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Algal Colonization of Submerged Carcasses in a Mid-Order Woodland Stream

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ABSTRACT: One of the primary goals of forensic pathology is the determination of time of death. In aquatic systems, one method to do this is to analyze the colonization of a corpse by algae. Algal communities typically follow a serial colonization pattern, therefore the taxa present at any given time may provide clues about postmortem submersion time. This study was undertaken to examine the algal colonization on rat carcasses in a medium-order woodland stream. Two habitats were studied: a low flow pool and a high flow riffle, with rats being removed from each site every 3 to 6 days over 31 days. The diversity of colonizing taxa increased at both sites as the study progressed, and after 17 days similar taxa were present (Sorensen's similarity index >60%) in each site. Some taxa, such as desmids (Chlorophyta), tended to increase in diversity throughout the study, making them possible indicators of submersion time. Diatoms were the most abundant taxa found in each site and accounted for 63 of the 92 total taxa identified. Due to their ubiquitous presence in nearly all streams, we suggest that diatoms may be the key organisms for the study of postmortem submersion in lotic systems.

KEYWORDS: forensic science, streams, phycology, algal colonization, freshwater, animal studies

The postmortem interval (PMI) is the time between the death and discovery of a corpse. In terrestrial systems, this time can be estimated by an examination of the invertebrate community inhabiting the corpse (1,2). Few studies, however, have dealt with invertebrate colonization of corpses in aquatic systems. These studies typically focus on submersion in lentic, or still bodies of water (3,4). Researchers looking at lotic, or flowing systems, have been able to use the presence of midge larvae as indicators of submersion interval (5). One problem with using aquatic insects, however, is the significant temporal variability of their occurrence (6).

One of the ubiquitous components of lotic ecosystems is the algal community (7). As such, this community presents an excellent assessment tool for determining PMI. While major algal taxa (i.e., divisions) experience some temporal heterogeneity, the algal community is virtually always omnipresent. Some researchers have previously employed algae in forensic studies. Typically, certain taxa, most notably diatoms, have been employed to provide evidence for drowning (8–10). Diatoms have also been employed to estimate the general vicinity of drowning by comparing taxa from the lungs of victims and the river diatom community along a spatial gradient (10). Similarly, the presence of algae from specific habitats has been used to link criminals to crime scenes in forensic investigations (11).

The purpose of our investigation is to document the algal colonization of submerged carcasses from a stream in order to elucidate the submersion interval (SI). We are not aware of any studies which sought to qualify the whole algal community colonizing submerged bodies. To that end, we placed immature dead rats in a pool and riffle area of an undisturbed stream and examined them every 3 to 6 days. In addition, we sought to determine whether there was a significant difference between two distinct stream habitats, namely pool and riffle areas. In this way, we hope to establish a general pattern of algal colonization which may be useful for determining the SI in lotic systems.

Methods

The study was conducted in East Fork Queer Creek, a fourth-order stream located near Ash Cave State Park, Hocking County, Ohio. The particular stretch of stream used was bordered by a relatively undisturbed riparian zone and only partially shaded by a mixed riparian canopy, dominated by river birch (*Betula nigra*), red elm (*Ulmus rubra*), and sycamore (*Platanus occidentalis*). The stream and surrounding area have been extensively studied for biotic surveys, including qualification of the algal community.

Two ca. 1 m × 1 m wooden cages were constructed to house small mammalian carcasses for the experiment. Cages consisted of a wood frame to which fine metallic mesh (0.5 cm gap size) was affixed. Both cages were constructed with a ca. 45° slope on one end to facilitate water flow and reduce buildup of debris. The cages were affixed with a removable lid from which samples were withdrawn. Both cages were secured with iron rebar and weighted with heavy stones from the stream.

Immature rats were utilized to simulate a colonizable substrata similar to human skin. Twenty-four immature rats were obtained postmortem from a local pet store and placed in each of the two cages. Rats were positioned at 8 cm intervals and individually tied to the bottom mesh by use of plastic ties. One cage was completely submerged in a pool (ca. 1.5 m depth) and the other cage was placed in a fast-flow riffle 10 m downstream. The lids of both cages were secured with plastic ties to prevent any disturbance by animals.

Cages were placed on March 20, 1999, and an individual rat from each site was removed after Days 3, 8, 12, 17, 21, 25, and 31. Rats were collected by cutting the plastic ties and immediately placing the rat into a scintillation vial with 2.5% calcium-carbonate buffered gluteraldehyde to preserve the algae for identification. Care was taken not to dislodge any organisms during collection. Afterwards, the sloping section of the riffle cage was scraped clean of any accumulated growth or debris.

