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# A PROTOCOL FOR USING SURFACE-FLOATING PUPAL EXUVIAE OF CHIRONOMIDAE FOR RAPID BIOASSESSMENT OF CHANGING WATER QUALITY

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ABSTRACT Documentation of biological responses to changing sediment and stream water quality conditions require repeated sampling over extended periods of time. Consequently, significant budgetary and personnel resources must be devoted to maintenance of biomonitoring networks. Alternative methods for monitoring biological responses that are more cost-effective than traditional benthic sampling approaches are of interest to agencies charged with measuring or managing water quality. In recent years we have refined an alternative approach for biomonitoring that relies upon collection of surface-floating pupal exuviae of Chironomidae to augment or replace benthic sampling. Our results indicate that collections of surface-floating pupal exuviae are more efficient at determining patterns of chironomid community composition in routine field sampling. Collections are easily standardized using timed-effort approaches, show high statistical precision and require approximately one-third the time to process compared to benthic samples. Increased taxonomic resolution is generally achieved with pupal exuviae relative to benthic collections of larvae. Our collection protocol is described and sampling efficiency, efficacy and economy discussed.

## INTRODUCTION

Biological sampling can be effectively used to generate information that is useful for making water quality management decisions. Because of the relatively low dispersal capabilities and long life spans of aquatic macroinvertebrates, collections of these organisms allow one to make inferences about the recent past history of the water quality conditions of specific water bodies. Thus the data generated by collecting aquatic macroinvertebrates adds a temporal dimension to water quality assessments that complements the point-in-time assessment that is generated by chemical analysis of water samples. Taken together, biological samples and water samples provide a more comprehensive information base upon which management decisions can be made.

Unfortunately, the more traditional approach of taking quantitative benthic samples for aquatic macroinvertebrates is time consuming and expensive. An alternative method for generating biological information about a particular body of water involves the collection of surface floating pupal exuviae of a group of aquatic flies of the family Chironomidae. This method has been employed in a basic research mode for projects of the Kansas Biological Survey where it has proven to be useful for assessments of enrichment by domestic sewage effluents in eastern Kansas streams. In an attempt to refine and standardize the methodology for impact assessment we have performed a series field exercises to evaluate the efficiency, efficacy and economy of the methodology. Here we present the results of these exercises and, where appropriate, contrast our methodology with results obtained by using the commonly used d-net sampling procedures.

