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Reduction of sampling effort assessing macroinvertebrate assemblages for biomonitoring of rivers

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ABSTRACT

Key-words:
stream invertebrates, effort reduction, biomonitoring

Biomonitoring methods based on macroinvertebrate assemblages are widely developed in streams and rivers. However, the use of invertebrates has been criticized due to the long time and expense of processing samples. Therefore, we evaluated the effectiveness of reducing the sampling effort from 20 to 5 samples to assess the stream macroinvertebrate community. In six streams in the Basque Country (North of Spain) 20 kick nets were collected following a multihabitat stratified sampling design. The macroinvertebrates were identified to family level and a smoothed family accumulation curve fitting the Clench function to the data was calculated for each stream. Richness was lower in 5 than in 20 samples. However, in general, the percentage of richness estimated with the subsampling may be considered representative of the existing taxa richness. Therefore, the study of five samples may be adequate for biomonitoring Basque streams, greatly minimizing time, effort and costs.

RÉSUMÉ

Réduction de l'effort d'échantillonnage pour l'évaluation des assemblages de macroinvertébrés pour la biosurveillance des rivières

Mots-clés :
invertébrés, cours d'eau, réduction de l'effort, biosurveillance

Les méthodes de biosurveillance basées sur les assemblages de macroinvertébrés sont largement développées dans les ruisseaux et rivières. Cependant, l'utilisation d'invertébrés a été critiquée en raison du long temps de collecte et du coût de traitement des échantillons. Par conséquent, nous avons évalué l'efficacité de la réduction de l'effort d'échantillonnage de 20 à 5 échantillons pour évaluer la communauté des macroinvertébrés d'une rivière. En six rivières du Pays Basque (nord de l'Espagne) 20 filets à main Kicker ont été recueillis suivant un plan d'échantillonnage stratifié multihabitat. Les macroinvertébrés ont été identifiés au niveau de la famille et une courbe de cumul de famille lissée de la fonction de Clench ajustée aux données a été calculée pour chaque rivière. La richesse était plus faible dans 5 échantillons que dans 20. Cependant, en général, le pourcentage de la richesse estimée avec le sous-échantillonnage peut être considéré comme représentatif de la richesse des taxons existants. Par conséquent, l'étude de cinq échantillons peut être adéquate pour la biosurveillance des rivières basques, minimisant considérablement les efforts de temps et les coûts.

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INTRODUCTION

Streams and rivers are among the most threatened habitats in the world (Malmqvist and Rundle, 2002). Since they provide important ecosystem services (Thorp *et al.*, 2010), it is crucial to understand the consequences of human perturbations on these ecosystems to preserve or restore their integrity (Meybeck, 2003). Therefore, assessment and biomonitoring programs are carried out widely by public authorities. In river biomonitoring, the aquatic macroinvertebrates are the most commonly studied group (Bonada *et al.*, 2006) since they are sensitive to multiple ecological alterations (Johnson and Ringler, 2014). In particular, taxa richness has been widely used because it is a key measurement to assess the structure of biological assemblages (Gotelli and Cowell, 2001). However, the use of invertebrates has been criticized due to the long time and expensive costs of sampling, sorting, counting and identifying them (Ciborowski, 1991). Therefore, since the effectiveness of biomonitoring protocols depends mainly on the time required and on the overall costs, techniques that optimize the cost-benefit have been developed (Marini *et al*., 2013; Pinna *et al.*, 2013, 2014). Nevertheless, caution is needed since the technique and the way in which samples are collected and processed may influence the description of the studied community (Boonsoong *et al.*, 2009; Di Sabatino *et al.*, 2014). In fact, reducing effort and costs is not the only aim of subsampling methods, but also of paramount concern is the need to gain information not substantially biased by the procedure and capable of answering research questions (Barbour and Gerritsen 1996).

In Spain, as in other states in Europe, the methodology focused on benthic macroinvertebrates established by the Water Framework Directive, WFD (EU, 2000), for river biomonitoring is multihabitat stratified sampling (Barbour *et al.*, 1999; AQEM, 2002). However, the large number of kick samples required in each stream increases the time, effort and costs, making the application of this approach in programs with a great number of monitored systems nearly impossible. Therefore, the goal of this work is to test if a significant reduction in the sampling effort from 20 to 5 samples, that would minimize time, effort and costs, allows for the collection of representative information about the richness of macroinvertebrate communities for biomonitoring.

MATERIALS AND METHODS

> *STUDY SITE*

The study was conducted in six streams in the Basque Country (North of Spain) flowing into the Atlantic Ocean. The study sites differ in the basin area, but not in the water physicochemical characteristics. The main land uses of the catchments are native vegetation, conifer plantations and farming, percentages varying among sites (Table I). The climate in this region is oceanic, with cool winters (mean winter temperature around 9 °C) and warm summers (mean summer temperature around 21 °C), and with a mean annual rainfall of 1000–1200 mm, evenly distributed throughout the year.

