CASE REPORT

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Forensic Limnology: The Use of Freshwater Algal Community Ecology to Link Suspects to an Aquatic Crime Scene in Southern New England

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ABSTRACT: During July, 1991, two young boys were brutally attacked by multiple teen-aged assailants while fishing at a suburban Connecticut pond. After being accosted at knife point, the victims were bound with duct tape, beaten with a baseball bat and dragged into the pond to drown. One victim managed to free himself, rescue his colleague, and summon help from local residents. An exhaustive investigation led to the rapid apprehension of three suspects.

In an effort to link the suspects to the crime scene, sediment encrusted sneakers were seized from both assailants and victims, and analyzed for aquatic microorganisms. Numerous species of diatoms and scaled chrysophytes (planktonic algae) were recovered from the sneakers and from reference samples of pond sediment. The marked similarities in the algal communities present on the sneakers indicated exposure to a common freshwater habitat, most probably the crime scene pond. Additional analyses revealed that *Mallomonas caudata* was the dominant scaled chrysophyte species in each sample, and that there was no significant difference in the ratios of three species of the diatom *Eunotia* between all samples examined. These findings further supported the idea that all of the samples originated from a common, if not the same, locality. This case further exemplifies the applicability of aquatic community ecology to forensic investigations.

KEYWORDS: forensic science, limnology, diatom, scaled chrysophyte, algae, ecology

Fundamental to forensic science, is the ability to form definitive associations between evidentiary items of documented origin (known samples) and those of unknown source

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(questioned samples) [1]. Forming such linkages frequently constitutes the basis for successful case resolution and has given rise to the concept of the "evidence environment" [2]. Simply defined, the evidence environment is the totality of characteristics that render any scene unique. Forensic investigators routinely evaluate scene uniqueness and subsequently sample scene-specific items deemed most easily transferable to transient persons or property.

Historically, the natural sciences have proven useful in forming evidentiary linkages particularly at outdoor scenes. Plants (botany), pollen (palenology), fungi (mycology), insects (entomology) and animal hairs (mammology) have been successfully used to associate suspects, victims, or property with specific locations [3–9]. Here, we report on the successful employment of another organismal group (algae) and biological discipline (limnology) to link three subjects to a freshwater crime scene in southern New England.

Case History

During July, 1991, two young boys were brutally attacked by multiple teen-aged assailants while fishing at a suburban Connecticut pond (Fig. 1). After being accosted at knife point, the victims were bound with duct tape, beaten with a baseball bat and dragged into the pond to drown. Fortunately, one victim managed to free himself, rescue his injured colleague, and summon help from local residents. The apparent motive for the attack was the theft of the victims' bicycles. An exhaustive investigation led to the rapid apprehension of three suspects. When questioning the suspects, investigators noted the water-soaked, mud-encrusted footwear and clothing.

In an effort to place the suspects at the crime scene, investigators obtained numerous items of physical evidence left at the scene by the assailants. These included footwear impressions, duct and electrical tape fragments, and a submerged baseball hat. Additionally, investigators accessed bioenvironmental scene characteristics and sampled unique items deemed most likely to have been unknowingly carried away from the scene by the attackers. These included reference samples of pond water, sediment, and aquatic vegetation that were obtained from the pond in the area where the victims had been submerged and from suspected routes of entrance and egress.

Water-soaked clothing and footwear (sneakers) were subsequently obtained from both the victims and the suspects in the hope that linkages could be made based upon a comparative analysis of adhering pond biota. A visual examination of these items revealed sediment of similar color on the inner and outer surfaces of the footwear. The sediment encrusted sneakers seized from the victims and suspects were forwarded, along with a reference sample of pond sediment, to the limnological laboratory of Connecticut College, New London, CT for examination.

Limnological Methods

Following visual and light microscopic examination in the laboratory, mud from a suspect's sneaker and a victim's sneaker was removed by scrapping and placed into individual glass beakers. Mud from the pond reference sample was added to another beaker. Sulfuric acid and potassium dichromate were added to each beaker and the resultant slurries were allowed to settle for 24 hours. Each slurry was then transferred to a centrifuge tube, placed into a water bath and boiled for one-hour in order to complete the oxidation of organic matter [10].

