events.

METHODS

Experimental design

The experiments were conducted in a set of metal flumes situated on the riparian zone of the Fersina Stream (46° 04’ 32” N, 11° 16’ 24” E) at 577 m asl. The Fersina Stream is a 2nd order stream, originating at an altitude of 2005 m, and joins the Adige River at Trento (Trentino Province, Northeastern Italy) at 191 m asl. It is a snowmelt-fed gravel-bed stream, approximately 37 km long, with a 171 km^2 watershed, receiving the contribution of numerous small streams that descend from lateral valleys. The mean annual discharge of the Fersina in 2012, measured at its joining to the Adige in Trento, was approximately 3 m^3 s^{-1} (downloaded from http://www.floods.it/public/PreDati.php). The experimental setting consists of five 20 m long, 30 cm wide metal flumes (bottom surface area: 6.0 m^2); flumes A, B, C are 30 cm deep; flumes D and E are 50 cm deep. Each flume has an adjustable longitudinal slope and feeding discharge. They are connected to a loading tank that is directly fed by water diverted from the stream, and benthic invertebrates can freely colonize the flumes by downstream drift and egg deposition. The flumes are filled to the same depth with two layers of cobbles of approximately 10 cm diameter, and a deposited fine layer of silt/sand/gravel has collected around the stones. The flumes had been run continuously at a baseflow of 0.005 m^3 s^{-1}, velocity 0.4 m s^{-1}, from the beginning of April 2013 for other simulations (Bruno *et al*., 2016), and reset on September 10th, and the flumes left undisturbed until the experiment day, occurring on 22nd October 2013.

Biological sampling

Two benthic samples were collected one day before the experiments in each of the flumes (downstream of the inlet, upstream of the outlet) with a Hess sampler (23.5 cm diameter, 100 µm mesh). During the experiment, the upstream entrance to the flumes was netted-off to prevent incoming drift from upstream. Drift samples were collected by filtering the whole volume of water leaving the flumes with 350 µm mesh drift nets inserted at the downstream end of each flume. We entrained benthic invertebrates in the flow by manually moving the substratum, as uniformly as possible, inside a frame of 22x22 cm (surface 0.0484 m^2). The disturbance technique has been used previously in similar studies (McLay, 1970; Larkin and McKone, 1985; Lancaster *et al*., 1996).

Basedrift (*i.e*., behavioural active drift, and background passive drift occurring naturally, not associated with any disturbance events) was assessed by filtering each flume for 60 min prior to conducting the simulations. For each flume, we collected drift samples by disturbing the substrate at 1.5 m intervals, at increasing distance from the downstream end: 1 m, 2.5 m, 4 m, 5.5 m, 7 m, 8.5 m e 10 m. A total of 7 disturbances were created in each flume, with five replicates (*i.e*., five flumes) for each disturbance. Disturbance lasted 1 min, and drifting invertebrates were collected for 10 min from the beginning of each disturbance. Each disturbance was performed 30 min after the previous one, each set of 7 samples took 3 h and 10 min to be completed. Samples in the different flumes were staggered by 5 min. Sampling took place from 10 a.m. to 1.30 p.m. After collection, all samples were fixed on the field in 75% ethanol. All samples were sorted in the laboratory and organisms identified to the lowest possible taxonomic level following Campaioli *et al*., 1994, 1999; Fochetti *et al*., 2009. Several early instars of insects could not be identified further than family; we classified all nymphs as *juvenile nymphs* (first and second instars, size generally <0.5 cm) or *nymphs* (later instars, up to mature nymphs). We excluded adult insects from the analysis, since they are accidentally present in drift. For the analysis, we focused only on Ephemeroptera, Plecoptera and Trichoptera (EPT) which are often good indicators of environmental conditions in streams (Lenat, 1988; Barbour *et al*., 1996; Resh and Jackson, 1993), and we chose to use only the EPT taxa whose total density in drift was ≥%, *i.e*., the Ephemeroptera *Baetis* spp., *Epeorus* spp., *Ecdyonuru*s spp., *Rhithrogena* spp., the Trichoptera *Hydropsyche* spp., *Sericostoma* spp., *Rhyacophila* spp., the Plecoptera *Nemoura* spp., *Leuctra* spp., *Isoperla* spp.

Statistical analysis

Invertebrate drift densities were expressed as number of individuals (N. ind.) m^{-3} by adjusting the numbers of collected individuals for the filtered volume of water, calculated based on sampling time (600 s) and discharge (0.005 m^3 s^{-1}).

Changes in drifting community over drifting distances

Firstly, as preliminary step, we assessed if the flumes had been colonized by the same benthic communities, *i.e*., if the drift samples collected in the different flumes were replicates. In fact, if the density and composition of the drift source (*i.e*., the benthos) were similar among flumes, then drift abundances, and their differences over distance from the disturbance, could be used for the purpose of this research without bias. Benthic density was measured by calculating the mean densities per flume, and expressed as N ind. m^{-2} by testing for significant differences in densities of each taxon and of the entire community overall (one-way univariate and multivariate ANOVA, factor FLUME), and in composition of the benthic community (one-way PERMANOVA, factor FLUME). Secondly, because the analysis showed that the flumes had indeed been colonized by similar assemblages (see results for details), we used the drift densities (calculated as the total number of animals in the drift at each distance from the disturbance over the whole sampling time interval, and expressed and N ind. m^{-3}) for the remaining analysis. Drift densities were log(x+1) transformed, and the Bray-Curtis index was used to calculate the similarity matrix for all PERMANOVA analyses. We tested if the drift samples collected in the different flumes were replicates, by testing for significant differences in density of each drifting taxon (one-way PERMANOVA, factor FLUME), and in composition of the drifting community (one-way PERMANOVA, factor FLUME), among flumes. Thirdly, we assessed the effectiveness of our simulations by testing for significant differences in abundance and composition (non parametric Kruskall-Wallis test, and PERMANOVA, respectively) between drift samples conducted during the manipulations and the basedrift for each distance and for each taxon, on a log (x+1) transformed data matrix. Fourthly, we investigated drifting and settling distances for the entire community. We looked for patterns in the drift at different distances from the source in the unconstrained multivariate space using the Principal coordinates analysis (PCO, also known as MDS), and we assessed significant differences in abundance and composition (one-way ANOVA and PERMANOVA, respectively) followed by pairwise comparisons (HSD Tukey's test and PERMANOVA pairwise comparisons, respectively). To evaluate changes in community composition with increasing settling distance (*i.e.,* distance from the disturbance), we used a ranked species cumulative abundance plot to visualize species abundance distributions, and the Bray-Curtis dissimilarity index calculated comparing the most distant drift sample (10 m from disturbance) with progressively closer ones.

