

2010

Using Algae to Estimate Postmortem Submersion Interval in a Louisiana Bayou

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USING ALGAE TO ESTIMATE
POSTMORTEM SUBMERSION INTERVAL
IN A LOUISIANA BAYOU

A Thesis
Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Arts

in

The Department of Geography and Anthropology

by
Sophia Renke
B.A. (Hons.), University of Alberta, 2007
May 2010

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ACKNOWLEDGMENTS

I begin by acknowledging the financial support I have received for this research. It was a tremendous honor to receive the Forensic Sciences Foundation Acorn Research Grant from the American Academy of Forensic Sciences and I am thankful for the opportunities this has given for my research, its publication, and its presentation. I also thank the Louisiana State University Department of Geography and Anthropology for the West Russell Travel Grant, the Research Materials Grant, and the HAAG Award. I thank the LSU Department of Graduate Studies for two Graduate Travel Awards which covered travel costs to present at the 61st and 62nd Conference of the American Academy of Forensic Sciences where I was able to speak with experts about my research.

I have had help in many different forms for this thesis. Michelle Whipp was instrumental in the formulation of my research plan; she was my sounding board, my knot expert, and my coast guard. I also have to thank Arnold Modlin who took me on an emergency shopping trip for supplies. I thank Lauren Pharr, not only for suggesting my site but for allowing me to become her roommate so that my research was easier to conduct. Nicole Harris deserves a special thank you for her calming words after my first encounter with a Louisiana water snake. Marc Massom went above the call of friendship, donning a scuba suit to help me when my site flooded. Leighanne Lawton not only secured my pigs, she helped me out on a crucial day and identified my insects; I am incredibly grateful.

Stephanie Crider was my resident seamstress and bone cleaner; she and Nicki Smith trekked through weeds in the early morning to help with preliminary studies as well as being chauffeurs on numerous occasions. I am thankful for the help Mikeal Blackford and Marc Massom gave to combat chest wader leak emergencies. I must also thank Jonathon and Teresa

Wilson for helping dilute my chemicals. I am incredibly thankful to Adam Shanks with the United States Army for donating the parachute rope—it was, without a doubt, my most important piece of equipment! I also thank the Louisiana State Arthropod Museum for kindly donating last-minute insect pins when I ran out in the fall. Dr. John White at the Wetland Aquatic Biogeochemistry Laboratory deserves a special thank you for processing my water nutrient samples free of charge.

I sincerely thank Ross Del Rio, Jessica Czubakowski, and Cris Garcia in the Harmful Algae Bloom lab. They patiently explained how to use the equipment and were always enthusiastic about having an anthropologist in their laboratory. Cris deserves a special thank you for keeping me company all night at the microscope; her love of algae was contagious and she was always excited to take a “quick” look at my samples.

Dr. Rebecca Saunders and Dr. Ginesse Listi were wonderful committee members, being positive, helpful and full of enthusiasm for my research. Dr. Wayne Kramer deserves thanks for supplying my fetal pigs and for giving much insight into scientific research. I also owe thanks to Dr. Gail Anderson for introducing me to Dr. John Wallace who, without ever having met me, gave guidance, inspiration, and incredible insight on the use of algae for estimating postmortem submersion interval. It was research conducted by both Dr. Anderson and Dr. Wallace that inspired this master’s thesis and I thank them both sincerely.

I am forever in debt to Dr. Sibel Bargu Ates, my algae expert. She took a chance on an anthropology student with no algae experience to become a crucial member of my thesis committee. She gave me unlimited access to her algae lab and taught me everything I know about algae. Dr. Bargu Ates was always enthusiastic and helpful—this thesis could not have occurred without her expertise and support.

Ms. Mary H. Manhein has been a true inspiration. The very first forensic lecture I heard by Ms. Manhein inspired me to investigate water decomposition, and she has been a constant supporter of my research in addition to kindly donating crucial chemicals. I am so thankful for the two years of graduate assistantship funding Ms. Manhein provided and the amazing opportunities she has given me as a graduate research assistant; I could not have asked for a better experience as a Canadian in the Deep South!

I also have family members to thank for their support. My grandparents, Teresa and Joseph Hajduk, sent letters, cards, and pictures. My little sister Rachel kept me upbeat, my brother Alex drove from Canada to visit, and my parents helped me see the light at the end of the tunnel.

Lastly, I have to thank John Carlson, my love. Even though he was thousands of kilometers away, his constant love and support were felt most strongly of all.

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ABSTRACT

While algae are often used in forensic science for the determination and confirmation of death by drowning, their utility for the estimation of postmortem submersion interval (PMSI) has been underutilized. Algae are present in all water systems and will grow upon decomposing matter; yet, very little published literature exists on their use in PMSI estimation. Because PMSI is difficult to predict due to the variable nature of water, the reaction of the submerged body within water, and the lack of truly sarcophagous aquatic insects, algae are a potentially invaluable tool for the forensic anthropologist. This research investigates the utility of algae as an indicator of PMSI in a Louisiana bayou, considering both seasonality and clothing as factors. Fetal pigs (*Sus domestica* L.) were placed in water in both spring and fall, some clothed and some unclothed. Algae samples were collected from two pigs and two control tiles per season and analyzed for chlorophyll *a* concentration. Biomass removal was also measured on two similar pigs in order to quantify decomposition. Results indicate that chlorophyll *a* concentration conforms to a positive linear relationship with time in both spring and fall and on all substrates, being especially dense on clothed spring substrates. Thus, algae growth can be used to estimate PMSI. Additionally, a clothed body will decompose slower than an unclothed body, and decomposition is more rapid in spring than fall. This research adds to the academic knowledge of the utility of algae for estimation of PMSI and brings attention to the growing need for collaboration between multi-disciplinary scientists investigating forensic cases.

