STANDARD OPERATING PROCEDURE

No. FW130A

COLLECTION AND IDENTIFICATION OF SURFACE FLOATING PUPAL EXUVIAE OF CHIRONOMIDAE FOR USE IN STUDIES OF SURFACE WATER QUALITY

BY

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This SOP not currently approved or in use by EPA Region 7

A. PURPOSE

The purpose of this standard operating procedure (SOP) is to establish uniform procedures for the collection and identification of surface floating pupal exuviae of Chironomidae as a method of gathering biological data for use in support of surface water quality studies conducted by personnel of the Water Quality Section, Environmental Monitoring and Compliance Branch (EMCM), Environmental Services Division (ENSV).

B. APPLICATION

The procedures contained in this SOP are applicable to all personnel of the Water Monitoring Section, Environmental Monitoring and Compliance Branch (EMCM) responsible for collecting, analyzing and interpreting biological information in support of surface water quality studies.

C. GENERAL CONSIDERATIONS

Rapid assessment protocols that are non-destructive to macroinvertebrate communities in surface waters are desirable if biological sampling is to remain as an integral part of surface water quality monitoring. Collections of surface floating pupal exuviae offer several distinct advantages that make them well suited for large monitoring networks. This document describes the sampling procedures and discusses advantages of the methodology as related to water quality monitoring. In addition it presents several of the more common concerns that have been raised about possible limitations of the methodology and briefly discusses some of the research activities recently conducted in order to evaluate the suggested limitations.

D. BACKGROUND INFORMATION AND DESCRIPTION OF SAMPLING PROTOCOL

1. 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.

2. Unfortunately, the more traditional approach of taking quantitative bottom 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 recently been shown to provide rapid, accurate and cost-effective evaluations of surface water quality conditions in the Rhine River of Germany and and the Thames River in England. This method has been employed in a basic research mode for projects of the Kansas Biological Survey and it has proven to be appropriate for assessments of enrichment by domestic sewage effluents in eastern Kansas streams. However, since the methodology has not been extensively used by regulatory agencies in North America this section is included in order to provide detailed background information of the procedures and underlying assumptions.

3. 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; Fittkau, 1962; Brundin, 1966). 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 evtensively for surface water quality monitoring (McGill et al., 1979; Ruse and Wilson, 1984; Wilson, 1977, 1980; Wilson and Bright, 1973; Wilson and McGill, 1977; Wilson and Wilson, 1983) In North America the methodology has been successfully used in ecological studies of phenology (Coffman, 1973, 1974; Wartinbee and Coffman, 1976: Wartinbee, 1979), ecology and community composition (Kavanaugh and Ferrington, 1986), microbial decomposition (Rao, McKinney and Ferrington, 1986) and assessment of the effects of point sources of enrichment and pollution by heavy metals (Coler, 1984; Coler and Ferrington, in preparation; Ferrington and Schmidt, 1986; Ruse, 1986; Ruse, Schmidt and Ferrington, 1987). The following paragraphs provide a brief description of aspects of the methodology that are common to all of the above applications.

4. 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

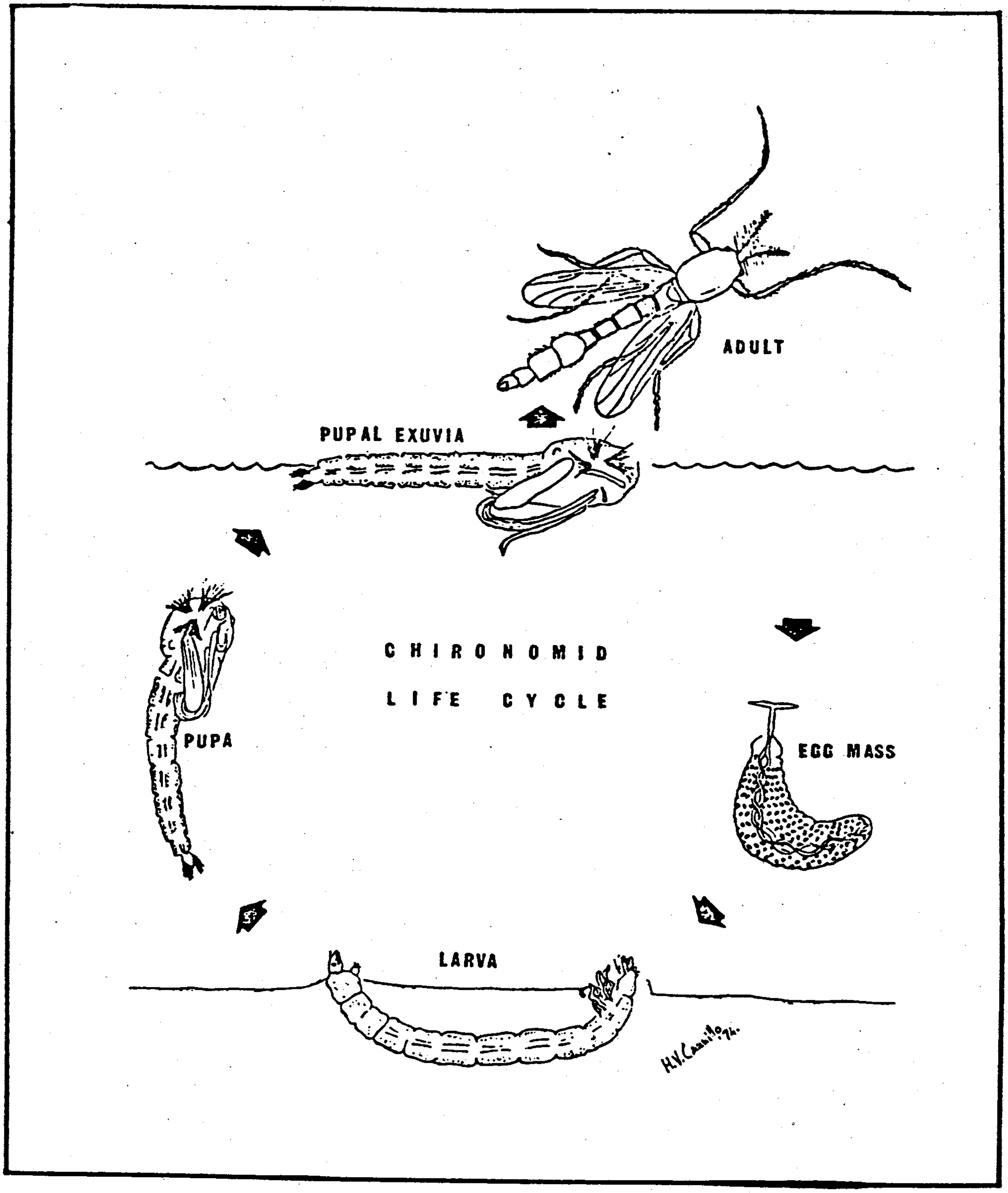

Figure 1. A Generalized Representation of the Chironomid Life Cycle.

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.

5. 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 microns. 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.

6. Using this field sampling approach, the smallest "sample" that can be consistently taken is a single dip. In practice, however, most researchers have taken many dips and accumulated them 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 are estimated with a high degree of accuracy. While this assumption is probably valid, the approach does not allow for an estimate of precision for the sample, nor does it allow for a statistical evaluation of the effect of sample size on estimated emergence frequencies. It is therefore recommended that several smaller samples be taken at each sample site when statistical analyses are to be performed on the resulting data (Ferrington and Kavanaugh, 1984).

7. For certain applications it is often necessary to collect samples in a consistent, standardized manner so that comparisons can be made among several sample sites or over time at a given sample site. In order to standardize the collection technique an equal effort approach can be used, with a predetermined period of time being spent at each site. In practice 10 minutes is usually sufficient for biological monitoring purposes during spring and summer months. While this standardized unit of effort based upon time does not yield strictly quantitative data such as numbers per unit area, it does result in order of magnitude evaluations of emergence density and relative emergence for individual taxa. Samples from different sites often yield numbers of exuviae that vary by as much as 100X or 1000X, and previous studies have shown

that this roughly correlates with standing crop densities at the corresponding sample sites. Ten minutes has also been shown to be a sufficient period of time to provide adequate evaluations of emergence frequencies in sewage enriched environments in Kansas (Coler, 1984; Ferrington and Kavanaugh, 1984).

