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Midge Larvae (*Diptera: Chironomidae*) as Indicators of Postmortem Submersion Interval of Carcasses in a Woodland Stream: a Preliminary Report

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ABSTRACT: Data on colonization of rat carcasses by aquatic insects in riffle and pool areas of a small woodland stream were obtained to elucidate patterns potentially useful for determining the postmortem submersion interval of corpses in flowing water habitats. After 39 days, the carcasses had no visual signs of deterioration in the absence of large scavenging animals. Midge larvae (*Diptera: Chironomidae*) were the dominant insects colonizing the carcasses. No patterns in numbers of larvae over time were evident, but the diversity of genera increased after 29 days in the riffle. Also, *Orthocladius* larvae did not begin to colonize the carcasses until after 13 days of submersion in the riffle and after 20 days of submersion in the pool. Although separated only by 20 m, the riffle and pool rats had dissimilar faunal assemblages. This suggests that different indices for determining the postmortem submersion interval of corpses based on midge larvae colonization should be developed for these two habitats. This investigation does not provide replicated data, but does shed light on what may happen to mammalian carcasses placed in a stream at a particular time of the year.

KEYWORDS: forensic science, forensic pathology, forensic entomology, chironomidae, aquatic insects, submersion interval, streams

The morbid remains of a corpse in a terrestrial setting often contain the components of a story elucidating its death and subsequent postmortem history. These components are members of the arthropod community inhabiting the body. The time between death and corpse discovery, or postmortem interval (PMI), can be determined by an investigation of the composition and age of this invertebrate assemblage (1–3). Dipteran larvae frequently are the principle component of the assemblage inhabiting vertebrate animals (including humans), and they tend to be the focus of the forensic entomologist in most scenarios (4). Although criminal investigations of corpses found in terrestrial settings are consistently assisted by arthropod data, no comparable data are available to determine how long a corpse has been submerged in a body of water.

A review of the literature found few studies pertaining to dead bodies found in water (5). Pig carcasses exposed to the invertebrate fauna of a freshwater lake were colonized by 20 insect species, mites (Acari), and amphipods (*Crustacea: Amphipoda*) (6). Other investigators who placed mammalian carcasses in aquatic environments only observed the terrestrial insects (e.g., blow flies [*Diptera: Calliphoridae*]) associated with the body after it bloats and rises to the surface due to gasses created by bacterial metabolism (7). Oviposition by terrestrial flies does not occur on a completely submerged carcass. It has been suggested that there is a potential for the use of algae, sediment, and aquatic insect larvae for determining the submersion interval (5). In this study, we placed euthanized adult rats in a riffle and pool of a northeastern Ohio stream. Our objective was to describe any patterns of benthic macroinvertebrate colonization or utilization potentially useful for helping determine the postmortem submersion interval (PMSI) of a corpse found in a running water environment. We define macroinvertebrates as organisms belonging to the kingdom Animalia which lack backbones and are large enough to be seen with an unaided eye.

Methods

The study was conducted in Bixon Creek, a third order stream that flows into Michael J. Kirwan Reservoir in West Branch State Park, Portage County, Ohio. The study section of Bixon Creek (41° 07′ 11 N, 81° 09′ 31 W) flows through a secondary growth beech/maple forest (*Fagus grandifolia/Acer rubrum*), has fluvial substrate, and is relatively unimpacted by man. A diverse fauna of aquatic insects (over 40 genera) is known to inhabit the stream. A riffle and pool approximately 20 m apart were chosen as our sampling stations.

Twenty adult rats, approximately 120 days old, were euthanized with carbon dioxide 24 h prior to the beginning of the experiment and refrigerated at 10°C. Two cages were constructed to hold the rats submerged in the stream, and to keep out scavengers such as large crayfish and raccoons. Each cage had a wooden frame, and was covered on all sides and bottom with coarse screening (1.25 cm mesh) (Fig. 1). The top of each cage was covered with $^1/_4$ in. (6 mm) thick plywood, and hinged to allow access to the rats. The upstream-facing side of each cage was sloped 45° to prevent debris from building up. Ten rats were placed in both cages with each rat individually tied to the bottom with string to permit removal of individual rats without disturbing the others. One cage was placed in the riffle and the other in the pool such that the rats were completely submerged. The front of each cage was weighted down with 3 stream rocks approximately 10 cm in diameter which were scrubbed clean of algae and invertebrates. Two 1.3 m lengths of iron rebar were weaved through the screen on each side of both cages, and were pounded into the substrate to hold the cages in place.