CHAPTER 1: INTRODUCTION

The forensic anthropologist is trained in the examination of bones to determine race, sex, ancestry, stature, pathology, and trauma in order to create the biological profile of an unidentified set of human remains. Additionally, a major task for the forensic anthropologist is the determination of postmortem interval (PMI)—the time elapsed between death and discovery of a body. When a body is found in a terrestrial setting, the forensic anthropologist has a wide array of taphonomic information available to estimate PMI. The stages of decomposition can be determined visually, taphonomic factors can be analyzed, and insects can be collected and studied for their patterns of succession. Through consultation with other forensic experts, the forensic anthropologist has the ability to estimate how long a body has been decomposing on land.

However, decomposition in water is far less understood, with the estimation of postmortem submersion interval (PMSI) more difficult to determine. While insects are a principal contributor to the data on terrestrial decomposition, few truly sarcophagous aquatic insects have been documented (Wallace et al. 2008). Water is a highly variable habitat, being affected by numerous factors such as sunlight, temperature, wind, pollution, and geographic location. The position of a body within water is also highly variable, with some bodies floating or sinking naturally while others are trapped or deliberately weighted down to remain submerged. Depth, salinity, pH content, current, and micro and macro-organisms all affect how quickly a body will decompose, and disarticulation coupled with fluvial transport make submerged cases particularly challenging.

While the presence of aquatic insects is variable on a submerged body, organisms that repeatedly have been observed in association with aquatic decomposition in all water systems are

algae. Algae are not an active decomposer of the body but rather are attracted to the nutrients a cadaver excretes during decomposition (Haefner et al. 2004). The association of algae with the cadaver can be studied much like insect succession on land; yet, very little research exists on its use for the determination of PMSI. Rather, algae's main contribution thus far has been the identification and confirmation of drowning through analysis of soft tissue by the forensic pathologist.

The purpose of this thesis is to investigate the utility of algae growth for determination of PMSI. The water system chosen for this experiment is Bayou Fountain in Baton Rouge, Louisiana. This bayou was chosen not only for its urban location, but also for its proximity to the Mississippi River. The Mississippi River is an important location due to its high number of casualties from occupational and recreational accidents, suicides, and homicidal body dumpings (Basset and Manhein 2002). But the Mississippi River is a poor location for research due to its rapidly changing water levels, volatile currents, high water traffic, and uninhibited public access. Bayou Fountain, while not directly comparable to the massive river, is a good compromise, for it has near constant depth, slow current, and areas accessible only through a gated, private community.

The research examines three questions: (1) will a decomposing pig have different algae growth than a non-decomposing object; (2) does a clothed substrate have different algae growth than an unclothed substrate; (3) is algae growth in spring different than algae growth in fall? Algae are measured in two ways, with the amount of algae quantified through chlorophyll *a* concentration and type of algae qualified through microscopic analysis. The research was conducted twice in the same location, once in spring and again in fall. Thus, algae growth as an estimator of PMSI is examined considering season and presence or absence of clothing.

Replicate unsampled pigs are measured for biomass loss to examine the effect of algae sampling on the rate of decomposition.

The results of this research are not just academic in nature. Due to the frequency of deaths occurring in water, it is the intention of the author to inspire a dialogue between forensic anthropologists and algologists for a partnership in the determination of PMSI in forensic cases. Aquatic insects associated with the bodies and the inanimate object were also collected and examined, and scavenger activity was documented.

The research was inspired by and closely mimics that done by Haefner et al. (2004), Zimmeran and Wallace (2008), and Hobischak and Anderson (2002), but with new considerations. Experimentation on the use of algae for PMSI estimation has not been conducted in the unique environment of Louisiana, making this thesis an important source of new data for the emerging field of forensic algology. Additionally, the impact of clothing on the use of algae for PMSI has not been considered in published research.

This thesis is not only the first research of its kind conducted in Louisiana, it is the only source of data on the impact of clothing on the use of algae to estimate PMSI. Environments and factors not considered in previous research (such as insect and scavenger activity, natural floating and sinking patterns, the impact of clothing on algae growth, and the unique conditions of a bayou habitat) are examined, solidifying algae as an important resource for the forensic anthropologist. While the results of this thesis contribute to any case involving water decomposition, they have particular value to the investigation of neonaticide due to the use of fetal pigs as experimental mediums.

