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The Development and Application of a Diatom-Based Quantitative Reconstruction Technique in Forensic Science

ABSTRACT: Diatoms are a group of unicellular algae that have been recorded and classified for over 200 years and have been used in a range of applications in forensic science. We have developed a quantitative diatom-based reconstruction technique to confirm drowning as a cause of death and localize the site of drowning in two recent, high-profile, case studies. In both case studies we collected diatom samples from the local and/or regional area to act as a control in the examination of diatom assemblages associated with lungs and clothing. In Case Study 1 the modern analog technique suggested that all lung and clothing samples have statistically significant similarities to control samples from shallow water habitats. In Case Study 2, the analog matching suggested that the majority of lung samples show a statistically significant relationship to samples from a pond, indicating that this was the drowning medium.

KEYWORDS: forensic science, diatoms, drowning, quantitative reconstruction technique

The basic principle of the diatom test in drowning is based on inference that diatoms are present in the medium where the possible drowning took place and that the inhalation of water causes penetration of diatoms into the alveolar system and bloodstream, and thus, their deposition into the brain, kidneys, and other organs (1–6). The application of diatom analysis in determining whether drowning was the cause of death has proved to be a valuable tool in forensic science. Pollanen (2) supported the validity and utility of the diatom tests for drowning through the analyses of 771 cases of drowning. In 90% of cases in which the sample of drowning medium was available, diatom in bone marrow matched assemblages in the drowning medium.

Diatoms are unicellular algae with chrysophyte-like photosynthetic pigments. The cell wall is silicified to form a frustule, comprising two valves, one slightly larger than other and both fitting together like a box and lid (6). Diatoms are microfossils and have been recorded and classified for over 200 years. Classification is predominantly based on the structure of the valve, its shape, intricate patterning, and ornamentation (6,7). In the late 1890s the systematic and taxonomic investigations of modern and fossil diatoms began to be supported by studies of distributional ecology (8). Living diatoms are distributed in almost all aquatic and damp terrestrial habitats. However, it was not until the 1920s that diatom analysis was recognized as a valuable tool in reconstructing ecological changes (9). Different diatom species are highly sensitive to water quality and many species are habitat-specific. Diatom assemblages are well preserved, easily detectable, and occur in high numbers (100–200/cm^3) in sediment and water, thereby providing a good statistical base for ecological interpretations (10–12). However, many interpretations are based on models developed in the 19th century, involving a simple classification of species into freshwater, brackish or marine forms, and provide only qualitative estimates of ecological conditions. Given this paucity of information, ecologists have recently adopted a quantitative approach to ecological reconstruction (13,14). This involves the collection of a training dataset of diatom counts and associated ecological data from modern surface sediments. This dataset is used to calibrate a numerical model, or transfer function, that quantitatively describes the relationship between species and ecological conditions. Once calibrated, the transfer functions can be applied to other assemblages to derive quantitative estimates of key ecological variables such as salinity, substrate, location, etc. This approach has been successfully applied in ecology, limnology, geology, oceanography, and water quality monitoring (10–14).

In this paper, for the first time, we use these quantitative reconstruction techniques in two recent, high-profile case studies of drowning. We aim to correlate samples from the study area and diatoms recovered from clothing and lung tissue/fluid to (a) reinforce drowning as a cause of death and (b) localize the site of drowning.

Classification of Diatoms

Diatoms have been classified in several ways, such as salinity preference, life form, pH preference, trophic classification, and habitat preference. Hudstedt (15,16) classified diatoms within a salinity tolerance spectrum. Polyhalobous species thrive in fully marine conditions, with a salt concentration exceeding 30 practical salinity units (psu). Mesohalobous diatoms thrive in salt concentrations of between 0.2 and 30 psu. Oligohalobous diatoms generally occur in salt concentrations less than 0.2 psu. Vos and de Wolf (17) and Juggins (18) further divided this category into oligohalobous-halophilus, which have an optimum in weakly brackish waters, and oligohalobous-indifferent, which show a preference for fresh water, but are tolerant of slightly brackish conditions. Halophobous diatoms are highly intolerant of salt and are found exclusively in fresh water.

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The classification of life form is based on Denys (19). Euplanktonic diatom species only live in the planktonic habitat, i.e., metabolize and reproduce in the water column. Tychoplanktonic diatoms occur in the plankton, but are derived primarily from other habitats. Epontic diatom species are sessile and normally live firmly attached to substrata. For example, epiphytic live attached to plants and episammic species live attached to sand grains. Benthic diatoms live within the sediment, but not attached to it.