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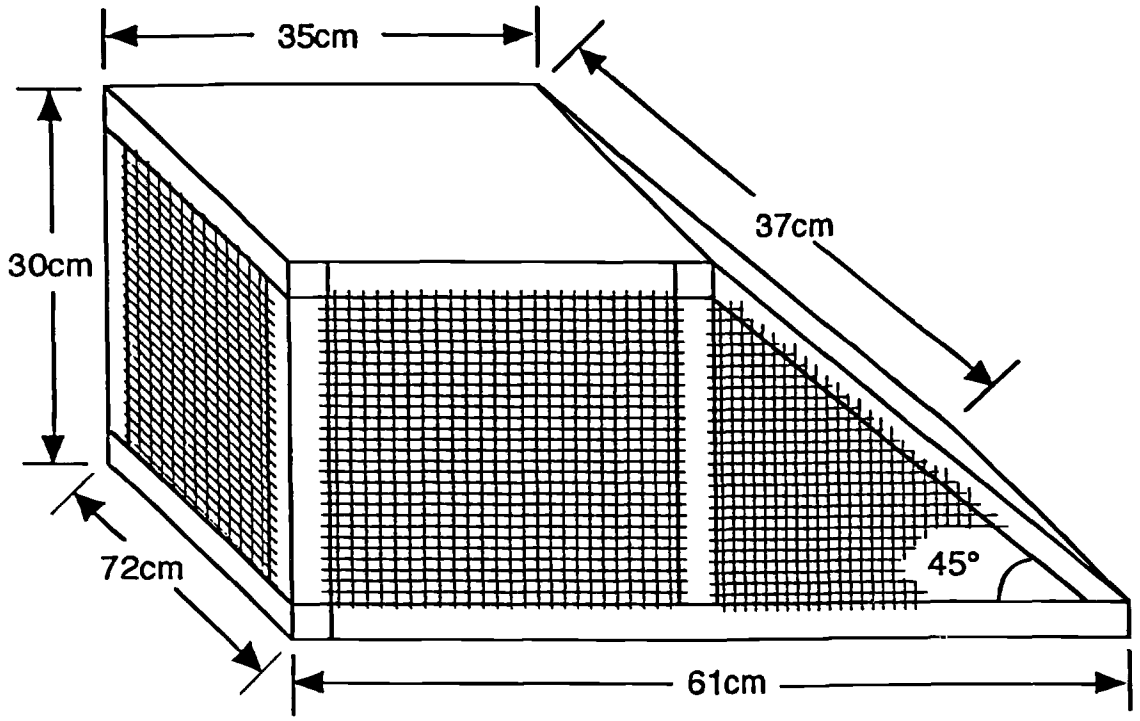

FIG. 1—*Dimensions of the cages in which carcasses were placed during submersion.*

Cage placement occurred on 20 March 1995. One rat was removed from each cage after 3, 6, 9, 13, 16, 20, 24, 29, 34, and 39 days. Collection was done by opening the top of the cage, placing an aquarium net (1 mm mesh) immediately downstream of the rat, cutting the tethering line, and lifting out the remains. The aquarium net prevented the loss of organisms dislodged during removal. Each rat was placed in a quart (0.946 L) jar containing 95% ethanol, and the net contents rinsed into the jar with a squirt bottle containing 95% ethanol. Within 24 h of collection, the alcohol was replaced with 70% ethanol for storage. During each collecting trip, water and air temperature, water depth, and weather conditions were recorded, and any buildup of debris on the front of the cages was cleared after removing the rats.

To remove invertebrates from a carcass, each rat was removed from its jar, held over a 0.5 mm mesh sieve, and rinsed with a strong stream of water. The ethanol remaining in the jar was then poured through the sieve and the jar rinsed. The contents of the sieve were examined for macroinvertebrates, which were removed and put into labeled 2 dram vials containing 70% ethanol. Each rat was then placed in a white-bottomed tray containing about 2 cm of water and examined externally for macroinvertebrates, as were all body openings.