Taxa-specific responses

Based on the working hypothesis that we manually displaced a portion of the residing benthos of approximately the same amount/composition for each disturbance, and that part of the drifting organisms would return to the substrate while travelling along the flumes, and hence would not be collected at the flume outlet, we calculated a *settling index* for each taxon and for the overall community (*i.e*., calculating over the total drift) at each distance. We used the drift collected at 1 m from the disturbance to standardize the remaining samples, based on the assumption that 1 m is not a distance long enough to allow animals to settle at that water velocity (based on the travelling time and distances reviewed in Elliot, 2002). We calculated the percentage of possible drifters as follows:

$$SI_d=100-(dr_d/dr_{1m} *100) \qquad \text{(eq. 1)}$$

where
d=distance from the disturbance;
dr=drift abundance (expressed as n ind. m^{-3});
dr_d=drift collected at distance d;
dr_{1m}=drift collected at 1 m.

For each taxon, we then calculated a settling index by averaging the 6 indices, as follows:

$$SI=\text{mean}\ (SI_{2.5}, SI_4, SI_{5.5}, SI_7, SI_{8.5}, SI_{10}) \qquad \text{(eq. 2)}$$

For the whole drifting community, we calculated a mean settling index for drifting intervals of about 3 m (2.5-4 m, 4-7 m, 7-10 m). The reason behind the choice of this interval will be explained below.

Data were analyzed in Primer 6 ver. 6.1.12 (PRIMER-E Ltd, 2009), and STATISTICA (data analysis software system) ver. 9.1. (StatSoft, Inc. 2010).

RESULTS

The flumes had been colonized in a similar way prior to conducting the experiment. In fact, benthic samples did not differ among flumes in species composition overall and for all pairwise comparison between flumes, nor in abundance overall and for each taxon density (Tab. 1). Hence, we could assume that the five flumes represented five replicates, and that approximately the same (in density and composition) component of the EPT assemblages was induced to drift by each manipulation. Flumes did not differ in species composition of the drifting assemblages, nor did they differ in drift density for each taxon and for the total density (Tab. 1). We therefore used the flumes as replicates in the subsequent statistical analysis.

Changes in drifting community over drifting distances

The catastrophic drift induced by manipulations differed significantly from the basedrift in composition and abundance overall and for each taxon density (Tab. 1). The ratio of the total drift collected for each sample and flume to the basedrift collected in that same flume, was always >1 for each flume and settling distance, and as average decreased exponentially with increasing settling distances from 126 (1 m settling distance) to 12 (10 m settling distance) (Tab. 2). Hence, the simulations induced a massive catastrophic drift, always higher than the behaviourally-occurring basedrift, and the amount of drifting animals that did not settle, and continued drifting catastrophically decreased over distance.

The catastrophic drift when ordinated in a principal coordinates analysis showed distinct composition according to a gradient of decreasing distance from disturbances (*i.e.*, with decreasing settling distances) (Fig. 1) on the primary axis, which explained 69.7% of the total variation; the secondary axis, which explained 9.7% of the total variation, represented a gradient due the drift collected after the invertebrates had 10 m to settle. Drift densities significantly changed with distance (Tab. 1, Fig. 2), with the drift collected at increasing settling distances being respectively 1.4 (drift collected after 2.5 m), 2.4 (4 m), 3.7 (5.5. m), 5.5 (7 m), 6.6 (8.5 m), and 9.6 (10 m) times less abundant than the one collected after 1 m. All taxa decreased in density with increasing settling distance (Fig. 3).

The drift abundance and composition showed a sig-

Tab. 1. Result of the statistical analyses.

		PERMANOVA			Univariate ANOVA			Multivariate ANOVA		
	Factor	df	Pseudo-F	P (perm) overall	P pairwise	df	P	df	F	P
Benthos	FLUME	4	0.97	0.467	Always >0.05	4	Always >0.05 for each taxon	20	2.25	0.21
Drift	FLUME	4	1.50	0.175	Always >0.05	4	Always >0.05 for each taxon	44	1.96	0.005**
Drift	Basedrift *vs* catastrophic drift	1	40.26	0.001**				28	8.78	<0.001***
Drift	Drifting distance	6	6.81	0.001**				60	2.44	<0.001***

*df, degree of freedom; *P=0.01 to 0.05 (significant); **P=0.001 to 0.01 (very significant); ***P<0.001 (extremely significant).*

Tab. 2. Ratio of total drift collected during the experiment to basedrift.

	Flume A	Flume B	Flume C	Flume D	Flume E	Mean
Drift 1 m	106	205	39	160	121	126
Drift 2.5 m	80	193	27	100	84	97
Drift 4 m	42	48	13	65	73	48
Drift 5.5 m	36	48	9	41	35	34
Drift 7 m	21	55	9	22	11	24
Drift 8.5 m	21	18	7	31	13	18
Drift 10 m	16	14	5	16	8	12

nificant change over distance overall (Tab. 1), and pairwise comparisons in composition (Tab. 3) indicate that samples collected with 1 and 2.5 m settling distance were similar but differed from all those collected with longer drifting distances. These samples, collected from 4 to 10 m from disturbances, differed among groups including 3 m of travelling distance, starting from 4 m (for instance, drift samples collected at 10 m were similar in composition only to those collected at 8.5 and 7 m; samples collected at 8.5 m only to those collected at 7 and 5.5. m; samples collected at 7 m only to those collected at 5.5 and 4 m; and samples collected at 5.5 m were similar only to those collected at 4 m).

