

Sampling of the ephemeran community in an intermittent Mediterranean stream by volunteers (The Buèges, southern France)

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In a temporary Mediterranean river, 29 volunteers sampled Ephemeran community. A total of 16 taxa were recorded. List of species and water geochemistry are reported.

The results suggest that species richness of samples was related to the number of microhabitats sampled. On the site studied, rarity of species seemed to be related to the frequency of microhabitats. Species with low abundances colonised microhabitats sampled with low frequencies. A low number of taxa were recorded by each observer. The variability in taxonomic richness of the samples was correlated with the experience of the volunteers. Thus, the experience of collectors should be taken into account when samples from different observers are compared.

Récolte d'éphémères sur un petit cours d'eau méditerranéen intermittent, la Buèges (Hérault, France). Importance du rôle du collecteur.

Mots-clés : Ephémères, macroinvertébrés, assèchement, cours d'eau temporaire, méditerranéen, Hérault, prospection, collecteur bénévole, biais, échantillonnage.

Un groupe de 29 collecteurs bénévoles a échantillonné les communautés d'Ephéméroptères d'un cours d'eau méditerranéen temporaire, la Buèges (Hérault, France). La liste des espèces ainsi que des données physico-chimiques sont présentées. Les résultats suggèrent que le nombre d'espèces recensées est relié au nombre de microhabitats prospectés. L'abondance des espèces semble liée à la fréquence des microhabitats. Les espèces rares sont celles qui colonisent des habitats faiblement prospectés. Globalement, une faible proportion des espèces présentes sur la station a été recensée par chaque bénévole. Le nombre d'espèces détectées augmente avec l'expérience des bénévoles. Aussi, ce critère doit être pris en considération lorsque des relevés provenant de différents observateurs sont comparés.

Introduction

Species richness and species composition at different locations are frequently used in management and conservation to identify population dynamics or to assess natural or anthropogenic effects (WIENS 1996).

If there are too few samples, extraneous variation is high enough to obscure the presence of any long-term trends. The probability of obtaining a statistically significant result, given that there is a real biological effect in the studied population, increases with increasing sample size (THOMAS & KREBS 1997). One important goal of monitoring programs is thus to increase spatial and temporal sampling effort (SKELLY *et al.* 1999, BOWARD 2001). However, there are few studies in which workers have acquired many years of demographic data on species at multiple sites covering a large area (KOENIG 1999).

Volunteer monitoring teams are the key to producing sufficient data to make a difference in decision making (BURTON & DESANTE 1995, USGS & SURVEY 1998). For example, collaborative efforts with government agencies, conservation organisations and volunteer groups in structured aquatic monitoring programmes provides baseline information on the biodiversity of stream fish, amphibians and invertebrates (XERCES 2001). State and local agencies may use volunteer data to screen for water quality problems, identify and monitor streams impacted by poor management and to establish trends in waters quality. However, volunteer recruitment, training, and administration requires substantial co-ordination and support. Nevertheless, the time and financial resources invested in volunteer management is rewarded by the high volume of data collected (DAMBERG & NELSON 1995, WEST 1995).

Although many official reports and scientific publications include volunteer data (MATTSON *et al.* 1994, BROWN *et al.* 2001), many managers and scientists question the quality and reliability of this data source (GREGORY *et al.* 1995, FORE *et al.* 2001). Estimating samples bias is necessary when the data are recorded by a large number of volunteers (WEIDINGER 2001).

