J Forensic Sci, July 2008, Vol. 53, No. 4
doi: 10.1111/j.1556-4029.2008.00748.x
Available online at: www.blackwell-synergy.com

Kathryn A. Zimmerman,[1] *B.Sc. and John R. Wallace,*[1] *Ph.D., D-ABFE*

The Potential to Determine a Postmortem Submersion Interval Based on Algal/Diatom Diversity on Decomposing Mammalian Carcasses in Brackish Ponds in Delaware*

ABSTRACT: Recently botanical evidence has been studied to determine if it is useful in forensic investigations**.** This study was performed to examine stillborn piglet decomposition in a brackish water environment and to semi-quantitatively document stages of decomposition, degree day accumulation per stage as well as the algal/diatom diversity useful in determining a postmortem submersion interval (PMSI). Piglets and ceramic tiles were submerged in brackish ponds and sampled on a regular basis to document algal diversity and succession between substrates and stages of decomposition. Significantly greater weight was lost from piglet carcasses during the early floating and advanced floating decay stages. Seasonal effects were observed in degree-day accumulations. Diatom diversity was significantly greater on piglet carcasses compared to tile substrates. Algal diversity decreased over time on the piglet carcasses as well as the stage of decomposition. A significant relationship and strong correlation between algal diversity found on the piglet substrate with time was observed. Our results indicate that more research is needed to examine the potential to use diatoms in not only determining manner of death but also the duration of time (PMSI) a victim may have been immersed in an aquatic environment.

KEYWORDS: forensic science, postmortem submersion interval, brackish water environment, diatoms, algae

In death scene investigations, the determination of time-since-death or the postmortem interval (PMI) is critical for criminal investigators. Because terrestrial insects have evolved to feed on carrion, this life history strategy has facilitated their use to estimate a portion of the PMI (1). However, in aquatic systems such as streams and rivers, aquatic insects have not evolved this feeding strategy and consequently their direct association with a corpse correlated to any specific time is difficult to establish (2). Casamatta and Verb (3) used algal succession to estimate a postmortem submersion interval (PMSI), i.e., the time period the body was submerged to time of discovery. In addition, recent research has shown that algal production in the form of chlorophyll *a* can be quantified and lends a solid quantitative approach to estimate a PMSI (2).

Algae are found everywhere in aquatic systems and can be identified using a light microscope. Moreover, diatoms are present throughout the entire year (3,4). Diatoms and other algae have been found useful in a few criminal investigations; however, due to their complex population dynamics and ecology, diatoms have great, but underutilized forensic significance (4). For example, some forensic cases have identified diatoms inside the organs (e.g., brain and liver) of drowning victims, as well as on suspects/victims, or their clothing, thereby linking them to specific aquatic crime scenes (2,4).

A unique taxonomic division of algae is represented by unicellular plants called diatoms (Order Bacillariophyta). These algae are single celled and can be characterized by the environment in which they live, e.g., some genera of diatoms inhabit only freshwater, others only saltwater (5). Diatoms are abundant in naturally occurring waters such as lakes, ponds, rivers, oceans, and salt marshes and are typically used as good indicators of water quality (6).

While diatoms have been documented from some crime scenes (4), their application in a quantitative fashion to establish a PMSI has been wanting. Specifically, in lentic systems such as ponds or lakes (freshwater or marine), no research has examined diatomology in these types of aquatic environments. The purpose of this study was to develop a semi-quantitative approach to estimate a PMSI based on species diversity over time. The objectives of this study were the following: (1) to determine the rate of decomposition of a mammalian carcass in a brackish pond, (2) to compare the algal/diatom diversity on a mammalian carcass during five stages of decomposition to an artificial substrate such as a ceramic tile, and (3) to develop a semi-quantitative approach to estimate a PMSI using algal diversity.

Materials and Methods

Study Sites

This study was conducted from May to July 2004 in two brackish water ponds in Smyrna, Delaware. Two ponds (*c.* 0.5 hectares in size) were selected based on the similar salinity content. The ponds were located within the Delaware State Aquatic Research Education Center (AREC) facility. Both ponds were open to direct sunlight and were bordered by grasses and shrubs.