The Settling Index calculated over the total drift collected at each distance increased with increasing distance (Tab. 3), and after 10 m, 90% of the drifting animals had settled. More interestingly, the moving SI average, calculated for drifting intervals with no differences in composition, *i.e.*, of about 3 m (2.5-4 m, 4-7 m, 7-10 m), increases linearly (R^2=0.967) with distance from 43 ind. m^{-3} for 2.5-4 m to 71 ind. m^{-3} for 4-7 m, to 85 ind. m^{-3} for 7-10 m.

The ranked species cumulative abundance plot (Fig. 4) shows a progressive decrease in evenness and taxa richness with increasing distance from the disturbance (*i.e.*, less taxa reached the end of the flume, and they are less equitably-distributed because several individuals reached the bottom), and the Bray-Curtis dissimilarity increases linearly (R^2=0.98) when comparing the drift with no settling (1 m from disturbance) with progressively increasing settling distance (Tab. 3), *i.e.*, the longer the settling distance, the more dissimilar the drifting communities become, because more taxa and individuals regain the bottom.

Taxa-specific responses

The taxa with highest total catastrophic drift were, in decreasing order: *Baetis* spp. (40.5 % of the total, and representing 42.8% of the benthic community as assessed with kick sampling), *Hydropsyche* spp. (36.6 %of catastrophic drift, 26.4% of benthos), *Nemoura* spp. (8.5 % and 10.3%), *Leuctra* spp. (7.2 % and 7.1%), *Sericostoma* spp. (2.0 % and 1.3%), *Epeorus* sp. (1.2 % and 5.2%), *Isoperla* spp. (1.1 % and 1.3%), *Ecdyonurus* spp. (1.1 % and 1.8%), *Rhithrogena semicolorata* (1% and 2.7%), *Rhyacophila* spp. (0.8% and 1.0%). All taxa (except *Leuctra* spp. for which the percentage of juveniles could not be determined, since we did not identify the specimens to the species level, and the genus varies in size, even at the late larval stage) were represented by mainly young instars, whereas the percentage of young instars present in the benthic assemblages was generally lower (Tab. 4), es-

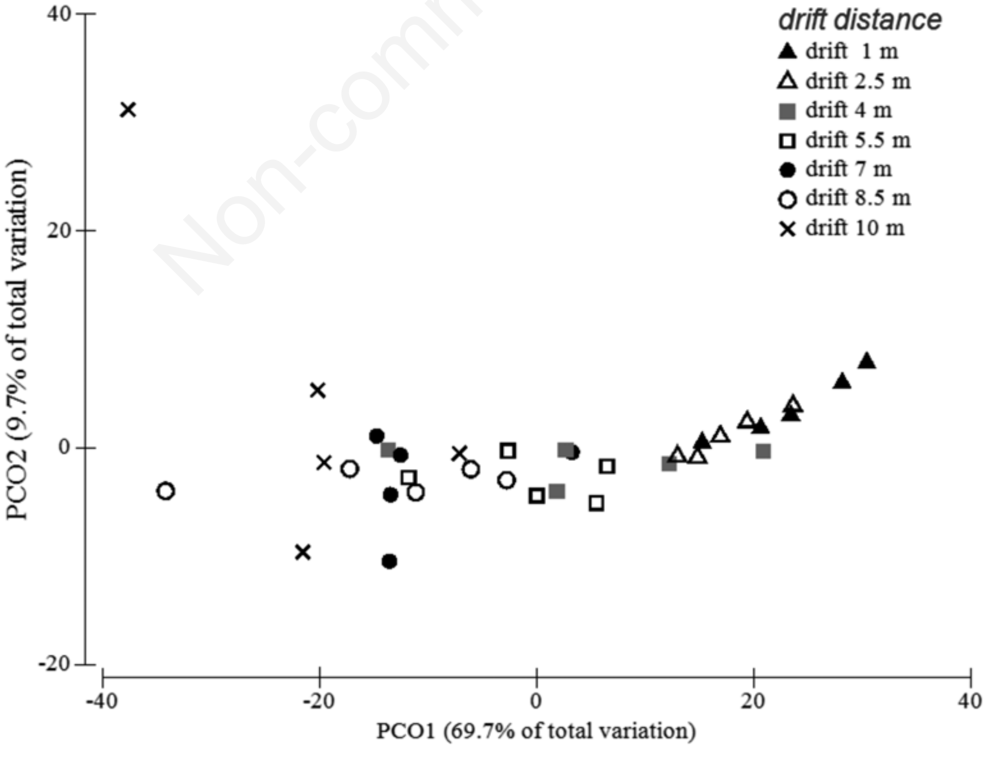

Fig. 1. Principal coordinates analysis based on Bray-Curtis Similarity Index of drifting invertebrate communities collected at each distance, for the five replicates (*i.e.*, flumes). Data log (x+1) transformed.

pecially for Heptageniidae and all Trichoptera except Rhyacophilidae. For all taxa, the percentage of young larval stages drifting during basedrift was very high, representing about 100% of the total, with the exception of *Epeorus* sp., which had a similar and very low percentage of young larval stages drifting with baseflow and during the catastrophic event (Tab. 4). The comparative analysis of the ratio of catastrophic drift to basedrift, the mean catastrophic drift, the drift for each sample, the mean basedrift, the mean settling index and the settling index for each increasing distance (Tab. 4, Figs. 3 and 5) provides information on the drifting behavior and settling ability of each taxon. The taxa with settling index scores in decreasing order were (Tab. 4): *Epeorus* sp., *Rhithrogena semicolorata*, *Isoperla* spp., *Sericostoma* spp., *Ecdyonurus* spp., *Nemoura* spp., *Leuctra* spp., *Baetis* spp., *Hydropsyche* spp., *Rhyacophila* spp.