> *FIELD PROCEDURES*

Benthic macroinvertebrates were sampled in late spring 2012 in three of the six streams (S1, S2, S3) and in late spring 2013 in the other three (S4, S5, S6). At each site, multihabitat, stratified and semiquantitative sampling was carried out, collecting 20 kick nets (25×20.5 cm; 500 μm) within a 100-m-long reach (Barbour *et al.*, 1999; AQEM, 2002). With each kick an estimated stream bottom area of 0.05 m^2 (semiquantitative) was sampled. The habitats that represented $\geqslant 5\%$ of the total surface were sampled (multihabitat). The number of samples taken in each habitat depended on the percentage that each habitat represented of the total study reach surface (stratified). The habitats considered included rocky substrates, cover

Table I
Location, water physicochemical characteristics and basin land-use percentages of the studied streams.

	S1	S2	S3	S4	S5	S6
Watershed name	Barbadun	Arratia	Altube	Oria	Altzolaratz	Urumea
UTMX	488 696	518 568	504 944	561 781	564 110	587 359
UTMY	4 790 874	4 783 160	4 776 385	4 763 504	4 788 455	4 786 268
Basin (km^2)	47.9	137.7	192.9	59.8	21.9	156.7
Water temperature (°C)	14.3	14.3	14.5	13.8	14.3	13.2
pH	7.8	7.9	8.1	8.0	7.9	8.1
Conductivity ($\mu S \cdot cm^{-1}$)	363.7	397.4	379.3	577.6	310.9	60.4
Total nitrogen ($mg \cdot L^{-1}$)	1.97	2.26	1.39	1.61	2.07	1.00
Total phosphorous ($mg \cdot L^{-1}$)	0.09	0.26	0.21	0.07	0.04	0.04
O_2 saturation (%)	86.7	91.1	92.9	94.3	93.1	111.9
Land use (%)						
Native vegetation	36.9	24.1	50.8	46.2	55.8	77.0
Conifer plantations	20.9	57.5	31.9	29.6	10.6	18.4
Farming	40.2	17.4	16.1	23.4	33.3	4.6
Rocky	1.9	0.3	0.7	0.6	0	0
Urban	0	0.7	0.4	0.4	0.2	0

vegetation (aquatic bryophytes and algae), macrophytic vegetation (emerged, floating and submerged macrophytes) and riparian vegetation (roots, vegetation over the channel and vegetal detritus).

> LABORATORY PROCEDURES

The macroinvertebrates were preserved in 4% formaldehyde for subsequent processing. In the laboratory, each sample was divided into multiple subsamples. The fauna was studied in a subsample, identifying the individuals to family level (Oligochaeta and Ostracoda to class) following Tachet *et al.* (2002). The size of the analyzed subsample depended on the sample size (following AQEM, 2002). The total number of individuals in each sample was estimated by multiplying the number of each taxon by the inverse of the portion. From the portion of the sample not analyzed, taxa previously not encountered were extracted *a visu*.

> COST CALCULATIONS

The cost of 20 and 5 samples' collection and processing was calculated both in terms of time and money, considering a price of 12 € per hour spent doing the field and laboratory work.

> STATISTICAL ANALYSIS

For each study site, a smoothed family accumulation curve was calculated using EstimateS (100 randomizations; Colwell, 2000). This program computes sample-based rarefaction curves for family richness estimation, presenting the mean number of random sample re-orderings, and thus removing the possible effects due to the order by which the samples have been listed. Using STATISTICA, we fitted a Clench function to the data of each study site to assess the precision of our estimates of family richness. The equation of the Clench function is $S_n = an/(1 + bn)$, where S_n is the observed family number for a given number of samples, n is the number of samples, a is the new families' increase rate in the first stages and b is a parameter related to the curve form.

Table II
Values of the Clench function fitted to family accumulation curves for each stream and the representativeness of the estimated richness of the richness percentage collected with 20 and five kick samples.

Stream	R^2	*a*	*b*	Final stage slope	% taxa richness collected	
					20 kicks	5 kicks
S1	0.97	37.34	0.60	0.12	96.0	75.0
S2	0.98	15.88	0.35	0.24	91.5	64.0
S3	0.97	18.65	0.41	0.21	93.4	67.5
S4	0.98	24.15	0.75	0.09	96.5	79.0
S5	0.96	37.87	0.72	0.15	96.0	78.2
S6	0.99	42.41	0.99	0.01	97.0	83.3