Physical/Chemical Analysis

Water temperature was recorded at continuous 10-min intervals for the duration of the study using an OnSet® Tidbit temperature probe (Onset Computer Corporation, Bourne, MA). The temperature probe was secured to a piece of rebar that was submerged securely in the pond. Very little data exist on the calculation of

[1]Department of Biology, Millersville University, Millersville, PA 17551.

*Presented as a poster at the 50th Annual Meeting of the American Academy of Forensic Science in New Orleans, LA, February 2005.

Received 4 Mar. 2007; and in revised form 21 June 2007; accepted 20 Oct. 2007.

degree-day requirements for aquatic plants or more specifically, diatoms. However, Montagnes and Franklin (7) have indicated that at 8°C, diatoms have continued access to carbon and nitrogen necessary for growth/development, and Spencer and Ksander (8) have also used a minimum threshold of 8°C to calculate degree days for other aquatic plants, e.g., *Hydrilla*. Therefore, we calculated degree days with a minimum threshold of 8°C for this study. The salinity of water was recorded at each site using a digital refractometer (Pocket Pal-1; ATAGO U.S.A, Inc., Kirkland, WA). On every sample date, three salinity measurements were recorded and averaged for the day.

Decomposition Analysis

To simulate a mammalian substrate and control the impact of large scavengers, stillborn piglets (average weight = 1–1.5 kg prior to submersion) were used, obtained from the Penn State University Swine Research Facility. Piglet carcasses were placed on plastic trays inside Havahart® traps (Havahart®; Animals B-Gone, Orrstown, PA) (0.61 × 0.18 × 0.18 m). The cages were submerged and secured to the bottom of the pond (*c*. 0.75 m depth) using rebar (2). To serve as a control, unglazed ceramic tiles (7.62 × 7.62 × 0.635 cm each) were cemented to the surface of concrete blocks which were then submerged on the opposite side of the pond. The ceramic tiles were used to simulate a rock substrate that would naturally be found in the pond.

Stillborn piglets were weighed using an Ohaus® portable digital scale (Ohaus Corporation, Pine Brook, NJ); on every sample date, weights were recorded to determine decomposition rate as a function of weight loss over time. In Pond 1, samples were taken from piglets and tiles every 3 days for a 15-day period ($n = 5$ sample dates) during May 2004. Because decomposition occurred much faster than anticipated during the first trial with Pond 1, samples were collected every 2 days in Pond 2 to obtain a greater number of samples. Therefore, Pond 2 samples were taken from submerged stillborn piglets and tiles every 2 days for a 12-day period ($n = 6$ sample dates) during July 2004. The length of each sampling period was determined by the rate of decomposition. Samples were collected until no carcass remained.

Stillborn piglets and unglazed ceramic tiles were used as substrates for the colonization of algae. Five piglets and six blocks containing 12 tiles glued on each block were submerged at both study sites. Using a modified sampling device described by Steinman et al. (9), the same technique was used to collect algal samples from the surface of the stillborn piglet carcasses and tiles. Algal samples collected from the piglet carcasses were sampled from areas on the carcasses unsampled prior to each date. Each algal sample was placed into a glass jar and transported to the laboratory and preserved using concentrated HCl and Lugol's solution (iodine/potassium iodide) (ratio of 1:100). The samples were stored at room temperature in the dark, away from light to help decrease the possible degradation of preserving solution until analysis.

Algal Diversity Analysis

To document diatom diversity in each sample, five tiles were sampled and slides per algal sample were made for pigs ($n = 5$) and tiles ($n = 5$). Each sample was mixed using a vortex mixer (The Lab Depot, Dawsonville, GA) for 30 sec. A 0.1-mL liquid sample of this slurry was placed on a glass slide and heated with a hot plate until all liquid material evaporated and most of the organic material had burned off. The slides were immediately viewed using a light microscope (Nikon Eclipse E400, Melville, NY), and digital images were recorded to use later for diatom identification. Algae/diatoms were identified with a key from Patrick and Reimer (18) and later confirmed by a diatomologist, Dr. Marina Potopova from the Pennsylvania Academy of Science, Philadelphia, PA.