The pH optimum of the diatom flora used in the diatom analysis was based on Hudstedt (20). He classified the diatoms into the following categories: Alkalibiontic diatoms occur in waters of pH value exceeding 7; Alkaliphilous diatoms occur at pH values of about 7, but with the widest distribution at $>$pH 7; Circumneutral diatoms occur equally above and below a pH of 7, with their optimum at or close to pH 7; Acidophilous species occur at pH values of about 7, but with the widest distribution at $<$pH 7; and Acidobiontic diatoms occur at values of $<$pH 7, with optimum distribution at pH 5.5 and under.

The trophic status or productivity of water body from the diatom assemblage follows Naumann (21). In this scheme "irrelevant" implies the species has no clear optimum throughout the trophic spectrum. In simplified terms, diatoms showing a preference for oligotrophic waters have optima in waters lacking plant nutrients, often with a large amount of dissolved oxygen. Mesotrophic waters have low-to-moderate levels of plant nutrients. Eutrophic waters are rich in mineral and organic nutrients that promote a proliferation of plant life, especially algae, and may have a reduced oxygen content. Dystrophic waters are poor in plant nutrients, usually very acidic, and often have a high humic acid content.

The habitat preference of the diatoms (19) is very simple. Diatoms are either classified as "aquatic" or "unknown." Aquatic diatoms are subdivided into those species commonly found in periodic water or wet subaerial habitats, moist subaerial habitats, and dry subaerial habitats. Subaerial indicates the diatoms are found immediately above the surface.

Methods

We collected a sample of surface sediment consisting of approximately 5 cm^3 volume (5 cm^2 surface sample by 1 cm thick) from control stations within the local/regional area. We were provided with diatoms slides from the lungs and clothing by Crown Prosecution Service of England and Wales. In Case Study 1 the diatom slides were prepared from fluid in the cadaver's lungs, whereas in Case Study 2 the diatom slides came from lung tissue taken at the original autopsy. All diatom samples for investigation were prepared following standard methodology (5,10,22). Briefly, the samples were digested in 70–100 mL of 20% H_2O_2 by heating gently in a water bath for up to 24 h, or until all organic matter was removed from the sample. Two and five drops of digested sample were pipetted onto two cover slips with 10 drops of distilled water and dried on a warm hotplate. Cover slips of differing concentration were then inverted and placed onto a glass slide using Zrax, which is a high refractive index medium mountant. After further gentle heating and cooling we counted a minimum of 250 diatoms at a magnification of 1000 times using the keys of Hartley (23) and van der Werff and Huls (24).

Quantitative Analysis

We used cluster analysis of the diatom assemblages to detect, describe, and classify the control samples taken from the local/regional study area into more or less homogeneous clusters (25). Many methods create nested series of clusters, which can be represented as a hierarchy or dendrogram (12–14,25). One of the main usefulness of cluster analysis is grouping of microfossil samples. For example, Horton and Edwards (14) and Zong and Horton (22) showed using cluster analysis that the surface microfossil assemblages from the saltmarshes of the British Isles fell into groups, which closely corresponded with the vegetational zones from which the samples were taken.

We also employed a transfer function, known as modern analog technique, to compare the control samples with the lung and clothing samples. The basic idea of modern analog techniques is to compare numerically, using an appropriate dissimilarity or similarity measure, the diatom assemblage in a lung or clothing sample with the diatom assemblages in all available control samples. Having found the control sample(s) most similar to a lung or clothing sample, the local environment for the latter is inferred to be the locality of the analogous control sample(s) (26). Examples of this simple use of a modern analog technique include Le (27), Bartlein and Whitlock (28), and Hayward et al. (29). Furthermore, the modern analog technique is an important means of evaluating the likely reliability of ecological reconstructions (13,14). Horton and Edwards (13) used the technique to assess the significance of spatial variability for transfer function development. We calculate the dissimilarity between a lung and clothing sample and the most similar control samples, using the squared chord distance as the dissimilarity coefficient (30). The 10th percentile of the dissimilarity range calculated between control samples is an approximate threshold value to indicate a "good analog" (13,14,26). Thus, the reconstructed location for lung and clothing samples was assumed to be reliable if a "good analog" (dissimilarity coefficient $\leq$ 10th percentile) was indicated while the estimates associated with "no close analog" (dissimilarity coefficient $>$ 10th percentile) samples should be treated with caution. Cluster analysis and modern analog technique were conducted using the TILIA (31) and C2 (32) programs, respectively. For all statistical analyses we removed all species groups that contributed less than 2% of any assemblage (10–14,22).