Baetis spp. has a very high drift rate, both behavioral (basedrift) and catastrophic, with comparable drift rates (in fact, the catastrophic drift was only 16 times higher than the basedrift), and catastrophic drift decreases exponentially (R^2=0.9965). The mean settling index scores 70, and increases linearly (R^2=0.9271) with increasing settling distances, suggesting a strong ability to settle. When the set-

Tab. 3. P values for pairwise tests, PERMANOVA factor *drift distance* fixed, average Bray-Curtis dissimilarity with sample 1 m, sand ettling index.

	Drift 1 m	Drift 2.5 m	Drift 4 m	Drift 5.5 m	Drift 7 m	Drift 8.5 m	Average Bray-Curtis dissimilarity with sample 1 m	Settling index
Drift 2.5 m	0.095						8.92	28
Drift 4 m	0.020*	0.049					21.17	58
Drift 5.5 m	0.009**	0.010*	0.751				27.85	73
Drift 7 m	0.015*	0.008**	0.087	0.129			36.70	82
Drift 8.5 m	0.008**	0.009**	0.043*	0.086	0.730		40.80	85
Drift 10 m	0.010*	0.016*	0.013*	0.025*	0.123	0.394	48.13	90

**P*=0.01 to 0.05 (significant); ***P*=0.001 to 0.01 (very significant); ****P*<0.001 (extremely significant).*

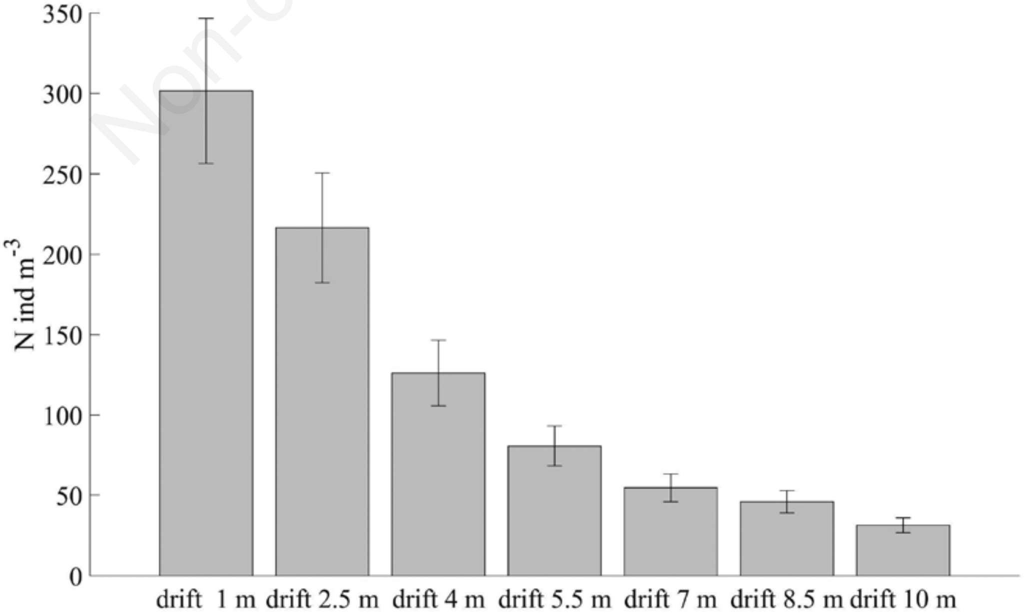

Fig. 2. Total drift for each settling distance (calculated as the sum of the mean value over five replicates for each taxon). Whiskers: standard error of the mean.

tling distance is 10 m, only 11% of the animals (as recorded in the 1 m sample) are still drifting. *Hydropsyche* spp. has a very low basedrift (does not tend to drift behaviourally), but very high rates of catastrophic drift (about 448 times the basedrift). The mean settling index scores 67, very similar to *Baetis* spp., but settling has a slightly weaker correlation than *Baetis* (linear regression, R^2=0.7788), and occurs mainly for distances from 4 m, and at a settling distance of 10 m, 9% of the animals are still drifting. The Heptageniidae *Ecdyonurus* spp., *Epeorus* sp. and *Rhithrogena semicolorata* all have low basedrift and low tendency to drift catastrophically. The ratio to basedrift is low and similar for the three taxa. The settling scores are among the highest of all taxa, with *Epeorus* sp. scoring 85, followed by *Rhithrogena semicolorata* (81) and *Ecdyonurus* spp. (71). Settling occurs more similarly for all distances for *Epeorus* sp., is more irregular for *Ecdyonurus* spp. but generally higher from 5.5. m on with a peak at 10 m, and for

Tab. 4. Values of basedrift, catastrophic drift, ratios, settling index, percentage of juveniles over the total of each taxon, calculated as mean over 5 flumes.

	Mean basedrift (ind. m^{-3})	Mean catastrophic drift (ind. m^{-3})	Ratio catastrophic drift to basedrift	Settling index	Juveniles in drift (%)	Juveniles in basedrift (%)	Juveniles in benthos (%)
Baetis spp.	3.12	50	16	70	99	100	100
Hydropsyche spp.	0.1	45	448	67	99	100	86
Nemoura spp.	0.38	10	27	70	98	100	100
Leuctra spp.	0.06	9	146	70	n.a.	n.a.	n.a.
Isoperla spp.	0.02	1	67	80	98	100	100
Epeorus sp.	0.14	1	11	85	53	50	34
Ecdyonurus spp.	0.04	1	33	71	83	100	68
Rhithrogena semicolorata	0.04	1	31	81	86	100	70
Sericostoma spp.	0	2	Not drifting	72	100	Not drifting	90
Rhyacophila spp.	0	1	Not drifting	56	73	Not drifting	67

n.a., not available.