Statistical Analysis

A one-way ANOVA was used to compare the daily mean water temperature of Pond 1 and Pond 2. Stages of decomposition were determined by piglet weight loss over time. Degree days for each stage of decomposition were calculated based on the daily mean water temperature.

The Sorenson's index summarizes the similarity of the communities and was used to compare the similarity of diatom species in Pond 1 versus Pond 2, as well as comparing the similarity of piglet versus tile substrates in both ponds. A Sorenson's value of 1.0 indicates complete similarity, whereas a value of 0.0 indicates complete dissimilarity. A Spearman's rho correlation coefficient was calculated to determine a relationship between the number of diatom species and stages of decomposition. This test is used to show if a relationship exists; it does not determine cause and effect, but examines the association between rank orders, in this case, stages of decomposition. Algal diversity between pig versus tile and among stages of decomposition was statistically compared using a one-way ANOVA. Linear and polynomial regressions were used to analyze the correlation between algal diversity and substrate over time. Statistical analysis was completed through Microsoft Excel (Microsoft Corporation, Redmond, WA) and SPSS (Chicago, IL).

Results

Physical/Chemical Analysis

The average salinity of both ponds was 0.14 ppt (SE = 0.024). In Pond 1, the average daily water temperature ranged from 20.6°C to 27.1°C. In Pond 2, the average daily water temperature ranged from 25.3°C to 27.82°C. The average daily water temperature of Pond 2 was significantly greater than Pond 1 ($F = 5.135$; $p < 0.05$). The mean water temperature and degree days of each stage of decomposition were calculated for both study sites (Table 1). Piglets submerged in Pond 1 required a greater number of degree days for complete decomposition in comparison to Pond 2.

Decomposition Analysis

Five stages of decomposition were determined and described for both Pond 1 and Pond 2 (Fig. 1). There was a strong negative correlation of weight loss with the progression of time ($R^2 = 0.99$) (Fig. 2). There was a significant amount of weight loss between the early floating stage and the advanced floating decay stage of decomposition ($F = 6.87$; $p < 0.05$).

Algal Diversity Analysis

Diatoms and other algae were identified to genera and species (Table 2). The most abundant genera observed in both Pond 1 and 2 were *Amphora, Nitzchia, Pinnularia, Navicula,* and *Cyclotella*. In addition to diatoms, desmids and other algae were observed in both ponds, such as *Scenedesmus, Closterium,* and *Cosmarium*. The Sorenson's index was calculated to measure the similarity of diatom species between Pond 1 and Pond 2 (C = 0.58).

TABLE 1—*Mean temperature and total degree-days per stage of decomposition in Pond 1 and Pond 2.*

	Temperature (°C)			
	Pond 1	Pond 2	Total Degree Days	
Stage of Decomposition	Mean	Mean	Pond 1	Pond 2
Submerged fresh	25.72 (0.48)	25.11 (0.22)	70.86 (11.65)	51.33 (9.82)
Early floating	26.00 (0.24)	27.10 (0.76)	161.97 (16.50)	152.62 (17.25)
Floating decay	24.75 (0.52)	26.09 (0.78)	117.27 (13.39)	90.47 (12.24)
Advanced floating decay	24.03 (0.54)	26.68 (0.74)	208.40 (17.83)	130.77 (15.62)
Sunken remains	22.84 (0.60)	26.68 (0.74)	148.44 (13.60)	130.77 (15.62)
		Total	706.94	555.96

Standard errors of the mean temperatures are in parentheses. Average degree-days per stage are in parentheses and calculated based on a minimum threshold of 8°C.

