INFLUENCE OF SAMPLING METHODS ON DETERMINATION OF MAYFLY (EPHEMEROPTERA) SPECIES RICHNESS IN A COASTAL PLAIN STREAM

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A 19-month study was conducted to compare the efficacy of the modified Hester-Dendy multiplate artificial sampler and the dipnet methods for determining mayfly (Ephemeroptera) species richness in a moderate-sized coastal plain stream system flowing from South Georgia to North Florida. The results indicated that the dipnet method significantly showed both a higher frequency of collecting mayflies and a higher number of species compared to the Hester-Dendy method. The present study also suggests that we need to be mindful of the behavior and biology of various benthic taxa that we deal with prior to the adoption of a standardized method of sampling.

INTRODUCTION

The suitability of artificial substrates for use in biomonitoring of the quality of surface waters has been the subject of numerous investigations in recent years (MASON *et al.*, 1973; LAMBERTI & RESH, 1985; MORIN, 1985; DE PAUW *et al.*, 1986; BOOTHROYD & DICKIE, 1991; CLEMENTS, 1991; DOBSON, 1991; GESSNER & DOBSON, 1993). Central to the use of artificial substrates for water quality monitoring is the supposedly greater replicability and reliability than collections from naturally heterogeneous substrates. It is a formidable task to decide which artificial substrate to use considering the patchy nonrandom distribution of benthic organisms, including mayflies, in streams.

The dominant and most common substrate of a given stream often dictates the type of artificial substrate to sample benthic macroinvertebrates for water quality bioassessments. In the shifting sand bottomed, Coastal Plain streams, particularly in northern Florida, submerged coarse woody debris, or snags, comprise the dominant substrate and are heavily colonized by aquatic insects (BENKE *et al.*, 1984; HUBBARD & SWADLING, 1995). For biomonitoring of the water quality of streams and rivers the Florida Department of Environmental Protection has for a long time used, and still uses, the modified Hester-Dendy multiplate artificial sampler method (see description below) as the main sampling technique for collecting macroinvertebrates (FDER, 1987) and the dipnet method has been used only sparingly, even though the latter method is considered more cost efficient. Studies have shown that the number of taxa in introduced substrate and collections from naturally heterogeneous substrates is considerably less variable than abundance (MINSHALL & PETERSEN, 1985; CLEMENTS, 1991), and therefore species richness is often the method of choice for comparison of ecosystems. However, investigators have also noted that certain macroinvertebrate taxa may be under represented or over represented on artificial substrates compared to abundance in natural substrate (MINSHALL & MINSHALL, 1977; SHAW & MINSHALL, 1980). Another important consideration in choosing between the dipnet or artificial substrate is that the use of the dipnet may be difficult or impossible in habitats that are deep, rocky, or where accessibility is limited.

The study reported here was initiated to evaluate and compare the efficacy of the modified Hester-Dendy and dipnet methods on the determination of mayfly species richness in Little Attapulgus Creek and Attapulgus Creek, a moderate-sized stream system flowing from South Georgia to North Florida.

STUDY AREA AND METHODS

This study was conducted at three sampling sites (AC1, AC2, and AC3) on Little Attapulgus Creek and Attapulgus Creek, within the Ochlockonee River Basin (Fig. 1). Attapulgus Creek and its tributary, Little Attapulgus Creek, originate in southern Decatur County, Georgia. The streams joins very near the Florida Georgia border and from there the stream flows through Gadsden County, Florida where it is joined by several tributaries before emptying into Lake Talquin as Little River. Three sampling sites were established for this study, two (AC1 and AC2) from Little Attapulgus Creek and one (AC3) from Attapulgus Creek. Sampling site AC2 is approximately 5.0 km below sampling site AC1 and sampling site AC3 is approximately 7.1 km below AC2 (Fig. 1).