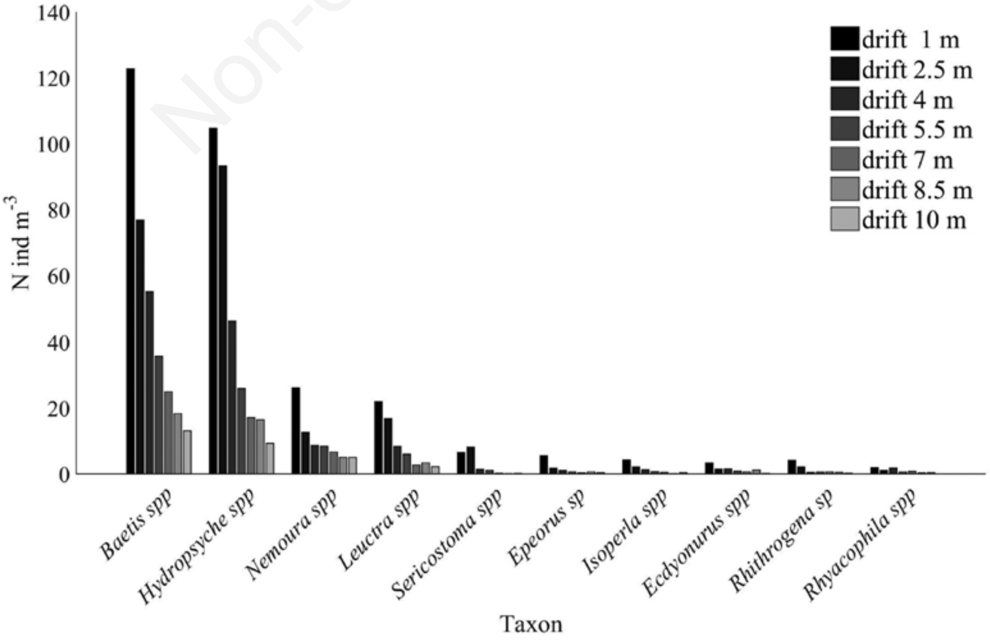

Fig. 3. Mean drift (calculated over five replicates) for each drifting taxon at each settling distance.

Rhithrogena semicolorata increases strongly for settling distances from 4 m; the percentage of animals still drifting at 10 m is respectively 7%, 4%, 21%. The stoneflies *Leuctra* spp. and *Isoperla* spp. have low basedrift while *Nemoura* spp. has higher basedrift rates; catastrophic drift is low for *Isoperla spp.*, higher and comparable the remaining two taxa (Tab. 4). The tendency to drift catastrophically is higher for *Leuctra* spp. (the ratio of catastrophic to basedrift scoring 146 for *Leuctra* spp., 67 for *Isoperla* spp., and 27 for *Nemoura* spp.). The settling index scores 70 for *Nemoura* spp. and *Leuctra* spp., and is higher (80) for *Isoperla* spp., but *Nemoura* spp. settling abilities do not change much with distance (settling index is similar over distances, especially from 7 to 10 m, at 10 m 19% of the animals are still drifting), whereas *Leuctra* spp. and *Isoperla* spp. settle more from 4 m on, and especially from 7 to 10 drifting m; at 10 m only 10% of the animals are still present in the catastrophic drift for *Leuctra* spp.and *Isoperla* spp. The caddisflies *Rhyacophila* spp. and *Sericostoma* spp. are not present in basedrift, and have very low tendency to drift catastrophically (higher for the caddis-bearing *Sericostoma* spp. than the free-roaming *Rhyacophila* spp.). The settling index scores 72 for *Sericostoma* spp., and has the lowest recorded value, 56, for *Rhyacophila* spp. The settling behaviour differs: the settling values by distance of *Sericostoma* sp. was negative at 2.5 m., indicating a higher number of drifting individuals than at 1 m, and possibly due to the patchy distribution of this rare taxon (the drift at 2.5 m was variable among flumes: 15, 8, 8, 4, 4, ind. m^{-3} for flumes A to E). Settling values were much higher for the remaining distances, among the highest recorded for all taxa, and almost 100% of the individuals had settled for distance over 7 m. *Rhyacophila* sp. was the rarest taxon in drift and benthos, tendency to settle was highly variable with a sharp decrease at 4 m, higher for all other distances and particularly from 8.5 m settling distance.

DISCUSSION

The flume system described in this study was successfully used to assess macrobenthos drift responses in previous simulations (Carolli *et al*., 2012, Bruno *et al*., 2013, 2016). Our flumes have the advantage of being freely colonized by the benthic fauna from the pristine Fersina