All invertebrates were identified to genus. Midge larvae (*Diptera: Chironomidae*) were cleared and mounted on microscope slides for identification (8). A random subsampling was done as follows: For samples with 1–9 midges, all were mounted for identification; for samples with 10–20 midges, 50% were mounted; for samples with more than 20 midges, 20% were mounted. The subsample data were then extrapolated to reflect the actual number of midges in each sample.

To compare the midge fauna of the riffle and the pool, Sorenson's index of similarity (S) was used as follows:

$$S = 2C/(A + B)$$

where A is the number of midge genera in the pool, B is the number of midge genera in the riffle, and C is the number of genera common to both (9).

Results

After 39 days in the water, the rats were remarkably intact. No large scavenging organisms such as crayfish or raccoons had gained access to the rats. There was no evidence of boring, shredding, or other physical degradation of the flesh, although the hair sloughed off easily and the putrid odor of decay was present. Qualitatively, the rats showed a gradual increase in algal coverage over time. Rats collected after three and six days had little biofilm accumulation in contrast to those from day 39 which were virtually unrecognizable due to algal growth. Amongst the rats' hair and strands of filamentous algae were macroinvertebrates, but none were found in body cavities.

Midge larvae were the dominant taxa found. Of the 457 invertebrates collected from the 20 rats, only 10 were not midges. Indeed, most of the rats examined had nothing but midge larvae present with the exception of the riffle rat collected after 24 days of submersion upon which no invertebrates were found. Other taxa (e.g., planarians) were curiously absent. Therefore, we focused our efforts on developing a method of submersion interval determination based on midge larvae colonization. All midges were identified to the lowest taxon possible (Tables 1 and 2). Larvae of this family are often difficult to identify due to the small structures used in diagnosis (9). Despite this, we were able to determine 93% to genus. Sixteen genera were collected from the pool (Table 1) and 18 genera were collected from the riffle (Table 2). In each habitat, 12 genera were encountered on only one or two carcasses. No reliable species keys are currently available for larval Chironomidae.

The number of midge larvae per rat did not follow any noticeable pattern in the riffle (Fig. 2). With the exception of day 16, the total

TABLE 1—*Succession of midge genera colonizing the rats in the pool.*

Subfamily	Genus	Day 3	6	9	13	16	20	24	29	34	39	Total
Chironominae	*Microtendipes*							5				5
	Paratanytarsus										1	1
	Polypedilum									1		1
	Tanytarsus										1	1
Orthocladinae	*Brillia*								2	1		3
	Cricotopus		1						1			2
	Eukiefferiella				5							5
	Hydrobaenus		3	5	15	1	5	5	10	4	2	50
	Metriocnemus		3	5		2	5	5			2	22
	Orthocladius						20	10	12	5		47
	Parakiefferiella		4	5	5	3	5	10				32
	Thienemanniella		1									1
Tanypodinae	*Conchapelopia*									1	1	2
	Meropelopia					1					1	2
	Natarsia									1		1
	Telopelopia								1		1	2
Undetermined		3	3		5				1	1	1	14
	Total	3	15	15	30	7	35	35	27	14	10	191

numbers in the pool followed a relatively smooth curve peaking at 20 d (Fig. 2). The low number of larvae on day 16 coincided with increased flow due to precipitation. This perturbation may have washed some larvae off the pool carcass, as a similar but less dramatic decrease occurred in the number of larvae on the riffle carcass as well.

The diversity of genera per rat over time in the pool experienced a modest increase towards the end of the investigation (Fig. 3). For the first 29 days, there were five or less genera present per carcass. On the last two collection dates (34 and 39 days after submersion), six and seven genera were found on each carcass respectively.

The diversity of genera per rat over time in the riffle exhibited a more dramatic increase towards the end of the investigation. For the first 24 days, there were four or fewer genera present per carcass. On day 29, five genera were present, on day 34, eight were present, and on day 39, 15 were present (Fig. 3).

Hydrobaenus larvae were consistently present during the study in both the pool (Table 1) and riffle (Table 2). In contrast, *Orthocladius* larvae were absent from the pool rats until 20 days of submersion, and did not colonize the riffle rats until 13 days of submersion (Fig. 4). This genus was consistently found on every carcass afterward with the exception of the final pool rat and the individual collected from the riffle on day 24 upon which no macroinvertebrates were encountered.

