Hydrobiologia 157: 69–75 (1988)

A method for subsampling unsorted benthic macroinvertebrates by weight

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Received 30 July 1986; accepted 6 February 1987

Key words: benthos, filamentous algae, dispersion, variability, drift samples, artificial substrates

Abstract

A simple method for subsampling unsorted benthic macroinvertebrates by weight is described for different types of samples obtained from lentic and lotic environments. It is especially useful for samples containing large amounts of filamentous algae that preclude the use of conventional subsampling methods. The method provided random dispersions of individuals in the original samples. Overall variability of the subsamples was low for artificial substrate and catastrophic drift samples. Variability was higher for regular drift samples, which had the lowest numbers of individuals of the three sample types. The method produced considerable savings in time spent sorting. Subsampling approaches for community level studies are discussed.

Introduction

A number of strategies are used to reduce the time and labor required to process samples of benthic macroinvertebrates (Resh *et al.,* 1985). One such strategy is subsampling (e.g. Hickley, 1975; Rosenberg, 1975). The basis of the many subsampling techniques available is volumetric, whereas weight has been virtually ignored (except see Van Ark & Pretorius, 1970, for light-trap catches of insects). It may be necessary to use weight as a basis for subsampling when working with samples containing large amounts of filamentous algae. The macroinvertebrates in such samples usually are entangled, making volumetric subsampling, as well as other processing methods such as elutriation or flotation, difficult to do.

A number of methods for processing samples of macroinvertebrates in algae have been reported. Klattenberg (1975) described a plexiglass box for subsampling *Cladophora* containing benthic macroinvertebrates, but he provided no data for which estimates of variability could be calculated. The vegetation elutriator of Cross & Minns (1969, p. 316) also was used on '...samples containing a high proportion of filamentous algae', but its efficiency was reduced. They reported '...good results... with a considerable time saving over hand-sorting provided the vegetation was teased apart...' and processed in small (20 g) batches. Once again, no data were presented to substantiate these claims. Pollard & Melancon (1984) developed an efficient field-washing method for recovery of benthic macroinvertebrates from algal mats and watercress, but their method requires live samples. Obviously, in some field situations, samples cannot be processed immediately, so they must be preserved and sorted later.

Preserved samples containing masses of filamentous algae usually have to be handpicked in their entirety, a long and tedious undertaking which taxes the resources of most benthic research programs.

The purpose of this paper is to describe a simple yet effective method for subsampling benthic macroinvertebrates by weight before sorting. Although primarily intended for samples containing filamentous algae, the method is also useful for samples that are free of extraneous debris. In addition, the paper considers approaches for dealing with the subsampling of rare species in community level studies.

Methods

Sample types

Tests were conducted on artificial substrate samples that had been colonized along newly flooded shorelines in the Southern Indian Lake reservoir, Manitoba (Resh *et al.,* 1983; Wiens & Rosenberg, 1984; and unpublished data), and on drift samples collected from an experimental methoxychlor addition to the Souris River, Manitoba (Sebastien, 1986). The artificial substrates were chicken barbeque baskets filled with freshly cut sticks of three species of tree commonly found along the shoreline of Southern Indian Lake (*Alnus rugosa* (DuRoi) Spreng., *Picea mariana* (Mill.) B.S.P., *Salix* spp.). A total of three basket samples was used in this test.

Drift samples of 4 h duration were collected from the Souris River using a Burton-Flannagan bomb sampler (Burton & Flannagan, 1976) equipped with a 500 μm mesh net. Two types of drift samples were used here: those taken as part of regular, diurnal monitoring (three sets of three samples each, comprising a total of nine samples), and those measuring catastrophic drift in response to the methoxychlor addition. Only one catastrophic drift sample was used in this test because of the extremely high numbers of invertebrates that were captured.

The artificial substrate and regular drift samples contained large amounts of filamentous algae, whereas the catastrophic drift sample was relatively free of extraneous debris. Samples used in the tests were selected arbitrarily from all those collected in the course of the two studies.