CHAPTER 2: REVIEW OF LITERATURE

2.1 Introduction to Algae

Algae are part of a large and diverse group of primarily aquatic plantlike organisms. They are classified in the kingdom Protista which consists of organisms that do not fall into other more well-defined kingdoms. While sharing similarities with both plants and bacteria, they have numerous physiological differences that require separate classification. Canter-Lund and Lund (1995) explain that most algae are autotrophs, some are heterotrophs, and others have characteristics of both. Sheath and Wehr (2003) define algae as prokaryotic or eukaryotic taxa which are aquatic and photosynthetic but without a vascular system or a sterile cell covering of their reproductive bodies. They are aquatic and subaerial, live in fresh, marine, and brackish waters, and they can survive in oligotrophic and eutrophic lakes (Bold and Wynne 1985). They can tolerate alkaline or acidic conditions and are able to grow in a wide range of temperatures, turbidity, and concentrations of dissolved oxygen and carbon dioxide (Bold and Wynne 1985).

Bold and Wayne (1985) categorize algae based upon habitat, both aquatic and terrestrial. The benthic algae live on the bayou floor attached to stones (epilithic), sediment (epipelic), plants (epiphytic) or animals (epizoic). The planktonic algae live suspended within the water column, sometimes forming blooms. The neustonic algae live on the surface where water meets atmosphere. Algae can be terrestrially epilithic, epipelic, epiphytic, and epizoic and can live in and on soil (edaphic), on tree bark (corticolous), or endozoically upon a host such as a coral, or as endophytes or endosymbionts of plants (Bold and Wynne 1985).

Algae occur as microalgae or macroalgae, ranging from two-tenths micrometer picoplankton to sixty meter kelp (Barsanti and Gualtieri 2006). Explained by Stevenson (1996), algae species are distinguishable from each other by their chemical differences in chlorophyll,

accessory pigments, cell wall, cell storage chemistry, form of motility, number of flagella, and number of membranes around their chloroplasts. In fact, the autotrophic algae groups are only similar in that they all contain chlorophyll *a* and they all produce oxygen as a product of photosynthesis (Canter-Lund and Lund 1995). Algae all exist in different forms, as explained by Sheath and Wehr (2003). Some are microalgae, existing as a single motile or nonmotile cell. Some are macroalgae, aggregating into organized, multicellular forms. Algae are seen visually in different forms, such as a single cell, chain, colony, or aggregate. Macroalgae can even exist in very plant-like form. These differing characteristics allow algae to be commonly classified into nine phyla. Table 1.1 briefly summarizes these nine phyla.

Stevenson (1996) identifies three characteristics used to describe algae: biomass, taxonomic composition, and chemical composition. First, biomass can be measured through area-specific masses of matter, chlorophyll *a* and pigment density, dry mass and ash-free dry mass, or microscopic examination of cell density and biovolume. The best approach for measuring biomass will depend on the sample numbers and available personnel. This kind of analysis will identify the amount, not the type, of algae present.

The second method for characterizing algae explained by Stevenson (1996) is through taxonomic composition. This is done by identifying and counting algae cells microscopically. Ratios of cell biovolume or density to total biovolume or density are used with these microscopic cell counts to identify the relative abundance of each algae taxon counted. Or, pigment ratios can be used to compare green algae, red algae, and diatoms. Lastly, autotrophic indices of the algae proportion of biomass can be compared to the greater algae community. A species' composition is then summarized by its richness and evenness of abundance and its diversity and similarity within the community through this autotrophic index (Stevenson 1996).

Table 1. 1 Algae Phyla. Summarized from Sheath and Wehr (2003), Canter-Lund and Lund (1995), Bold and Wynne (1985), and Bell and Hemsley (2000).

Phylum	Common Name	Habitat	Chlorophyll	Features
Cyanophyta	Blue-green, Cyanobacteria	Mostly freshwater; swamps, soil	a	prokaryotic; congregate in surface blooms; produce oxygen through photosynthesis; bacteria-like in that they do not possess a nucleus, nucleolus, or chloroplast; plant-like in that they have membrane bound organelles
Chlorophyta	Green Algae	Mostly freshwater; marine, brackish, subaerial	a, b	closely related to higher plants; present in many forms, from unicellular to tubular; most are green because of the predominance of green photosynthetic cell pigments
Charophyta	Stonewort, Brittlewort	Mostly freshwater	b	nonvascular hydrophytes; benthic, use rhizoids to anchor to sand, mud, and limestone; visible as bushy plants
Euglenophyta	Euglenoids	Mostly freshwater; standing water	a, b	in standing waters with abundant nutrients/organic matter; distinct motion involving flexion, contraction, and reexpansion; capable of changing cell shape
Phaeophyta	Brown Algae	99% marine; benthic in freshwater	a, c	flourish in cold ocean water; most diverse organisms of the temperate-subpolar regions; predominate the lower littoral-upper sublittoral zones; include kelp
Chrysophyta	Golden Algae, Diatoms	Mostly freshwater	a, c	six classes of algae with common food reserves, pigmentation, chloroplast endoplasmic reticulum, and predominance of carotenoids over chlorophylls
Pyrrhophyta	Dinoflagellate	Mostly marine	a, c	biflagellate unicellular organisms present in flagellate and nonmotile form; can form large blooms; can be toxic
Rhodophyta	Red Algae	97% marine	a, d	phycoerythrin pigment produces red color; not all are red; capable of chromatic adaptation; common as red seaweed
Cryptophyta	Cryptomonads	Marine, freshwater, brackish	a, c	periplast-bound asymmetric biflagellates; are different colors from each other; can change color over a lifetime; may have eyespots, most contain ejectosomes
<i>Cyanophora/Glaucocystis</i> *		Freshwater	a	colorless host cell with a permanently ingested pigmented prokaryotic endosymbiont

* Genus

Thirdly, Stevenson (1996) explains that chemical composition can be used to characterize algae, with resource ratios (such as N:C) used as indicators of cellular nutrient status. Ratio of phaeophytin to chlorophyll *a* can also be used to indicate community senescence.