E. SAMPLE SORTING AND IDENTIFICATION

1. As recommended earlier in this SOP, pupal exuviae samples should always be sorted in the laboratory. This is necessary since some species may be rather small, ranging from 3-6 mm in length, and lightly pigmented. Pupal exuviae are sorted by pouring aliquots of the sample into a petri dish and then inspecting the dish for exuviae using a dissecting microscope set at 12X magnification. Individual exuviae are picked from the dish and identified, counts recorded and exuviae placed into a small vial for future reference. It may not always be necessary to slide mount many exuviae for identification purposes. However, some specimens of each species must be slide mounted to provide voucher material for future studies. All exuviae can be identified to genus by sight recognition or using recently published keys by Coffman and Ferrington (1984) or Wiederholm (1986).

2. A record should be made of the amount of time required to sort and identify each sample. These data can then be used to evaluate the relative effort needed to process pupal exuviae samples versus the more standard type of d-net bottom sample.

F. DATA ANALYSIS

1. Collections of surface floating pupal exuviae provide estimates of species richness and relative abundance of individual Chironomidae species emerging from a stretch of stream. Exuviae have been shown to drift in smaller streams for distances no greater than 500 meters, with the majority being displaced 100 meters or less (Wilson and Bright, 1973). Ferrington and Kavanaugh (1984) estimated the average distance for which half of the specimens drifted in Mill Creek, Johnson County, Kansas to be 30 meters or less (see Section H for more details). Exuviae remain floating on the surface of the water for periods that have been estimated to be as short as 0.5 to 2.0 hours in a fast flowing mountain stream (Ferrington, 1981a) to as long as 15-48 hours (Coffman, 1973; McGill, 1981). Thus the typical collection of floating pupal exuviae probably provides a "moving average" of the Chironomidae that have emerged within the preceeding one day period from a short stretch of stream immediately upstream of a collection point.

2. Several parameters of interest in addition to species richness and relative abundance can be calculated from collections of floating pupal exuviae and these parameters should be used for interpretation of the data The parameters are (1) taxonomic composition and percentage composition hierarchies, (2) information content and evenness values for the sample collection and (3) estimates of the theoretical maximum numbers of species emerging at a given sample site.

3. Taxonomic composition deals with the relative numbers of species of a sample collection that are represented within the subfamilies Orthocladiinae and Tanypodinae and the Tribes Chironomini and Tanytarsini. General patterns of taxonomic composition have been empirically determined for selected streams in Kansas and these patterns provide a reference for evaluating the responses of Chironomidae to selected types of environmental stresses. Taxonomic composition is calculated by dividing the number of species contained in one of the above subfamilies or tribes by the total number of species within the corresponding collection and multiplying by 100 to express the final value as a percent. General trends that are expected for taxonomic composition of Chironomidae for streams in Kansas during summer months are detailed in Section H of this report.

4. Percentage composition refers to the percent of a given collection represented by a given species. It is useful to know the percent composition of selected species in a collection since their relative abundances may have a bearing upon the conclusions one would draw about the ambient water quality conditions of a given stretch of stream. For instance it is important to know the percentage composition of rheophilic, stenoxybiontic taxa in a sample since their presence in large numbers would suggest that oxygen concentrations and current characteristics of a particular sample site have not dropped below the minimum values required by these species during the larval lifespans of the species in question. Percentage composition is calculated by dividing the total number of specimens collected in a given sample into the number of specimens for the species being considered, then taking this value times 100 to express it as a percent.

5. Information content and evenness values for the sample collection can quickly be calculated by using standard computer programs. For instance a standard program (ECOMEAS) written by the staff of the Kansas Biological Survey calculates 10 different varieties of species diversity indices for collections of organisms and also calculates the corresponding maximum, minimum, equitability, evenness and redundancy values for selected indices. Brillouin's (H') Index is the most appropriate for analyzing information content of collections of floating pupal exuviae (Pielou, 1966), however other index values can be calculated and included in appendix form for reference purposes. Collections with high information content and moderate to high evenness are generally considered to be reflective of a community that is not experiencing substantial environmental stress.

6. The theoretical maximum number of species for a given sample site can be calculated and is often quite informative. For this purpose an algorithm was developed by the staff of the Kansas Biological Survey, based upon an equation given by Preston (1948). Species richness values are estimated for a given stretch of stream based upon a finite collection of all the floating pupal exuviae of the given stretch. Since the species richness estimate is based upon a subsample rather than a census of all the exuviae in a given stretch of river it is possible, or perhaps even likely that some rare species will not be included in the collection. This is generally the case with collections from communities with high species richness. Preston argued that empirically the distribution of relative abundances of species collected in samples from species rich communities generally follow a lognormal distribution, and species that are extremely rare do not appear in the sample collection because they are to the left of the zero abundance octave for a sample with a given number of specimens. He also argued that these rare species could be estimated by assuming that the different relative abundance octaves were symmetrically distributed around the modal octave, and the number of species could be calculated based upon the comparison with a lognormal model for the observed modal octave and corresponding standard deviation. In my research the efficiency of collecting efforts has been evaluated by estimating the theoretical maximum number of species that could occur given the modal octave and standard deviation values for the sample collections. The theoretical number is then compared with the observed value to determine efficiency. This should be done for each collection at each sample site on each sample date.

G. ADVANTAGES OF THE METHODOLOGY

1. The advantages of using collections of surface floating pupal exuviae to evaluate the composition of Chironomidae in streams have been summarized by Coffman (1973), Wilson and Bright (1973) and Wilson (1980). The discussions in these publications are extensive and need not be repeated in full here. Interested individuals should consult the original publications. In the paragraphs that follow the discussion is restricted to those advantages of using pupal exuviae that are most appropriate for biological monitoring applications.

2. Collections of floating pupal exuviae yield specimens of species that occur in a wide cross-section of microhabitats that are present in the stream being sampled. Typically, qualitative benthic samples using a d-net or kick-net have been the recommended approach for collecting invertebrates in water quality studies in recent years (Plafkin et al., in draft, and references therein). The objective of this approach is to intentionally sample a wide range of habitats and collect a large number of taxa that are representative of each microhabitat. In practice this approach works well for epilithic, rheophilic taxa

and for taxa that occur in the upper regions of sediments of pools. However, taxa that occur deep within the substrata of streams or in submerged wood or that bore into submerged hydrophytes are usually not collected. Taxa that grip tightly to rocks or are attached by coccoons or other pupational or filtering structures are usually missed or undercollected. Very small or unusual microhabitats may be overlooked during sample collection and, consequently, taxa that are specific to these microhabitats do not show up in the samples. Collections of floating pupal exuviae, however, yield specimens of all microhabitats, since emergence of adults of all aquatic midge taxa occurs at the air-water interface and water currents naturally concentrate the floating pupal skins.

3. Collections of floating pupal exuviae yield specimens of species that represent a wide range of size classes. The collecting efficiency of d-net or kick-net samplers is limited by the aperture size of the net mesh. Nets with mesh apertures of 500 microns or more are commonly used to collect macroinvertebrates. This results in overrepresentation of large species in the sample and underrepresentation or complete lack of small species (Ferrington, 1984). This is a particularly serious problem since many species of Chironomidae are small enough to pass through the 500 micron aperture even when they are of the maximum larval size class. In Kansas it has been empirically determined that Chironomidae of streams with good water quality include many small species, such as _Corynoneura_, _Thienemanniella_, _Eukiefferiella_, _Parakiefferiella_, _Tvetenia_, _Rheosmittia_, _Lopescladius_, _Nanocladius_, _Stempellina_, _Stempellinella_, _Labrundinia_, and _Nilotanypus_. Conversely, streams that are highly enriched by STP effluents are dominated by larger species such as _Chironomus riparius_, _Psectrotanypus dyari_, _Chironomus decorus_ gr. sp., _Goeldichironomus holoprasinus_, _Polypedilum illinoense_ and _Cricotopus bicinctus_. Using d-nets or kick-nets would cause small species to be underestimated or missed in clean water streams and would overestimate the larger taxa. This would cause a clean water stream to appear more similar to a sewage impacted stream than it really is and could result in a classification of a stream as having poorer water quality than actually occurs. Since it is recommended that a U. S. Standard Testing Sieve with an aperture of 125 microns be used for concentrating the sample of floating pupal exuviae there is no possibility of missing specimens of the smaller species that exemplify good water quality conditions.