Sample treatment

Individual artificial substrate and regular drift samples were thoroughly mixed in a 1 or 2 l beaker, to ensure a random distribution of the invertebrates, by stirring with a glass rod. Samples were then poured onto a pre-weighed sieve (U.S. Standard no. 70 mesh, 212 μm, ID 20.3 cm) and allowed to stand for 15–20 min until excessive preservative had drained off. The moist sample was stirred again while on the sieve, and was then weighed on a electronic pan balance to the nearest 0.1 g. Four approximately equal amounts by weight were removed at random. Thus, each portion comprised $\simeq 25\%$ by weight of the total sample. Total weights of artificial substrate samples varied from 8–41 g, whereas those of regular drift samples varied from 18–99 g.

The invertebrates in each quarter were sorted, identified, and enumerated. Organisms in the artificial substrate samples were identified as Chironomidae and other invertebrates, whereas organisms in the regular drift samples were identified to species whenever possible.

The catastrophic drift sample was treated in the same manner as the artificial substrate and regular drift samples. However, because of the high number of invertebrates involved, 10 subsamples, each only $\simeq 1\%$ by weight of the total sample, were removed. Thus, a total of $\simeq 10\%$ by weight of the original sample was subsampled. These subsamples as well as the remainder of the sample were sorted, and organisms were identified to species whenever possible.

Statistical treatment

Dispersion

In order to estimate the total number of organisms in each sample from subsample counts, it was first necessary to establish that the organisms were dispersed randomly in the original sample. The index of dispersion (I), which is based on the variance to mean ratio of the subsample and tests for conformity to a Poisson series (Elliott, 1977; Wrona *et al.,* 1982), was calculated for all samples. This index approximates a χ^2 distribution, allowing the following relationship to be used (Elliott, 1977):

$$I(n-1) \simeq \chi^2{}_{df=(n-1)} = \frac{s^2(n-1)}{\bar{x}} \text{ when } n<31$$

where
n = number of subsampling units taken
s^2 = sample variance
$\bar{x}$ = sample mean
df = degrees of freedom

Agreement to a Poisson series was checked by examining whether the calculated χ^2 approximation value occurred between the 0.975 and 0.025 probability levels of the χ^2 distribution (Elliott, 1977; Snedecor & Cochran, 1980).

Variability

Variability of the subsamples was measured by the coefficient of variation (Elliott, 1977):

$$CV = s\left(\frac{100}{\bar{x}}\right)$$

where
CV = coefficient of variation
s = sample standard deviation
$\bar{x}$ = mean number in the sample

Results and discussion

Dispersion

χ^2 values calculated for numbers of benthic macroinvertebrates in subsamples of the three artificial substrate samples used, indicated that Chironomidae and total numbers of invertebrates were randomly dispersed in the samples prior to subsampling (Table 1). Chironomidae were emphasized because they comprised >80% of total numbers in these samples, and were the taxon of greatest interest in the Southern Indian Lake study.

Chironomidae were also abundant in the drift samples (regular: ≃ 25–70% of total numbers; catastrophic: 11.5% of total numbers), but the Ephemeroptera, Trichoptera, and Amphipoda were emphasized because they were the taxa of interest in the Souris River methoxychlor-addition study. χ^2 values calculated for one of the three sets of regular drift samples are shown in Table 2. In only two instances (*Hyalella azteca* (Saussure), replicate 3; others, replicate 1) were the dispersions significantly different from random ($p \le 0.05$). In fact, the dispersions were uniform in these two instances. Most of the dispersions from the other two sets of samples also were random, and a summary of the results from all three sets (9 replicates) is shown in Table 3.

Table 1. Subsample counts for Chironomidae and total invertebrates in artificial substrate samples from Southern Indian Lake, Manitoba.