Case Study 1

Context

A body of a woman was found face down floating in a river. Postmortem examination found the death to be suspicious. Death was attributed to the asphyxial effects of drowning due to homicide; however, a key aspect of the subsequent investigation was the precise site of drowning. Thus, we collected 12 samples for diatom analysis from five sites along a 50 km length of the river, including the body recovery site (Fig. 1) to act as a control in the examination of diatom assemblages associated with lung fluid and clothing belonging to the accused (training shoe, socks, and T-shirt). At each sampling site, we collected diatom samples upstream of the local weir from a variety of river bed and river bank habitats to permit comparisons with the ecological conditions at the body recovery site.

Control Samples

We identified 99 different diatom species from the 12 control samples. The diatom assemblages are very similar and share many species. The dominant diatom species present include *Navicula radiosa*, *Cocconeis placentula* var *euglypta*, and *Achnanthes lanceolata*. However, there are variations in the presence or absence of minor species and changes in the proportions of

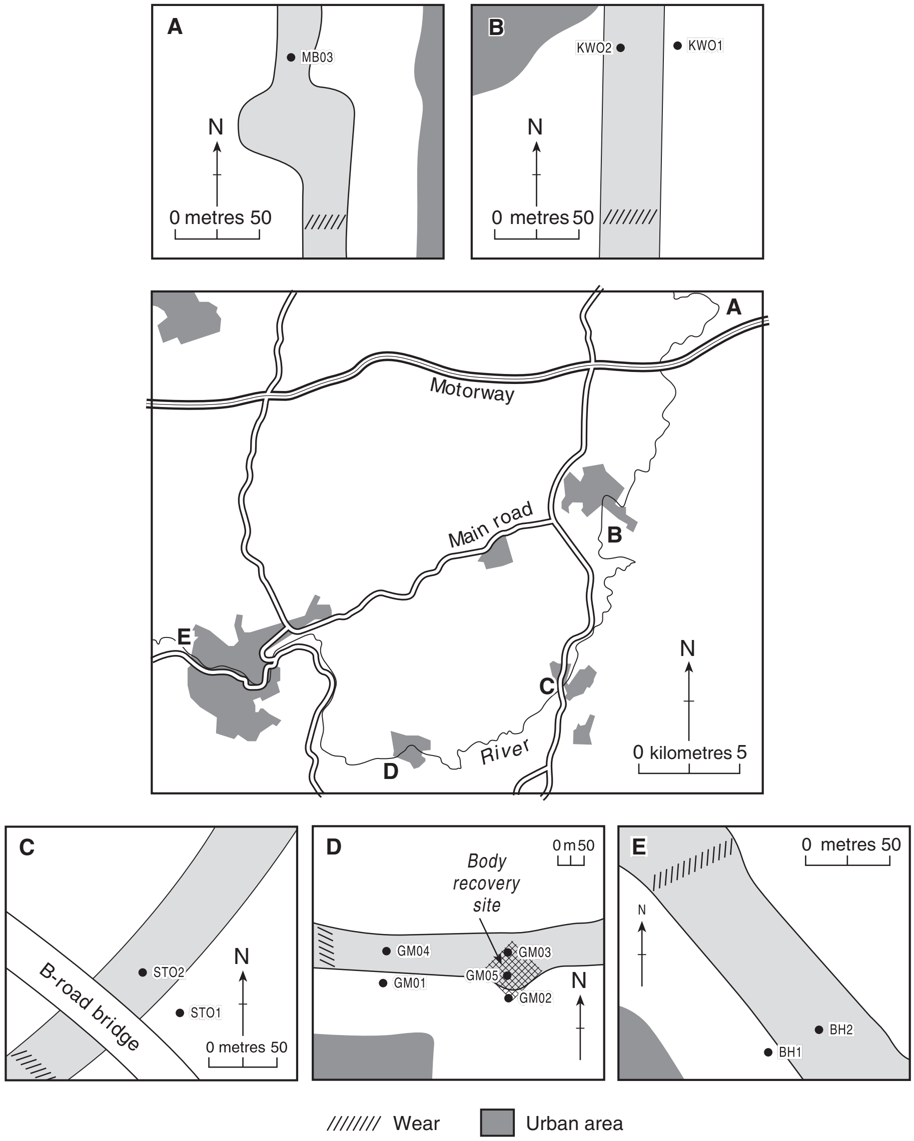

FIG. 1—*Location map for Case Study 1.*

the dominant species; this is expected when one considers the complexity of in-channel microhabitats. Figure 2 illustrates that the samples are divided into three reliable cluster zones:

- I. Samples GM/03, KW/02, BH/02, and GM/04 occupy zone I. All four samples are taken from in-channel habitats at a depth of 1.5 m or deeper with moderate flow. The zone is characterized by the benthic species *N. radiosa* and low percentages of the sessile/epontic species *C. placentula* var *euglypta*.
- II. The zone is comprised of five samples (GM/01, GM/02, ST/01, KW/01, and BH/01), which are taken from the river bank, above the water level. These samples differ from the in-channel habitats in zones I and III. They are characterized by having the lowest percentages of *N. radiosa* and higher percentages of sessile/epontic species such as *C. placentula* var *euglypta* and *A. lanceolata*.
- III. The zone has three samples (GM/05, ST/02, and MB/03), which are from shallow water habitats (less than 1.5 m deep) and slow water flow. Zone III is composed of relatively high percentages of *N. radiosa* and *C. placentula* var *euglypta*.

Comparison of Control Samples with Diatom Assemblages from the Lung Fluid and Clothing

The lung fluid (GCAF 16), training shoe (KP31D) and T-shirt (KP87D) are characterized by high percentages of *C. placentula* var *euglypta*, *Melosira varians*, and *N. radiosa*. Thirty-five of the 37 diatom species from the lung and clothing samples are found in the control samples. Subsequently the modern analog techniques suggest that all lung and clothing samples have a "good analog." The assemblages show statistically significant similarities to all control samples from shallow water habitats with slow water flow (GM/05, ST/02, and MB/03). However, the reconstructions suggest that the closest control analogy for the lung fluid, training shoe, and T-shirt is sample GM05 (Table 1). GM05 is from the body recovery site, 100 m upstream of a weir, from the edge of an embayment, near the river bank with a water depth of 0.5 m.

In contrast, the sample from the sock is characterized by a relatively low percentage of *N. radiosa* and the higher percentages of *C. placentula* var *euglypta*. The assemblage shows a strong sim-