Rare species, *i.e.* species that occur at a low frequency in samples, constitute an other important potential bias factor. Introducing or eliminating them in the analysis can lead to significantly different results (CAO *et al.* 1998). Some authors have suggested that rare species must be eliminated because : (i) their presence is accidental and without any obvious link to the considered habitat, and (ii) they generate a very high number of zeros in matrices and so constitute a bias in statistical analysis (NOVOTNY & BASSET 2000). On the other hand, some studies have indicated that rare species represent a important part of community (NOVOTNY & BASSET *ibid*). Moreover, rare species which are usually connected with specific or less impacted habitats have high sensitivity to environmental change and so constitute particularly good indicators of habitats status. However, whether rarely recorded species are the result of specific absence-presence patterns or to artefacts inherent in sampling methods remains unresolved. Their low detection can be due to inherent characteristics (*e.g.* cryptic species), to low local abundance and to a particular geographical distribution (*e.g.* clustered distribution) (LAWTON 1993, GASTON 1996, JOHNSON 1998). The generalist-specialist theory hypothesises that habitat specificity trades off with local abundance (FOX & MORROW 1981, FUTUYUMA & MORENO 1988, REY BENAYAS *et al.* 1999). A species can be restricted to specific habitats and be abundant in this habitat (MAY 1988, SIMONS & NAT 1996). For non-specialised species, if one habitat is sampled by a low number of collectors (so this habitat is sampled with a low frequency), its specialised species will then appear as rare species in the whole set of records (*i.e.* with a low occurrence frequency).

The purpose of this study was to evaluate the precision of data collected by volunteers. We hypothesised that the microhabitats (defined as a three-mensional space whose axes are facies, posi-

tion and substrate) sampled by each observer determined the taxonomic richness of samples and the presence of rare species in the matrices. We also tested whether observer identity significantly influenced detection probabilities of species. The ephemeropteran community was chosen for this study. The macroinvertebrate fauna, particularly many of the Ephemeroptera, are commonly used as bioindicators of water quality because they are extremely sensitive to various types of perturbation and pollution (LANDA & SOLDAN 1991, METCALFE-SMITH 1994, ZAMORA-MUNOZ & ALBA-TERCEDOR 1996, BUFFAGNI 1997).

Study site

The Hérault river is located in the south of France and drains a basin of 2500 km² at its outlet to the Mediterranean Sea. This study was conducted on the Buèges stream, a tributary of the Hérault located to the north of Montpellier. The climate is typically Mediterranean and characterised by a considerable irregularity in rainfall. Precipitation events are often short, but very intensive, inducing flash floods (PALOC 1967). The Buèges stream drains a karst area, composed of limestones dolomites, clays and calcareous marls (PETELET et al. 1998). It is therefore difficult to precisely evaluate the catchment area of the Buèges stream, but this latter has been estimated at Saint-Jean-de-Buèges between 30 and 40 km². Downstream of Saint-Jean-de-Buèges, the river suddenly disappears underground and flows in karstic aquifers (LAURÈS 1947).

This intermittent Mediterranean stream present a high contrast between minimum discharge and flood flow. Estimates of the average total annual discharge and the average monthly dry weather flow range between 25 and 30 L/s and 3 to 4 L/s, respectively. The chemical characteristics of the water are closely related to the drainage lithology. Ca, Mg, HCO₃⁻ concentrations are high (Table 1, PETELET et al. 1998). During the study period conductivity and pH were 0.45 mS/cm and 7.8 respectively. The minimum discharge occurred in August, when no surface flow was observed and it just still ponds remained.

The Buèges valley is an area characterised by a high faunae and florae diversity. Two oak species (*Quercus ilex* and *Q. pubescens*) and ground-box (*Buxus sempervirens*) are dominant. Arid and rocky slopes are also colonised by a typically Mediterranean flora with some rare species (VERNET 1981).

To avoid variation in taxonomic richness across sites (CAO et al. 2002), only one collecting site was chosen. The collecting site was a reach about 200 m long and 5 m wide at base flow, close to the village of Saint-Jean-de-Buèges (Long. 03° 37' 06 ; Lat. 43° 49' 46 ; ED50). This site was selected because of its diverse mayfly community and its accessibility. Maximum water depth at the station was about 1 m. Deciduous trees formed the main riparian vegetation.

Materials and methods

Sampling design

To assess the effect of a previous stream knowledge on the sampling efficacy, we first sampled twelve other sites to determine the ephemeropteran taxa present along the stream and to determine a list of potential microhabitats. We defined microhabitat using a combination of three parameters : habitat type, transverse position, and substrate (Table 2).