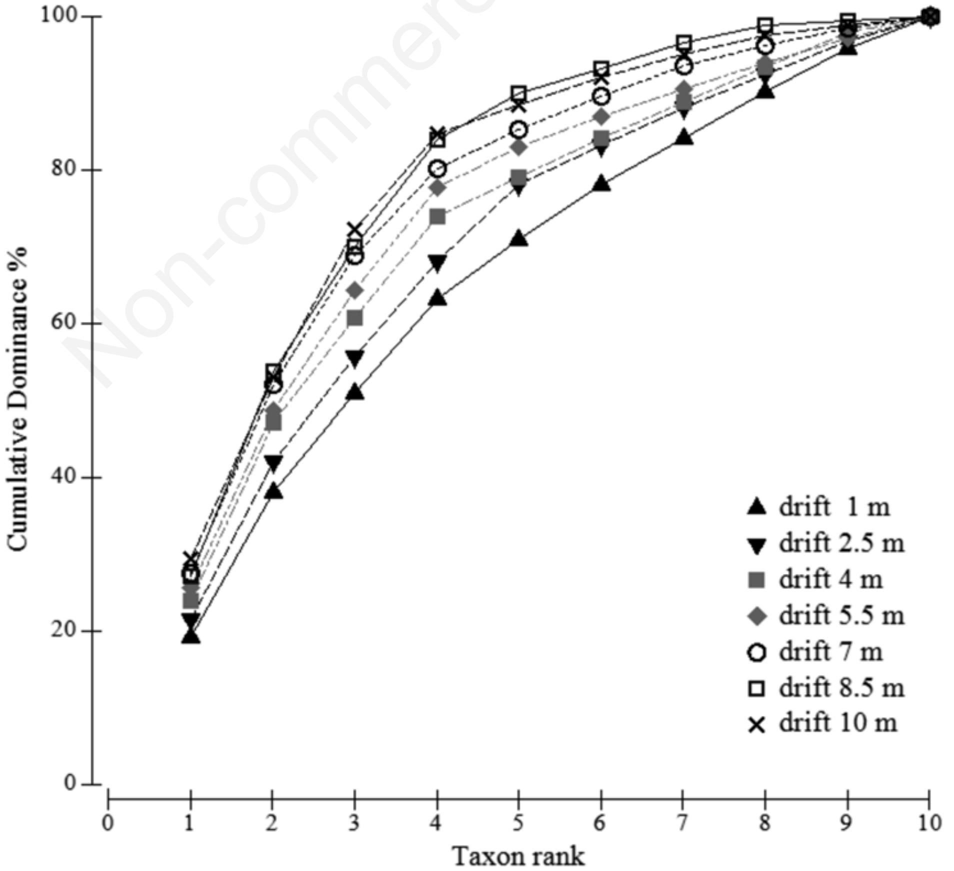

Fig. 4. Ranked taxa cumulative abundance plot for each settling distance.

Stream *via* incoming drift from upstream (mainly) and egg deposition by adult (likely occurring, but never observed directly), and thus they approximately represent the benthic assemblages present in the area. The benthic communities were left undisturbed at baseflow, with natural day-light cycle and subject to natural daily changes in water temperature for a period of time which allowed colonization by benthic invertebrates, and reaching the highest taxa diversity and abundance. These near-to-natural conditions are in contrast to the small flumes in laboratory conditions (10 m or less; Allan and Feifarek, 1989; Ciborowski, 1983) used in most of the studies on drift distances, where pre-selected taxa were introduced in the drift rather than eliciting the responses of the whole naturally-occurring community. Therefore, the number of drifting nymphs involved in our experiment was higher, and the drifting community was more diverse in comparison with other studies, which often referred to observations on single or few individuals of one or few species (Allan and Feifarek, 1989; Ciborowski, 1983; Lancaster *et al*., 2006; Oldmeadow *et al*., 2009). To our knowledge, the most comparable study setting is discussed in Lancaster *et al*. (1996), where benthic macroinvertebrates were disturbed from the stream substrate and caught in drift nets at various distances downstream, at four selected water velocities. However, the authors investigated a very different benthic community (dominated by Plecoptera, whereas in our flumes Ephemeroptera and net-spinning Trichoptera were dominant).

The species-specific habitat requirements of benthic invertebrates determine distinctive assemblages or densities of different taxa associated with the hydraulic and geomorphic characteristics of stream mesohabitats. Our flumes are a simplified system, *i.e*., there are no riffle-pool sequences or other mesohabitats. This obviously reduces the macrobenthos diversity by selecting more reophilic taxa, and/or taxa or life stages that can find hydraulic refuges on the bottom. However, the use of such simplified system is particularly relevant considering that Oldmeadow *et al*. (2009) showed that constraints on movement and settlement might be important drivers of distribution patterns within streams. The small-scale distribution patterns of some benthic taxa within a stream would therefore correspond to their drift behaviour and ability to access various hydraulic patch types, and the observed distribution patterns may reflect primarily the results of departure and settlement processes (Oldmeadow *et al*., 2009). The geomorphologically uniform setting of our flumes, therefore, allowed us to test only the tendency to settle and the distance travelled by drift of the investigated taxa, by removing the hydraulic variability created by riffle-pool sequences.

Changes in drifting community over drifting distances

Entrainment of zoobenthos along with sediment at high flows has a strong contribution to drift (Bond and Downes, 2003; Gibbins *et al*., 2005), and previous studies in artificial channels and in natural streams have con-

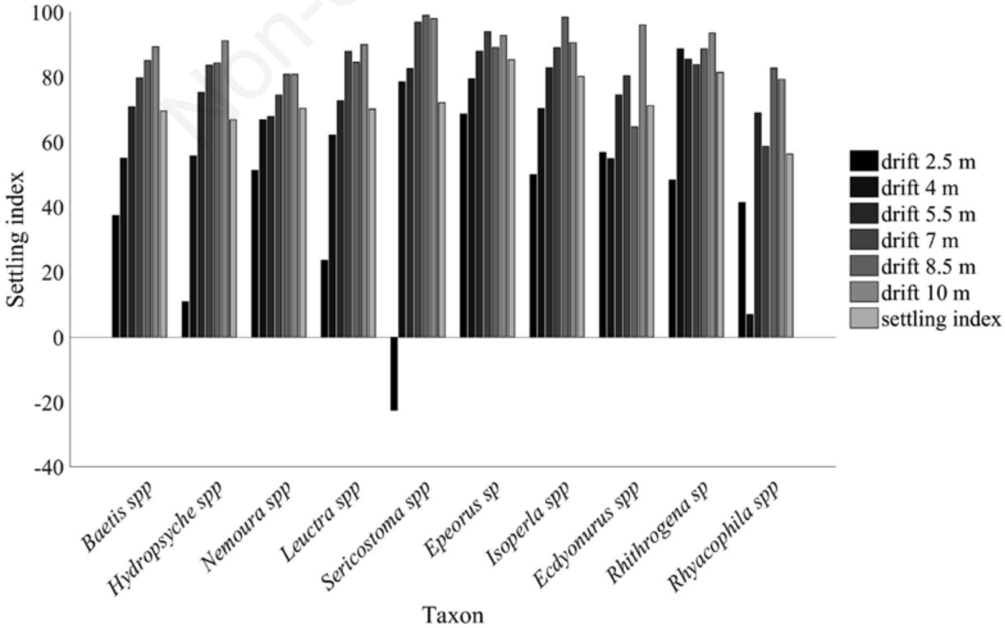

Fig. 5. Settling index for each distance and means settling index, for each taxon.