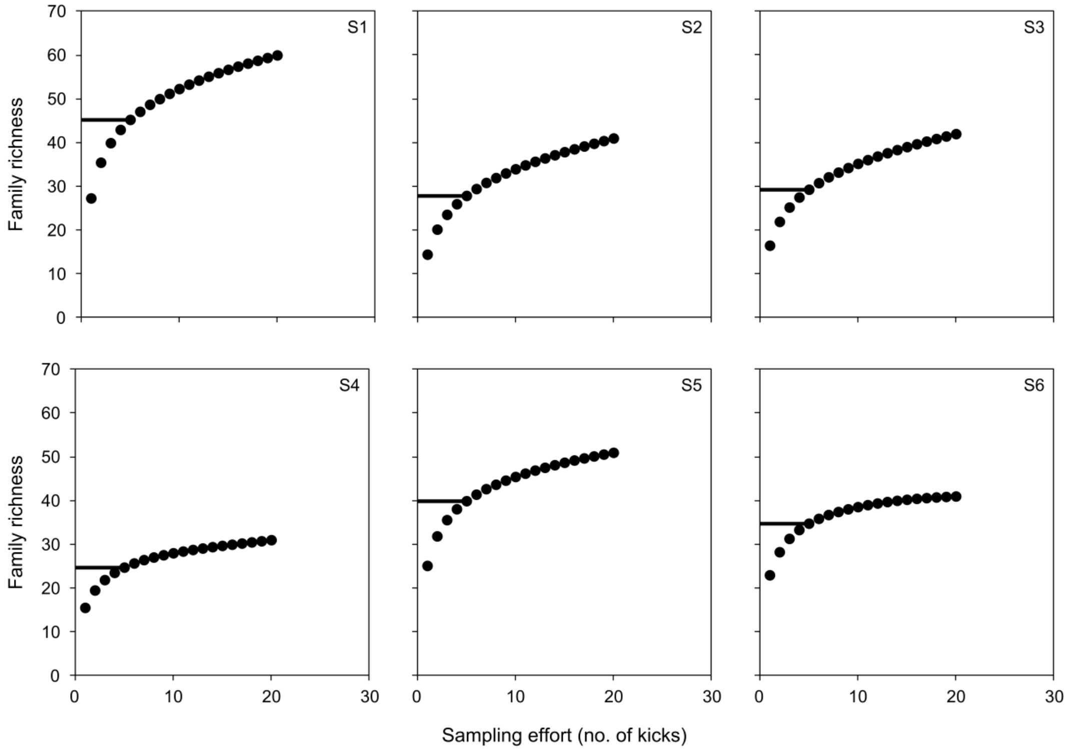

Figure 1
Family accumulation curves for each stream. Black bars indicate the estimated family richness, taking into account five samples.

RESULTS

The Clench function estimate of the number of families based on 20 samples represented from 91.5% (S2) to 97.0% (S6) of the total estimated richness (Table II). In comparison, the number of families estimated by collecting just five samples was lower (Figure 1), representing from 64.0% in S2 to 83.3% in S6 of the total estimated richness (Table II).

From the practical viewpoint, the collection and processing of 5 samples is 3.3 times less time-consuming and cheaper than for 20 samples (Table III).

DISCUSSION

Estimating lower taxa richness is common when attempting to minimize sampling efforts, either when subsampling within the same technique or using simpler techniques. In this study, reducing the sampling effort from 20 to 5 samples led to estimating lower taxa richness, but the diminution was not significant. According to Moreno and Halffter (2000), the inventory of a

Table III
Cost in terms of time and money required to collect and identify macroinvertebrate assemblages with 5 and 20 samples.

	Person number	5 samples	20 samples
Habitat identification (h)	2	0.33	1.00
Sample collection (h)	2	0.42	0.75
Sample processing (h)	1	2.00	8.00
Total time (h·person^{-1})		3.50	11.50
Total price (12 € ·h^{-1}·person^{-1})		42	138

particular group is considered representative of its existing species richness when the number of sampled species reaches at least 70% of the estimated number of species present. In this study, four of the six cases were over this threshold, and in the other two (S2 and S3) the values were very close. Additionally, in these two streams, the percentages collected from the estimated maximum family richness value with 20 samples were also lower than those in the other four streams, the richness value not reaching the asymptote of the curve. These lower values were attributed to the river bed substratum and not to differences in water physicochemical properties, basin size or land uses. In fact, the river bed was covered by boulders and bedrock in these two streams, which complicated the collection of samples with the kick net.

Moreover, despite losing taxa richness, it has been shown that subsampling within the same sampling technique allows obtaining a representative view of the structure of benthic macroinvertebrate communities and that the differences among systems remain, their application being acceptable in biomonitoring (Marini *et al.*, 2013; Pinna *et al.*, 2013, 2014). In contrast, using different techniques to minimize effort can lead to bias in the information on the structure of biological communities (Di Sabatino *et al.*, 2014).

In conclusion, the reduction of the sampling effort from 20 to 5 samples may provide a representative view of the macroinvertebrate community composition in these streams. Therefore, the collection of five samples may be sufficient for biomonitoring Basque streams, allowing savings on time, human effort and costs. These savings, simplifying the spatial sampling effort, would provide greater temporal effort with a similar cost estimate. Thus, managers and policy-makers could carry out more exhaustive monitoring in systems where steps to improve the ecological status are being applied.

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