Secondly, the site at Saint-Jean-de-Buèges was sampled. To minimise the temporal difference, all sampling was undertaken during a 3 weeks period in June 2000, during which, depth and water temperature did not vary significantly. We sampled the collecting site before the 29 volunteers who had never undertaken any sampling on the river. All volunteers sampled the invertebrates independently and under the same conditions. For each volunteer, three variables were recorded :

<i>Major elements (mmol/l)</i>	
Ca ²⁺	2.62
Mg ²⁺	0.58
Na ⁺	0.46
K ⁺	0.02
Cl ⁻	0.55
SO ₄ ²⁻	0.31
NO ₃ ⁻	0.02
HCO ₃ ⁻	4.47
<i>Trace elements (nmol/l)</i>	
Rb	6
Sr	1235
Ba	66
Pb	0.1
U	1.2
Sum of dissolved cations (meq/l)	6.88
Sum of dissolved anions (meq/l)	5.67
PH	7.64
Conductivity (mS/cm)	0.485

Table 1. Chemistry of the Buèges river. Sampling was done by PETELET et al. in March 1995 during low flow.
Tableau 1. Chimie de la rivière Buèges. Prélèvement effectué par PETELET et al. en mars 1995 par basses eaux.

Facies	1-cascade; 2-riffle (depth inf. 30cm); 3-pool (depth 30-80 cm); 4-profundal zone (sup. 80cm); 5-adjacent ponds (not directly reliated with stream channel)
Position	1-littoral zone (inf. 50cm du bord); 2-channel (sup. 50cm du bord)
Substrate	1-xylal (trunks, dead wood, branches...); 2-coarse to fine gravel (sup 2mm); 3-sand (2mm-6microm); 4-fine substrat (silt, loam, clay inf. 6mm); 5-emergent macrophyte; 6-submerged macrophyte

Table 2. Microhabitat typology. The microhabitat noted «223» is constituted by sand in channel in a pool zone.
Tableau 2. Typologie des microhabitats. Le microhabitat désigné «223» est constitué par du sable dans une zone stagnante.

- the sampling time, limited to a maximum of 45 minutes,
- the total number of sampled microhabitats,
- the total number of techniques used.

Five techniques were identified :

- disturbing and removing submerged macrophytes,
- disturbing and removing logs and snags,
- rubbing large stones by hand to dislodge clinging organisms,
- disturbing the substrate upstream of a net,
- filtering fine substrate with a handnet.

Volunteers were asked about the habitats they selected. A categorical variable «strategy» with four modalities was defined (1- random sampling ; 2- more sampling on habitats where more larvae were found ; 3- sampling across the whole set of habitats identified by the collector ; 4- sampling in a particular habitat with assumed high species richness). We assigned the «quality» of volunteers into two groups according to their experience in collecting biota. One group comprised volunteers without experience, and the second group volunteers with at least one experience in a previous study. After each collection, we checked that species not recorded by collectors were actually present at the site. Macroinvertebrates were sampled using a circular hand net (30 cm diameter, 0.5 mm mesh). Collected mayflies were fixed and stored in 80 % ethanol. Larvae were identified to species in the laboratory, except for *Rhithrogena*, *Epeorus* and *Habrophlebia* which were identified to genus (the two first taxa because of their taxonomic complexity and the third one because only one individual was recorded). To reduce the bias linked to identification, each sample was identified by the two authors, twice. When there was a discrepancy, a third person (Brulin of the French inventory of Mayflies-) made a third identification. We then calculated the total number of taxa per sample.

Statistical analyses

We considered the presence or absence of taxa in 29 samples. We excluded our own sample in the statistical analysis.