firmed that some taxa actively exit the drift by behavioural control (Ciborowski and Corkum, 1980; Campbell, 1985; Otto and Sjökström, 1986; Allan and Feifarek, 1989; Elliott, 2002; Oldmeadow *et al*., 2009). Studies to assess drifting samples have been conducted by manually introducing selected larvae in artificial or natural streams and recording the numbers present in drift after a certain distance (Elliott 1971, 2002; Ciborowski and Corkum, 1980; Campbell, 1985; Otto and Sjökström, 1986; Allan and Feifareck, 1989; Oldmeadows *et al*., 2009); or forcing animals into the water column by disturbing the substratum and inducing catastrophic drift (McLay, 1970; Larkin and McKone, 1985; Lancaster *et al*., 1996). It has been argued by Elliott (2002) that by using the latter method, there is no control over the number of invertebrates entering the drift, or the different taxa in the drift. In our experiment, we chose to manually disturb the benthos nonetheless, since we were interested in evaluating the impact of settling distance on the drift densities recorded in a community in near-natural conditions. We assessed if the drift collected at the end of the 20 m flumes represents a correct estimate of the animals drifting as response to a disturbances, or an underestimate since a fraction of the drifting animals would have had time to settle while travelling along the flumes. The final aim was to widen the knowledge of the mechanisms involved in drift and settlement of EPT nymphs. As remarked by Oldmeaedow *et al*. (2009), *understanding settlement processes is critical to understanding, for example, how substrate- and habitat-specific associations arise, and how dispersal influences population structure*. We therefore were more interested in the distance travelled than in the time spent drifting as in other works, and in the responses of the EPT drifting community over all, rather than a single or few selected taxa. However, from the distance intervals and water velocity (0.4 m s^{-1}), the drifting time of an object carried by the water flow can be estimated in 2.5, 6.25, 10, 13.75, 17.5, 21.25, 25 seconds respectively for distances of 1, 2.5, 4, 5.5, 7, 8.5, 10 m. These values are roughly comparable with published data, as drift time may be prolonged or reduced by altering body posture or by swimming, and drifting invertebrates do not behave like passive particles (Oldmeadow *et al*., 2009). Most benthic invertebrates drift for short distances before regaining contact with the substrate, and the distance travelled in the drift varies with different species. In studies conducted in flumes at velocities of 0.24 m s^{-1}, most drifting invertebrates regained the bed within 1 m of their release point (Gibbins *et al*., 2007). In natural streams the drift distance of most invertebrates, and of *Baetis* in particular, were derived from <2 m upstream of the sampling point (Townsend and Hildrew, 1976; Elliott, 2002) at velocities of respectively 0.2 and <0.3 m s^{-1}.

Several studies showed a clear negative exponential relationship between drift catches and distance travelled from the source of the disturbance (McLay, 1970; Elliott, 1971; Ciborowski, 1983; Larkin and McKone, 1985; Allan and Feifarek, 1989, Lancaster *et al*., 1996). In our flumes, every 3-m interval (corresponding to a travelling time of approximately 7.5 seconds) a significant part of the drifting assemblage settles, creating discontinuous changes in the drift communities, which become more dissimilar and less equitably-distributed due to the different responses of the drifting taxa.

Taxa-specific responses

Most of the animals drifting in our experiment were early nymphal instars, which have been reported in other alpine streams in the same watershed of our experimental site to use the hyporheos as a refuge area to complete their development and growth (Silveri *et al*., 2008) or to avoid catastrophic drift during sudden discharge peaks (Bruno *et al*., 2009). In addition, younger instars have smaller body sizes, a factor that affects the time spent in the water columns: in laboratory studies Allan and Feifarek (1989) found that drift distance increased with decreasing body size for mayfly nymphs; the first instar of the case-building caddisfly *Potamophylax cingulatus* drifted more than ten times as far as the fifth instar (Otto, 1976).

According to Rader (1997), benthic invertebrate propensity to drift depends on several traits, including settling efficiency which, according to the author, depends on swimming ability, agility in regaining a foothold on the substrate, and sinking postures. The flume experiments showed that EPT taxa settle within a distance of 10 m and differ qualitatively in their behavioral responses during drift. This resulted in taxon-dependent settling distances, which can be compared with data from previous studies on the same genera of mayflies, stoneflies, caddisflies. Otto and Sjöström (1986), investigated drift behaviour in 25 species of mayflies, stoneflies and caddisflies in a laboratory stream. Although they recorded some differences in all orders, even between closely related species, as a general trend mayfly nymphs swam to reach the substrate as quickly as possible; stonefly nymphs prolonged the time spent in the water column by swimming, and caddisfly larvae were reluctant to swim.