4. Collections of floating pupal exuviae yield specimens of Chironomidae that are all of the same age class. Benthic collections of larvae, by contrast, usually include several instar classes. In practice it is often difficult to distinguish between different instars of the same species, and the same instar of different species. Larval Chironomidae (and other benthic insects) show varying degrees of age related polymorphism. Allometric growth of antennal segments, additions or decreases in the number of dorsomental or ventromental teeth, changes in mandible structure and differing locations of eyespots

or pigment patches often cause the inexperienced identifier to classify different instars of the same species as two or more "species". One instance is even recalled where an individual argued vehemently that he was dealing with two species because one had enlarged thoracic segments and two patches of circular pigmented regions near the occipital margin of the headcapsule, and the other "species" totally lacked all indications of these structures. In reality he had a single species of <u>Conchapelopia</u>, but was seeing the structures of the cephalothorax and eyespots of the developing pupa that were visible through the integument of the fourth instar larvae, but totally absent from the third instar larvae. Since collections of floating exuviae yield only the pupal stage there is no possibility of incorrectly defining species due to age-related polymorphism.

5. Pupal exuviae are much more easily slide mounted and prepared for identification than are larval specimens. There is no need to clear the exuvium and the correct structure of diagnostic characters can usually be seen even if the specimen is mounted up-side-down. This is not the case with larval specimens. It is often necessary to clear the specimen and flatten the headcapsule in order to see the diagnostic characters of the larvae and many times the mandible will obscure the mentum or pectin epipharyngis, or the antennae will be broken or missing or the mandible or mouthparts worn so badly that one cannot readily determine the state of the character of interest.

6. Samples of pupal exuviae are usually cleaner and quicker to sort than d-net or kick-net samples. If necessary they can be easily subsampled, and with a little bit of experience it is quite possible to sight identify many of the taxa that are collected. By contrast, subsampling and sight identification of larval samples is not easy to accomplish.

H. <u>COMMON CONCERNS ABOUT POSSIBLE LIMITATIONS OF THE METHOD</u>

1. Over the past few years the author has presented the interim results of several research experiments dealing with efficiency and efficacy of pupal exuviae collections at annual meetings of the North American Benthological Society and at several more informal meetings and seminars attended by benthic biologists and professional water quality managers. As a result of these presentations quite a bit of feed-back has been received regarding the reservations that the practicing professionals have regarding use of pupal exuviae for monitoring surface water quality. As a result of the feedback field experiments have been designed to address the concerns and provide additional insight into the effectiveness of pupal exuviae collections for evaluating water quality. Most of the research results given in subsequent paragraphs of this section are recently derived and as

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yet have not appeared in published format. By necessity the data are given here as personal communication and should be cited as such until they appear in final published form.

2. The format for the remainder of this section consists of a series of questions that have been commonly asked about pupal exuviae collections , followed immediately by responses in paragraph form.

3. QUESTION: If these are collections of free floating exuviae, how far do they float?

a. This is the very first concern about the methodology that is typically voiced. Since a common objective of surface water quality monitoring is to determine the impact of point sources of pollution it is, of course, necessary to be sure that one is collecting exuviae of Chironomidae that have emerged from within the suspected zone of impact. As mentioned in Section F of this protocol, field evidence suggests that in the extreme case pupal exuviae are displaced downstream no more than 500 meters (Wilson and Bright, 1973). Fifty percent or more of the exuviae do not displace more than 100 meters and the displacement curve is generally a negative exponential decay function. Therefore if one is collecting pupal exuviae a distance of 400-500 meters downstream from a suspected point source of pollution it is likely that the specimens being collected are the pupal skins of Chironomidae that have completed their life cycle within the suspected impact zone.

b. Of course there are several additional factors that need to be considered regarding displacement distances. An obvious consideration deals with the potential for washin of exuviae from small feeder streams that confluence with the stream of concern downstream of the point of suspected impact. Ideally, one would like to sample upstream of any confluence points, but 400-500 meters downstream of the suspected impact zone. If this is not possible then one may want to sample for exuviae in the tributary near its confluence with the stream of concern and delete or neglect the species collected from this stream if they occur in large numbers in the stream of concern.

c. In smaller streams of Kansas where current velocities are reduced or where there is a lot of riparian vegetation, dense growths of filamentous algae, brush in the water, logjams, etc., it is unlikely that exuviae are transported more than one pool/riffle segment downstream. In Mill Creek, Johnson County, Kansas, it was possible to detect differences in the emergence composition of Chironomus riparius and Cricotopus bicinctus between adjacent pool/riffle stretches. In one pool/riffle area that consisted of approximately 80% pool habitat and 20% bedrock and cobble riffle habitat the percentage of Chironomus riparius exuviae was statistically significantly higher than collections from an adjacent downstream pool/riffle habitat that was approximately 50% pool habitat and 50% riffle habitat. Larvae of C. riparius are more common in the softer sediments of the pools than in the riffle areas, and one would expect to find more exuviae of C. riparius at the site with a greater pool to riffle ratio if exuviae are entrapped and retained within a short floating distance.

d. By contrast, exuviae of Cricotopus bicinctus showed a reciprocal pattern of abundance in the two areas that were sampled. Larvae of C. bicinctus occurred almost exclusively on rocks in riffle habitats and pupal exuviae of this species were more common in the pool/riffle area that had the higher percentage of riffle habitat. Based upon this study it was inferred that the average displacement distance for 50% of the exuviae was approximately 30 meters.

4. QUESTION: How long do pupal exuviae float on the surface of the water?

a. After emergence of the adult the exuvium fills with air and remains on the surface of the water until bacterial and fungal action degrades the surface waxes and chitin of the exuvium or until it fragments due to turbulence of the water surface. The degradation process is temperature dependent and may vary slightly depending upon the nutrient content of the water. High water temperatures and high nutrient concentrations facilitate the most rapid growths of bacteria and fungi and result in the shortest float times in laboratory experiments (Rao et al, 1986). Estimates of float times vary from 0.5 to 2.0 hours in very turbulent mountain streams to 48 hours for exuviae from deciduous forest stream during summer months. In winter, exuviae may float for longer periods, perhaps as long as 7 days, but probably are fragmented due to physical abrasion before bacterial and fungal decomposition is extensive. The low float time estimate for the turbulent mountain stream also reflects the affects of abrasion, breakage and subsequent sinking that occurs in fast flowing streams.

5. QUESTION: How can one relate the number of specimens collected to a unit area of stream since the collections are obviously not quantitative?

a. This question reflects the benthic biologist's preoccupation with density related measurements. The implication is that "quantitative" (in the sense of numbers per unit area) data are necessary in order to measure a potential impact. This is not usually the case. In most preliminary impact studies data that are non-density related will be sufficient to determine if there is a community response in the suspected impact zone (Ferrington, 1981a). Taxonomic composition, species richness and relative abundance are usually sufficient data upon which to base such a decision. All of these parameters can be calculated from collections of floating pupal exuviae. It should be emphasized that the d-net or kick-net sample that is often used as the method of choice for impact assessment is not a quantitative measure of density either.