Species richness

Counts tended to follow a Poisson-like distribution in which the variance was proportional to the mean, so a log-linear model analysis (GENMOD, SAS Institute) was used to determine the influence of the number of studied microhabitats and the number of collecting strategies used by each collector on species richness. Volunteer's quality, which showed a high correlation with the variable «number of studied microhabitats» was removed from the model to avoid multicollinearity and examined separately. The logarithm of the variable «collecting time for each collect» was included in the model as an offset to insure sampling effort did not induce bias in the results. Independent variables in models included these main effects and the interaction between them. Significance was determined using likelihood-ratio F-statistics, assuming a Poisson distribution (type 3 options) with corrections for possible overdispersion. The backward elimination procedure was used to delete unimportant variables, one at a time. Starting from the full model, we began to eliminate the two-way interactions (AGRESTI 1996). A variable was removed from the model when the F-statistic was not significant. At each step, the AIC criterion (AKAIKE 1983) was calculated to retain the best model (SHTATLAND et al. 2000).

«Rare» species

We investigated the relation between the rarity of species and the rarity of the microhabitats. We defined «rare» species and «rare» microhabitats as species and microhabitats with low observed frequency in matrices.

We calculated the new variable F_s by weighting each sample as expressed as follow :

$$F_{sj} = \sum (H_{ij} / f_{s_i})$$

with : F_j = weighting of the sample j , $H_{ij} = 1$ if the species i is present in the sample j else $H_{ij} = 0$, f_{s_i} = frequency of the species j in the whole set of samples.

In the same way, the samples were weighted by the observed frequency of the microhabitat to defined the new variable, F_m :

$$F_{mj} = \sum (H_{ij} / f_{m_i})$$

with : F_{mj} = weighting of the sample j , $H_{ij} = 1$ if the microhabitat i is found in the sample j else $H_{ij} = 0$, f_{m_i} = frequency of the microhabitat i in the whole set of samples.

To estimate the degrees of association between F_s and F_m , we calculated a Pearson correlation coefficient.

Volunteers «quality»

We investigated if there was a relation between collectors quality and the number of recorded species with a one-way ANOVA. Because the data were not normally distributed, the signification level of R and F statistics was estimated by a randomisation procedure (MANLY 1997).

Results

Sampling

A total of 16 taxa were recorded at the site (Appendix 1). The Rank-frequency diagram of taxa sampled (Figure 1) showed that three abundant species were found frequently (*Serratella ignita*, *Baetis rhodani*, *Habroleptoides confusa*). In contrast, 62 % of taxa were found in less than 50 % of the samples and four taxa (*Ephemera danica*, *Electrogena* sp., *Cloeon dipterum*, *Habrophlebia* sp.) were found by less than three observers.

Species richness sample was highly variable between volunteers. The number of taxa recorded by each volunteers varied between 2 to 13. The average number of inventoried taxa was 6 ($\mu = 6.6$; $SE = 0.45$) whereas the number of taxa in the control sample was 16. The total number of taxa recorded increased with the number of samples. For each value of number of samples (x), 5000 sub-samples of x samples were randomly selected and the mean of taxonomic richness were calculated. As suggested by Ferry & Frochot (FERRY & FROCHOT 1970), the cumulative total of taxa recorded was plotted against sampling effort (Figure 2). The number of taxa increased rapidly, but new taxa continued to be added with increased sampling effort.

Species richness

After removing non-significant interactions and main effect terms, the final model contained only one predictor of species richness, the number of sampled microhabitats (parameter estimate = 0.02 ± 0.006 , $F = 10.53$, $df = 27$, $P = 0.0031$; Figure 3). The value of deviance (28.4) divided by the degrees of freedom was close to 1 and examination of deviance residuals plotted against predicted values did not reveal any systematic departures, suggesting a good model fit. There was no consistent influence on the strategy used by each collector on sample taxonomic richness. The

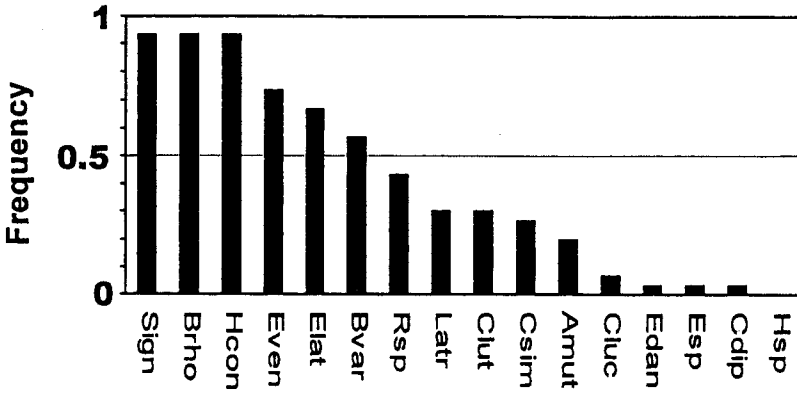


Fig. 1. Rank-frequency diagram of taxa sampled.