After oxidation was completed, each sample was rinsed several times in distilled water. An aliquot of each resulting mixture was dried onto a glass coverslip and mounted onto a glass slide with Hyrax. Each slide was examined using phase contrast microscopy (1000X). Qualitative and quantitative analyses of two groups of microscopic freshwater

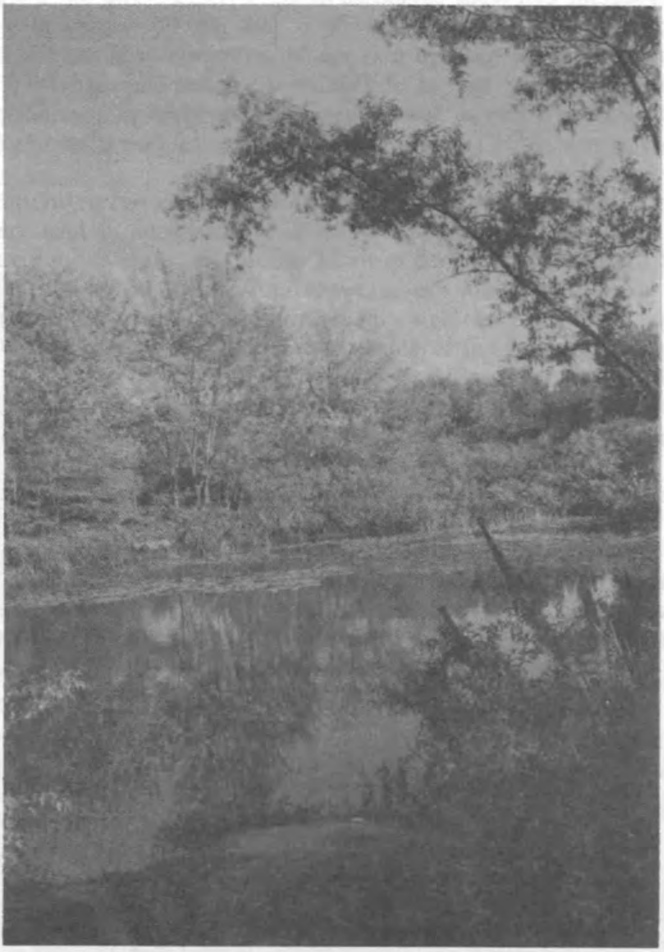

FIG. 1—*Photograph of the crime scene.*

algae, the diatoms and scaled chrysophytes, were conducted. These groups were chosen because of the rigorous nature of their species-specific, silica cell walls and their aquatic diversity [*11–14*]. The purpose of the analysis was to determine if the mud removed from each sneaker originated from within a body of water (as opposed to a yard, street, or other habitat), and it so, to determine how similar each was to the known sample of pond sediment.

In each case a qualitative assessment of the diatom and scaled chrysophyte species was made. Additionally, relative counts of three species of *Eunotia* were enumerated for each sample and a statistical analysis was employed to quantify similarities between the algal population ratios encountered [*15*].

Results and Discussion

All samples contained numerous specimens of both diatoms and scaled chrysophytes. Diatoms and scaled chrysophytes are microscopic types of algae that form highly sculptured, species-specific cell walls composed of silica [*11,12*]. The species-specific components of the siliceous cell walls, known as frustules (diatoms) and scales (scaled chrysophytes), become incorporated into aquatic sediments once the cell dies. Aquatic habitats often contain many species of diatoms and scaled chrysophytes (Fig. 2). Each lake or pond develops a unique floral community comprised of specific species populations. Bodies of water with similar physical and chemical characteristics develop similar but not identical algal communities [*16*].

Diatoms grow in practically all bodies of water. Many, however, are quite restricted and survive in only particular habitats [*17*]. In a given pond or lake, certain diatom species are found growing attached to rocks, weeds, and other debris (periphytic diatoms). Other species (planktonic diatoms) grow only in open water. In general, the shallower a body of water and the higher the density of vegetation, the greater will be the ratio of periphytic (as opposed to planktonic) diatoms [*16*]. Conversely, the deeper a waterbody, the greater the percentage of planktonic organisms.