6. QUESTION: If the samples are not quantitative, how can comparisons among sample sites be made?

a. This is another aspect about "quantitative" sampling that is often confused or misapplied. The implication of this question is that density data must be generated in order to make meaningful comparisons among sample sites. As indicated in the previous paragraph, density data may not be required, and decisions can usually be made by comparisons of taxonomic composition, species richness and relative abundance. In order to make meaningful comparisons among sites it is only necessary to have consistency in terms of sample collection procedures and representativeness in terms of data generated.

b. Consistency relates to sample collection techniques, and subsequent sorting and identification activities. Consistency in sample collection technique is easily achieved by repeating the same collecting procedures for the same amount of time at every sample site. In practice this means that one determines ahead of time the unit of effort to be applied during each sample collection and that the unit of effort does not vary from site to site being investigated. As indicated earlier in this SOP, it has been found that 10 minutes collecting time per site is sufficient for biological monitoring purposes.

c. Representativeness is a little more difficult to guarantee. Representativeness means that the species in the community being sampled occur within the sample collection in direct proportion to their relative occurences in the area being sampled. Since the pupal exuviae collection technique relies upon finding and collecting exuviae in areas where stream currents have naturally accumulated the exuviae it is assumed that exuviae of different species accumulate in these areas in direct proportion to their emergence frequency. Since the collector does not have to choose specific areas of stream to sample from, but rather attempts to pan and sieve exuviae from the natural accumulation points, there is little or no bias resulting from collector activities.

d. Relative to d-net or kick-net sampling the collection of floating pupal exuviae should result in much greater representativeness of species in the sample collection. Both d-net and kick-net sampling require that the collector select the area to be sampled. Of course it is recommended that a conscious effort be made to sample all microhabitats, and this should result in increased "representativeness" of species in the sample collection. However in order to have high representativeness the microhabitats that are sampled must be sampled in direct proportion to their frequency of occurence at the sample site. This is rarely attempted, and probably never achieved.

e. Because representativeness requires that species occur in the sample collection in the same frequency as they occur in the area being sampled, the collecting efficiency of the sampling device is also a critical factor. D-nets and kick-nets with large mesh sizes consistently underestimate smaller size classes of most aquatic insects. They will also result in underestimates of species richness if the the benthic community being sampled contains small species. These two factors further complicate the representativeness aspects d-net and kick-net samples, but do not come into play when pupal exuviae samples are collected and concentrated using a U. S. Standard Testing Sieve with fine mesh.

7. <u>QUESTION</u>: Intuitively it seems logical that pupal exuviae samples have high representativeness, but what experiments have been done to test this assumption?

a. In order to test the representativeness of a pupal exuviae sample one would have to take a standard sample from a defined stretch of stream, then collect all the remaining exuviae in the same stretch of stream. By adding the counts of the two samples together a census of the total species richness and relative abundances of all the emerging species would be determined for the stretch of stream being investigated. One would then have to compare the estimates of species richness and relative abundances of individual species in the standard collection with the census data. This, of course, cannot be done and other more practical experiments must be constructed to draw inferences about representativeness of pupal exuviae samples.

b. Sample representativeness can be decomposed into two distinct components, and these two components can be measured in the field. The components of sample representativeness are sample efficiency and sample efficacy. Sample efficiency is defined as the ability to detect species that occur in the community being sampled. Sample efficacy is defined as the ability to consistently produce the sample results.

c. In field experiments sample efficiency has been tested by comparing the number and types of species collected in pupal exuviae samples versus the combined numbers and types of species collected in both pupal exuviae and bottom samples for larvae. The sample efficiency is defined as the number of species collected in a pupal exuviae sample divided by the combined total number of species collected as pupal exuviae and larvae from the same sample site, times 100 to express the value as a percent.

d. Two important trends result from the tests of sample efficiency for collections of pupal exuviae. These trends indicate that sample efficiency is not a constant, but varies in a predictable manner.

e. Trend number 1 relates sample efficiency to stream size. The experimental design and results are presented in more detail in the appendix to this SOP, but quickly summarized, the estimated efficiency of pupal exuviae samples increases from first to third order streams. Exuvial collections were only 54% efficient in a spring-fed first order stream with a maximum width of one meter, but were 92% and 94% efficient for third order streams with widths of 4 to 8 meters.

f. Trend number 2 deals with the relative efficiency of pupal exuviae collections for detecting species as species richness increases. The experimental design and results of this experiment are also presented in the appendix, but what was seen is that pupal exuviae efficiency increases as species richness increases. At the extremes, pupal exuviae collections were between 50% and 100% efficient when 10 or fewer midge species occured at a given site. For sites where 11 or more species were collected the pupal exuviae collection efficiency varied from 81.9% to 100%.

g. The efficiencies of benthic sampling for midges can be calculated in a similar manner, and they too are not constant with regard to stream size and midge species richness. The experimental results show that efficiency declines from first to third order streams and also with increasing species richness. Benthic sampling was 87% efficient in the first order stream, but declined to 57% in the third order streams. Benthic sampling efficiency ranged from 33.3% to 85.7% when 10 or fewer species were detected, but dropped to 23.1% to 69.2% when 11 or more species were present. While neither sampling approach is 100% efficient, it is obvious from these results that in the larger streams where most impact assessment activities are performed the pupal exuviae collections are more efficient.

h. Sample efficacy, or the ability to produce the same result, was tested in two different ways. One test of efficacy was to determine how the estimated emergence frequency of <u>Chironomus riparius</u> varied as successive samples were taken at a given sample site. <u>Chironomus riparius</u> is a good indicator of severe enrichment by STP effluents, and can be readily distinguished and counted in the pupal stage. For this experiment a single dip was considered to comprize a sample, rather than the 10 minute timed effort that has been suggested in this SOP. A single dip is considered to be the smallest "sample" that can be taken in a consistent manner. By taking many small samples it was possible to process considerably more samples and obtain greater statistical precision in emergence frequency estimates for C. riparius.

i. The experimental design and results are presented in more detail in the appendix of this SOP. It was found that 12 single dip samples provided an estimate of emergence that was within ± 8% of the final value obtained after 20 single dip samples were taken. In practice one can usually collect 20 dips or more in 10 minutes, therefore it was concluded that high efficacy would be obtained in 10 minutes sampling for the stream in which the experiments were performed. Equally as important, it was observed that the standard error of the emergence frequency estimate did not change substantially after 8-10 samples. Therefore it follows that sample efficacy, as defined as the ability to accurately determine the emergence frequency of Chironomus riparius, will be high when 20 or more single dips are combined into a single 10 minute "sample".

j. A second test of sample efficacy was to determine the ability of pupal exuviae samples to produce the same species richness estimate at the same site, but when samples were taken on 2 different sample dates, and from two different sides of the stream at a given sample site on the same day. These experiments were conducted on Indian Creek, 16 July 1987 and 21 August 1987. Norm Crisp and Franz Schmidt assisted with the field collection and data analysis. The experimental design is not included in the appendix but, briefly, consisted of taking one standard 10 minute pupal exuviae sample and one standard 10 minute d-net sample from the stream at a site near Leawood Park on 16 July, and one set of pupal exuviae and d-net samples from each of the north and south banks at the same site on 21 August.

k. Species richness estimates for pupal exuviae samples were 12, 12 and 15 respectively for 16 July, 21 August (North bank) and 21 August (south bank). The corresponding values for species richness based upon midge larvae and pupae collected by d-net were 9,9 and 8. In both sampling approaches the sampling efficacy was relatively high.

l. Based upon the analysis of sample efficiencies and sample efficacies it can be concluded that the representativeness of pupal exuviae is high. It is possible, of course, that there is some internal sample bias that has not been measured by the experimental designs, but since 2 of the 3 experiments that were conducted required comparisons with benthic sampling it seems unrealistic to expect that the same bias would be present in both sampling approaches. It can also be concluded that the efficiency and efficacy components of sample representativeness for pupal exuviae samples are at least equivalent to or exceed those obtained for d-net or kick-net samples.