Fig. 1. Diagramme rang-fréquence des taxons prélevés.

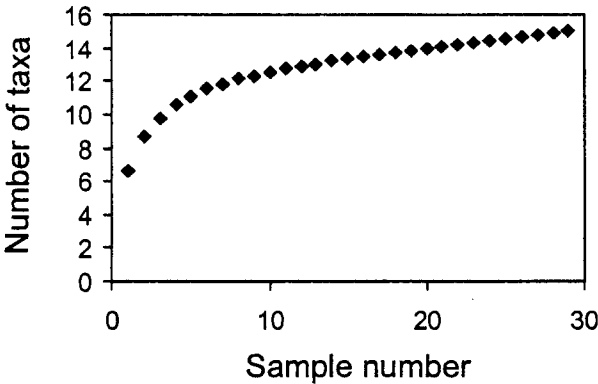


Fig. 2. Cumulative taxonomic richness curve. The graph shows the cumulative total of the number of taxa collected as a function of the number of samples taken into account.

Fig. 2. Courbe de richesse taxonomique cumulée. Le graphique montre le total cumulé du nombre de taxons récoltés en fonction du nombre de relevés pris en compte.

strategy parameter did not show any significant correlation with the number of sampled habitats (Spearman correlation : $R_{obs} = 0.4$; NS)

«Rare» species

The presence of species with low observed frequencies in samples was associated with habitats sampled by a low number of volunteers. The two variables, F_s and F_m , showed a high positive correlation. None of the Pearson correlation coefficients (R_{rnd}) computed on the 5000 data sets created by resampling was greater than the correlation coefficient (R_{obs}) computed from the real data sets (Pearson correlation : $R_{obs} = 0.74$; $R_{rnd} > R_{obs} = 0$; $R_{rnd} < R_{obs} = 5000$). This demonstrated that this observed correlation coefficient was statistically highly significant.

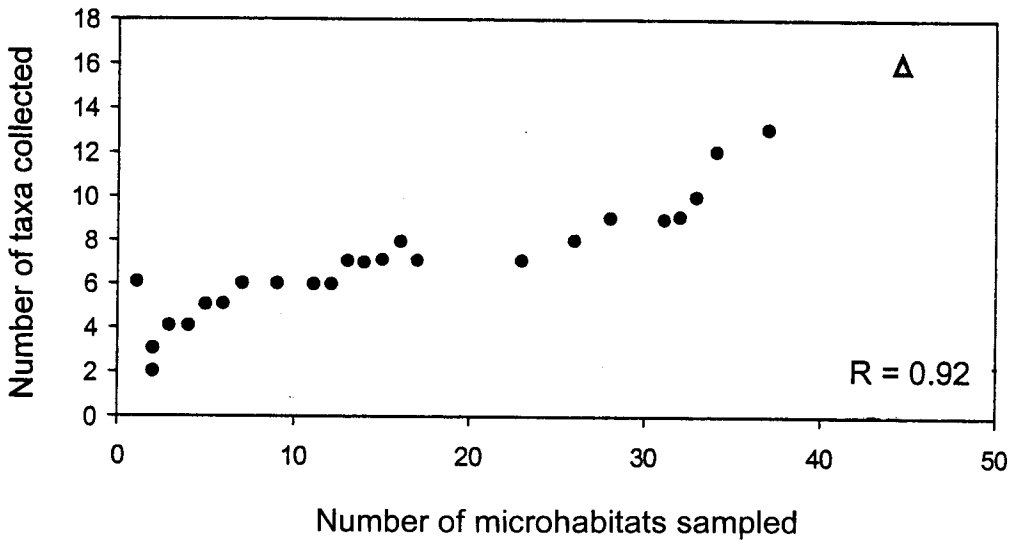


Fig. 3. Positive correlation between the number of taxa collected and the number of microhabitat prospected. Pearson correlation coefficient was calculated on samples realized by volunteers (black circle). Our own sample is plotted on the graph (triangle).