Sorenson's Similarity Index produced a value of 0.77 on a scale of 0.0 to 1.0 (0.0 = complete dissimilarity and 1.0 = complete similarity). This is an intermediate value suggesting that the riffle and pool have different assemblages of midge genera.

Discussion

A carcass in a warm terrestrial setting decomposes quickly due to arthropod and microbial action (2–3). This was not the case in

TABLE 2—*Succession of midge genera colonizing the rats in the riffle.*

Subfamily	Genus	Day 3	6	9	13	16	20	24	29	34	39	Total
Chironominae	*Micropsectra*									1	2	3
	Paratanytarsus										1	1
	Polypedilum									1	2	3
Orthocladinae	*Brillia*									1	1	2
	Cricotopus									4	1	5
	Eukiefferiella										1	1
	Euryhapsis	1										1
	Hydrobaenus		1	6	5	2	5		6	1	1	27
	Metriocnemus					4				1	1	6
	Orthocladius				25	12	40		50	18	1	146
	Parakiefferiella			2	5	2	5		16			30
	Parametriocnemus			2							1	3
	Psilometriocnemus								1			1
	Rheocricotopus										1	1
	Thienemanniella	1							1		2	4
Tanypodinae	*Conchapelopia*										1	1
	Meropelopia				5					1	3	9
	Telopelopia										1	1
Undetermined		1	1	2		2			1	1	3	11
	Total	3	2	12	40	22	50	0	75	29	23	256

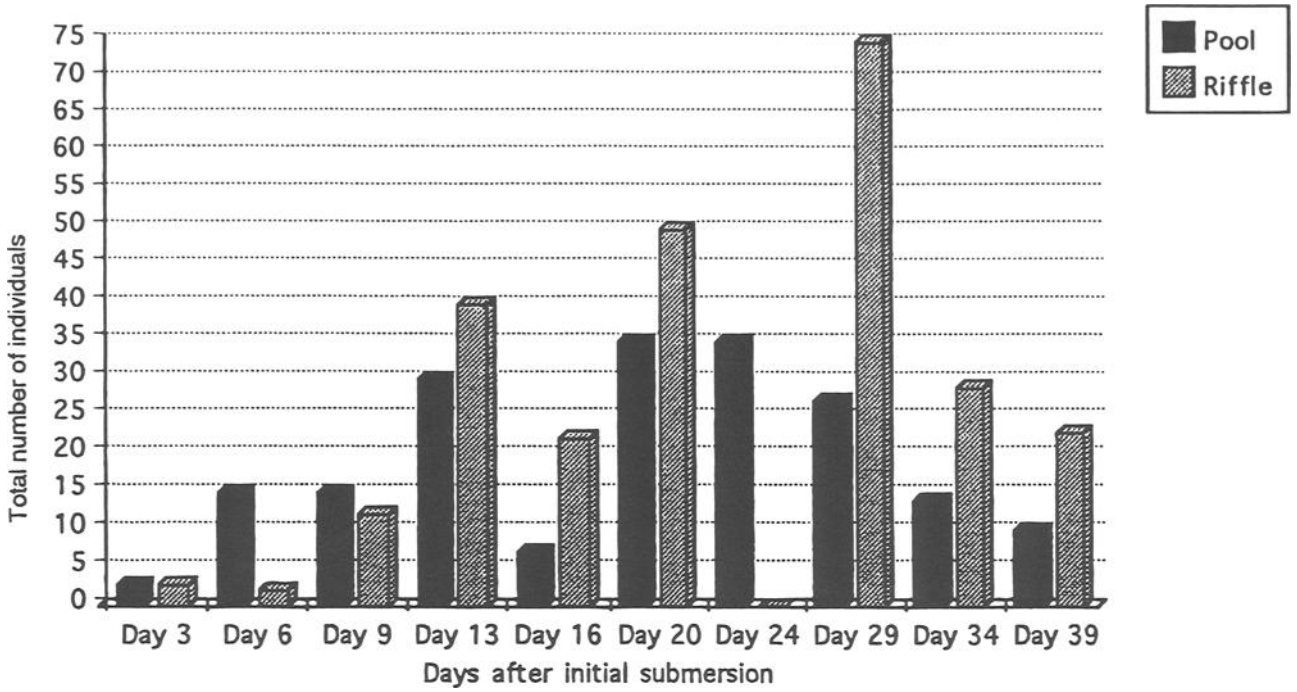

FIG. 2—*Total number of midge larvae collected from each carcass.*

Bixon Creek during the spring of 1995. The rats experienced temperatures in the stream of ca. 12°C, and no burrowing invertebrates increased the surface area of the carcasses for colonizing bacteria. Although it may take a matter of days for a body to decompose in a warm and humid environment, a corpse in a cold water stream could take months in the absence of large scavenging animals. Therefore, long term studies of insect colonization of carcasses are necessary for generating indices usable for determining the PMSI.