Algae growth is dependant on the availability of species specific nutrient levels in the water, particularly phosphorous, nitrogen, carbon and silica (Tilman et al. 1982; Lund 1972). The ability of a particular species to successfully reproduce and grow depends on its specific nutrient requirements, called its optimal nutrient ratio (Stelzer and Lamberti 2001). The required nutrient which is least available in the water is known as a species' limiting nutrient. In order for two algal species to co-exist, they must have either completely different or nearly identical nutrient requirements in order for equilibrium to be established (Tilman 1981). Otherwise, one species will use up the limited nutrient, leaving the other species with inadequate nutrient supplies. Environmental change which alters water nutrient levels will result in changed community structure due to competition for the limiting nutrient (Stelzer and Lamberti 2001).

Water nutrient levels are altered through the process of eutrophication, an addition of nutrients and organic matter to water (Lund 1972; Nixon 1995). This addition can be desirable and harmless or undesirable and polluting (Lund 1972). Anthropogenic eutrophication can be through urban sewage run-off, agricultural waste and fertilizers, industrial waste, detergents, and fossil fuel combustion (Lund 1972; Nixon 1995). Fairchild et al. (1985) examined the effect of eutrophication on algae growth by adding varying amounts of phosphorus and nitrogen to submerged clay flower pots. They found that the nutrients increased algae growth as measured through chlorophyll *a* concentration. A decomposing organic substance, such as sewage, similarly would enrich the nutrient content of water and promote algae growth.

Algae are incredibly important in Earth's ecosystem for numerous reasons. They are the most basic energy source in the marine food chain (Barsanti and Gualtieri 2006). Many algae

are capable of fixing atmospheric nitrogen, being soil fertilizers as well as stabilizers (Bold and Wynne 1985). Amazingly, algae are responsible for producing nearly half of Earth's oxygen (Barsanti and Gualtieri 2006). However, algae can also be harmful, causing plant and animal illness and death as harmful algae blooms (HABs) (Bold and Wynne 1985). HABs can be harmful through non-chemical or chemical means (Smayda 1997). Non-chemical harm can be through mechanical damage of other organisms (such as when sharp algae pierce the gills of fish) or through sheer biomass which causes starvation and death in competing species (Smayda 1997). Chemical harm can be through phytotoxin production (such as domoic acid), with other organisms directly ingesting the toxin, being directly exposed to the toxin, or indirectly ingesting the toxin through food web vectoring (Smayda 1997). Even a large, non-toxic bloom can cause toxic harm by altering the water chemistry to be anoxic or hypoxic (Smayda 1997).

Algae have yet another important role: forensic indicators. While often overlooked, algae are capable of establishing cause of death, location of death, and even PMSI.

2.2 Algae as Forensic Indicators

2.2.1 Death by Drowning

Diatoms are one of the most successful and dominant microalgae groups, inhabiting all freshwater habitats (Sheath and Wehr 2003). Diatoms are found in both standing and flowing water and can be planktic or benthic (Sheath and Wehr 2003). Shkrum and Ramsay (2007) summarized that diatoms are unicellular, have silica cell walls, and are composed of over 100,000 species. Due to their resilience and abundance in water systems, analysis of diatoms can be used in the investigation of deaths by drowning because water is aspirated into the lungs. Diatoms present in the water enter the alveolar capillaries and then move into the circulatory system. The presence of diatoms in organs such as the lungs, heart, kidney, liver, brain, and

bone marrow can be indicators of death by drowning, especially when compared to diatoms in the nasal sinuses, stomach fluid, and water source. Shkrum and Ramsay explained that diatoms in the organs not only confirm drowning but can also implicate drowning as cause of death in cases not originally believed to be drowning. Algae can also link a victim to the location of drowning. Therefore, diatoms have significant forensic value for cases involving death by drowning.

Studies have been conducted to test the merit of the most popular methods of diatom detection in human tissue. Ludes et al. (1994) compared three techniques of diatom extraction used to determine drowning, beginning first with the collection of water samples from the site of drowning in Strasbourg, France. These techniques were chemical digestion using nitric acid, enzymatic digestion method using proteinase K, and the ashing method using a muffle furnace. Ludes et al. found that the proteinase K method was the fastest, most reliable, and least hazardous method for diatom detection and the determination of death by drowning. Ming et al. (2007) examined the use of nitric acid with hydrogen peroxide, proteinase K, nitric acid in a Disorganization Can, and Soluene-350. Like Ludes et al. (1994), they concluded that proteinase K is the best method for diatom detection.

Yoshimura et al. (1994) used the solubilization method using the tissue solubilizer Soluene-350 to detect green algae and diatoms in human organs. Looking at liver, lung, and kidney pieces from victims found in the Yodo River, Japan, they were able to detect phytoplankton in the tissues of three victims of drowning. Their controls—one submerged but not drowned victim and one not submerged motor vehicle accident victim—did not have algae present in their tissues. Yoshimura et al. were therefore able to conclude that the presence of algae in human tissue could be used as an indication of drowning by extraction of diatoms using Soluene-350.