Fig. 3. Corrélation positive entre le nombre de taxons récoltés et le nombre de microhabitats prospectés. Le coefficient de corrélation de Pearson a été calculé sur des prélèvements réalisés par des volontaires (cercles noirs). Notre propre prélèvement est représenté sur le graphique (triangle).

Volunteer quality

The number of detected species from collectors (we have already participated in other faunae or florae inventories) was significantly greater than the number of species sampled by other collectors. The value of the F statistic calculated from our data was never reached by any of the F values calculated from the resampling generated data sets (ANOVA : $F_{\text{obs}} = 26.90$; $F < F_{\text{obs}} = 0$; $F < F_{\text{obs}} = 5000$).

Discussion

Ephemeroptera communities

In low flow Mediterranean streams, drought leads to reduction of water flow and habitat quality. As habitat diminishes, predation by terrestrial animals may increase and cause a decrease of aquatic biota (SIH et al. 1985, SEMLITSCH 2000). However, our results do not support the negative pattern observed in other studies of the low flow effect on benthic macroinvertebrates (JACKSON 1997). In the present study the ephemeropteran community was highly diversified with 16 taxa collected. Spatial and temporal variability in drying patterns clearly maintain a dynamic and diversified mosaic of habitats (POFF et al. 1997).

The biota of temporary waters possesses various strategies to survive drying (BOULTON et al. 1992). And at the catchment level, reproductive surplus from productive habitats may maintain po-

pulations in sink habitats (PULLIAM 1988). Ephemeroptera are common members of the drift (ELLIOTT 1967). At local levels, stream biota possesses an array of survival mechanisms to deal with drought (LAKE 2000). In August a sediment core was taken where no surface flow was observed. Larvae of *Habroleptoides confusa* and *Serratella ignita* were observed in damp sediments, just beneath the dry surface substrate. Temporal streams submitted to severe drying periods may indeed support an abundant and diverse aquatic fauna (BOULTON et al. 1992). Higher levels of disturbance can however alter such patterns and increased water abstraction can reduce flow and exacerbate droughts. Especially if critical thresholds are exceeded (WITH & CRIST 1995).

Sampling

The 29 volunteers collected a total of 15 of the 16 taxa recorded at the station. 62 % of the taxa were found in less than 50 % of the whole set of samples. None of the volunteers added any new species compared to our own control sample. The cumulative total of taxa recorded with increased sampling effort, did not reach a plateau although our results suggested that taxonomic richness was highly dependent on sampling effort (LI et al. 2001). Comparisons between sites or streams consequently must thus be normalised for sampling effort.

Species richness

At the volunteer level, species richness increased significantly with the number of sampled microhabitats. Streams are frequently characterised by high habitat diversity. As in other taxa, ephemeropteran species are mostly associated with specific environmental conditions and nymphs show a high morphological and ecological differentiation (SOWA 1975). Nymphs of varying size can occupy different substratum types and interstitial spaces of different dimensions (SHELDON 1969, BUFFAGNI et al. 1995). During periods of flow variation, the spatial niche of species may also change. During the short study period flow was more or less constant and taxa were always found in the same microhabitats. Surprisingly, there was no relation between the number of microhabitats sampled and the sampling strategy used by volunteers. Volunteers who tried to maximise the number of sampled habitats did not sample more microhabitats than those who used other strategies. We suggest that the former distinguished different habitats on non-pertinent criteria and thus did not develop a correct microhabitat typology. As suggested by FORE et al. (2001), selection of volunteers must be undertaken before any inventory study.