Submerged Fresh	Early Floating	Floating Decay	Advanced Floating Decay	Sunken Remains
Fresh; still sunken: stage ends when body floats to surface	Floating on surface; bloated; cage indentations on top of carcass; algal growth visible	Minor decay apparent; disarticulation of soft tissues; loss of muscle mass; identity of carcass still evident	Major decay apparent; exposed bones, including skull; loss of bones; identity of carcass becoming difficult to distinguish	Remains sunken at bottom of cage; "soup-like" consistency of remaining skin; mostly bones remaining in cage
				

FIG. 1—*The physical description of the stage of decomposition, along with a photograph of a representative stillborn piglet carcass for each stage of decomposition.*

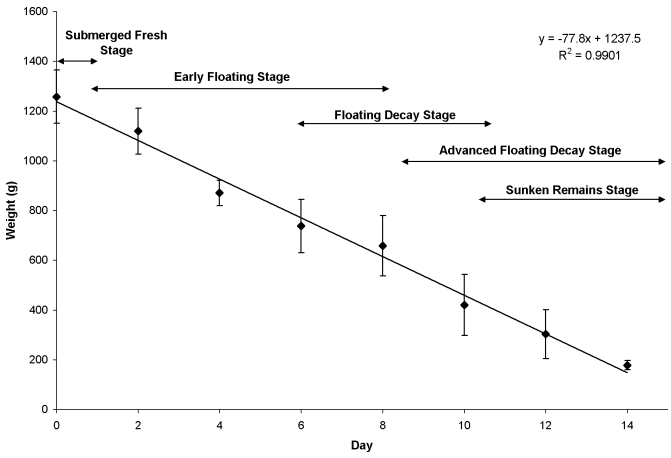


FIG. 2—*Linear regression comparison of the mean weight loss of piglets per sample date. Data represent averages between ponds. Average duration of each stage of decomposition is indicated with arrows.*

In Pond 1, diatom diversity was greatest during the early floating stage of decomposition for both piglets and tiles (Fig. 3). However, in Pond 2, diatom diversity was greater on the stillborn piglet substrate during the early floating stage of decomposition compared to the tile substrate, the latter substrate contained a greater amount of diatom diversity during the floating decay stage of decomposition. In Pond 1, there was a significantly greater number of species of diatoms found on the piglet substrate in comparison to the tiles (control) ($F = 8.67$; $p < 0.05$). Conversely, overall diatom diversity did not differ between piglet and tile substrates later in the season in Pond 2 ($F = 0.75$; $p > 0.05$). In Pond 1, the Sorenson's index was calculated to measure the similarity of diatom species between piglet substrate and tile (C = 0.35), whereas in Pond 2, the Sorenson's index was greater (C = 0.69), indicating a greater similarity in diatom assemblages between piglet and tile substrates in the latter. The Spearman's rho correlation coefficient was significant at the 0.01 level (two-tailed) for an inverse relationship between the number of diatom species and stages of decomposition ($\rho = -1.000$) in both Ponds 1 and 2. A significant negative correlation was observed between algal diversity found on the piglet substrate over time in both ponds (Pond 1: $p < 0.05$; $R^2 = 0.90$; Pond 2: $p < 0.05$; $R^2 = 0.97$) (Figs. 4*A* and 4*B*). No significant relationship was found between algal diversity found on the tile substrate with time in either pond (Pond 1: $p > 0.05$; $R^2 = 0.61$; Pond 2: $p > 0.05$; $R^2 = 0.59$) (Figs. 4*A* and 4*B*). The polynomial regression analysis revealed a very strong correlation between algal diversity

TABLE 2—*Diatoms found in algal samples identified to genera and/or species for Pond 1 and Pond 2.*