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Identification of all algae was performed using an Olympus BX40 light microscope, at ×200 to ×1000 magnification. Samples were obtained by scraping rats and collecting the dislodged materials. At least four slides were prepared from each sample. In addition, diatoms were clarified as described elsewhere (12). Algal identifications followed standard authorities (13–18).

To compare the algal community similarity between the pool and riffle sites, Sorenson's index of similarity was employed (19):

$$S = \frac{2C}{(A + B)}$$

where A equals the number of taxa in the pool, B the number of taxa in the riffle, and C the taxa common to both sites. Values range from 0 to 1, with 0 being no taxa in common to 1 being all taxa found in each site.

Results

After immersion in water for one month, the rats were in very poor condition. By Day 25 the rats had become very fragile due to decomposition from a thick mat of fungi growing epizooically (upon an animal substrate). Seventy-three algal taxa were observed from the pool and riffles samples, with a total of 92 taxa total from both sites (Table 1).

As the study progressed, the number of different taxa recovered increased steadily (Table 2). The pool site had the greatest number

TABLE 1—*Taxa listed by algal division identified during each sampling date. "P" represents those taxa from pool samples, "R" taxa from riffle samples.*

Taxa	Day 3	Day 8	Day 12	Day 17	Day 21	Day 25	Day 31
Bacillariophyta							
Achnanthidium minutissimum		P	P, R	P, R	P, R	P, R	P, R
Amphipleura pellucida					P	P, R	
Amphora ovalis						R	R
Amphora veneta				R			
Brachysira vitrea			R		P, R	R	R
Caloneis ventricosa				P, R		R	R
Craticula cuspidata						P	
Cymbella affinis							P
Cymbella naviculiformis					P	P	R
Diploneis elliptica			P		R	R	R
Encyonema silesiacum			P, R	P, R	P, R	P, R	P, R
Encyonema lange-bertalotii			P	P, R	P, R	P, R	P, R
Encyonema minutum					P, R	P, R	R
Epithemia adnata							R
Eunotia exigua			P		P		
Eunotia muscicola					P, R	P	P
Fragilaria capucina			P, R	P, R	P, R	P, R	P, R
Frustulia rhomboides						P, R	P, R
Frustulia vulgaris			P		P, R	P	R
Gomphonema acuminatum					P		R
Gomphonema angustatum				R	P, R		
Gomphonema gracile						P	R
Gomphonema minutum				P, R	P, R	P, R	P
Gomphonema olivaceum					R	P	
Gomphonema parvulum			R	P, R	P, R	P, R	P, R
Gomphonema sarcophagus					P, R		
Gomphonema sp. 1					R	P, R	R
Gomphonema sp. 2			P, R	R	P, R	P	P
Gomphonema sp. 3					P, R	P	R
Gomphonema truncatum			P				P, R
Luticola sp.			P		P, R	P, R	P, R
Melosira lineata				P	P, R	P, R	R
Melosira varians					P, R		
Meridion circulare	P	P	P, R	P, R	P, R	P, R	P, R
Navicula capitata						R	
Navicula cryptocephala					P, R	P, R	P, R
Navicula cryptotenella						P	P
Navicula halophila cf				P,		P, R	
Navicula lanceolata							R
Navicula rhynchocephala					P, R	P	P, R
Navicula viridula					R		P, R
Nitzschia acicularis			R	P	P, R	P, R	P, R
Nitzschia capitellata					P, R	P	P, R
Nitzschia clausii					P		
Nitzschia dissipata				P, R	P, R	P, R	P, R
Nitzschia linearis					R	R	P, R
Nitzschia palea			R	R		P, R	
Nitzschia paleacea				R			
Nitzschia pusilla					P	R	