Species of tree used in artificial substrate	Subsample				Calculated value of χ^2	CV (%)
	A	B	C	D		
A: Alder (*Alnus rugosa* (DuRoi) Spreng.)						
Numbers of Chironomidae	766	731	731	785	2.87	3.6
Total numbers of invertebrates	917	878	897	924	1.43	2.3
B: Spruce (*Picea mariana* (Mill.) B.S.P.)						
Numbers of Chironomidae	983	997	942	1010	2.65	3.0
Total numbers of invertebrates	1161	1156	1121	1197	2.50	2.7
C: Willow (*Salix* spp.)						
Numbers of Chironomidae	924	930	919	861	3.38	3.5
Total numbers of invertebrates	1023	1014	1025	962	2.63	3.0

Each subsample is 25% of the total weight of the sample. $I(n-1) \approx \chi^2{}_{3df,\alpha=0.05} = 0.22-9.35$, i.e. if χ^2 value lies between 0.22–9.35, agreement with a Poisson series is accepted at the 95% probability level. CV = coefficient of variation (see text for definition of terms).

Table 2. Subsample counts for benthic macroinvertebrate species in regular drift samples from the Souris River, Manitoba.

Taxon	Replicate no. 1 Subsample counts						Replicate no. 2 Subsample counts						Replicate no. 3 Subsample counts					
	A	B	C	D	χ^2	CV (%)	A	B	C	D	χ^2	CV (%)	A	B	C	D	χ^2	CV (%)
Hyalella azteca (Saussure)	2	6	0	1	9.23	116.9	8	4	5	4	2.05	36.1	6	6	5	6	0.13	8.7
Isonychia sicca Walsh	3	2	2	3	0.41	23.1	1	9	5	4	6.90	69.6	2	6	2	9	7.33	71.7
Caenis tardata McDunnough	11	9	10	5	2.37	30.1	11	11	12	6	2.20	27.1	27	22	24	17	2.36	18.7
Leucrocuta maculipennis (Walsh)	6	4	9	6	2.04	33.0	7	7	9	6	0.65	17.4	23	17	16	12	3.65	26.7
Baetis spp.	46	47	27	38	6.51	23.4	48	36	31	39	3.97	18.6	35	42	39	30	2.22	14.2
Cheumatopsyche campyla Ross	2	4	2	0	4.01	81.7	2	1	3	0	3.34	86.1	1	0	2	1	2.01	81.7
Others*	1	1	1	1	0	0	6	4	0	1	8.27	100.1	1	2	1	3	1.57	54.7
Total	71	73	51	54	6.21	18.2	83	72	65	60	4.26	14.2	95	95	89	78	2.17	9.0

* Includes: *Ephoron album* (Say), *Psychomyia flavida* Hagen, *Stenacron interpunctatum* (Say), *Hydroptila ajax* Ross, *Hydropsyche recurvata* Banks, and *Polycentropus cinereus* (Hagen).
Each subsample is 25% of the total weight of the sample. $I(n-1) \simeq \chi^2_{3df, \alpha=0.05} = 0.22-9.35$, i.e. if χ^2 value lies between 0.22–9.35, agreement with a Poisson series is accepted at the 95% probability level. CV = coefficient of variation (see text for definition of terms).

Most species yielded high percentages of random counts. However, *Caenis tardata* McDunnough was a conspicuous exception to the general trend (Table 3), their dispersions in the original samples being contagious in four out of the nine replicates.

Subsamples of species in the single catastrophic drift sample consistently agreed with a Poisson series, thus confirming effective subsampling (Table 4). Note, however, that for *Isonychia sicca* Walsh $\chi^2 = 18.9$, a value very close to the upper limit of χ^2 values.

Unlike in some of the regular drift samples, *C. tardata* in the catastrophic drift sample appeared to be randomly dispersed in the original sample. Nymphs of this species may have a body shape or some morphological feature that predisposes them to entanglement in the filamentous algae that pervaded the regular drift samples. This may have prevented a random dispersion when some of these samples were mixed prior to subsampling.