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8. QUESTION: Can collections of floating pupal exuviae be used in a general sense to predict the responses of other benthic groups to a given type of pollution?

a. This question is simply another way of asking if a taxocene approach can be used to predict overall impact responses of the benthic community. In the recent literature one finds both yes and no answers to whether Chironomidae can be used effectively in a predictive sense for enrichment and heavy metals pollution. There are several potential reasons why the opposing viewpoints have been voiced: some reasons may be habitat related, others related to sampling efficiency, problems with level of identification, life stages collected, time of sampling, etc. The best way to answer this question is to recount the empirical data on hand for selected streams in Kansas and then judge whether pupal exuviae samples are likely to work in a broader spectrum of stream types in this region.

b. For streams that are severely enriched by STP effluents one sees a characteristic Chironomidae fauna and a drastic decrease in or total loss of Plecoptera, Ephemeroptera and Trichoptera species. The midges that are characteristic of the enriched sections of stream are *Chironomus riparius*, *Cricotopus bicinctus*, *Psectrotanypus dyari*, *Chironomus decorus* grp. sp., *Polypedilum illinoense* and *Goeldichironomus holoprasinus* (Coler, 1984; Ferrington, unpublished data. Also see Beck, 1977; Gower and Buckland, 1978; Oliver, 1971; Pinder, 1986). Other species may be represented, but only in very low frequencies. Species richnesses are generally substantially reduced, and in third to fourth order streams may be 20-33 species or lower (0-5 species in extreme cases), versus 70-80 species for streams of similar size but with good water quality. Species composition and relative abundances in the enriched streams are shifted to the Chironomini as opposed to the Orthocladiinae in unimpacted streams. The changes are predictable and readily measured. Based upon these characteristics it is concluded that collections of floating pupal exuviae provide a rapid mechanism to predict overall community responces by benthic taxa to severe enrichment.

c. Research conducted in the lead/zinc mined areas in and around Galena in Cherokee County, Kansas suggests that similar conclusion can be drawn regarding the ability of exuvial collections to provide an index of overall benthic responses. There is not sufficient empirical data to assess if exuvial collections would be appropriate for other types of pollutants such as VOC's or herbicides/pesticides, but it is expected that the approach would be at least as workable as using any other taxocene of benthic organisms to predict overall benthic responces.

d. There two distinct advantages to using Chironomidae to predict community responses. The first advantage is that midges tend to become increasingly dominant in terms of percent taxonomic composition and terms of relative abundance, as one moves along a gradient of increasing enrichment or heavy metals concentrations. This allows one to make distinctions among streams that are both heavily impacted, but differ in absolute amount of the pollutant. In cases of enrichment, for example, Plecoptera and Ephemeroptera are extirpated very early along the enrichment gradient and cannot be effectively used to rank or evaluate two or more streams or sample sites that are further along the enrichment gradient. Because of their total absence from the sample sites no further distinctions can be made unless information from other taxocenes is used. Generally, however, one finds midges that occur along most, if not all, of the enrichment gradient, and their compositional patterns can be used to distinguish between all but the most severly impacted sites.

e. The second advantage of using Chironomidae is that they invariably comprise a large proportion of the total aquatic insect species in all types of lotic habitats. For Kansas streams, research estimates vary from 55% to 70% of the benthic insects for streams with high water quality (Ferrington, unpublished data). Estimates of Chironomidae composition for sample sites on enriched streams vary from 82.7% for Mill Creek in Johnson County (Coler, 1984), to 46.6% to 77.8% for sites on Indian Creek, also in Johnson County, Kansas. At severely enriched sites in other streams all aquatic insects but midges have been extirpated. Because of the large percentage of midge taxa in most lotic habitats there is a high amount of autocorrelation when attempting to predict responses of community species richness based upon Chironomidae species richness. Chironomidae species richness should then function well as a predictor of overall community responses as judged by species richness criteria.

9. <u>QUESTION</u>: How well do species richness estimates based upon collections of pupal exuviae correlate with overall midge species richness estimates and with estimates of total macroinvertebrate richness?

a. Until this summer there were no empirical data that could be used to evaluate this question for Kansas streams. However, as a result of a reconnaissance study of surface water quality in the Dry Turkey Creek watershed near McPherson in McPherson County, Kansas and an intensive study of surface water quality in Indian Creek, Johnson County, Kansas, it was possible to generate limited data that provide some insight into these questions. Pupal exuviae and d-net samples were collected in July and August from sample sites on Bull Creek, Dry Turkey Creek, Turkey Creek, Indian Creek and Tomahawk Creek. Sites were selected so that good to very poor water quality conditions were represented. Each pupal exuviae and d-net sample was collected in a consistent manner for 10 minutes. Samples were sorted and identified in the lab and species lists were generated and reconciled for each approach at each site. A total of 85 aquatic insect taxa were collected in 25 pairs of samples from the sites. Thirty-six of the taxa were Chironomidae. The total number of insect taxa and the total number of midge species per site were determined from the reconciled list for both of the samples. Three correlation coefficients were calculated using the PROC CORR program in the SAS Statistical Package. One R-value was determined for the species richness estimates based upon pupal exuviae samples versus the species richness estimates for midges based upon d-net samples. The second R-value was determined for midge species richness estimates of the d-net samples versus the species richness estimates for all benthic taxa. The third R-Value was calculated for the midge species richness estimates based upon pupal exuviae samples versus the richness estimate for all benthic taxa.

b. The correlation between richness estimates for midges based upon pupal exuviae and d-net samples was R= 0.501 ($p<.01$, $n=25$). The R-squared value, which represents the amount of variation explained, is 0.25 or 25%. The R-value for richness estimates of pupal exuviae and total benthic richness estimates was R= 0.837 ($p< .0001$, $n=25$, R-squared=.70). The R-value for richness estimates based upon midge species in d-net samples versus total benthic richness was R= 0.629 ($p< .0008$, $n=25$, R-squared= .396). Based upon these results it can be concluded that Chironomidae species richness is statistically significantly correlated to total benthic community richness. A stronger correlation exists between the species estimates derived from pupal exuviae collections and the total benthic richness estimates than between the estimates derived from the d-net sampling approach, however both function satisfactorily for the sample sites in the streams examined this past summer.

c. The correlation between the species richness estimates based upon pupal exuviae samples and the richness estimates based upon d-net samples requires further comment. It was expected that this correlation would be low, and may not be statistically significant. The reasoning was as follows: The pupal exuviae samples only collect specimens of the portion of the midge community that are currently completing the larval portion of their life cycle and are in the process of adult emergence. One would expect that at any given point in time there would be additional species of midges that would be present in the community but not at a point in their life cycle where emergence activities would have commenced. These taxa would show up in the d-net samples, but not in the pupal exuviae samples. Taxa that are in the process of emergence would show up in both of the samples; as mature fourth instar larvae and pupae in the d-net samples and as exuviae in the pupal exuviae samples. One would therefore predict that species richness estimates for d-net samples would equal or usually exceed the species richness estimate based upon the pupal exuviae samples.

d. Results of the studies conducted this summer do not support these predictions. The estimate of species richness for d-net samples was less than the estimate based upon the pupal exuviae samples in 22 of 25 sets of samples that were collected. In several instances the d-net samples revealed 5 or fewer taxa while the corresponding pupal exuviae samples detected 10 or more taxa. Although sample redundancy values have not yet been calculated for these samples, it is expected that the degree of redundancy is significantly greater for the d-net samples.

e. These results were totally unexpected so the specimens were analyzed in more detail and it has been possible to determine why the d-net samples result in lowered species richness estimates. The taxa that showed up in the d-net samples were taxa that are large when mature. Furthermore, the specimens of the taxa that show up consistently in d-net samples are primarily fourth instar larvae, and many of the specimens exhibit the swollen abdomen and signs of developing pupal structures that are characteristic of the late fourth instar or pre-pupal stages of larval development. In other words, d-net samples only consistently collect larvae of midge taxa that are large and about ready to pupate. At the populational level most of the taxa will have some individuals that have already emerged and these taxa are detected in the pupal exuviae samples. Smaller species of midge are not, or only infrequently, retained in the d-net samples and are either missed or underestimated. Even if they are mature larvae, or if the population is currently in the process of emerging, these taxa are not detected in a representative manner by the d-net sampler, but are often detected by the pupal exuviae sampling. For instance, one of the most cdmmon taxa that was found to be emerging at the sample sites investigated this summer was Nanocladius. Although represented by several hundred specimens in the pupal exuviae samples, this taxon was not collected in any of the d-net samples. Other small taxa that were missed or underrepresented in the d-net samples were Corynoneura, Thienemanniella, Parakiefferiella, Harnischia, Tanytarsus, Eukiefferiella, Tvetenia, and Paratanytarsus. It is also possible that incomplete sampling of all available microhabitats by the d-net sampler contributed to the disparity in species richness estimates among the two sampling methods.