Calliphorid and sarcophagid flies provide crucial evidence for determining the PMI when a body is exposed to ovipositing adults in terrestrial habitats. Midge larvae have the potential to play this role in streams. A submerged substrate can be colonized by midge larvae in several ways. It can be oviposited on directly, as females of some species dive below the water to lay eggs. In other species, eggs are laid on the water's surface or on objects overhanging the stream allowing newly hatched larvae to fall directly into the water. Additional colonization occurs when larvae from an upstream

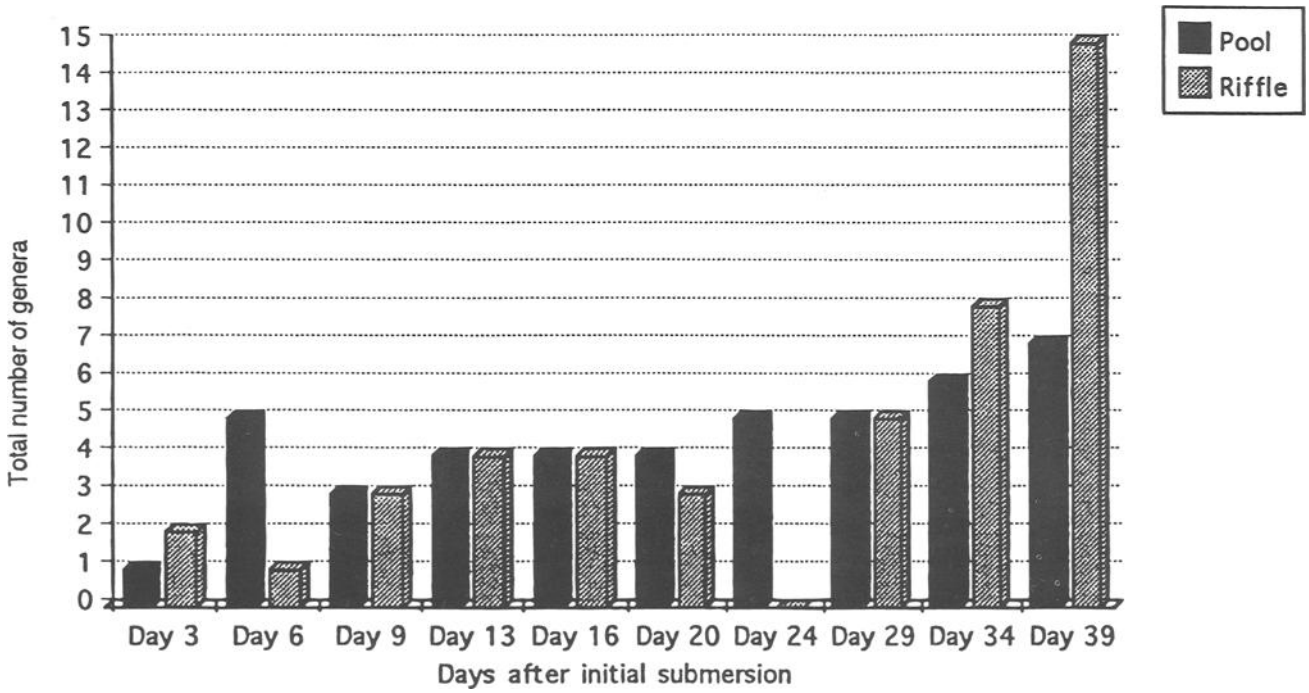

FIG. 3—*Number of midge genera collected from each carcass.*

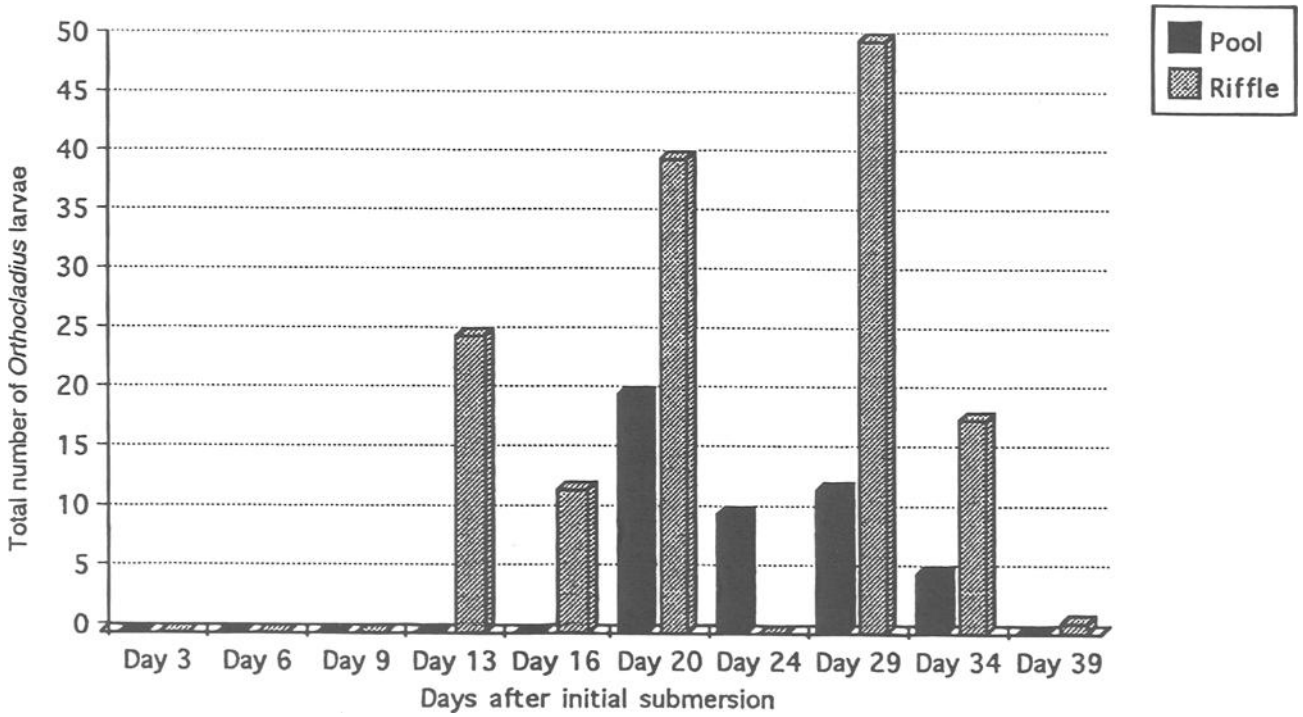

FIG. 4—*Number of* Orthocladius *larvae collected from each carcass.*

locality are caught in the current and settle downstream onto a new substrate (10). A corpse would make a suitable substrate to be colonized in similar manners. The midge larvae collected in this study most likely drifted onto the carcasses, because adult midges were not yet active in northeastern Ohio.

It has been demonstrated that final instar calliphorid fly larvae migrate from a corpse to the surrounding soil where they pupate (1). We found no evidence that midge larvae exhibit similar behavior, nor were any reports of this found in the literature. Some species of *Orthocladius* larvae build tubular cases attached to substrate within which they dwell (11). However, the larvae we encountered did not build tubes. Pupation occurs within the algae and sediment on benthic substrate where the basal portion of the insect is attached. We concluded the study prior to adult emergence. If the study were conducted through the time that pupation and adult emergence occurs, this would change the composition and size of the midge communities on the carcasses. The most obvious change would be a decrease in individuals present, although pupal exuviae would be left behind. This niche would then be underutélized, allowing new con- and heterospecific colonizers access. It is also possible that the scouring action of floods may devastate populations in the pupal stage. As larvae, insects are mobile and possibly could move more deeply into the biofilm or move to the downstream sides of rocks to avoid such catastrophes. Pupae are immobile and therefore more susceptible to unpredictable disturbances.

Other biological factors should be taken into account when analyzing the succession of midge larvae on a submerged carcass or corpse. These include territoriality, competition, and predation (12). A decrease in the number of larvae can occur from any of these phenomena, either from death or emmigration.