For the recorded taxa of mayflies, according to Rader (1997), *Baetis* is a good swimmer with rapid settling capacity, whereas Heptageniidae are weak swimmers with intermediate settling efficiency, although they quickly regain a foothold when they get in contact with the substrate. Based on the values reported by Elliott (2002) for the time spent in the drift by 75%, 50%, 10% and 1% of the drifting invertebrates and the mean drifting time value, recorded in an experimental stony, fast-flowing section of stream, we can extrapolate for comparison the following travelling distances at the velocity recorded in our flumes (*i.e.*, 0.4 m s^{-1}): a mean 6.6 m for *Ecdyonurus* (with 75% of the animals settling at 1.9 m) and 3.8 m for *Baetis* (with 75% of the animals settling at 1.1 m). Oldmeadow *et al*., 2009 in ramped-step flume mimicking bedforms that are common in coarse-grained, high-gradient streams, recorded drifting distances of about 2 m for *Baetis*, and 4 m for *Ecdyonurus* spp. These values are well-comparable with our results, *i.e.*, *Baetis* spp. starts settling immediately and regularly over increasing distances and *Ecdyonurus* spp. settles mainly from 5.5. m onwards. *Baetis* spp. and *Ecdyonurus* spp. have behavioural control over drift through swimming bursts and posturing (*i.e.*, maintaining a stable body orientation) (Oldmeadow *et al*., 2009), and *Baetis* spp. has been reported to actively exit the drift by orienting their body and actively swimming (Allan and Feifarek, 1989). Elliott (1971) assessed the distance travelled by drifting invertebrates in a shallow and turbulent stream, and recorded how *Rhithrogena semicolorata* nymphs were unable to make a firm contact with an exposed substratum in turbulent water, and returned to the bottom when they landed in zones with low water velocity, with a mean drift distance at 4.6 m s^{-1} of approximately 15 m. Low velocity areas are not present in our flumes, and in fact *Rhithrogena semicolorata* started to settle after 4 m, but at 10 m 21% of the nymphs were still drifting. To our knowledge, there are no studies on the settling ability or drift travelling distances for *Epeorus*, but this genus is reported as weak swimmers, rare in drift (catastrophic only) (Poff *et al*., 2006).

For caddisflies, Elliott (2002) reports a mean of 8.8 m travelling distance before settling for *Hydropsyche* (with 75% of the animals settling at 2.6 m), and these values are well-comparable with our results, where 91% of *Hydropsyche* spp. settled within 10 m, and started settling from 4 m. Net-spinning caddisflies often trail a silk thread to enhance their settling efficiency (Elliott, 1971; Otto and Sjöström, 1986). The remaining two caddisflies, *Rhyacophila* sp. and *Sericostoma* spp., are both rare in drift, respectively a free-living clinger preferring strong current, with weak swimming abilities, and a case-maker preferring slow currents with sandy deposits, with no swimming abilities. To our knowledge, there are no published data on entrainment distances for Sericostomatidae, but Lancaster *et al*. (2006) record for the limnephilid cased caddisflies *Potamophylax latipennis* in an experimental setting with water velocities similar to those of our flumes, a mean entrainment distance of approximately 9 m. These values are close to those we recorded for the nymphs of *Sericostoma* spp., which, although rare in drift, had all settled after 7 m drifting.

Stoneflies have perhaps been les investigated than mayflies and caddisflies for their swimming and sinking abilities. Elliott (1971) recorded how *Leuctra* spp. cannot make a firm contact with an exposed substrate in turbulent water, and can only land in a zone of low water velocity, with a mean drift distance of approximately 15 m. Lancaster *et al*. (1996) also found that the distance drifted by Leuctridae differed in two streams with different retention properties (*i.e.* physically determined drift distance), but was constant for Nemouridae and therefore drift may be under behavioural control in the nemourids but more physically determined in the leuctrids. In fact, both genera are reported as common in drift, *Leuctra* without swimming abilities, and *Nemoura* with weak swimming abilities (Poff *et al*., 2006). Moreover, in a low-velocity (0.1 to 0.4 m s^{-1}) stream, Leuctridae had higher settling rates values than Nemouridae; the rates were similar in a higher velocity (0.3 to 0.8 m s^{-1}) stream (Lancaster *et al*., 1996). In our flumes, *Leuctra* spp. and *Nemoura* spp. had a high catastrophic drift rate, with higher tendency to drift for the former, which settled after drifting from 4 m on, and especially from 7 to 10 m, whereas the settling abilities of *Nemoura* spp. did not change much with distance, with a higher percentage of nymphs still drifting after 10 m, thus confirming the trend recorded by Lancaster *et al*. (1996). *Isoperla* spp., is common in drift and with weak swimming abilities (Poff *et al*., 2006) but had a low catastrophic drift rate in our flumes; similarly to *Leuctra* sp., this taxon tended to settle after 7 m drifting.

CONCLUSIONS

In this experiment, we simulated a point disturbance represented by a sediment movement that triggered catastrophic drift. In real case scenarios (such as floods, hydropeaking events) the disturbance is, however, diffuse. In previous experiments we conducted in the same flume system (Carolli *et al*., 2012; Bruno *et al*., 2013, 2016), we simulated hydropeaking or thermopeaking waves which elicited a drift response from the benthos occupying the entire flume substrate. Hence, the results of this and our previous studies cannot be compared. Nevertheless, as pointed out by Gibbins *et al*. (2007) field studies aimed at monitoring drift during periods of elevated discharge associated with sediment movement should correctly assess the point from which the drifting animals originate in order to distinguish between hydrological events that represent ecological disturbances (those resulting in catastrophic drift) and those that do not. We have shown, in accordance with numerous other studies, that entrained EPT nymphs travel only short distances before returning to the substratum, and that the actual distance travelled while drifting and the total time spent in drift varies between species.

On a large scale (*i.e.*, stream length or reaches), these taxon-specific factors are important because they define the space/period over which a drifting invertebrate is available, for instance, to drift-feeding fish, or to recolonize previously depleted areas. On a smaller scale (*i.e.*, flume simulations), the study can provide insights on how to extrapolate results obtained in artificial flume systems to support evidences from field studies.

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