Variability

Relative variability of subsamples from the artificial substrates was only ≃3% (Tables 1 and 5). However, CVs were usually higher and more variable for the regular drift subsamples due to the relatively low numbers in these samples. CVs for one of the three sets of regular drift samples are shown in Table 2, and values for all of the sets are summarized in Ta-

Table 3. Number of times subsample counts were outside the value of $I(n-1) \simeq \chi^2_{3df, \alpha=0.05} = 0.22-9.35$ for regular drift samples from the Souris River, Manitoba (the maximum number of times = 9 unless otherwise indicated).

Taxon	No. of times table values were outside of χ^2	% of counts random
Hyalella azteca	1	88.9
Isonychia sicca	0[a]	100.0
Caenis tardata	4	55.6
Leucrocuta maculipennis	0	100.0
Baetis spp.	1	88.9
Cheumatopsyche campyla	0[b]	100.0
Others[c]	1	88.9
Total	1	88.9

[a] Eight replicates used.
[b] Seven replicates used.
[c] Includes six species (see Table 2, footnote).

Table 4. Subsample counts for species occurring in a catastrophic drift sample from the Souris River, Manitoba.

Species	Subsample											χ^2	CV (%)	Total no. in entire sample
	1	2	3	4	5	6	7	8	9	10	Σx			
Hydropsyche recurvata	34	41	39	40	44	41	43	28	46	43	399	6.3	13.3	4462
Cheumatopsyche campyla	232	243	251	248	274	227	281	266	266	288	2576	14.8	8.0	28458
Polycentropus cinereus	2	2	5	2	5	4	2	3	4	4	33	4.3	37.9	418
Psychomyia flavida	121	108	116	98	126	102	131	125	128	134	1189	11.8	10.5	12689
Hydroptila ajax	23	18	19	21	24	25	23	19	24	29	225	4.5	14.9	1920
Isonychia sicca	97	91	113	98	127	126	131	133	121	127	1164	18.9	13.4	9230
Caenis tardata	107	101	123	118	126	131	116	128	124	130	1204	7.4	8.3	11210
Leucrocuta maculipennis	105	103	113	113	127	125	127	130	141	138	1222	12.7	10.7	14126
Stenacron interpunctatum	34	41	39	40	43	32	46	45	52	44	416	7.4	14.0	3215
Ephoron album	27	35	37	32	30	28	24	31	26	28	298	5.0	13.6	2854
Baetis spp.	142	152	155	136	163	147	160	149	172	167	1543	7.5	7.4	18270
Hyalella azteca	94	90	92	94	93	72	99	95	109	119	957	14.1	12.8	9011

Each subsample is 1% of the total weight of the sample. I = index of dispersion ($I(n-1) \approx \chi^2_{9df, \alpha=0.05} = 2.7 - 19.0$, i.e. if χ^2 value lies between 2.7 – 19.0, agreement with a Poisson series is accepted at the 95% probability level). CV = coefficient of variation (see text for definition of terms).

ble 5. Although 13 of 24 CVs for the set of regular drift samples shown in Table 2 were ≤30%, eight of the values exceeded 50%. Most of the CVs for subsamples in the catastrophic drift sample were ≤15% (Tables 4 and 5), indicating a relatively low variability. The highest CV in this sample was recorded for *Polycentropus cinereus* (Hagen) (37.9%), a taxon that was present in the lowest numbers in each subsample. Although variablity was highest in samples with low numbers of invertebrates, values of χ^2 indicated that dispersions usually were not significantly different from random in both large and small samples (see above).

Subsampling approaches

A central problem of subsampling in community oriented studies is the strategy that should be used in dealing with rare species. Basically, there are two approaches that can be taken to deal with this problem, depending on the objective of the study (Wrona *et al.*, 1982). The first approach would be adapt the numbers counted to the desired accuracy. Once randomness has been demonstrated, the accuracy of the estimated total count for a given taxon depends on the total number of individuals counted rather than the number of subsampling units taken (e.g. at least 100 individuals would be required to yield an accura-

Table 5. Summary of frequencies of occurrence of coefficients of variation (CV) for subsamples of artificial substrates (AS), regular drift samples (RDS), and catastrophic drift samples (CDS).