	Early Floating Stage		Floating Decay Stage		Advanced Floating Decay Stage	
Genera/Species	Pig	Tile	Pig	Tile	Pig	Tile
Pond 1						
Amphora pediculus	•	•	•			
Amphora veneta or copulata	•	•	•		•	
Amphora sp. 1			•		•	
Cocconeis placentula	•		•			
Cocconeis sp. 1	•	•	•	•		•
Cyclotella sp. 1	•	•	•			
Fragilaria capucina	•					
Frustulia rhomboides	•		•			
Navicula gregaria	•					
Navicula lanceolata	•					
Navicula trivialis	•		•		•	
Navicula sp. 1	•	•	•	•		•
Navicula sp. 2	•		•			
Navicula sp. 3		•		•		
Nitschia capitellata						•
Nitzschia sp. 1	•		•			
Nitschia sp. 2		•		•		
Nitschia sp. 3		•		•		
Plagiotropis lepidoptera	•				•	
Tryblionella acuminata	•					
Unknown 1	•		•			
Unknown 2			•		•	
Unknown 3			•		•	
Unknown 4		•		•		
Pond 2						
Amphora copulata	•					
Amphora pediculus		•		•		
Cyclotella	•	•	•	•	•	•
Navicula trivalis	•	•	•	•	•	
Navicula sp. similar to trivalis	•					
Navicula sp. similar to salinarum	•					
Navicula sp. 1		•		•		
Navicula sp. 2	•		•	•	•	•
Navicula sp. 3	•	•		•		
Nitzchia capilellata	•	•	•	•	•	
Nitzchia heufleriana	•					
Nitzchia reversa	•					
Nitzchia sp. 2	•	•	•	•		•
Nitzchia sp. 3	•		•		•	
Nitzchia sp. 4	•	•	•	•	•	•
Pinnularia subcapitata	•					
Pinnularia virardis	•	•	•	•	•	•
Pinnularia sp. 1	•	•	•	•		
Unknown 1	•	•	•	•		•
Unknown 2	•		•	•		•
Unknown 3	•		•			
Unknown 4	•		•			
Unknown 5	•	•	•	•		
Unknown 6		•		•		

found on the piglet substrate with time for both ponds (Pond 1: $R^2 = 1.00$; Pond 2: $R^2 = 0.98$) (Fig. 5).

Discussion

The importance of diatoms has been known and utilized in many areas of science. Diatoms are important primary producers and have been used to determine water quality, and recently they have been used in criminal investigations (4). Their use in case work, while limited, has included linking suspects to the crime scene and determining if a victim had drowned. Studies in freshwater and marine environments indicated that diatoms were the dominant

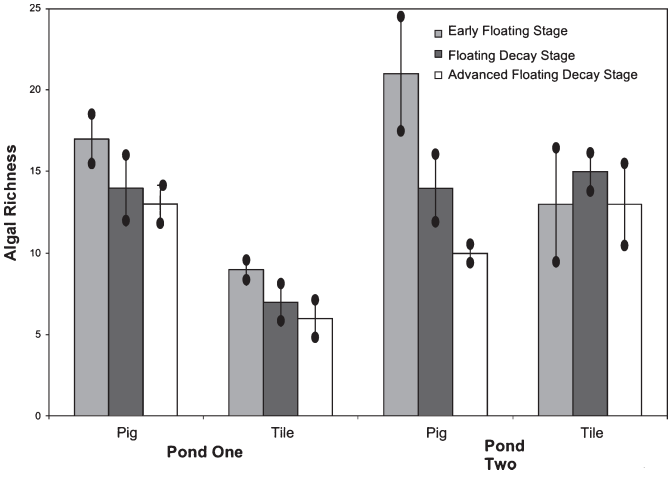


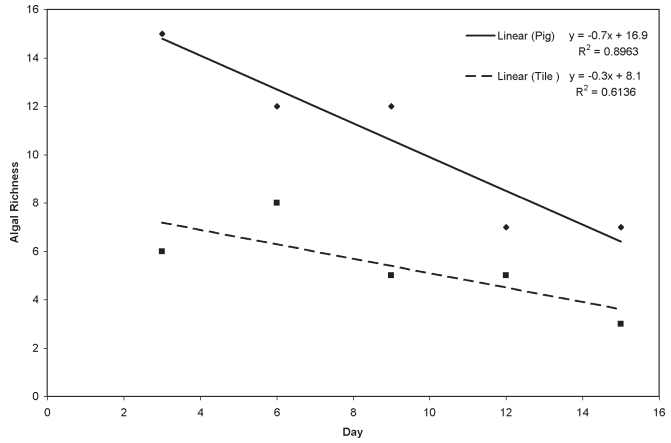
FIG. 3—*Algal richness in ponds 1 and 2 for each stage of decomposition.*