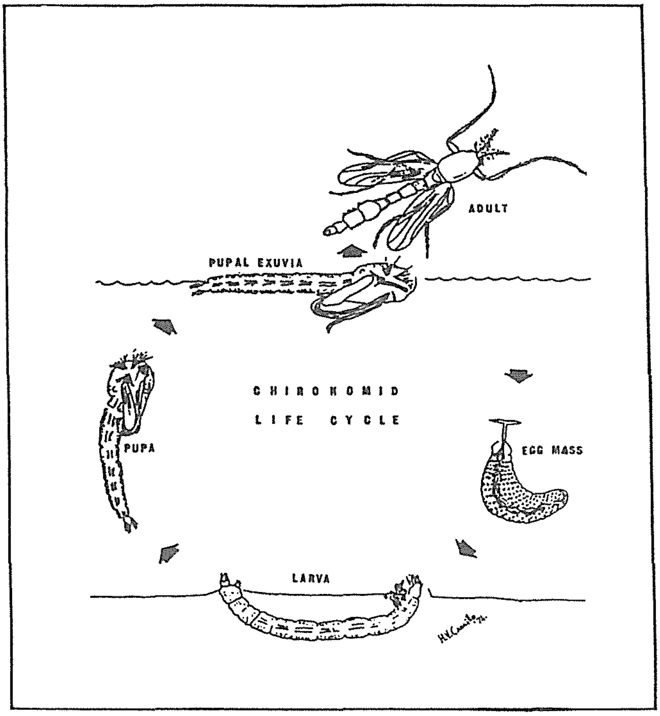


*FIG. 1 The Chironomid life cycle.*

## BACKGROUND AND DESCRIPTION OF THE METHODOLOGY

Collecting surface floating pupal exuviae is not a new approach for gathering information about Chironomidae communities. It was first suggested by Thienemann (1910), but was only occasionally used until recently (Humphries, 1938). During the last 20 years, however, there has been increasing use of pupal exuviae collections. Reiss (1968) and Lehmann (1971) used collections of pupal exuviae to supplement their larval collections when investigating Chironomidae community composition. In Western Europe and England collections of surface floating Chironomidae pupal exuviae have been used extensively for surface water quality monitoring (McGill *et al.*, 1979; Ruse & Wilson, 1984; Wilson, 1977, 1980, 1987, 1989; Wilson & Bright, 1973; Wilson & McGill, 1977; Wilson & Wilson, 1983) In North America the methodology has been successfully used in ecological studies of phenology (Coffman, 1973; Wartinbee & Coffman, 1976), ecology and community composition (Kavanaugh, 1988), microbial decomposition (Kavanaugh, 1988) and assessment of the effects of point sources of enrichment (Coler, 1984; Ferrington & Crisp, 1989). The following paragraphs provide a brief description of aspects of the methodology that are common to all of the above applications.

Figure 1 illustrates the life cycle dynamics of Chironomidae. Chironomid larvae live in soft sediments or on rocks and interstitial materials in stream beds. Upon completion of the larval life they attach themselves with silken secretions to the surrounding substrates and pupation occurs. When the developing adult matures the pupa frees itself from the silken chamber and swims to the surface of the water where the adult emerges from within the pupal skin (or exuvium). The exuvium fills with air and by virtue of an outer waxy layer of the cuticle (which has non-wettable properties) it remains floating on the water surface until bacteria begin to decompose the wax layer. Floating exuviae are concentrated by stream currents into eddy areas or into regions such as slack water areas downstream of rocks or points where riparian vegetation or fallen trees contact the water surface. By collecting exuviae from these "natural" collection points, one is able to rapidly evaluate the emergence of Chironomidae from a broad spectrum of microhabitat areas in the stream. Emergence frequencies are then calculated for the species that occur in the sample.

Field collection of floating pupal exuviae is accomplished by dipping an enameled pan into the water downstream of areas where pupal exuviae accumulate. Water, detritus and floating pupal exuviae flow in as one edge of the pan is dipped beneath the surface of the water. After the pan has filled with water, the contents are then passed through a U.S. Standard Testing Sieve with an aperture of 125 µm. Detritus and exuviae are retained by the sieve. The entire procedure of dipping and sieving is repeated until a large amount of detritus and exuviae is accumulated in the sieve. The contents of the sieve are then transferred to a sample jar and a field preservative (usually 80% ethanol) is added, along with a sample label. Exuviae are sorted from the detritus in the laboratory under 12X magnification in order to insure that all specimens are found and removed. It has been our experience that 10 min of collecting provides sufficient sample size for impact assessments in streams that are moderately to severely impacted by organic enrichment in eastern Kansas. This collection protocol has been developed as a Standard Operating Procedure for use in water quality investigations by the U. S. EPA (Ferrington, 1987).

## EFFICIENCY, EFFICACY AND ECONOMY IN ROUTINE WATER QUALITY ASSESSMENTS

To evaluate the effects that water quality conditions have on the aquatic fauna it is necessary to employ a sampling methodology that accurately measures the community of the aquatic fauna. Three criteria need to be considered when choosing a methodology-- efficiency, efficacy and economy. We define these criteria as follows:

(1) Efficiency— the capacity of the methodology to detect a significant proportion of the benthic community represented at the sample site.

(2) Efficacy— the capacity of the methodology to consistently measure the relative abundances of taxa that are detected at a given sample site.

(3) Economy— a measure of the cost of a given methodology relative to alternative methodologies that have similar standards of efficiency and efficacy.

In evaluating the efficiency, efficacy and economics of the pupal exuviae methodology we will compare this method relative to the more traditional approach of using random d-net approach for collecting benthic samples.