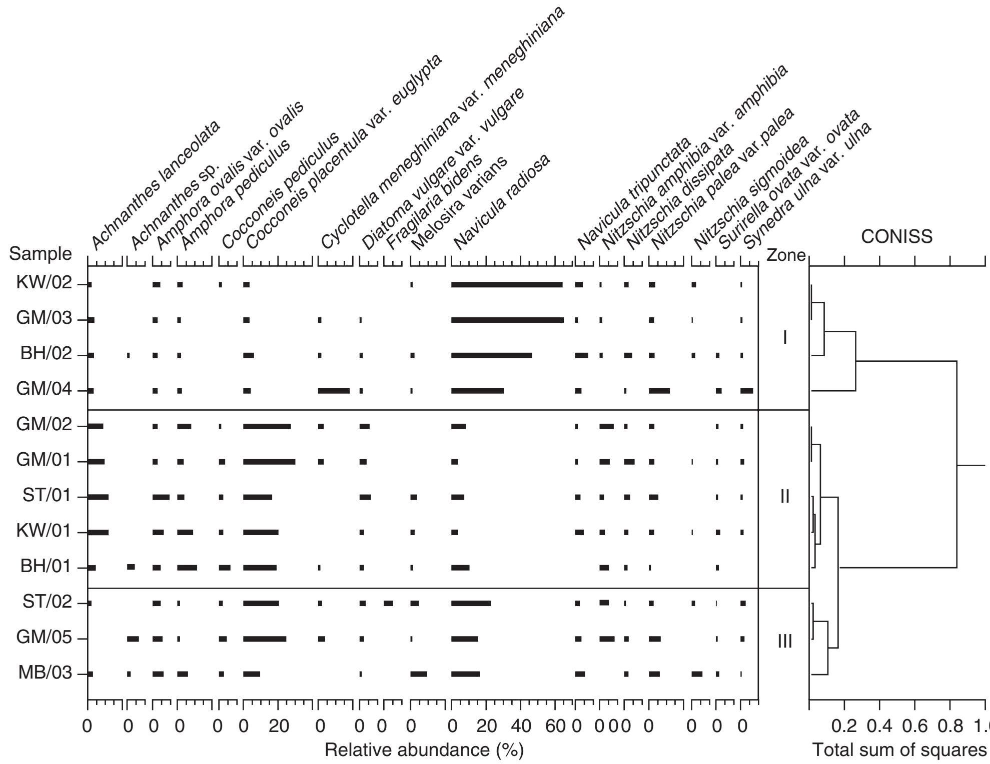

FIG. 2—*Unconstrained cluster analysis based on unweighted Euclidean distance of diatom assemblages from Case Study 1 (only species greater than 5% are shown).*

ilarities to control samples taken from the banks of the river, which all provide "good analogs" (GM/01, GM/02, ST/01, KW/01, and BH/01). The analog-matching technique suggests the sock has the closest analogy with control sample GM02, which is upstream of weir at the bottom of the ramp/slipway adjacent to the body recovery site, 10 cm above water level.

Case Study 2

Context

The body of a boy was found face down floating in a pond. The post mortem conducted at the time concluded that death was by vasovagal inhibition as a result of cold water immersion. The pathologist attributed death to fresh water drowning and concluded that death was not suspicious. However, the case was reopened as it was thought the drowning may have been homicide by the child's mother. It was suggested that drowning took place in a domestic bath, and that the body was subsequently placed in the pond. Thus, we collected 14 samples for diatom analysis from four transects around the circumference of the artificial pond and two samples of sediment from the center to act as a control in the examination of diatom assemblages associated with three lung tissue samples (Fig. 3).

Control Samples

We identified 37 different diatom species from the control samples of the pond. The dominant diatom species include *A. lanceolata*, *Achnanthes hungarica*, and *Navicula cryptocephala*. The results of the unconstrained cluster analyses show that the samples are divided into two reliable cluster zones (Fig. 4):

I. The zone consists of 33 species of diatom and is characterized by the relatively high percentages of *A. hungarica*, *A. lanceolata*, *C. placentula*, and *N. cryptocephala*. The *Achnanthes* species recorded are epontic diatoms that have an optimum in eutrophic water with pH values of about 7, but with widest distribution at a pH > 7, and are adapted to periodic/wet subaerial environments. *C. placentula* is an epiphytic species and its presence is most likely to be associated with the peripheral and emergent vegetation within the pond, whereas *N. cryptocephala* is a benthic species. The samples within zone I come from the bottom and edge of the pond.

II. The zone is characterized by low percentages of *A. hungarica* and one sample with a high percentage of *Hantzschia amphioxys* (SAM06). Zone II has a lower proportion of alkalibiontic to alkaliphilous, eutrophic–mesotrophic, periodically wet subaerial, and epontic diatoms than other zones. The samples within this zone are from ephemeral habitats at the edge or landward of the pond that are prone to frequent desiccation.

Comparison of Control Samples with Diatom Assemblages from the Lung Tissue and Clothing

It is difficult to speculate on the origin of the diatoms in the three lung samples and to postulate about their source without examining a sample of water from the pond at the time of drowning. The diatom flora of the control samples from the pond today are much more species diverse than the diatom flora in the lungs. Nevertheless, the diatom assemblages in the lungs comprised of many species that are also found in the diatom flora of the pond. The habitat preferences of the diatom species found in the lungs are likely to be a body of water with a pH of around 7 or slightly higher where the water is eutrophic or eutrophic–mesotrophic, that is prone to frequent desiccation around the periphery and/or changes in water depth. The aquatic habitat would also have a variety of substrates providing an array of microhabitats/ecological niches for benthic and epontic species. This inference is supported

TABLE 1—*The inferred location of the control sample generated by the modern analog transfer function as the closest analog.*