Little Attapulgus Creek at sampling station AC1 is a 2nd order stream and at AC2 is a 3rd order stream. The 1992 mean annual discharge for Little Attapulgus Creek was 0.56

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m^3/sec^{-1}, recorded at a USGS gauging station 1.9 km southwest of Attapulgus, Georgia (between AC1 and AC2). Sampling station AC3 was located on the main stream of Attapulgus Creek (4th order stream) below the junction with Little Attapulgus Creek. The stream is much wider and considerably deeper at this point, with the substrate consisting of considerable silt and muck.

Selected water quality data for Little Attapulgus and Attapulgus Creeks are presented in Table 1. Little Attapulgus Creek receives discharges from a fuller's earth and kaolin processing plant located between sampling stations AC1 and AC2. The comparatively high conductivity level in AC2 could very well be due to the industrial discharge from the plant that flows into the stream approximately 1 km above the sampling site. Previous reports also indicated elevated nitrate-nitrite concentrations below the industrial plant (GEORGIA DEPARTMENT OF NATURAL RESOURCES, 1984). Besides the industrial activities already mentioned, the majority of the surrounding land is forested, with some agricultural activity as well.

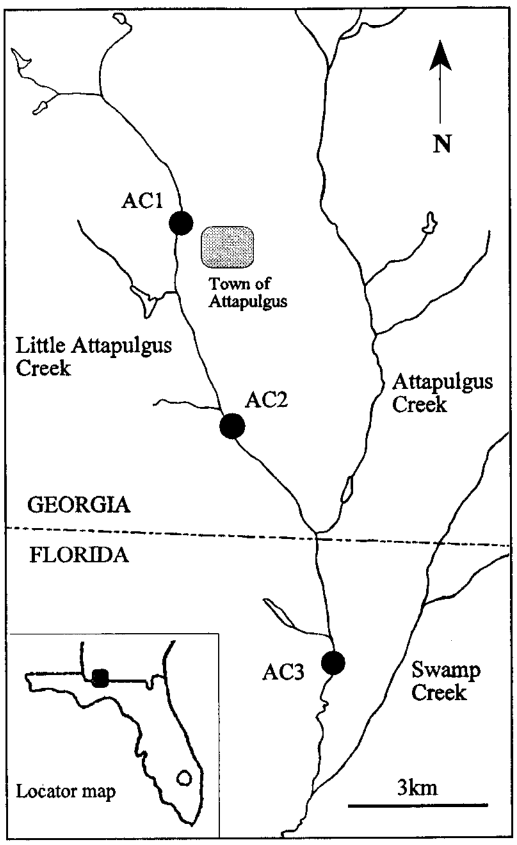

Fig. 1. Sampling sites on Little Attapulgus and Attapulgus Creeks.

Little Attapulgus and Attapulgus Creeks offer a diversity of microhabitats for benthic macroinvertebrates to utilize. Little Attapulgus Creek at sampling station AC1 is well-shaded by a dense understory of shrubs and has numerous snags, logs, and exposed roots that provide natural cover. Additionally, near the bridge where the canopy is broken, emergent vegetation and an area of exposed sand and gravel appears. Downstream, at sampling stations AC2 and AC3, the stream is wider and slower with somewhat unstable undercut banks. Water depth here is quite variable, greatly dependent upon the rainfall amounts. The riparian vegetation of AC2 and AC3 consists mainly of bottomland hardwoods, shrubs, and non-woody macrophytes. Numerous fallen trees and exposed roots along the undercut banks provide suitable habitat for a variety of benthic macroinvertebrate species.

The mayfly nymphs were collected bimonthly from December 1991 through June 1993, using modified Hester-Dendy multiplate samplers [consisting of four columns of masonite multiplate samplers mounted on a concrete block]. Each column of Hester-Dendy plates had a total surface area of approximately 0.09 m^2 (a detailed description can be found in HUBBARD & SWADLING, 1995). One block of Hester-Dendy multiplate samplers was installed at each sampling site. The Hester-Dendy samplers were incubated in the river for approximately 60 days. Upon retrieval, the incubated samplers were placed in plastic bags half-filled with stream water, and stored in ice while in transit. The samples were refrigerated in the laboratory and subsequently processed within 24 hours by dismantling and carefully brushing with tape water over a 0.71 mm mesh sieve. The sieved material was preserved in 75% ethanol and subsequently sorted, identified, and counted using a dissecting microscope.