10. QUESTION: How difficult is it to identify pupal exuviae?

a. Currently, it would be fair to say that the average practicing benthic biologist is more adept at larval identification than pupal identification. The reason for this is historical, and largely influenced by past emphasis that has been placed on d-net or kick-net sampling for impact assessment. Until recently there were not any comprehensive identification guides for pupae, and this is also partly to blame. Well illustrated keys to the genus level for pupae recently published by Coffman

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and Ferrington (1984) and Wiederholm (1986) have made identification attempts much easier. It is the opinion of the author that identification of pupal exuviae to the genus level is much easier for pupal exuviae than for larvae, especially within the Tanypodinae, Tanytarsini and Orthocladiinae. An example to illustrate this point is in the Thienemannimyia group and related genera of the Pentaneurini (Tanypodinae). Larvae of Thienemannimyia, Conchapelopia, Meropelopia, Helopelopia, Hayesomyia, and Telopelopia are very difficult to identify even to genus level in the larval stage. They are very commonly collected in many lotic habitats, and several species usually co-occur in the same samples. Larvae must be slide mounted, measured and the setae of the maxillary palp examined in order to facilitate identification of fourth instar larvae. Earlier instar larvae may or may not be identifiable to genus. In Contrast, genus level identification of pupae and exuviae can be readily accomplished using a dissecting microscope, and most specimens can be accurately identified to species without mounting or with only temporary mounting and inspection under low magnification using a compound microscope.

11. Question: How do the diversity values for pupal exuviae and d-net or kick-net samples compare?

a. This is another area for which the first evidence was collected during the past summer. Based upon the Brillioun Index values for information content of the collections it was observed that d-net samples had higher H' values than the pupal exuviae samples (T= 3.01, p=.0067, n=23). The pattern of the diversity index (DI) from station to station was very similar, however, with high DI for d-net samples being coupled with high DI values for the corresponding pupal exuviae samples and low DI values for d-net samples coupled to low DI values for the corresponding pupal exuviae samples. A few discrepancies occurred, but the differences were usually not great and probably represent sampling error effects.

b. Perhaps more importantly, a relationship was found between the amount of time required to sort and identify a sample and the resulting DI value. It was determined that samples that took longer to sort and identify generated higher DI values. Normalizing the DI's for time spent sorting and identifying, it was found that pupal exuviae samples yielded more information per unit of time than d-net samples (T= -3.46, p= .0032, n=23). This suggests that since the pattern of DI values for both sampling methods is similar across sample sites (even though the magnitudes differ), collections of pupal exuviae will provide the same level of site to site resolution with a smaller time committment. Alternatively, for a given amount of time committed, more pupal exuviae samples can be processed, thus providing a greater number of sample sites to be evaluated and generating greater longitudinal resolution of the responses by the midge community through out a stream system.

12. Question: How long does it take to sort a standard 10 minute pupal exuviae sample?

a. This question is best answered by comparing the time required to sort and identify a pupal exuviae sample versus a d-net sample. For a given sample, either d-net or pupal exuviae, there will be several factors that influence the sort and identification time. These factors include the amount of detrital material in the sample, the number of specimens collected, the size of the specimens collected, the difficulty in identification of the specimens, the identification skills of the person performing the identifications, etc. Although no attempt has been made to sort out all of these variables, the average time and range of times required to sort the 25 paired d-net and pupal exuviae samples collected this summer were determined.

b. D-net samples took an average of 121.5 minutes to sort and the range of sort times was from 30-300 minutes. Identification time averaged 68.6 minutes and ranged from 10-185 minutes. On the average the time required to sort and identify the average 10 minute d-net sample was 190.1 minutes.

c. Sample sorting and identification was accomplished concurrently for pupal exuviae samples. The average time to sort and identify the specimens in a sample was 52.7 minutes, and ranged from 12-115 minutes. 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.

d. Estimates of sorting time versus size for a second study are included in graphical form in the appendix. These data were generated by an inexperienced individual after receiving 20 minutes of instruction on how to sort samples. This individual was shown the exuvium of *Chironomus riparius* and was asked to count this species and determine its emergence frequency in each sample. No other species in the sample were identified by the individual, thus the graph shows the time required to sort and estimate the frequency of a midge species that has been shown to be an accurate indicator of organic enrichment. This allows one to determine the magnitude of effort that could be required to detect excessive enrichment based upon a given number of samples with a range of sizes.

13. <u>QUESTION</u>: Since the pupal exuviae sample procedure requires that Chironomidae are emerging in order to generate samples, how can the methodology be used in the winter when no emergence is occuring?

a. Empirical data that has been generated shows that there are species of Chironomidae in Kansas that develop as larvae in the stream and emerge during winter months (Ferrington, unpublished data). These hibernal taxa occur in streams that are of good to excellent water quality and are extirpated from streams that are severly enriched by domestic sewage effluents. Taxa included among the hibernal emerging groups are species of <u>Hydrobaenus</u>, <u>Oliveridia</u>, <u>Orthocladius (Euorthocladius)</u>, <u>Orthocladius (Orthocladius)</u>, <u>Chaetocladius</u>, <u>Diamesa</u>, <u>Potthastia</u>, <u>Sympotthastia</u>, <u>Cricotopus</u>, <u>Eukiefferiella</u> and <u>Diplocladius</u>. Collections of pupal exuviae can therefore be used in water quality investigations during winter months. In practice, however, not many evaluations are conducted during this time of the year.

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APPENDIX

RELATED RESEARCH

Efficiency, Efficacy and Economy of Using Pupal Exuvial Collections in Water Quality Assessments

In evaluating the efficiency, efficacy and economics of pupal exuviae collections it is necessary to compare this method relative to the more traditional approach of collecting benthic samples. Three basic questions need to be answered. These questions are:

1. Efficiency: How effective are collections of pupal exuviae in resolving species composition relative to larval collections of Chironomidae?

2. Efficacy: How consistent are individual collections in determining relative emergence composition?

3. Economy: How costly is the methodology relative to larval collections, given identical standards of efficiency and efficacy?

Research that I have performed to date has been directed toward answering these questions with regards to the pupal exuviae approach. Some of the original data from these studies are included in Tables 1 through 4 and Figures 2 through 6, which are discussed in the sections that follow.

Efficiency of Using Chironomidae Exuvial Collections in Water Quality Assessments

Table 1 shows the results of a study performed within a small watershed in southwestern Pennsylvania. Five sites were established on streams of differing order as follows: Site I - first order, maximum width of one meter; Site II - second order, maximum width 2 meters; Site III - third order, maximum width 4 meters; Site IV - third order, maximum width 8 meters; Site V - second order, maximum width 2 meters. Samples of larvae and pupal exuviae were taken concurrently at approximately monthly intervals for a period of one year. Identifications of larvae by Leonard Ferrington and pupal exuviae by William P. Coffman were performed independently. After all identifications were completed species lists were reconciled for each site and a master list compiled based upon both sampling approaches. The data were then summarized according to sample site and sampling approaches at both the species level (Table 1a) and the generic level (Table 1b). The relative efficiency of each sampling approach was calculated as the number of species detected by the given approach, divided by the total number of species found using both approaches.

As can be seen from the table, the highest efficiencies for larval sampling were obtained in the first order stream (87% of species detected, 85% of genera detected). The efficiency of larval collections then declines with increasing stream order (or overall size), being 66% or 61% efficient at the second order sites in detecting species, and 57% efficient in species detection at the third order sites. Summarized at the generic level, the corresponding efficiencies are 79% and 65% (second order) and 64% and 60% (third order).

TABLE 1. Results of year long inventory of Chironomidae of Bear Run Nature Reserve, Fayette Co., PA. Table 1a shows total number of species collected at each of five sites and the number of species and corresponding efficiencies for substrate and pupal exuviae collections. Table 1b shows the same values for numbers of genera collected.

TABLE 1a. Species Collected

Site	Surber	Exuviae	Total
I	27(87%)	17(54%)	31
II	34(66%)	44(86%)	51
III	50(57%)	83(94%)	88
IV	51(57%)	82(92%)	89
V	52(61%)	69(81%)	85
TOTAL	74(56%)	128(97%)	132

TABLE 1b. Genera Collected

Site	Surber	Exuviae	Total
I	22(85%)	16(62%)	26
II	27(79%)	26(76%)	34
III	33(60%)	52(95%)	55
IV	30(64%)	46(98%)	47
V	33(65%)	45(88%)	51
TOTAL	47(64%)	72(97%)	74

In contrast, collections of pupal exuviae were least efficient at the first order site, being 54% efficient at detecting species and 62% at detecting genera. These efficiencies shift to 86% and 81% (second order) and 94% and 92% (third order) for species detection, and 76% and 88% (second order) and 95% and 98% (third order) at the generic level.