Scaled chrysophytes are strictly planktonic organisms found in ponds and lakes. Like diatoms, many species of scaled chrysophytes display marked habitat preferences and reflect these requirements in their distributions [*14,18,19*]. In general, the shallower a pond or lake and the greater the weed density, the greater the ratio between periphytic diatoms and scaled chrysophytes.

A total of 25 species, representing 15 genera, were identified in the samples of mud examined. Of this number, 14 species were common to all of the samples. Table 1 lists the diatom and scaled chrysophyte species encountered and their relative abundances. All species found were indicative of a freshwater habitat. The presence of scales of *Mallomonas caudata* in each of the samples limited the originating freshwater habitat to

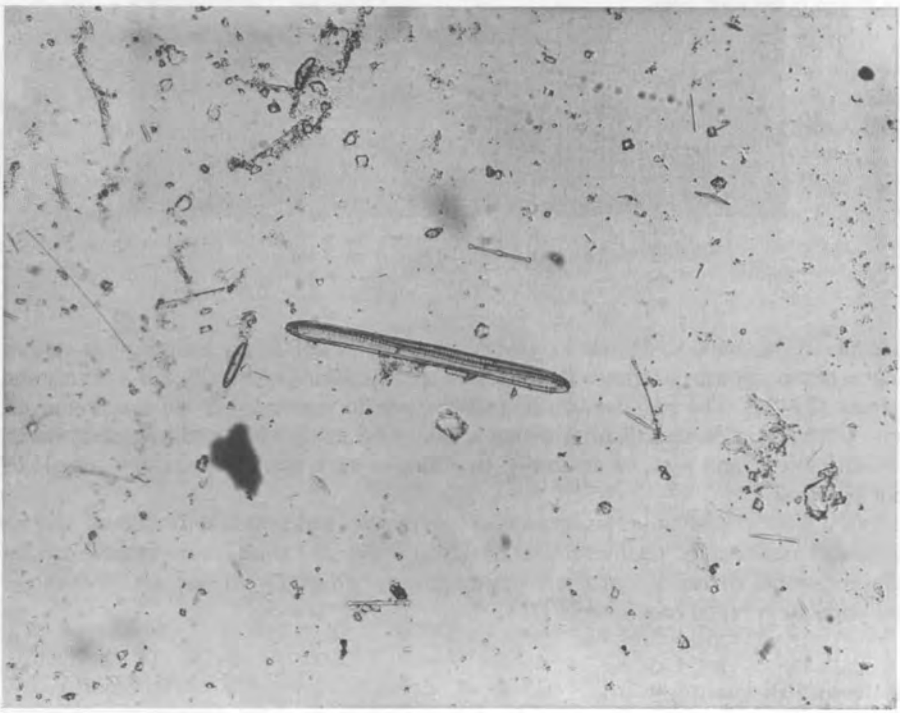

FIG. 2—*Photomicrograph (1000X) of the siliceous Cell Wall of the Diatom* Nitzschia *sp.*

TABLE 1—*Listing of diatom and scaled chrysophyte species recovered from evidentiary items submitted for limnological analysis.*

	Evidentiary item		
Algal species	Suspect's sneaker	Victim's sneaker	Pond sediment
Achnanthes minutissima	XX	XX	XX
Achnanthes linearis	X	X	X
Achnanthes sp 1	X	X	X
Amphora sp 1	X	...	...
Anomoeoneis sp 1	XX	XX	X
Cyclotella bodanica	X	X	X
Cymbella gracilis	X	X	X
Cymbella turgida	X	...	...
Eunotia incisa	XX	XX	XX
Eunotia pectinalis	X	X	X
Eunotia flexuosa	X	X	X
Fragilaria sp 1	...	X	X
Tabellaria fenestrata	XX	XX	XX
Tabellaria flocculosa	XX	XX	XX
Stauroneis phoenicentron	X	X	X
Stauroneis sp 1 (small)	...	X	X
Nitzschia parvula	X	X	...
Nitzschia sp 1	X	X	...
Frustulia rhomboides	X	X	...
Gomphonema angustatum	X	X	X
Gomphonema constrictum	X	...	...
Navicula radiosa	X	X	...
Pinnularia viridis	X	...	...
Pinnularia braunii	...	X	...
Mallomonas caudata	XX	XX	XX