form of algae in the first stage of algal succession (10–12). Diatoms are the initial colonizers in aquatic habitats and are ubiquitous, thereby they can start an underwater time clock to estimate a PMSI as well as provide a geographic link between the suspect and a potential aqueous crime scene through their ecological distribution. Understanding the importance of degree-day accumulation for each developmental stage of terrestrial insects is a critical component to calculate the PMI for death scene investigations. Likewise, degree-day associations with algal growth during the various stages of decomposition can be vital to PMSI calculations in aquatic systems. As diatoms colonize a substrate, the process of plant succession is initiated similar to how succession occurs and progresses in terrestrial ecosystems (13).

The collection and use of evidence from aquatic habitats such as arthropods or plants (e.g., algae or diatoms) is uncommon among case studies. Moreover, research efforts have been lacking with respect to the utilization, collection, precision, and accuracy of this type of evidence (14). However, this study has repeated a technique useful in the collection of algae and diatoms from either natural substrata such as rocks or possibly from corpses. Aquatic plants such as algae and diatoms have been used to link suspects to crime scenes (4); here, we provide new information to support the collection and use of this type of forensic evidence as a more semi-quantitative approach to estimate a PMSI.

In the brackish ponds, we observed five stages of decomposition consistent with that described by previous investigators (2,3), even though those studies were conducted in freshwater streams. However, Anderson and Hobischak (15) studied the decomposition of carrion in the marine environment of Howe Sound, British Columbia using freshly killed pigs that were submerged at two different depths. Our findings agreed with the five stages of decomposition observed by Anderson and Hobischak (15) except for the duration of each stage that were shorter than Anderson and Hobischak's study due to warmer water temperatures and smaller pig carcass size in our study compared to their findings. We used stillborn piglets and conducted the study during the summer months at a lower latitude. In addition, decomposition in aquatic environments can be hastened by scavenger activity, especially in marine or saline environments. Anderson and Hobischak (15) found starfish (*Pycnopodia helianthoides,* Brandt) and whelks (*Strongylocentrotus droebachiensis,* O. F. Müller), to be primary invertebrates in accelerating the decomposition process. In our study, we found scavenger activity to be limited to small fish, herons, possibly blue crabs (*Callinectes*

(A)



(B)

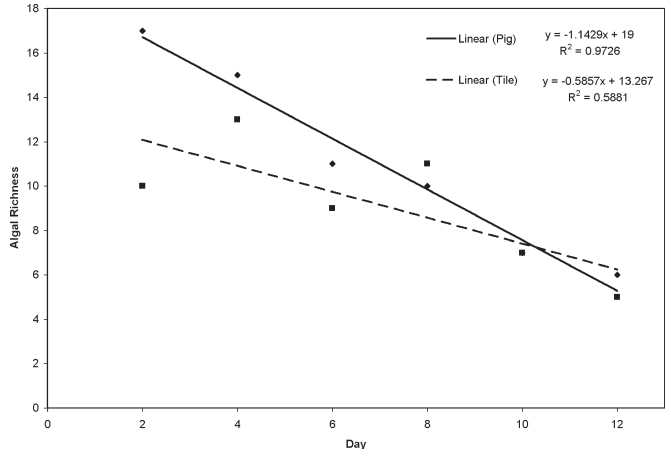


FIG. 4—*Linear regression analysis comparing the number of species of diatoms over time. (A) Pond 1, (B) Pond 2.*