Summarized for the entire watershed, collections of pupal exuviae were 97% efficient at detecting both species and genera, whereas larval collections were only 56% and 64% efficient, respectively. Thus it can be concluded that for equal field effort expenditures, the collection of pupal exuviae is a much more efficient method of gathering information about Chironomidae in streams that are of the size in which most biomonitoring efforts are concentrated.

The efficiencies of pupal exuviae collections and d-net collections relative to the species richness of Chironomidae was determined for two stream systems in Kansas during summer of 1987. A total of 25 sites were investigated in the Dry Turkey Creek watershed in McPherson County, Kansas and the Indian Creek watershed in Johnson County, Kansas. A pupal exuviae sample and a d-net sample was taken of each site. Each sample was sorted and the Chironomidae specimens identified, and a species total based upon both collecting methods was determined. The relative efficiencies of both collecting methods were calculated by determining the percentage of total taxa that were collected by the respective method. Table 2 shows the relative efficiencies versus total species richness values.

Water quality at individual sample sites varied from poor to good and the corresponding total numbers of species collected reflected water quality conditions. Total species collected per sample site ranged from 2 to 16. Several sample sites had the same number of species, and provide 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.

TABLE 2. 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	Relative d-net collection efficiency	Number collected as pupal exuviae	Relative pupal exuviae efficiency
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	26.7%	15	100.0%
16	7	43.8%	15	93.7%

D-net samples were less efficient at detecting chironomid species as overall species richness increased (Table 3). At sample sites with species richness 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.

TABLE 3. Average efficiencies of d-net samples and pupal exuviae samples versus ranges of species richness

Total midge species detected	Number of collections	Average d-net efficiency	Average pupal exuviae efficiency
2-5	4	65.0%	70.0%
6-10	7	57.2%	79.4%
11-14	11	47.0%	91.8%
15-16	3	45.7%	97.9%

Pupal exuviae samples were more efficient at detecting species as species richness 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.

Efficiency of Using Chironomidae Pupal Exuvial Collections in Water Quality Assessments

With regard to the question of efficacy of collections of pupal exuviae, the results of a series of experiments are summarized in Figure 2 through Figure 6.

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 the approach to answering these questions, the standard field procedures are detailed in the following paragraphs.

Field sampling 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 is filled with water, the contents are then passed through a U.S. Standard Testing Sieve with an aperture of 125 microns. 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.

Using this field sampling approach, the smallest "sample" that can be consistently taken is a single dip. In practice, however, most researchers have taken many dips and accumulated them 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.

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. 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.

Figure 2 is a histogram of the number of exuviae collected per single dip sample. 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.

Figure 3 is a histogram of the time (in minutes) required to sort the samples. The minimum time required to sort a sample was 2 minutes and the maximum time required was 80 minutes. Ninety percent of the samples were sorted in 30 minutes or less per sample.

Figure 4 is a graph showing the time required to sort a sample versus the number of exuviae per sample. The x-axis units represent approximate ten percentile intervals (i.e. ten percent of the samples had 0-2 specimens, ten percent 3-4 specimens, etc.). Each dot represents an average of time spent sorting samples of the given size on each of the three sample dates.

Figure 5 shows the effects 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. In fact the value calculated based upon 12 samples was with $\pm$ 8% of the value calculated after 20 samples for all three sample sites on all three dates.

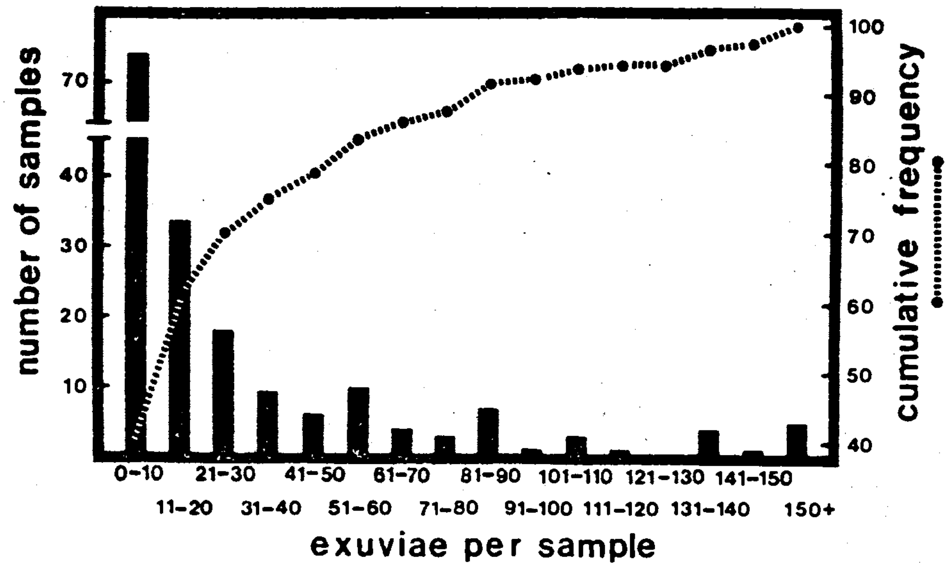

Figure 2. Histogram of number of exuviae per sample for 180 single dip samples taken from Mill Creek, Johnson Co., KS on three sample dates during summer of 1983.

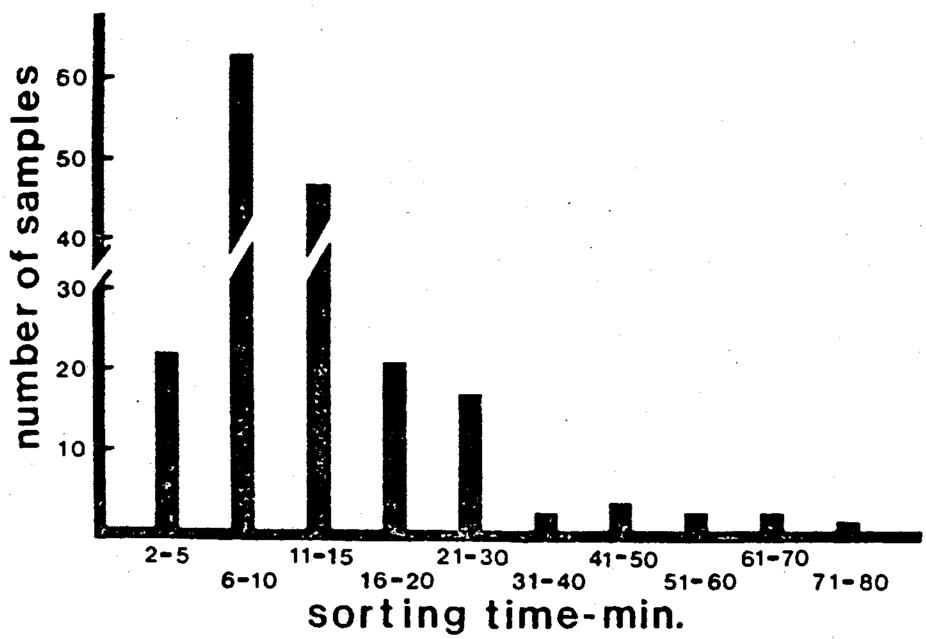

Figure 3. Histogram of time (in minutes) required to sort 180 single dip samples taken from Mill Creek, Johnson Co., KS on three sample dates during summer of 1983.