## METHODS

### Efficiency of Using Exuviae Collections in Routine Water Quality Assessments

The efficiencies of pupal exuviae collections and d-net collections for determining species richness of Chironomidae were tested as part of a routine study of water quality in two stream systems in Kansas during summer of 1987. The locations of the two stream systems are shown in Fig. 2. A total of 25 sites were investigated in the Dry Turkey Creek catchment in McPherson County, Kansas and the Indian Creek catchment in Johnson County, Kansas. In this study a pupal exuviae sample and a d-net sample were taken concurrently at each site. Each sample was sorted, the Chironomidae specimens identified, and a cumulative species total based upon both collecting methods was determined for each sample site. Relative efficiencies of both collecting methods were calculated by determining percentage of total taxa collected by each of the respective methods.

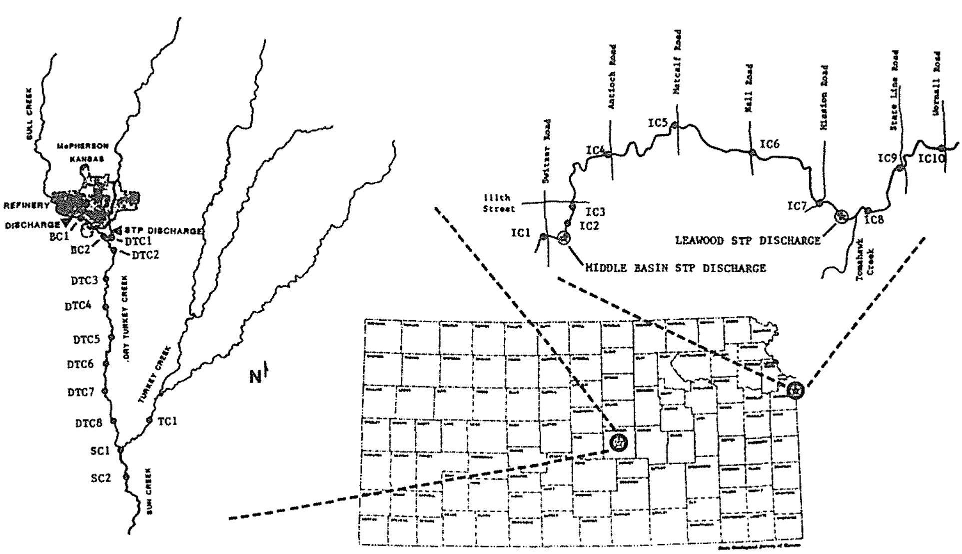


*FIG. 2 Locations of catchments and sample sites.*

### Efficacy of using Exuviae Collections in Routine Water Quality Assessments

The basic questions that these experiments were designed to address were related to sample size and statistical precision of emergence estimates based upon collections of exuviae. In order to provide background for understanding our approach to answering these questions, the Standard Operating Procedures recommended by Ferrington (1987) should be reviewed.

Using the field sampling approach recommended by Ferrington (1987), the smallest "sample" that can be consistently taken is a single dip of a pan into an area where exuviae are most likely to have accumulated. In practice, however, most researchers have taken many dips when collecting exuviae, and accumulated the contents of the dips into a "sample" that contains many hundred or even many thousand specimens of exuviae. In this approach, the assumption has been that by virtue of taking a very large "sample" of exuviae, the emergence frequencies of individual species within the sample are estimated with a high degree of accuracy. While this assumption is probably valid, the approach does not allow for a statistical evaluation of the effect of sample size on estimated emergence frequencies.