TABLE 1—*Continued.*

Nitzschia sublinearis							P
Pinnularia interrupta							R
Pinnularia lundii						R	
Pinnularia microstauron					P		
Pinnularia viridis							P
Planothidium lanceolatum					P		
Sellophora pupula			R	R	P	P, R	
Stauroneis anceps						P	P, R
Surirella amphioxys					P	P, R	P, R
Surirella minuta				P, R	P, R	P	P, R
Surirella ovalis					P	P	
Synedra delicatissima				P, R	P, R		P, R
Synedra ulna	P	P	P	P, R	P, R	P, R	P, R
Tabellaria flocculosa			P	P, R	P, R	P, R	P, R
Chlorophyta							
Ankistrodesmus falcatus							P, R
Chlamydomonas globosa			R				
Closterium littorale				P		P	
Closterium moniliferum			R	P	P		R
Closterium peracerosum							P
Closterium sp.							R
Cosmarium botrytis				P			R
Cosmarium moniliforme				P			
Euastrum validum				P			
Euastrum verrucosum				P	P		
Mougeotia sp.						R	P
Scenedesmus bijuga							R
Spirogyra sp.						R	
Staurastrum turgescens							P, R
Stigeoclonium sp.					R	R	
Cyanophyta							
Oscillatoria sp.	P	P					
Oscillatoria anguina			P				
Oscillatoria angustissima	P, R	R	P, R	P, R		P, R	P
Oscillatoria animalis					R		
Oscillatoria hamelii							P
Oscillatoria subbrevis				R			
Oscillatoria tenuis						R	
Phormidium ambiguum					R		
Pseudanabaena sp.	P	P				P	
Rhabdoderma lineare			P, R				
Spirulina cf. Nordstedtii				R			
Synechococcus sp.			P, R				
Euglenophyta							
Euglena sp.						R	R
Trachelomonas cf. *Dybowskii*				P			P,R

TABLE 2—*Distribution of algal taxa found from rat scrapings for each sampling date.*

	Sampling Date							
	Day 3	Day 8	Day 12	Day 17	Day 21	Day 25	Day 31	Total
No. of pool taxa	5	5	17	24	40	39	36	73
No. of riffle taxa	1	1	15	22	35	36	45	73
Total taxa (both sites)	5	6	24	32	48	52	56	92
Sorenson's value*	0.34	0	0.5	0.61	0.72	0.61	0.62	

* Sorenson's value refers to Sorenson's index of similarity, describing the similarity value between the pool and riffle taxa on each day (0 = complete dissimilarity, 1 = complete similarity).

of taxa present after Day 21, and decreased in diversity afterwards. Conversely, the riffle samples continued to increase in diversity until the end of the experiment at Day 31. After an initial lag phase, both sites showed an increase in the total number of taxa as well as number of taxa common to both sites. Sorenson's index of similarity values were typically ca. 65%. The first two sampling dates had very low values, but there were also very few taxa at the time. After a substantial increase in the number of taxa after Day 12, the similarity values tended to stabilize.

The number of different algal taxa by division for each site are presented in Figs. 1 and 2. In both the pool and riffle area, diatoms were the most species-rich division after Day 12. Diatom richness

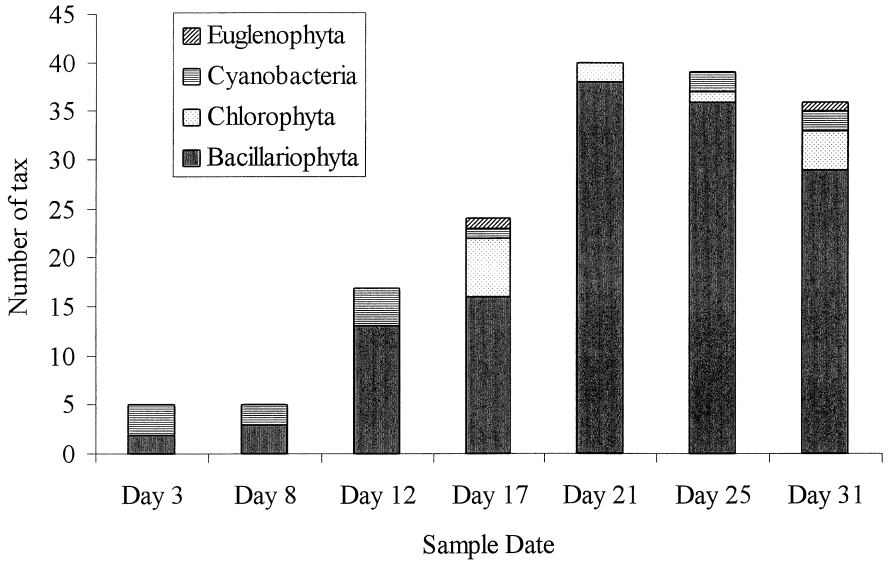

FIG. 1—*Comparison of the number of algal taxa from each division encountered during the sampling period for pool samples.*