This solubilization method of using Soluene-350 was tested by Sidari et al. (1999). They found that while the method was effective for the examination of fresh water diatoms in human tissue from the Rosandra Stream, Italy, Soluene-350 destroyed the less silicized frustules of Adriatic Sea diatoms. By using the Soluene-350, Sidari et al. could not find evidence of drowning in the human flesh of those drowned in the sea, even though drowning was confirmed by medico-legal data. They concluded that while the method works well for fresh water, it is ineffective in investigations of drowning in sea water. Ming et al. (2007) further agreed that Soluene-350 is unusable for sea water cases.

Some phytoplanktons are too small to be distinguished from bacteria microscopically, rendering them useless in the investigation of drowning. By means of dilution and cultivation, Kane et al. (1996) isolated picoplankton 16S rDNA from human lung tissue. With this isolation, they were able to locate microplankton and nanoplankton in human lung tissue not detectable by microscope. Thus, Kane et al. offer a molecular biological method of the examination of phytoplankton for the determination of drowning. Using a DNA perspective, Suto et al. (2003) use polymerase chain reaction to identify algae in the tissue of drowning victims. They found plankton DNA in lung, liver, and kidney tissue, introducing the polymerase chain reaction method as an alternative to the more hazardous diatom tests.

2.2.2 Location of Drowning

Ludes et al. (1999) recognized that while the presence of diatoms in lung tissue is an indication of drowning, it does not tell the investigator where the drowning occurred. For this, they used data from a river monitoring program from the area of Strasbourg, France, to complete a taxonomic profile of the fresh water system. By comparing the diatoms found in the lung tissue with diatoms known to inhabit the river, Ludes et al. had one hundred percent

concordance; they concluded that the drowning had occurred in that particular river. Ludes et al. showed that in order to have a complete investigation of drowning, postmortem findings, police investigation, and histological analysis must be consulted so that tissue samples can be compared with the supposed location of drowning. Similarly, Auer and Möttönen (1988) wrote that quantitative diatom analysis is crucial, especially when drowning is not supported by other forensic evidence. By analyzing the diatoms, they were able to link the victim back to the water environment in Finland where the drowning occurred. Bhatia et al. (1971) looked at the distribution and proportions of diatoms in Lake Hussain Sagar, India. While they did not experiment on drowned remains, they used their results to infer that diatoms can be used to determine the location of drowning.

Horten et al. (2006) were also concerned with linking the victim to the site of drowning, using algae present in the lungs and on the clothing of the deceased. Through the modern analogue technique, they positively identified the river site of drowning of a high profile case. They were also able to determine that drowning had occurred in a pond rather than a bathtub in a child death investigation. Then, He et al. (2008) looked at 16S rDNA with PCR-DGGE, experimenting on 30 rabbits in two Chinese lakes. With this method, they were able to link the drowned victim back to the location of drowning.

Pachar and Cameron (1992) recognized that a complete and uncontaminated taxonomic profile of the diatoms within the tissues and the location of drowning is crucial for a valid determination of drowning. They used scanning electron microscopy to identify seawater diatoms from the kidney and fresh water diatoms from the lung. These were then compared to the diatoms from the site of drowning, acting as a positive indication of drowning..

While the liver and kidney are often used to investigate the presence of diatoms for the determination of drowning, algae found in femoral bone marrow has also been used successfully.

Using bone marrow, Pollanen et al. (1997) and Pollanen (1997) confirmed the use of the diatom test as indicative of death by drowning, location of drowning, and even season of drowning. However, they concluded that the diatom test using bone marrow was relatively insensitive, with diatoms being detected at a low twenty-eight percent rate. Then, Pollanen (1998) used the diatom method to diagnose drowning in six homicide cases not initially believed to be drowning deaths. Gruspier and Pollanen (2000) found diatoms in femoral bone marrow of disarticulated bones found in Lake Ontario, Lake Erie, and the Niagara River, Canada. They were able to diagnose death by drowning followed by taphonomic dismemberment as opposed to deliberate dismemberment and dumping. In all four of the studies the state of decomposition was undefined. It is unknown how the femur bone marrow diatom test compares for fresh, decomposed, or skeletonized remains.

Lunetta et al. (1998) examined a different aspect of the use of algae for determination of drowning. Rather than focusing on the utility of algae for diagnosis of drowning or its ability to determine location of drowning, they focused on unstudied locations of algae in the body. Specifically, they looked at the presence of algae in the alveolo-capillary barrier using scanning electron and transmission electron microscopy. They found that scanning electron microscopy was best used for detecting algae in the airway and alveolar wall, while transmission electron microscopy was best for alveolar spaces. However, Lunetta et al. admitted that their results were not applicable to human drowning situations for two reasons. First, they used a highly contrived algae concentration in their water sample that was unrealistic in a natural setting. Second, their experiment was conducted on rats which have an alveolo-capillary barrier unrelatable to humans.

While these studies have looked mostly at diatoms, Diaz-Palma et al. (2009) used the diatom tests to identify non-diatom algae in the tissues of drowning victims in Chile.

Microalgae, such as dinoflagelletes, silicoflagelletes, and cholorophytes were best discovered using the proteinase K method combined with chemical digestion in body tissue, which was then compared to water from the site of drowning. Thus, Diaz-Palma et al. showed that algae other than diatoms also were forensically significant in the determination of drowning.