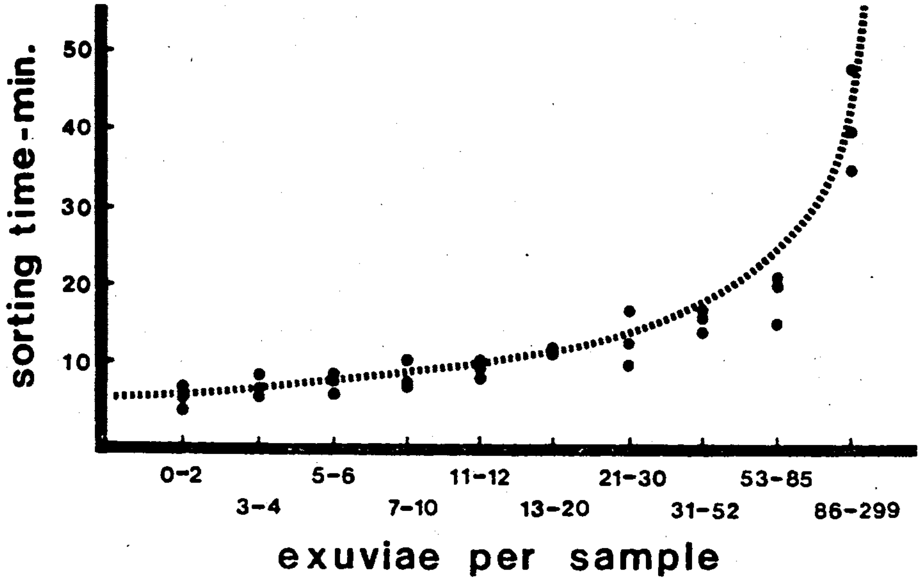

Figure 4. Relationship between number of exuviae per sample and time (in minutes) required to sort sample. Each point on graph represents average time to sort samples of indicated size range for each of three sample dates.

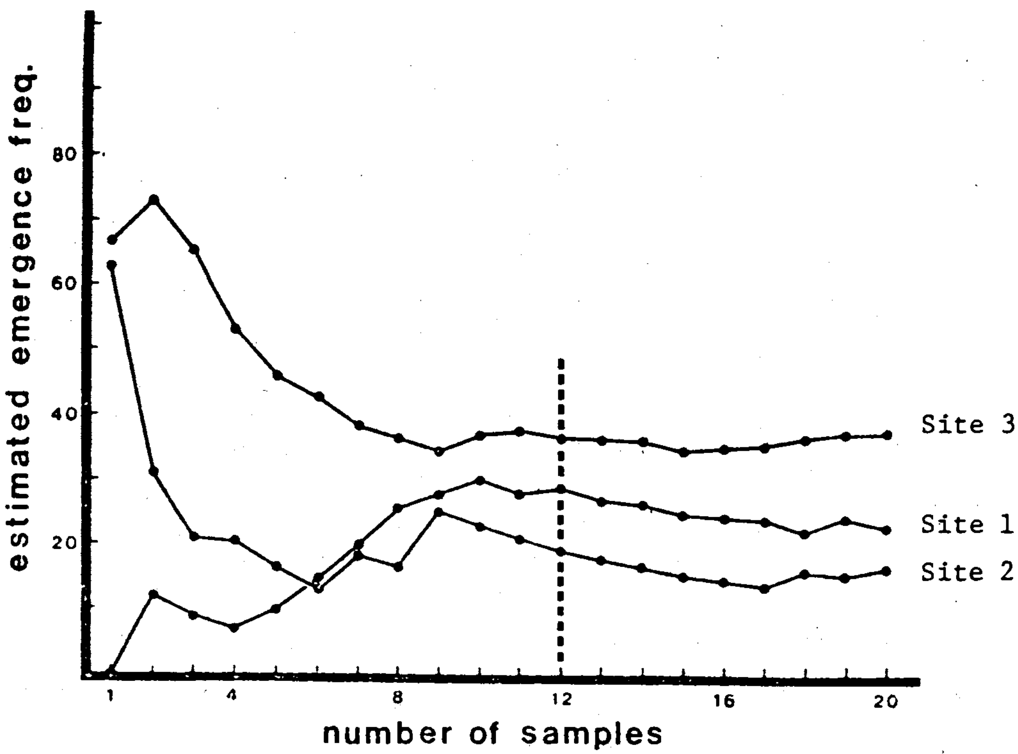

Figure 5. Graph showing the effect of increasing the number of samples on the estimated emergence frequency of <u>Chironomus riparius</u> from each of 3 sites on one sample date. Points on graph indicate average of indicated number of samples. Points for individual sample sites connected by solid line.

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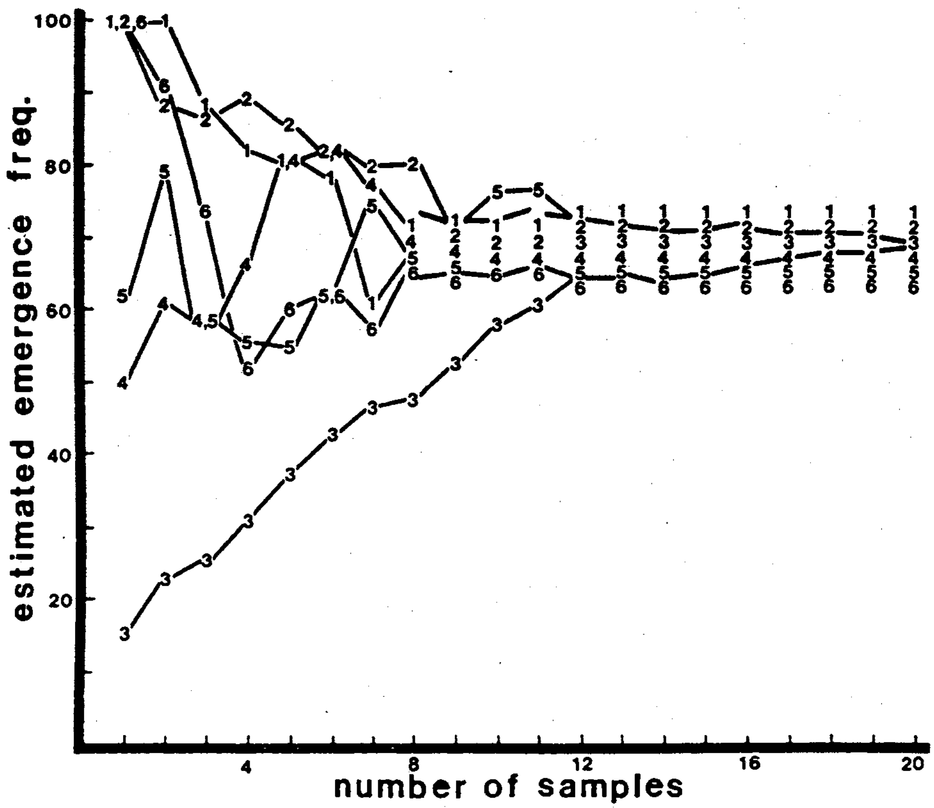

Figure 6. Results of 6 randomized simulations of the estimated emergence frequency of Chironomus riparius at one site on one sample date. Numbers indicate estimate of emergence frequency for a given simulation. In each simulation the 20 samples were averaged in random manner without replacement.

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Figure 6 shows the calculated emergence frequency estimates of *C. riparius* 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 of *C. riparius* is relatively stable.

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 is achieved. Approximately 12 samples are required for a precision of ± 8%. If 90% of the samples collected required 30 minutes or less to sort then 90% of the time it will require 360 minutes or less of sorting time to achieve a target precision value of ± 8% for emergence of *C. riparius* in Mill Creek.

Economy of Using Chironomidae Pupal Exuvial Collections in Water Quality Assessments

I do not have a comparable data set for the time required to achieve a similar degree of precision based upon collection of larvae, but based upon past experience with benthic samples I predict that it would require many more benthic samples than pupal exuviae samples in order to achieve similar degrees of precision.

In certain preliminary assessments of potential 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 specimens 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 minutes at each of 20 sample sites during the summer of 1987. 1 The sample sites were included in the Dry Turkey Creek watershed in McPherson County, Kansas and the Indian Creek watershed in Johnson County, Kansas and included stretches of stream with poor to good water quality. The processing time are given in Table 4.

TABLE 4. Time required to process 20 paired d-net and pupal exuviae samples. Each sample represents 10 minutes of field sampling effort. All sorting times given in minutes.

Total species detected	Sorting times for d-net samples	Identification times for d-net samples	Sorting & identification times for pupal 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
15	120	43	108

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

D-net samples took an average of 121.5 minutes to sort and the range of sort times was from 30-300 minutes. Identification time averaged 68.6 minutes and ranged from 10-185 minutes, excluding one sample that contained only larvae of Chironomus riparius, which were identified as they were sorted. On the average the time required to sort and identify the average 10 minute d-net sample was 190.1 minutes.

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 minutes, ranged from 12-115 minutes. 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.

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 within d-net sampler.