The fur on the body of a rat probably provides a substrate different than human skin for arthropod and algal colonization, as well as detrital deposition. However, we noticed the hairless tail garnered a coating of algae and sediment which appeared similar to that on the rest of the body. Midge larvae were taken from the tail. Because of this, we suspect a human corpse would develop a similar coating.

The widespread and ubiquitous nature of midge larvae should allow development of accurate indices for determining the postmortem submersion interval of corpses in specific geographic locations. Determination of the PMI does not pinpoint the time of death, but supplies a window of opportunity as to when death occurred. This would also be the case for PMSI determination.

As mentioned above, this study concluded in late April, which is slightly prior to pupation and mass emergence of midges from Bixon Creek (J. B. Keiper, unpublished observations). The presence of exuvia with pupae and larvae of the same species could give a more accurate time of how long a body has been submerged if it is known how long the pupal stage lasts for that particular species. Also, the presence of midge exuvia but not larvae could provide useful data if the emergence and oviposition period for the colonizing species is known.

The rat from the riffle cage collected on day 24 had no midge larvae attached, as mentioned above. We are unable to explain this. It is doubtful that human error in collection could have been the case, as at least a few larvae would be expected to cling to the carcass. All rats were of the same age and similar size, and they all came from the same batch reared in the laboratory. It is unlikely that some condition unique to this rat was responsible for the lack of colonization by insects. There were no unusual weather phenomena noted which would have removed all insects from this individual. Precipitation did cause the stream to rise, but this occurred on other collection dates as well.

The results of Sorenson's Similarity Index with respect to midge genera suggest that separate indices for riffles and pools should be used for determining the PMSI. An example of such an index is presented in Table 3, and was generated from the data gathered from the riffle carcasses. The data from the pool were not resolute and we refrained from creating an index for this habitat. Whether

TABLE 3—*An index for determining the postmortem submersion interval of a corpse found in a riffle of Bixon Creek based on midge larvae colonization.*

Evidence Collected from a Body	Postmortem Submersion Interval
Little or no biofilm, no *Orthocladius* present	<7 days
Biofilm heavy, no *Orthocladius* present	7–13 days
Biofilm heavy, ≤4 genera present, *Orthocladius* present	13–28 days
Biofilm heavy, >4 or more genera present, *Orthocladius* present	>28 days

this index can be applied to similar riffles during periods other than spring in the Portage Co., OH area and elsewhere needs to be tested.

Conclusions

It was not the purpose of this investigation to provide replicated data on midge larvae colonization to be used directly in criminal investigations. Our goal was to see what happens to mammalian carcasses placed in a stream at a certain time of year. We found that: 1) midge larvae are the dominant taxon of colonizing macroinvertebrate; 2) patterns in midge colonization are detectable; 3) no insect larvae shredded or bored into the carcasses as they do in a terrestrial setting; and 4) algae appear to colonize carcasses as well.

This investigation provides preliminary information on what may happen to carcasses in a pool and riffle habitat of a small woodland stream when large scavengers are excluded. Results would probably be different if large scavengers had access to the carcasses. For instance, maggots burrow into mammalian bodies in terrestrial environments, thereby increasing the surface area for bacterial action. Exposure to crayfish, for example, probably produces a similar situation in streams. A mammalian body may then begin to be used by stream insects as a food source after such conditioning. However, a human corpse would be a large object for crayfish to condition via shredding. Therefore, the body would be a substrate for algae and midge larvae colonization for some time. A next step in investigating this topic would be to allow large scavengers access to carcasses, and then to repeat the study with larger carcasses such as pigs. Furthermore, these and other studies should be conducted during all seasons of the year as species numbers and composition will undoubtedly be different. The data garnered in this study should not be used in criminal investigations without the benefit of further research.

Determination of the postmortem submersion interval may never be as resolute as determination of the PMI in a terrestrial setting. Currents may carry bodies between riffles and pools. A corpse may become washed up on shore for a period of time and then may be swept up in the current again during a flood event. However, data obtained from a submerged body should not be ignored, as it may provide insights and supporting data as to how long it had been under water. Midge larvae may be another tool for the forensic entomologists of tomorrow just as blow flies and carrion beetles are tools for the forensic entomologist today.

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