Despite these algae detection methods, an unambiguous determination of death by drowning is a very difficult conclusion for the forensic pathologist to make. Piette and De Letter (2006) examined the literature from the last twenty years of all the available techniques for the determination of drowning. They concluded that the diatom method remains the standard even though there are two problems with it. First, algae are sometimes present in the tissues of victims without drowning as known cause of death. Krstica et al. (2002), studying in Macedonia, were able to account for this problem, describing how algae can enter the body through the air, food, water, and contamination during autopsy of non-drowned cadavers. Yen and Jayaprakash (2007) studied the possibility of diatom fustules in bone marrow as a result of food ingestion rather than drowning. By investigating non-vegetarian foodstuffs prevalent in Kota Bharu, Kelantan, Malaysia, they found that enough diatoms are present in ingested food such as clams and prawns to be interpreted as drowning. To combat the problem of autopsy contamination, Hürlimann et al. (2000) collaborated with the medicolegal community to set forth techniques for obtaining tissue samples during autopsy that did not result in algae contamination.

The second problem recognized by Piette and De Letter (2006) is that algae are sometimes absent in tissues of known drowning victims. Krstica et al. (2002) believe this occurs because death can be too rapid for diatom intake, there are not enough diatoms present in the water to be transferred to the body tissue, or the individual simply had not died by drowning as proposed.

2.2.3 Linking Suspect to Crime Scene

Besides their use as an indicator of drowning, algae have also been used to link a suspect to a crime scene. In Siver et al. (1994), algae was used to connect three suspects to a Connecticut pond by evidence left on the suspects' shoes. Investigators qualitatively analyzed the algae species found in the pond with mud samples from the shoes, followed by quantitative analysis of the proportions of populations present. Through statistical analysis, they determined that the algae collected from the suspects matched the algae found in the pond, leading to conviction. Siver et al. therefore showed that algae are exceptionally valuable to forensic investigation due to characteristics of richness, diversity, and resilience.

2.2.4 Postmortem Submersion Interval

A third use of algae is the determination of PMSI. The pioneering investigation into the use of algae for PMSI was done by Casamatta and Verb (2000). They recognized three qualities of algae that make it particularly useful for forensic investigation. First, it is present in almost all lotic systems regardless of geographic location. Second, algal communities remain present throughout all seasons of the year. Third, identification of algal species is inexpensive, quick, and easy. They studied the use of algae to determine PMSI on immature rats in an Ohio Creek and found that because diatoms did not appear in large quantities until after three weeks, lack of alga growth can be used to identify a short submersion period. They were then able to identify early and late colonizers, concluding that after a three-week period, the diversity of taxa present can be used to determine PMSI.

Haefner et al. (2004) used algae to determine PMSI in an entirely different realm than the previous study undertaken by Casamatta and Verb (2000). Haefner et al. were uninterested in the species of algae taxa present on decomposing pigs and tiles; they were concerned with

concentrations of chlorophyll *a* as a quantitative indicator of algae accumulation. Their study emphasized the importance of the scientific method, statistical analysis, and quantitative data. Working in Pennsylvania streams, they placed tiles and pigs in two separate marine environments and compared the Chlorophyll *a* concentrations between them. The two streams showed little difference for Chlorophyll *a* concentration comparatively. The most important factor for algae growth was the presence of a decomposing carcass because the submerged body offered a source of nutrients not present on the tiles. Haefner et al. also examined the pH and oxygen level of the water, the accumulated degree days of decomposition, the changing biomass of the pigs, and the water current levels in order to collect a wide array of taxonomic data. They provided immense detail on the method of deposition, collection, examination, and analysis of their sample, and emphasized that decomposing flesh is imperative to creating a realistic environment.

Zimmerman and Wallace (2008) conducted the most recent research on the use of algae for determining PMSI. Using a combination of Haefner et al. (2004) and Casamatta and Verb's (2000) techniques, they proposed a semi-quantitative approach based on species diversity. Using piglet carcasses in brackish Delaware ponds, they monitored water temperature to determine degree days and analyzed the decomposition process by weighing the carcass. Samples of algae were taken every three days for 15 days or every two days for twelve days, analyzed under a light microscope for species diversity, and examined statistically. A significant inverse relationship was observed between algae species diversity and time submerged—the longer the body decomposed, the fewer types of algae were observed. Zimmerman and Wallace believe this occurred because high levels of decomposition-related nutrients decrease algae species abundance and disarticulation of the body decreases available surface area for algae colonization.

They also recognized that temperature and current were the most important factors affecting rate of decomposition, noting that these variables should be monitored carefully. Zimmerman and Wallace conclude the same as their predecessors—algae can be used to estimate PMSI.

2.2.5 Association with Forensic Entomology

One method for determining PMSI is with the use of aquatic insects. Numerous studies have been published regarding the use of aquatic insects for the determination of PMSI, but many do not mention algae (see Wallace et al. (2008); Davis and Goff (2000); Vance et al. (1995)). However, Haskel et al. (1989) examined the variables affecting underwater decomposition, mentioning algae in addition to aquatic insects. First, they note that some aquatic insects feed on algae; second, the progression of algae development may be usable for PMSI. They also mentioned that algae presence will be dependent on the same factors as insect presence, such as depth and current, with seasonal measurements needed for accurate PMSI estimation.