Case Study	Sample	Dominant Diatom Species	Location	Min. DC	Analog
1	Lung fluid	*Cocconeis placentula* var *euglypta* *Melosira varians* *Navicula radiosa*	GM05	0.14	Good
	Training shoe	*Cocconeis placentula* var *euglypta* *Melosira varians* *Navicula radiosa*	GM05	0.07	Good
	T-shirt	*Cocconeis placentula* var *euglypta* *Melosira varians* *Navicula radiosa*	GM05	0.14	Good
	Sock	*Cocconeis placentula* var *euglypta* *Melosira varians*	GM02	0.03	Good
2	Lung tissue A	*Hantzschia amphioxy* *Nitzschia palea*	SAM06	0.12	Good
	Lung tissue B	Indeterimate pennate species	SAM07	0.13	Good
	Lung tissue C	*Navicula accomoda*	SAM06	0.28	Not close

The critical value (10th percentile) for the dissimilarity coefficients (min. DC) produced by the modern analog technique is 0.17 and 0.15 for Case Studies 1 and 2, respectively.

by the modern analog technique; two of the three lung tissue samples have a "good analog" in the control samples. The closest analogs for all three lung samples high percentage are SAM06 and SAM07 (Table 1). Samples SAM06 and SAM07 are from the landward edge of the longest transect consisting of five samples (SAM06–10). Sample SAM06 is taken from an area of mossy vegetation at the base of the bank and SAM07 is from the sediment overlying the pond liner and gravel (Fig. 3). These samples are inundated periodically in response to local climate and water table variations. Lung tissue C has a moderate abundance of *Navicula accomoda*, which does not occur in the control samples and thus a no analog situation occurs.

Discussion

The diagnosis of drowning is one of the most difficult in forensic pathology. Diatom analysis has been proposed to provide supportive evidence of drowning, but the reliability and applicability of quantitative and qualitative diatom analysis in the diagnosis of drowning is still disputed in the literature (1,2,5,6,34). Drowning is substantiated as a cause of death when the types of diatoms in human organs matches diatoms present in the putative drowning medium (1,2,34). The transfer function approach presented in this paper offers a quantitative method to provide an informal assessment of "reliability" of correlations between control samples and samples from organs and clothing. In simple terms, the greater the dissimilarity between an organ and clothing sample and all samples in the control dataset, the more the transfer function is forced to extrapolate and the more prone the resultant estimate will be to error. For our two case studies, all organ and clothing samples except one (Case Study 2, Lung tissue C) have matching analogs in the modern training set, reinforcing drowning as the cause of death.

The analog matching provides further information on the location of drowning. Several circumstances arise that make localization of the precise site of drowning an important medicolegal issue (1,2,6,35). One situation is the differentiation of drowning in a domestic bath vs. a naturally occurring body of water when a body is recovered from the latter. In cases such as this, the modern analog technique can compare the diatoms present in the two sites with those occurring in organs and clothing samples. Results from Case Study 2 suggested that two of the three available lung samples show a statistically significant relationship to samples from the pond, indicating that the pond was the location of drowning. This was an essential piece of evidence in the acquittal of the accused woman of drowning the boy in a