*TABLE 1 Relative efficiencies of d-net samples and pupal exuviae samples versus total midge species collected at each of 25 sample sites.*

| Total midge species collected | Number collected with d-net efficiency | Relative d-net collection efficiency (%) | Number collected as pupal exuviae | Relative pupal exuviae (%) |
|---|---|---|---|---|
| 2 | 1 | 50.0 | 1 | 50.0 |
| 4 | 3 | 75.0 | 2 | 50.0 |
| 4 | 3 | 75.0 | 4 | 100.0 |
| 5 | 3 | 60.0 | 4 | 80.0 |
| 7 | 6 | 85.7 | 5 | 71.4 |
| 7 | 5 | 71.4 | 4 | 57.1 |
| 8 | 4 | 50.0 | 7 | 87.5 |
| 9 | 3 | 33.3 | 9 | 100.0 |
| 10 | 5 | 50.0 | 10 | 100.0 |
| 10 | 5 | 50.0 | 9 | 90.0 |
| 10 | 6 | 60.0 | 5 | 50.0 |
| 11 | 7 | 63.6 | 9 | 81.8 |
| 11 | 6 | 54.5 | 9 | 81.8 |
| 11 | 3 | 27.3 | 11 | 100.0 |
| 11 | 3 | 27.3 | 11 | 100.0 |
| 12 | 5 | 41.7 | 12 | 100.0 |
| 12 | 3 | 25.0 | 11 | 91.7 |
| 13 | 9 | 69.2 | 12 | 92.3 |
| 13 | 3 | 23.1 | 13 | 100.0 |
| 14 | 9 | 64.3 | 12 | 87.5 |
| 14 | 9 | 64.3 | 12 | 87.5 |
| 14 | 8 | 57.1 | 12 | 87.5 |
| 15 | 10 | 66.7 | 15 | 100.0 |
| 15 | 4 | 16.7 | 15 | 100.0 |
| 16 | 7 | 43.8 | 15 | 93.7 |

In order to test the effects of sample size on estimates of emergence frequencies, exuviae were sampled from three adjacent sample sites on Mill Creek on three separate dates during the summer of 1983. Each sample consisted of the smallest "sample" (i.e. a single dip of an enamel pan) that can be consistently taken. Exuviae collected in individual dips were sieved and preserved. Twenty single dip samples were taken at each sample site on each date, resulting in a total of 180 samples. Samples were sorted, specimens identified and the emergence frequency of *Chironomus riparius* was calculated for each single dip sample. The time required to take the samples and to sort the samples was also recorded.

Economy of Using Exuviae Collections in Routine Water Quality Assessments

In certain preliminary assessments of potential water quality impacts statistical precision may not be of concern, and often a single d-net sample may be sufficient to make an evaluation. In these instances the time required to sort the sample and identify the specimen is not usually excessive. However, if several sites need to be assessed, or if a large monitoring network is to be established, the cumulative number of samples will require a considerable amount of time to process.

In order to evaluate the amount of time needed to process d- net samples and pupal exuviae samples, we recorded the times required to sort paired d-net and pupal exuviae samples collected concurrently for a period of 10 min at each of 20 sample sites during the summer of 1987. The sample sites were included in the Dry Turkey Creek catchment in McPherson County, Kansas and the Indian Creek catchment in Johnson County, Kansas and included stretches of stream with poor to good water quality.

## RESULTS AND DISCUSSION

Efficiency of Exuviae Collections

Table 1 shows the relative efficiencies versus total species richness values by sample site. In this study water quality at individual sample sites varied from poor to good and the corresponding total numbers of species collected reflected water quality conditions (Ferrington & Crisp, 1989). Total species collected per sample site ranged from 2 to 16. Several sample sites had the same number of species, and provided a range of efficiency estimates. Estimates of the relative efficiencies of d-net samples ranged from 85.7% to 23.1%. Estimates for pupal exuviae ranged from 100% to 50% efficiency.

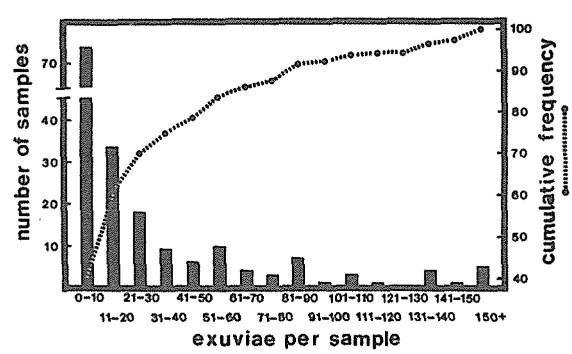


*FIG. 3 Number of exuviae collected per single dip sample.*

D-net samples were less efficient at detecting chironomid species as overall species richness increased At sample sites with species richnesses of 2-5 species the average d- net efficiency was 65%. At sample sites with 6-10 species, 11-14 species and 15-16 species the d-net efficiencies dropped to 52.2%, 47.0% and 45.7% respectively.