NOTE: X = Present in sample.
XX = Abundant in sample.

a pond or lake, rather than a stream or river, as this species is restricted in it's distribution to freshwater ponds/lakes [*14*]. The large percentage of periphytic diatoms (as opposed to planktonic diatoms and scaled chrysophytes) suggested a shallow, vegetated freshwater site. Marked similarities in the species compositions encountered in each sample strongly suggested a common source, that source being the site where the reference sediment sample was obtained. Thus, a qualitative analysis of the diatom and scaled chrysophyte communities present in the mud samples collected strongly suggested a common freshwater source, most likely a shallow, vegetated pond or lake.

In addition to the qualitative analysis described previously, a quantitative assessment of three species of *Eunotia*, a common freshwater diatom, was undertaken. Fifty specimens of *Eunotia* were counted and identified to species in mud samples from the suspect's sneaker and pond scene sediment, respectively. Eighteen *Eunotia* specimens, from the victim's sneaker mud, were similarly treated. The smaller sample size obtained from the victim's sneaker was dictated by the comparatively small amount of mud obtained from this item.

Table 2 lists the numbers of *E. incisa, E. pectinalis*, and *E. flexuosa* found in each of the samples examined and the population ratios of these three species in each sample. A Chi square statistical analysis [*15*] revealed no significant difference between the population ratios in any of the samples. The quantitative analysis of the populations of the three species of *Eunotia* encountered in each of the sediment samples further substantiated the likelihood of a common freshwater source. Rarely are the ratios of these

TABLE 2—*Relative abundance and population ratios of three species of* Eunotia *recovered from case samples submitted for limnological analysis.*

Species	Number of Specimens		
	Victim's sneaker	Suspect's sneaker	Pond sample
Eunotia incisa	10	28	26
Eunotia pectinalis	4	14	14
Eunotia flexuosa	4	8	10
Population ratio[a]	2.5:1:1	3.5:1.8:1	2.6:1.4:1

[a]Chi square goodness of fit test ($P > 0.05$) no significant difference between population ratios.

three species of a common diatom genus *Eunotia* similar between communities from different freshwater habitats. Finding distinct freshwater habitats containing only the same three identical *Eunotia* species, especially in the same relative abundance, is highly improbable.

It is quite rare to find *Mallomonas caudata* as the only dominant species of scaled chrysophyte in a waterbody from the northeastern United States [*14*]. However, *M. caudata* was the most abundant species of scaled chrysophyte on the suspect's sneaker, the victim's sneaker and in the pond reference sample, further indicating that each sample most likely originated from the same locality.

Although the similarities in the floras from all samples examined were striking and clearly indicated exposure to a similar freshwater locality, some diatom species were found in one sample, but not in another (Table 1). Such a scenario is common in the analysis of diverse diatom communities from the same locality. For example, if two counts of 300 individuals each were made from the same mud sample, the relative percentages of the more abundant species would be expected to be similar, but it would not be uncommon for each count to contain specimens of rarer species not found in the other count. If the numbers of specimens counted was increased, more of the rarer species would be encountered in each sample. In our study, the observation of the same dominant and subdominant species in each sample examined clearly indicated that they originated from a similar waterbody.

In May 1992, all three suspects entered guilty pleas to a variety of felony charges stemming from their involvement in this case. Each was sentenced to a lengthy period of incarceration. While a variety of investigative efforts and forensic analyses resulted in the successful resolution of this matter, the unique role that aquatic community ecology played in linking victims and suspects to a common freshwater source is noteworthy. The richness and diversity characteristic of freshwater diatom and scaled chrysophyte communities, when coupled with the resilient nature of their siliceous cell walls, render them ideal for forensic application. Forensic investigators should remain cognizant of the valuable role which limnology and other biological disciplines can play in terrestrial, aquatic and marine scenarios and should seek professional advice, when appropriate, in a timely fashion.

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