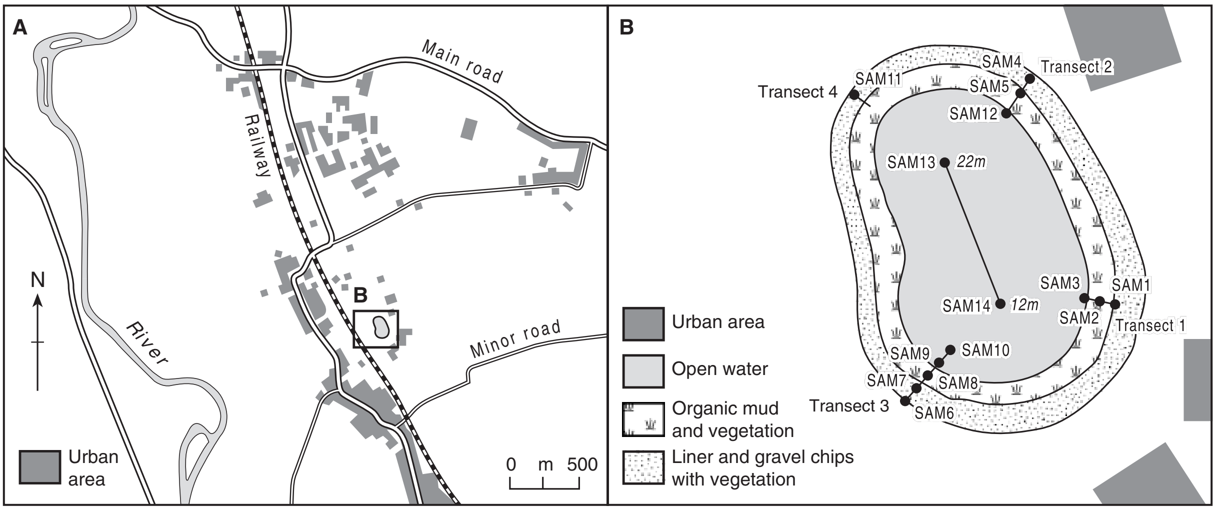

FIG. 3—*Location map for Case Study 2.*

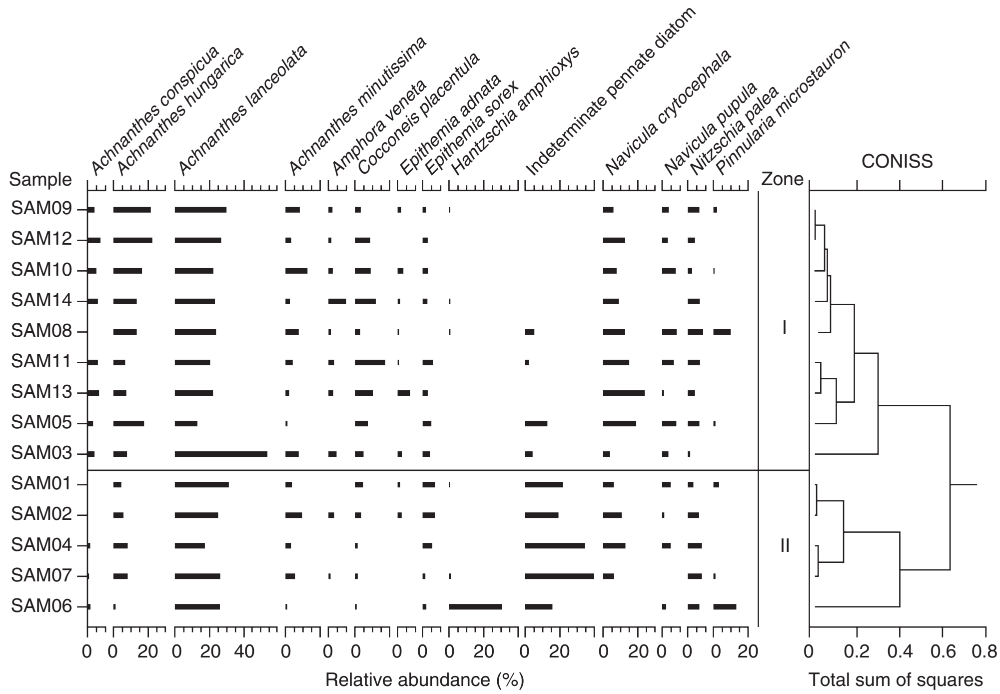

FIG. 4—*Unconstrained cluster analysis based on unweighted Euclidean distance of diatom assemblages from Case Study 2 (only species greater than 5% are shown).*

bathtub. In Case Study 1 the diatom-based reconstruction technique suggests strong similarities between lung and clothing samples, and control samples from shallow water habitats. Furthermore, analog matching implies that the site of drowning was at the body recovery site. These conclusions were pieces of evidence that assisted in the conviction of a man for murder.

While diatom-based transfer functions have the potential to produce reliable, high-resolution ecological reconstructions that correlate the diatoms present in the organs with those found in the putative drowning medium and enable valuable conclusions regarding the possible place of drowning, a number of limitations are also associated with their use. Firstly, the composition of the modern training set (the control samples) has the potential to significantly influence the performance of the resulting transfer function. Important questions regarding training set compilation include what constitutes an appropriate sample size and the spatial range over which samples should be collected. To control the random component arising from sampling variability, the sample size should be as large as practical (affordable in terms of collection time, analysis, and cost). To our knowledge, there are no clear guidelines indicating a minimum sample size for transfer function training sets. Predictions based on smaller training sets will be more prone to error, as the range of modern analogs will be restricted and spatial variability is likely to be under represented. Secondly, temporal variations in microfossils, such as diatoms, have been documented in many studies and may reduce the precision of the transfer functions (14,36). Therefore, a modern assemblage sampled at any one occasion may or may not be in equilibrium with the environment or be typical of assemblages over a longer time period. This is an important issue for any study that seeks to use surface assemblages as modern analogs to confirm drowning as a cause of death and/or localize the site of drowning. Horton and Edwards (36) conclude that an investigation of modern microfossils that recovers a complete set of samples in the winter, spring, summer, and autumn will provide the best quality data for use in environmental investigations. If only one set of measurements can be obtained, sampling in the winter months, with their lack of biological productivity, may represent the most reliable alternative. The accuracy of the quantitative technique is also hindered by spatial diatom variations. The magnitude of the errors are relatively high, especially if measurements are taken only once from a single transect (37).