In an experiment on the decomposition of submerged rats, Keiper et al. (1997) compared a riffle area to a pool area of an Ohio woodland stream. They used the Sorensen Index of Similarity to determine how alike the insects collected at both sites were to each other, determining that they are significantly dissimilar. They conclude that data cannot be extrapolated to other seasons and, for a more realistic study, scavenging should not be prevented. Additionally, they noted that by day 39, the rats were almost unrecognizable due to algae growth. The algae grew not only on the rat hair, but also on the skin of the tail; they extrapolated that it would also likely grow on human skin. They stated that algae colonized the carcass in the same manner as insects.

Tomberlin and Adler (1998) also found algae while conducting entomological experiments on rats in South Carolina. They placed rats in containers of water and directly on

land in summer and winter, examining the insects associated with decomposition. They noted that the submerged bodies were completely covered with algae by day 46 in their winter session. Tomberlin and Adler found decomposition to occur faster on land and faster in the summer, with slower decomposition in water and in the winter. However, they cautioned that because the experiment occurred in an artificial setting, the results could not be extrapolated to natural water systems.

Another study on aquatic insects that mentioned algae was performed in British Columbia, Canada, by Hobischak and Anderson (2002). Using clothed pigs in a pond and a stream habitat, they examined the aquatic insects associated with decomposition for one year. They noted that season, water temperature, water acidity, biotic factors, and scavengers played a role in decomposition, as well as the presence of clothing. Pigs were clothed over fifty percent of their bodies and four carcasses were either submerged or partially submerged in each habitat. They sampled from three of the four carcasses, using the fourth as a controlled, unsampled specimen. Insects were collected and the visual state of decomposition described. While Hobischak and Anderson expected an increase in carbon dioxide from the decomposition process, none was measured. They believed this was a result of the heavy growth of algae on the pig carcasses.

In another experiment, Anderson and Hobischak (2004) studied underwater pig decomposition as a factor of depth in British Columbia, Canada. Pig carcasses were anchored at two depths, were allowed to naturally sink and float but not drift away. The aquatic insects were monitored for five months, with algae observed as stain on the bones and as a general accumulation. Anderson and Hobischak concluded that the most important factor affecting the rate of decomposition was not the depth but rather if the body floated or sank to the bottom. If sunken, the type of sediment at the bottom played an important role in the rate of decomposition.

Merrit and Wallace (2001) proposed that since no truly sarcophagous aquatic insects that feed on human flesh alone exist, other factors such as algae could be used for the determination of PMSI. While there are no models of succession for aquatic insects mirroring terrestrial insects, algae appear to occur in a similarly successional manner, thus having the potential to be used for estimation of PMSI. A final point by Merrit and Wallace concerning algae was their role as nutrition for insects. In the early floating stage, algal or periphyton growth will significantly increase, giving aquatic insects, such as caddisflies, a food source for growth and reproduction.

2.3 Stages of Decomposition

These entomological experiments involve numerous situations and habitats. Yet, all research on aquatic insects has an element in common: they all monitor the visual stages of decomposition. Almost all cite Payne and King (1972) as the basis for their stages of underwater decomposition as follows:

Stage One—Submerged Fresh: The initial placement of the pig underwater, usually sinking to the bottom but sometimes floating. The pig is no longer considered fresh once bloating starts, causing a rise to the surface (lasting one to two days in the summer, two to three weeks in the winter). Bubbles of blood may rise to the surface, but no insects except the hydrophilids are present.

Stage Two—Early Floating: The distended abdomen projects above the surface of the water and is covered by blowfly eggs. Gases bubble from the natural openings accompanied by a pungent decay odor. The most exposed areas, including the neck and abdomen, rapidly discolor from pink/green to dark blue.

Stage Three—Floating Decay: The blowfly eggs hatch (24 hours after being laid, approximately the third day since body deposition) with maggots crawling around the exposed

skin. Predacious insects attempt to feed on the maggots, and no more Diptera eggs are laid. Beetles are present on the exposed body, being especially active nocturnally.

Stage Four—Bloated Deterioration: Increased maggot activity is present with a constant covering of froth over the body. Most of the exposed tissue is gone by day seven, and maggots will feed on the underwater portion (with spiracles remaining above water). The head, shoulders, abdomen and hind quarters may be gone, with numerous predacious insects feeding on the maggots. Half of the carcass remains floating even after most of the maggots leave.

Stage Five—Floating Remains: Few insects remain on the carcass and many dead maggots float in the surrounding water. Stage five lasts a variable amount of time, from four to fourteen days, ending when the carcass sinks.

Stage Six—Sunken Remains: Lasting anywhere between ten and thirty days, bacteria and fungi finish the decomposition, rendering the carcass to skin and bones.

Payne and King (1972) found it important not only to examine the insects associated with the stages of decomposition, but also to monitor the temperature and weight of the pigs. The internal pig temperature was similar to the water and never rose higher than the surrounding air, and the pig lost weight beginning in stage two.