Pupal exuviae samples were more efficient at detecting species as species richnesses increased. Pupal exuviae samples were 70% efficient at sample sites with 2-5 species. Sample sites with 6-10 species, 11-14 species and 15-16 species yielded pupal exuviae efficiencies of 79.4%, 91.8% and 97.8%, respectively.

Efficacy of Exuviae Collections

Figure 3 is a histogram of the number of exuviae collected per single dip sample in Mill Creek during summer of 1987. Individual samples contained from zero to 299 specimens; however more than 60% of the samples contained 20 or fewer specimens. Less than 10% of the samples contained more than 90 specimens.

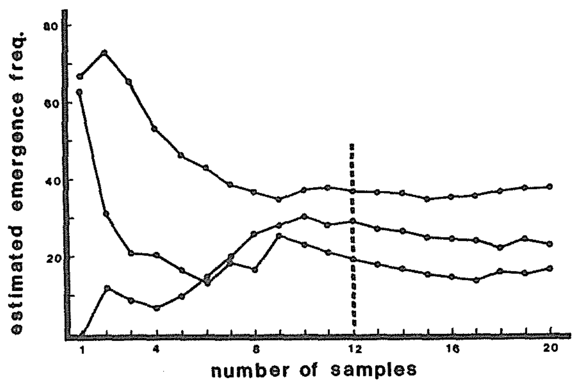


*FIG. 4 Estimated emergence frequency of Chironomus riparius.*

Figure 4 shows the effect that increasing the number of samples has on the estimated emergence frequency of *C. riparius*. This graph shows the estimated emergence from the three sites on a single sample date. Estimates were calculated as follows: Samples were averaged in the sequence in which they were collected in the field. The emergence frequency estimates for each site are connected by the solid line. For instance, sample number one at site 1 contained no exuviae of *C. riparius*. Sample number two contained specimens. The second dot thus represents the average of the emergence estimates of the first two samples for site 1. Individual samples were averaged until all samples were included -- the final point on the graph thus represents the average of all twenty single dip sample estimates for each site. As can be seen from the graph, the emergence "estimate" does not vary considerably after twelve samples are analyzed, and the value calculated based upon 12 samples was within + 8% of the value calculated after 20 samples for all three sample sites on all three dates.

Figure 5 shows the results of step-wise simulations of emergence frequency estimates of *C. riparius* derived for a single site on a single date. In this analysis the sequence in which samples were averaged was randomized (with no replacement for a single randomization) and the results were plotted versus the number of samples averaged. A total of 6 simulations of randomized averaging is included in this figure. Again one can see that at or about 12 samples, regardless of the sequence in which samples are analyzed, the emergence estimate of *C. riparius* is relatively stable. Figure 6 is a histogram of the time (in min) required to sort the samples. The minimum time required to sort a sample was 2 min and

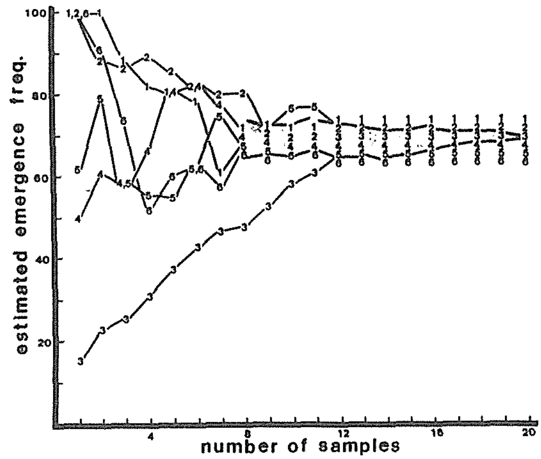


*FIG. 5 Step-wise simulations of emergence frequency estimates of Chironomus riparius.*