Our results suggested that a total list of the species present at the station requires sampling all potential microhabitats, even if some of them are poorly represented compared with others. For the purpose of comparisons between stations, the habitat complexity should be taken into account because species located at the station, as well as the sampling variance, may differ depending on the landscape structure (BOULINIER et al. 1998). Furthermore this study highlights the importance of sampling appropriate substrates (MCKIE & CRANSTON 2001) implying the need to use various collection techniques appropriate to each microhabitat. Some species are restricted in specific microhabitats and their absence in samples may be due to inefficient collecting methods (ELLIOTT 1967). For example, the use of samplers for particular microhabitats like the Hester-Dendy samplers do not sample the entire mayfly fauna (MASON et al. 1973, HUBBARD & SWADLING 1995). Their use is justified only if the comparison of a part of benthic macroinvertebrate populations is required. The use of many techniques (e.g. hand-net, artificial substrates, driftnets, traps) could in many cases give better results than one of these methods alone (BATTEGAZZORE et al. 1994). Thus, macroinvertebrate sampling should be optimised using a flexing strategy depending on the study objectives (STATZNER et al. 1998).

«Rare» species

Species with low detection probability represent the major part of the recorded taxa. Except for *Caenis*, which may have a low detectability because of its small size, the low abundances can be explained by a low spatial density at the station level and/or by the fact that they are spatially localised. Our results confort the latter hypothesis. Rare species are well correlated with rare habitats and some observations revealed that larvae were generally numerous in their respective microhabitats.

The frequency of a microhabitat determined the frequency of the species associated with a particular microhabitat. Drift may lead to a redistribution of individuals (NOVOTNY & BASSET 2000), but the same species was found in the same microhabitats throughout the study period. This may be linked to the low flow rates during the study.

Volunteer quality

The averaged number of taxa found by volunteers was 6, 37 % of the total number of taxa being recorded at the study site. However, there were major differences between collectors. Their experience was well correlated with taxonomic richness of collections. This suggested that taking into account experience of collectors is necessary when samples taken by different observers are compared.

In field studies, collector identity and experience have been recognised as influencing detection probability of species (SAUER et al. 1994, KENDALL et al. 1996), (NICHOLS et al. 2000). It is possible to weight each collection in relation to volunteer experience.

Conclusion

This study showed a great variability between collectors. Comparing samples from different observers could thus lead to an overestimate of natural variability between samples and cause erroneous conclusions. This bias could be reduced by producing an appropriate microhabitat typology and then by sampling the whole set. It is also possible to weight each sample by the observer experience.

However, the samples collected by volunteers, when pooled together, can produce regional taxonomic lists. Samples obtained from local studies can be compared with the regional list of species to assess the quality of samples, to determine the detection probability of species, and to avoid other identified bias.

The large volume of data, that must be collected for such regional lists, requires the use of volunteers.

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Appendix I. List of species recorded at the study site.

Annexe I. Liste des espèces recensées à la station d'étude.

Amut	<i>Alainites muticus</i> (Linné, 1758)
Brho	<i>Baetis rhodani</i> (Pictet, 1843-45)
Bvar	<i>Baetis vardarensis</i> Ikonomov, 1962
Cdip	<i>Cloeon dipterum</i> (Linné, 1761)
Cluc	<i>Caenis luctuosa</i> (Burmeister, 1839)
Clut	<i>Centroptilum luteolum</i> (Müller, 1776)
Csim	<i>Cloeon simile</i> Eaton, 1870
Edan	<i>Ephemera danica</i> Müller, 1764
Elat	<i>Electrogena lateralis</i> (Curtis, 1834)
Esp	<i>Epeorus</i> sp.
Even	<i>Ecdyonurus venosus</i> (Fabricius, 1775)
Hcon	<i>Habroleptoides confusa</i> Sartori & Jacob, 1986
Hsp	<i>Habrophlebia</i> sp.
Latr	<i>Labiobaetis atrebatinus</i> (Eaton, 1870)
Rsp	<i>Rhithrogena</i> sp.
Sign	<i>Serratella ignita</i> (Poda, 1761)