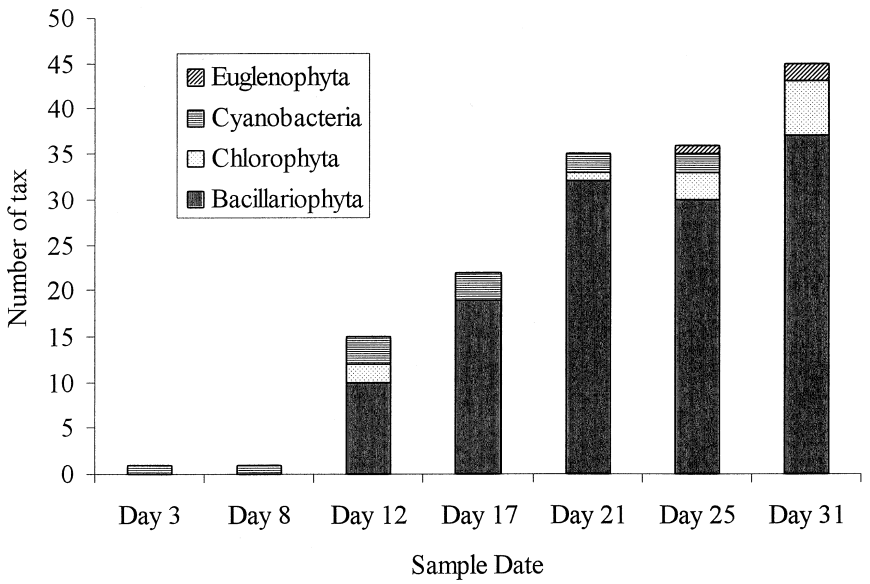

FIG. 2—*Comparison of the number of algal taxa from each division encountered during the sampling period for riffle samples.*

was so high that after Day 12 they accounted for more distinct taxa than all other algal divisions combined.

The diatom community was initially dominated by *Achnanthidium minutissimum, Fragilaria capucina, Meridion circulare,* and *Synedra ulna.* Throughout the colonization period of the corpses the species richness of the algal community continued to increase, but these four taxa remained the main constituents (relative abundance range for these four species 10% to 48%).

Discussion

While diatoms dominated the diversity of taxa, cyanobacteria and chlorophytes were also present. Cyanobacteria were nearly always filamentous forms, which are extremely common in most lotic and lentic systems. *Oscillatoria angustissima,* the most common taxon, is a typical component of streams and ditches in the U.S. Midwest. Nine out of the 15 chlorophyte taxa were desmids while the others were common aquatic algae occurring in a wide range of habitats. Therefore, chlorophyte taxonomic composition is of little informative value. On the other hand, it is interesting to note that it was not until Day 17 that many chlorophytes were encountered at either site, implying that corpses require time to become conditioned by algae. It is also possible that their delayed appearance was a result of chlorophyte's slower growth rate compared to some other taxa.

Diatoms had both the greatest diversity and actual numbers of cells among any algal division (R. Verb, personal observation). This is not surprising, as diatoms are probably the most prevalent component of stream algal communities (7). As such, diatoms have also been used as indicators of stream water quality (7). This could

be very important in forensic investigations, as the quality of a stream can have profound effects on the organisms which are capable of colonizing corpses. For example, in streams heavily impacted by acid mine drainage the richness of the diatom community is typically very reduced (20). Therefore, some of the common colonizers which are acidophobic may not be present in the typical serial succession.

Diatoms are commonly basal cells in developing periphyton mats. As colonization proceeds with subsequent reproduction, diatoms may alter the existing microhabitat thereby making the substrate more usable by other algal taxa (21). Therefore, some taxa potentially useful for PMI estimates, such as desmids or euglenophytes, may not occur until after extensive diatom colonization and conditioning of the substrate, a useful tool in PMI estimates.

In our study, there was a lag phase when little colonization occured until Day 12. Afterwards, colonization proceeded rapidly, doubling in the total number of taxa by Day 21. Therefore, the low number of diatom epiphytes prior to three weeks submersion may be an excellent indicator. After three weeks, however, the number of new taxa appears to decline, whereas certain key taxa, such as desmids and some diatom species, appeared.

The dominant diatoms *Achnanthidium minutissimum, Fragilaria capucina, Meridion circulare,* and *Synedra ulna* have been noted to be early dominant immigrants on freshly placed tile substrates in a stream (22). In addition, these taxa may represent colonizers of early successional stages of the algal community on the corpse. *Achnanthidium minutissimum* has been noted to be an important initial colonizer of lotic systems (i.e., 23,24). However, it may prove difficult to precisely date submerged corpses using the relative abundance values of the dominant taxa. This is due to high levels of heterogeneity between sample dates. There does appear to be a general pattern of decline in the relative abundance values of these diatoms from Day 3 to Day 31. For instance, *Meridion circulare,* by far the most dominant diatom species in this study, was found to have a relative abundance of 45% to 48% (riffle and pool), which had decreased to 12% to 18% by Day 31. In addition, while abundance values appeared to regress downward with time, there appeared to be an inverse relationship with species richness in the algal community (Figs. 1 and 2). These patterns and differences in species richness values may prove to be more useful tools for determining PMI with diatoms.