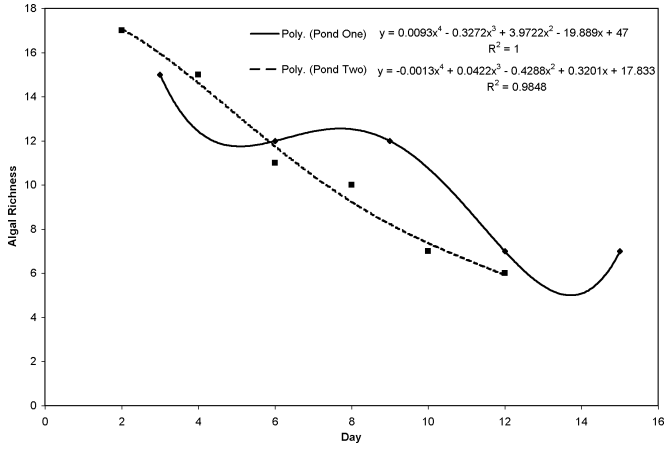


FIG. 5—*Polynomial regression analysis comparing the number of species of diatoms found on the piglet substrate with time for both ponds.*

sapidus Rathbun) and snapping turtles (*Chelydra serpentina serpentina* L.). Such scavenger activity may have accelerated decomposition rates if carcasses had not been placed inside cages.

Recent studies have addressed different methodologies to determine a PMSI using aquatic insects (14). These studies focused on the stages of decomposition as well as on the presence/absence of aquatic insects in a freshwater environment. Merritt and Wallace (14) noted that the determination of the PMSI has proven to be more problematic for corpses found totally submerged in aquatic environments. Temperature and current (or lack of) are two of the main factors that may affect the rate of decomposition and that was evident in this study along with previous studies (2,3,15). The idea of using algae had been proposed by previous investigators that have tried using aquatic insects to determine a PMSI (2,16,17). Sorg et al. (16) recommended determining time intervals needed for certain growth phases of aquatic plants that could then be used to estimate a PMSI.

It is difficult to distinguish between ecological and geographical barriers in terms of the overlap in the distribution of algae (18). There are chemical (e.g., salinity, pH, nitrogen, and calcium content) and physical factors (e.g., light, temperature, and turbulence) that affect the distribution of diatoms. Most algal genera have broad tolerance to these types of chemical and physical variables and consequently have survived over time and become broadly disseminated into appropriate habitats around the world (19). There is no sharp geographic distribution for diatoms as well, because they occur in the habitat in which their chemical and physical needs are fulfilled (20). For example, a species of diatom can be found in a freshwater pond during the winter and the same species found in a freshwater stream during the summer. However, not all species are as versatile. For example, some diatoms have a narrow range of physiochemical tolerance to temperature, pH, mineral content, and salinity; thus, a given species has specific niche requirements regarding these environmental factors, and the interaction of these factors is what produces seasonal fluctuations and sporadic blooms (21). We found considerable overlap among certain taxa between the two ponds; thus, this may not be due to pond, but rather due to temporal (seasonal) overlaps.

Diatom diversity in brackish water typically consists of a mixture of freshwater and marine taxa (22). The genera found in this study, as observed in Table 3, consisted of a mix of both freshwater and marine species. Patrick and Hohn (22) noted that the number of diatom species that make up an estuarine community appears to be less then riverine assemblages, even though diatom community structure in these two habitats is very similar. This was observed in our study in that fewer taxa were found in either pond compared to results from Casamatta and Verb (3), a study that was conducted in a freshwater stream. In this case, the structure of the aquatic communities (i.e., the way colonization occurs along with stages of decomposition in rivers, streams, and

TABLE 3—*Genera of diatoms found in algal samples in this study and the types of aquatic systems in which each is found.*

Taxa	Freshwater <0.5 ppt	Brackish 0.5–30 ppt	Saline 30–50 ppt
Amphora	x	x	x
Cocconeis	x	x	x
Cyclotella	x	x	x
Fragilaria	x	x	x
Frustulia	x	x	x
Navicula	x	x	x
Nitzschia	x	x	x
Pinnularia	x	x	
Plagiotropis	x		
Tryblionella	x		

estuaries) is similar, the difference being in the species that occupy that specific habitat.