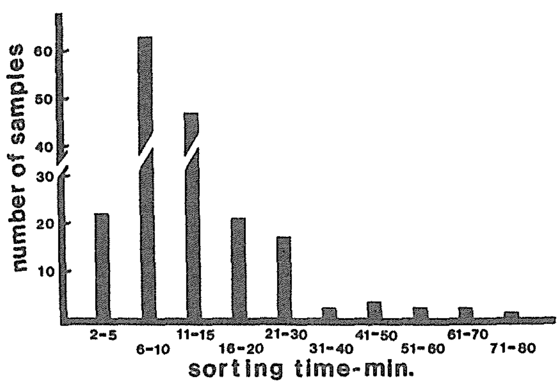


*FIG. 6 Time required to sort samples of pupal exuviae.*

the maximum time required was 80 min. Ninety percent of the samples were sorted in 30 min or less per sample.

Synthesizing the results of this experiment, the following can be concluded. A high degree of statistical precision can be achieved by taking a large number of very small (single dip) samples. Samples can then be sequentially sorted and processed until a target precision value of + 8%. If 90% of the samples collected required 30 min or less to sort then 90% of the time it will require 360 min or less of sorting time to achieve a target precision value of + 8% for emergence of *C. riparius* in Mill Creek.

## Economy of Exuviae Collections

The processing times for samples collected in 1987 from the Dry Turkey Creek catchment and the Indian Creek catchment are given in Table 2. D-net samples took an average of 121.5 min to sort and the range of sort times was from 30-300 min. Identification time averaged 68.6 min and ranged from 10-185 min, excluding one sample that contained only larvae of *Chironomus riparius*, which were identified as they were sorted. The average time required to sort and identify an average 10 min d-net sample was 190.1 min.

Sample sorting and specimen identification was accomplished concurrently for pupal exuviae samples. The average time to sort and identify the specimens in a sample was 52.7 min, and ranged from 12-115 min. On the basis of these results it can be concluded that 3 to 4 pupal exuviae samples can be processed in the lab for every 1 d-net sample.

*TABLE 2 Time required to process 20 paired d-net and pupal exuviae samples. Each sample represents 10 min of sampling. Sorting times given in min.*

| Total species detected | Sorting times for d-net samples | Identification times for d-net samples | Sorting & identification times for exuviae samples |
|---|---|---|---|
| 2 | 30 | 10 | 15 |
| 4 | 150 | *0 | 42 |
| 4 | 160 | 30 | 70 |
| 5 | 60 | 10 | 15 |
| 7 | 80 | 47 | 32 |
| 10 | 70 | 26 | 61 |
| 10 | 50 | 35 | 58 |
| 11 | 45 | 50 | 50 |
| 11 | 200 | 30 | 65 |
| 11 | 115 | 75 | 75 |
| 12 | 80 | 90 | 85 |
| 12 | 150 | 135 | 45 |
| 13 | 105 | 70 | 30 |
| 13 | 140 | 34 | 62 |
| 14 | 85 | 85 | 100 |
| 14 | 300 | 48 | 38 |
| 14 | 105 | 17 | 55 |
| 15 | 255 | 185 | 115 |
| 15 | 130 | 41 | 93 |
| 16 | 120 | 43 | 108 |

*Sample contained only 1 species, which was identified as it was sorted.

## SUMMARY

Samples of pupal exuviae appear to have high degrees of efficiency for assessing chironomid species richness and high degrees of efficacy for estimating emergence frequencies of indicator species. They also appear to be cost-effective, at least when compared with the more traditional approach of sampling benthic communities with a d-net sampler. Collections of pupal exuviae can be used to augment benthic samples in studies of biological responses to changing water quality conditions, or could be substituted for benthic sampling when large-scale analyses with repeated sampling over extended periods of time are conducted.

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