Finally, there are potential problems associated with the data from tissues and organs. Diatoms have been found in victims who were not drowned (38) and this raised a whole series of questions relating to how diatoms can come to be present in body tissues. Certain species are thought to be lifted up into the air and transported great distances, therefore, providing a source of diatoms that can be inhaled (39). Schneider (40) suggested that diatoms frustules in human tissues can be introduced from food and drinking water. Auer (41) states that diatoms may come from laboratory glass and reagents used during the analysis. In contrast, diatoms may be absent or present in very low numbers in tissues from victims who did drown. Sidari (42) suggested that this may be due to certain diatom preparation techniques. Their experiments showed that the siliceous frustule of sea water diatoms is solubilized by Solune-350 while fresh water diatoms are resistant to the treatment. Krstic (1) suggested that rapid death could prevent the penetration of diatoms into the bloodstream and their subsequent deposition in the organs, whereas Geertinger (43) concluded that drowning may have occurred in water deprived of diatoms. Diatom assemblages are also further to postdepositional changes as the result of diagenetic processes in human tissues and lungs. These include selective preservation of diatoms, which depends on frustule composition and structure, and transportation of frustules away from (loss) and into the assemblage (mixing) (10,13,22). Problems can further arise when analyzing the diatoms present on clothing. When individuals run or walk through a garden pond or a stream, or indeed any body of water, particulate material in the water (including diatoms) can remain on clothing afterwards (5). Thus, there is a potential problem of population super-position (i.e. the analytical effects of exposing an item of clothing to several successive environments before recovery). Ultimately, it is important that the analog matching reconstructions are not considered in isolation, but are evaluated by comparison with other supporting forensic data.

Despite these limitations, ecological reconstructions using a diatom-based transfer function have advantages in terms of precision, speed of response and applicability over traditional diatom methods currently employed in forensic research. With an increasing demand for high-resolution studies, particularly those seeking to identify the link between human organs and the drowning medium, it is imperative that the new generation of quantitative techniques employed are of the highest possible precision and accuracy. A vital step toward the realization of this aim is the collection of more surface data to improve the range of modern analogs covered by modern diatom datasets and the investigation of the role that seasonal and spatial variability may play.

Conclusion

The most frequent use of diatoms in forensic science is the diagnosis of death by drowning. In this paper, we use, for the first time, a relatively new quantitative diatom-based reconstruction technique in two recent, high-profile case studies to confirm drowning as a cause of death. Furthermore, we use the quantitative model to correlate control samples from the study area and diatoms recovered from clothing and lung to localize the site of drowning.

In Case Study 1 a body of a woman was found face down floating in a river. We collected samples for diatom analysis from sites along the length of a river, including the body recovery site, to act as a control in the examination of diatom assemblages associated with lung fluid and clothing belonging to the accused. The modern analog technique suggests that all lung and clothing samples have statistically significant similarities to control samples from shallow water habitats. The reconstructions suggest the site of drowning was the body recovery site.

In Case Study 2 the body of a boy was found face down floating in a pond. The case was reopened because of a suspicion that drowning had occurred in a domestic bath and the body transferred to the pond. We collected samples for diatom analysis from transects around the circumference and center of the pond in which the deceased was found. Although it is difficult to speculate on the origin of the diatoms in the lung samples without examining a sample of water from the pond at the time of drowning, the analog matching suggested that the majority of lung samples show a statistically significant relationship to samples from the pond, indicating that the pond was the location of drowning.

Diatoms have a number of characteristics, including their widespread occurrence, sensitivity to environmental water quality, good preservation, easy detection, and prevalence in high numbers (100–200/cm^3) for a good statistical base for quantitative interpretations, to suggest that they have further use in forensic investigation.

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