The Sorenson's index indicated that the diatom diversity found on the piglet substrate compared to the tile was not completely similar, suggesting that a tile or rock substrate cannot be used to simulate a mammalian carcass in an aquatic environment. However, diatom samples can be obtained from these substrata to compare with those collected of human remains. As there was not complete similarity between both ponds, this supports the rationale for the use of diatoms as indicators of a crime scene.

The correlation coefficient (ρ) for both ponds indicated a significant inverse relationship, i.e., as the stages of decomposition progressed the numbers of diatoms species decreased. Allan (5) tested the effects of growth of benthic autotrophs with nutrient releasing substrates, and found that the addition of low levels of nutrients increased periphyton abundance compared to the addition of high levels that decreased the periphyton abundance. The decline in number of diatom species in the later stages of decomposition in this study could be a result of large amounts of nutrients released from the pig carcass, along with less surface area available for colonization due to disarticulation. Plant succession of periphyton has been documented in many types of aquatic habitats (23), and because of the fast decomposition rate in this study, it is not known if the number of species on the tile substrate (control) would have reached equilibrium or would have kept decreasing.

Algal diversity as a function of time was significantly correlated in this study. An understanding that there is a significant relationship between algal diversity and time has been suggested as a methodology to estimate a PMSI (3,24). Polynomial regressions were used to predict unknown values of X or Y (25), therefore, the equation generated in this study has the potential to be used to predict a PMSI; however, more work is needed to be able to definitively use this in actual casework. For example, if a submerged body was found in a similar microhabitat as in this study and the algal diversity was extrapolated from the body, then that number can be inserted into the polynomial regression equation to calculate an approximate number of days the body was submerged.

To date, this is the first study to semi-quantitatively measure algal diversity on a mammalian carcass in a brackish water habitat. As generalized methodologies to estimate a PMSI among all aquatic systems are lacking, our work shows that diatom succession and diversity have the potential to provide useful methodologies to establish a timeline for a PMSI in a variety of aquatic habitats, e.g., freshwater, estuarine, or marine. The importance of determining a submersion interval would aid forensic scientists and police with investigations in which a submerged body was found. Because PMSI estimates utilizing aquatic insects lack precision to some degree (14), the use of semi-quantitative and quantitative approaches with aquatic plant models in the future may enhance PMSI estimation precision. Perhaps with additional studies, forensic investigators will be able to estimate a more accurate PMSI using a combination of knowledge on the stage of decomposition and relevant degree-day information for each stage and the extrapolation algal diversity present on human remains upon discovery. Specifically diatoms may be helpful in determining not only manner of death (4) but also the duration of time (PMSI) a victim may have been immersed in any aquatic environment.

Acknowledgments

We acknowledge the Catherine Keever and Alex Henderson grants of the Department of Biology at Millersville University for funding. A genuine thanks to Mr. Mark Kreidler of the Penn State University Swine Research Facility for supplying the stillborn pigs needed for the study and to the Delaware Aquatic Research Education Center for providing the study location. We would especially like to thank Mr. Wayne Lehman for his assistance in site location and salinity testing, as well as Dr. Marina Potopova (Pennsylvania Academy of Science, Philadelphia) for her help with diatom identification. KAZ would like to thank Drs. Ambler, Cosentino, Hardy, and Piperberg for their assistance with supplies and guidance with this project. We would like to express our extreme appreciation to Erin High for her hard work during the sampling period. We would like to acknowledge Dr. Barb Zimmerman and Mr. David Zimmerman for assistance in the field.

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Additional information and reprint requests:
John R. Wallace, Ph.D., D-ABFE
Department of Biological Sciences
P.O. Box 1002
Millersville University
Millersville, PA 17551-0302
E-mail: john.wallace@millersville.edu