In addition, the presence of certain individual taxa can be used as an indicator of specific conditions within the habitats. For example, after Day 17 the pool samples had a preponderance of desmids (Desmidiaceae, Chlorophyta) which typically occur under acidic conditions (25). These acidic conditions probably arose from the decomposition of the rats, and subsequent release of organic acids by action of the extensive fungal coat covering the rats. When allocthonous (originating outside of the stream) carbon sources appear, opportunistic fungi may quickly colonize and degrade the available material (26). The water in the pool had slow flow rate (D. Casamatta, personal observation), and as a result, a buildup of acids may be expected. Conversely, presence of desmids in the riffle was delayed, possibly because of dilution of acidic products.

The similarity of the taxa between the sites was higher than expected. Extremely different algal communities may develop on different types of habitats. For example, artificial versus natural substrates may have significantly different epiphytes (27), although in streams there is some evidence that epiphytes may be correlated with the individual substrate's structure and not composition (28). Still, the Sorenson's values were quite high given the difference between the sites (i.e., depth, flow, light levels, etc.). These factors appear to favor the algal community as an excellent estimator of the submersion time of a corpse. On the other hand, some taxa were recorded from either the pool or riffle habitat. Table 3 lists taxa unique to either site. These taxa may be used to determine movement of a corpse as a result of a spate event (flood) or other disturbance (such as movement resulting from scavengers).

TABLE 3—*Some important taxa unique to either pool or riffle habitats for estimating SI based on occurrence on two or more dates.*

Pool	Riffle
Eunotia exigua	*Stigeoclonium* sp.*
Navicula cryptotenella	*Euglena* sp.*
Surirella ovalis	
Closterium littorale	
Euastrum spp.	
Pseudanabaena sp.	

* Represents taxa observed only during the latter half of the study.

Conclusions

From our study, it appears that the algal community has several factors that make it an excellent method of assessing submersion interval values. First, algae are a ubiquitous component of lotic systems, being present in nearly any stream or river. Second, the algal community typically remains throughout the year, making it preferable to invertebrates, which may exhibit significant temporal variability. Third, identifications are relatively easy and inexpensive. Except for a few taxa which require sexual reproduction for identification (e.g., *Mougeotia*), most taxa can be readily identified to species with a light microscope.

Our identifications implied that the relative diversity of the colonizing algal community can be used to assess SI values. Relatively few taxa (i.e., <20) implies a relatively recent submersion prior to corpse discovery, whereas a greater number (i.e., >50) implies that submersion may have occurred for several weeks. Likewise, the presence of certain taxa, such as desmids, implies a certain time frame. Table 4 presents an index for estimating SI values

TABLE 4—*A descriptive index for estimating the SI from riffle and pool habitats.*

Riffle Index	Pool Index
Fragilaria absent = <12 d	*Fragilaria* absent = <12 d
Fragilaria present = 12–25 d	*Fragilaria* present
Euglena absent	*Navicula cryptotenella* absent = 12–25 d
Fragilaria present = 25–30 d	*Fragilaria*
Euglena present	*Navicula cryptotenella* present = >25 d
Ankistrodesmus absent	
Ankistrodesmus present = >30 d	

based on some key indicator taxa. Some organisms, such as *Meridion circulare* and *Synedra ulna,* while common in both pool and riffle areas, provide little information as they were present continually throughout the sampling period. However, a combination of taxa may prove very useful for SI determination, especially when their appearance is staggered. Therefore, an investigator interested in estimating SI should employ those taxa found sequentially through the study, using early and late colonizers as indicators.

Several caveats must be kept in mind with this study before definitive SI values can be created. First, this was only a single study with pseudo-replication. More studies employing different orders of rivers under various anthropogenic impact need be carried out. Second, a host of environmental factors may have influenced this study. For example, seasonality, with accompanying temperature and light level differences, may have an effect on colonization. Third, large animals were excluded from this study. In actual forensic cases, it is likely that corpses will be at least partially consumed by scavengers. How this affects algal colonization, with differential substrate structure and chemical composition, remains to be seen. Also, as corpses travel downstream this may affect the colonization process. Finally, immature rat corpses do not directly approximate human skin, and therefore the choice of substrate may influence algal colonization. Many questions still remain about using algae as PMI indicators. From this preliminary test, though, it appears that they may potentially prove a valuable tool in forensic studies of SI determination.

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