Dipnet collecting was carried out bimonthly for approximately three to five minutes, using a rectangular frame bottom kick net to sample the various types of habitats around the

Table 1. Mean values of selected water quality parameters from Little Attapulgus and Attapulgus Creeks (December 1991 to June 1993). N = number of readings.

Station	N	Temp (°C)	D.O. ($mg\ l^{-1}$)	pH (s.u.)	Conductivity ($\mu mhos\ cm^{-1}$)	Turbidity (NTU)
AC1	9	15.5	9.0	7.42	29.6	11.7
AC2	9	15.9	8.4	6.88	96.5	25.1
AC3	9	15.7	8.7	6.99	53.8	26.1

sampling sites. Dipnet collecting was carried out at the same time the Hester-Dendy samplers were retrieved and the same person was assigned to do the dipnetting to minimize process-driven biases. Dipnet samples were stored in 75% ethanol and later processed in a manner similar to the sieved material above.

Water chemistry data (Table 1) were collected bimonthly. Dissolved oxygen and temperature readings were taken in the field using a portable oxygen meter, while the specific conductivity, pH, and turbidity were analyzed in the laboratory using standard conductivity, pH, and turbidity meters.

The identifications of species was based primarily on mature nymphs using a dissecting microscope equipped with 50X magnification. If necessary, for better viewing of minute parts, slide mounts were prepared and examined under a compound microscope with at least 100X magnification.

The data obtained from the two sampling methods, the modified Hester-Dendy multiplate artificial sampler and the dipnet, were analyzed separately for each sampling station. The collection of at least one specimen of a species from that station's faunal list was categorized as a «positive» collecting event and failure to collect a species was categorized as a «negative» event. The objective was to compare the frequency of positive and negative events between the two methods to determine whether there is statistical relationship between them.

Because the data to be analyzed is nominal (positive or negative, which can be represented by 1 or 0), it is appropriate to use the nonparametric approach which requires no minimum sample size nor assumption of normality of the data. The Chi-square test of independence was used to determine whether the sampling frequencies were independent of the sampling methods. Rejection of the null hypothesis would imply that the sampling frequencies are not independent of sampling methods. However, when more than 20% of the expected frequencies in the cells of a contingency table were less than 5, Fisher's Exact Probability Test was used to strengthen the Chi-square results. The procedures for computing the Chi-square test and Fisher's Exact Probability Test are shown in Equations 1-2.

Equation 1. Chi-square test (χ^2)

$$\chi^2 = \Sigma \left[\frac{(O_{ij} - E_{ij})^2}{E_{ij}} \right] \text{ where}$$

O_{ij} = observed frequency and E_{ij} = expected frequency

Equation 2. Fisher's Exact Probability Test (F)

A	B	(A+B)
C	D	(C+D)
(A+B)	(B+D)	T

$$F = \frac{(A+B)!\ (C+D)!\ (B+D)!}{A!\ B!\ C!\ D!\ T!} \quad \text{where } T = (A+B+C+D).$$

A, B, C and D are values in contigency table, and ! = factorial.

RESULTS

The Chi-square analysis indicates that the frequency of positive collecting events appears to be significantly higher with the dipnet than with the Hester-Dendy plated [PR(χ^2 = 6.736) = 0.009] (Table 2). Of the 227 total number of paired collecting events, positive events occurred 62 times with dipnet compared to only 39 times with the Hester-Dendy plates. Also Chi-square analyses suggest a significant relationship between the frequency of collecting individual mayfly species and the type of sampling method (Table 3). A combined total of fifteen species of mayflies were collected over a period of 19-months; fourteen species were collected by dipnet compared to 11 species by Hester-Dendy plates [PR(χ^2= 23.539) = 0.052].