Sample type	% range					
	0 – 10	10 – 20	20 – 30	30 – 40	40 – 50	>50
AS (n = 6)	6	0	0	0	0	0
RDS (n = 24)	3	6	4	3	0	8
CDS (n = 12)	3	8	0	1	0	0

cy of ±20% at 95% confidence limits) (Elliott, 1977; Wrona *et al.,* 1982). Therefore, only enough subsamples woud be sorted to give sufficient numbers of an abundant taxon to achieve a desired accuracy, and thereafter these taxa would be ignored. In contrast, rare taxa would be counted in all subsamples and additional subsamples would be taken if the error terms for these rare taxa were still unacceptable. This approach would be used when accurate estimates of population density are required.

The second approach would be to standardize the number of subsamples taken, and to accept the substantial error associated with subsampling rare species. In a community level study such as one monitoring the effects of pesticide application on non-target organisms, continued counting of rare taxa defeats the original purpose of the subsampling, which is the saving of time and effort. Additionally, accuracy for rare taxa such as *P. cinereus* in the catastrophic drift sample (Table 4) can remain low even if substantial extra effort is expended in counting higher numbers. For some taxa present in samples with low numbers (e.g. regular drift samples), the error will be high even if all the individuals are counted.

Conclusion

Based on our results, and the time required to sort and identify each subsample (see below), only one subsample (25% by weight) was analyzed for artificial substrate and regular drift samples. Numbers of each taxon were multiplied by four to obtain an estimate of total numbers in each sample. This relatively large subsample size was chosen because it yielded relatively high numbers of individual taxa and lower variabilities than for a smaller subsample (cf. Van Ark, 1975; Madoni, 1984). Also, there was less probability of missing rare species with a larger subsample. These considerations were especially imporatant for the regular drift samples which had low total numbers of organisms.

For the catastrophic drift samples, only one subsample (1% by weight) was sorted and identified because of the high numbers of invertebrates in each sample, and numbers of each taxon were multiplied by 100 to yield an estimate of the total number in the entire sample. *P. cinereus* was the only taxon that had numbers too low in one subsample to obtain an accuracy of at least ±50% (i.e. a count of 16 at 95% confidence limits – see Hickley, 1975; Elliott, 1977; Wrona *et al.,* 1982) (Table 4).

The time saved by using the subsampling method was proportional to the size of the subsample. Thus, whereas the entire artificial substrate, regular drift, and catastrophic drift samples required approximately 40 h, 16 h, and 200 h respectively to sort, the subsamples only required approximately 10 h, 4 h, and 2 h respectively to sort.

Van Ark & Pretorius (1970) used mass as a basis for subsampling light-trap catches of insects, and Van Ark (1975) showed that mass was more reliable than volume. Although we have not compared the weight-based subsampling method presented here with one based on volume (samples with filamentous algae would preclude this comparison), the method presented appears to be a simple and reliable way to subsample collections of benthic macroinvertebrates, especially those containing filamentous algae.

Acknowledgments

Research support was provided to R. Brust from Agriculture Canada Grants 1013 and 1037, and to R.J.S. by a University of Manitoba Graduate Fellowship. The Department of Fisheries and Oceans, Western Region also provided considerable research support. B. Silver and V. DeJong provided technical assistance in developing the subsampling method and sorting the many samples involved. We are grateful to S. Kasian for statistical advice. Comments on drafts of the MS were provided by R. Brust, J. Culp, I. Davies, B. deMarch, N. Holliday, R. Hecky, S. Kasian, K. Mills, V. Resh, A. Salki, and F. Wrona. D. Laroque guided the production of the MS through its many drafts.

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