The collecting frequencies of various mayfly species using both sampling methods are presented in Table 4. The differences in the numbers of total frequency of collecting (see Total Frequency column) is function of the number of sites that the species was found to occur. For example, *Acerpenna pygmaea* and *Baetisca obesa* were collected only in AC1, hence the total frequency of 9 compared to a total of 23 for *Baetis intercalaris* and *Stenonema exiguum* which were found in all three sampling sites in the stream. Regardless of sampling sites, however, results of the Chi-square Analysis and the Fisher Exact Probability Test (FEPT) indicate that the choice between dipnet and Hester-Dendy plate samplers has a significant impact on the collection frequency of *Hexagenia limbata* and *Labiobaetis ephippiatus* (PR<0.01), and *Paraleptophlebia volitans* (PR<0.05).

Species richness and a comparison of frequencies of collecting mayflies between dipnet and Hester-Dendy plates from the three sampling

Table 2. Chi-square analysis comparing the frequency of positive and negative collecting events for mayflies (Ephemeroptera) by the Hester-Dendy and Dipnet methods from Little Attapulgus Creek and Attapulgus Creek. χ^2 = 6.736; df = 1; PR (χ^2 = 6.736) = 0.009.

Sampling Method	Frequency of Negative Events	Frequency of Positive Events	Total Frequency of Collecting Events
Dipnet	165	62	227
Hester-Dendy Plates	188	39	227

sites is shown in Table 5. The dipnet method was much more effective than the Hester-Dendy method at AC1, with 12 species collected with dipnet versus only 5 species with Hester-Dendy samplers. The number of species collected with the two methods varied to a lesser degree at AC2 and AC3. Of the 45 collecting events in AC1, there were 33 positive events by dipnet compared to 8 for Hester-Dendy plates. Both methods had almost equal frequency of positive collecting events at AC3. Results of the Chi-square analysis, however, indicate that the combined frequency of collecting mayflies from all three stations by dipnet was significantly higher than by Hester-Dendy plates [PR(χ^2 = 5.773) = 0.056].

The list of mayfly species collected from Little Attapulgus and Attapulgus Creeks during the study period is presented in Table 6. Four of the 15 species collected, *Acerpenna pygmaea, Baetisca obesa*, and *Hexagenia limbata*, and *Paraleptophlebia volitans*, were not collected by the Hester-Dendy multiplate samplers, and one heptageniid species, *Heptagenia flavescens*, was not collected by the dipnet method.

DISCUSSION

One of the limitations regarding the use of artificial substrates for sampling benthic communities is sampling selectivity (Rosenberg & Resh, 1982; Khalaf & Tachet, 1980; Clements, 1991). This study attempts to test the selectivity hypothesis of artificial substrates on mayflies, using the Hester-Dendy multiplate artificial sampler and compares species richness results with those of the dipnet method.

A 19-month study period may not be sufficient to provide data comprehensive enough to draw a definitive conclusion. The results however, demonstrate the effectiveness of the dipnet as a method of sampling mayflies in Little Attapulgus Creek and Attapulgus Creek in terms of species richness.

Table 3. Chi-square analysis comparing the frequency of collecting individual mayfly (Ephemeroptera) species by the Hester-Dendy plates and Dipnet methods from Little Attapulgus and Attapulgus Creeks. χ^2 = 23.539; df = 14; PR (χ^2 = 23.539) = 0.052. Note: More than 20% of cells have expected values less than 5 and χ^2 results may be unreliable.

Taxa	Frequency of Collecting		Total Frequency of Positive Events
	Dipnet	Hester-Dendy	
Acerpenna pygmaea	2	0	2
Baetis intercalaris	6	2	8
Baetisca obesa	2	0	2
Caenis amica	1	1	2
C. diminuta	3	1	4
C. macafferti	1	1	2
Eurylophella doris	8	4	12
Heptagenia flavescens	0	1	1
Hexagenia limbata	7	0	7
Isonychia arida	4	7	11
Labiobaetis ephippiatus	8	1	9
Paraleptophlebia volitans	4	0	4
Stenacron interpunctatum	4	5	9
Stenonema exiguum	2	4	6
S. smithae	10	12	22
TOTAL	62	39	101

Except for the heptageniid species *Heptagenia flavescens*, all mayfly species found in the streams through out the duration of the study were collected by dipnet. *Heptagenia flavescens* is a very uncommon mayfly in Florida, and only one nymph was found in the Hester-Dendy plate samples. Like most heptageniids, the nymphs of *H. flavescens* are clingers and primarily snag-dwellers, and it is no surprise that the nymph was collected by the Hester-Dendy plates.

Of the four species that were not collected by Hester-Dendy plate samplers, *A. pygmaea* and *P. volitans* have been collected by Hester-Dendy plate samplers from the other streams in which we are presently conducting research, although not as frequently as in the dipnet. Both species are common in AC1 but not

Table 4. Chi-square analysis and Fisher exact probability test comparing the frequency of success and failure of collecting Mayfly (Ephemeroptera) species by Hester-Dendy Plates and Dipnet methods from three sampling sites from the Little Attapulgus and Attapulgus Creeks. *significant at the 0.05 level; **significant at the 0.01 level; F = Probability of Fisher's Exact Test (2-tailed).

Taxa	Sampling Methods	Frequency of non-collecting	Frequency of collecting	Total Frequency	Probability (χ^2) Values
Acerpenna pygmaea	Dipnet H-Dendy	7 9	2 0	9 9	0.134
Baetis intercalaris	Dipnet H-Dendy	17 21	6 2	23 23	0.120
Baetisca obesa	Dipnet H-Dendy	7 9	2 0	9 9	0.134
Caenis amica	Dipnet H-Dendy	6 6	1 1	7 7	1.00
C. diminuta	Dipnet H-Dendy	13 15	3 1	16 16	0.285
C. macafferti	Dipnet H-Dendy	6 6	1 1	7 7	1.00
Eurylophella doris	Dipnet H-Dendy	8 12	8 4	16 16	0.144
Heptagenia flavescens	Dipnet H-Dendy	7 6	0 1	7 7	0.766
Hexagenia limbata	Dipnet H-Dendy	9 16	7 0	16 16	0.003** (0.0007)F**
Isonychia arida	Dipnet H-Dendy	10 7	4 7	14 14	0.246
Labiobaetis ephippiatus	Dipnet H-Dendy	8 15	8 1	16 16	0.006** (0.016)F*
Paraleptophlebia volitans	Dipnet H-Dendy	14 18	4 0	18 18	0.034** (0.104)F
Stenacon interpunctatum	Dipnet H-Dendy	19 18	4 5	23 23	0.710
Stenema exiguum	Dipnet H-Dendy	21 19	2 4	23 23	0.381
S. smithae	Dipnet H-Dendy	13 11	10 12	23 23	0.555

Table 5. Species richness and Chi-square analysis comparing the frequency of success of collecting mayflies at three sampling sites from Little Attapulgus and Attapulgus Creeks. χ^2= 5.773; df = 2; PR (χ^2 = 5.773) = 0.056 (significant at the 0.10 level).

Sampling Sites	Dipnet		Hester-Dendy Plates		Total Frequency
	Frequency	Species Richness	Frequency	Species Richness	
AC1	33	12	12	5	45
AC2	12	7	8	5	20
AC*	17	8	19	8	36
Total	62		39		101

abundant, and the nymphs are good and active swimmers, particularly those of *A. pygmaea*. The chance that these species swam away or were dislodged from the Hester-Dendy samplers during retrieval is a possible reason for their infrequent collection by this method.

The habitat preferences of the nymphs of the other two species not collected by the Hester-Dendy, *Baetisca obesa* and *Hexagenia limbata*, could well explain the reason for their not being collected by the Hester-Dendy plates during the study. The habitat of *B. obesa* in Florida differs from the others of the genus as *B. obesa* has not been found in or on sand but lives deep within vegetation mats and in rather slow flowing water (BERNER & PESCADOR, 1988). The nymphs of *H. limbata* on the other hand are burrowers, and there is little chance for the nymphs to colonize the Hester-Dendy plates unless the plates are buried in the substratum due to spates. We have observed this event in some of the streams that we are presently sampling, where a few nymphs of *H. limbata* were collected by Hester-Dendy plates buried in the substratum.

The study certainly supports the utility and usefulness of the dipnet method in determining species richness of mayflies in Coastal Plains streams. Of particular importance is the ability of the dipnet method to collect those species that, for one reason or another, are not usually collected by artificial samplers, resulting in a significantly higher frequency of collection of mayfly species. Although we did not record the time to quantify the efforts involved in processing the samples of both methods, we found out that it took us approximately one-third less time to sort the specimens from dipnet samples than those of the Hester-Dendy's. Moreover, the specimens that were collected by dipnet were very much more free of damage or mutilation, making identification easier. However, from our experience, accessibility to areas to be sampled is a serious drawback. Gulf Coastal Plain streams like the ones in Florida are often heavily vegetated along the banks which make it difficult to get access to various undisturbed reaches of the streams or are too deep or have rock substrata which limit the ability of the dipnet to adequately collect the fauna.

The advantages and disadvantages of using artificial substrates versus collections from natural substrates have been thoroughly discussed elsewhere (ROSENBERG & RESH, 1982; DE PAUW

Table 6. Data summarizing the mayfly species collected in 19 months of sampling using the Hester-Dendy and Dipnet samplers from Little Attapulgus and Attapulgus Creeks (0 - = not collected; X = Collected).

Taxa	Sampling Methods	
	Dipnet	Hester-Dendy
Acerpenna pygmaea	X	0
Baetis intercalaris	X	X
Baetisca obesa	X	0
Caenis amica	X	X
C. diminuta	X	X
C. macafferti	X	X
Eurylophella doris	X	X
Heptagenia flavescens	0	X
Hexagenia limbata	X	0
Isonychia arida	X	X
Labiobaetis ephippiatus	X	X
Paraleptophlebia volitans	X	0
Stenacron interpunctatum	X	X
Stenonema exiguum	X	X
Stenonema smithae	X	X

et al., 1986; CLEMENTS, 1991) and most need not be repeated here. The most common problems that we have encountered in Florida in the use of modified Hester-Dendy plates is tampering, and instances where either the Hester-Dendy plates were buried in the substrate during spates or exposed during drought.

Because of the greater cost-effectiveness of the dipnet method, it often could be considered the method of choice over the modified Hester-Dendy multiplate artificial sampler in determining species richness in Florida-Georgia coastal plain streams. We must emphasize however, the paramount importance of access to perform the process in various reaches of the stream, otherwise, complementary methods, such as the use of artificial substrates, are a must in order to have a significant representation of the actual mayfly pool in the stream.

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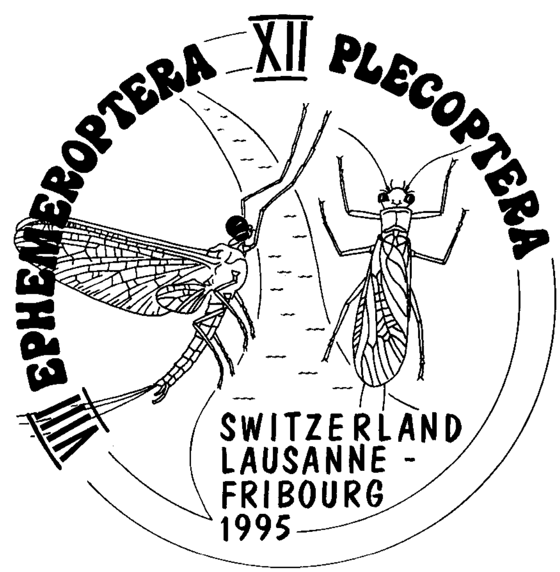

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