Adding to this early work by examining published research and cases, Rodriguez (1997) explained that a submerged body will decompose at a rate approximately half that on the surface due to lower temperatures and less insect activity. The bacterial content also plays an important role, for example with decomposition in a stagnant swamp occurring faster than in a clean lake. In the floating stage, the head and limbs will hang beneath the surface and will accumulate more lividity than the trunk. Additionally, while looking mainly at the sea water environment of the Gulf of Maine, Sorg et al. (1997) included microalgae in a table of sessile benthos which may modify human remains during the advanced decay and skeletonization phases. By settling on

bone, tissue, and clothing, Sorg et al. explained that sessile invertebrates can be indicators of PMSI, but only if their specific reproductive cycles and growth rates are known.

Boyle et al. (1997), examining deaths in Monterey Bay, California, recognized one of the first changes caused by decomposition is “washerwoman’s skin,” a wrinkling of the skin in longitudinal lines. Like Payne and King (1972), they offered stages to characterize underwater decomposition, but modify them to four:

Stage One—Fresh: Livor and rigor mortis may be present but, otherwise, no significant bloating or discoloration. This stage lasts zero to two days in freshwater.

Stage Two—Early Decomposition: With the departing of rigor, discoloration as marbling or greenish-violet is significant. The body may become fully bloated and skin slippage is prominent, especially in the hands and feet. The soft tissues of the face may be completely eaten away and readily noticeable autolysis will be present. This stage can last from two days to one week.

Stage Three—Advanced Decomposition: The facial features are gone, with bleaching, sagging, and discoloration of exposed skin. Adipocere begins to form. This stage can last from one week to one month.

Stage Four—Skeletonization: The body is reduced to bones, with significant adipocere formation. This stage can be from one month and beyond.

Hurst (2001) also found four stages of decomposition in a lacustrine environment. By examining the decomposition of docked, buoyed, and weighted pigs in a freshwater lake in Louisiana, Hurst identified a fresh, bloat, reduction, and desiccation phase. The pigs progressed from fresh to completely desiccated in 94 days for the weighted pig, 73 days for the buoyed pig, and 106 days for the docked pig. Hurst noted that during the reduction stage of the docked pig, green algae were present on areas of the body at the waterline.

In an extensive literature review, Haglund and Sorg (2002) looked at numerous aspects of human decomposition in water. They examined the factors that make a body sink or float, the position of a floating body, decomposition, disarticulation, fluvial transport, and bone modification. They demonstrated how this information is useful not only to the forensic taphonomist but also the archaeologist and paleontologist, saying that while water is perhaps the most ubiquitous of taphonomic agents, it is one of the least understood.

Adipocere formation also contributes to the estimation of PMSI. Adipocere, defined by Mant and Furbank (1957, in Micozzi 1991), is a postmortem fatty acid formed on a cadaver through the hydrolysis and hydrogenation of body fat. O'Brien and Kuehner (2007) conducted an experiment to monitor adipocere formation in a controlled environment at the Anthropology Research Facility in Knoxville, Tennessee. They found that adipocere only forms when conditions are "just right" (known as the Goldilocks Phenomenon), being most affected by temperature. In their literature review, they noted that a range of temperatures allowed for the growth of adipocere. They cited Cotton et al.'s (1987) finding adipocere growth at 21°C; Kahana et al. (1999) at 10-12°C; Payne and King (1972) at 27°C; Mellen et al. (1993) at 15-22°C; and Sledzik and Micozzi (1997) at 4°C. From their experimentation, O'Brien and Kuehner concluded that ideal temperatures for adipocere growth are between 21°C and 45°C. Water temperature can thus contribute to qualitative estimations of PMI through adipocere formation. Taking a quantitative approach, Yan et al. (2001) used chromatographic analysis to examine the rate of formation and decomposition of acids within adipocere tissue, providing a new approach to the use of adipocere for determination of PMI.

O'Brien (1997), doing research in Lake Ontario, Canada, summarized the literature on adipocere, showing that it can be present anywhere from three weeks to fourteen weeks on a submerged body. In addition to adipocere, he demonstrated that abrasion by rocks is a factor for

decomposition, stressing that meteorological data about air temperature, wind speed, and wind direction are crucial for taphonomic interpretation.

2.4 Impact of Clothing on Decomposition

Taphonomic research demonstrates that the presence or absence of clothing affects the rate of decomposition. Janaway (2002) specifically studied the decomposition of fabric in a forensic and archaeological context. Examining buried fabric, Janaway concluded that individual fabrics decompose based on the type of material, kind of dye, presence of chemical rotproofing or mothproofing, association with metals, soil type, and the presence of a decomposing body. Undyed cotton disintegrates the fastest, especially in the presence of acids. Gordon and Manhein (2003) looked at the rate of decomposition of fabric specifically for the determination of PMI. They found that such variables as insect and scavenger activity, the disruption of weave structure, color fading, and tensile strength can all be used to examine the PMI of clothing on a body, especially as related to temperature.

However, very little research exists as to the effects of clothing on the PMSI. Haglund (1993) recognized that clothing is a major factor affecting the disarticulation and movement of bone, with footwear and long-sleeved shirts being especially effective in preventing bone dispersal. He asserted that the decomposition of fabric in water requires much more study. Also, in a study of the use of temperature to determine time since death in shallow water using five statistical methods, Karhunen et al. (2008) recognized that wet clothing had the potential to inhibit temperature loss and thus affect PMI. Boyle et al. (1997) explained how the presence of clothing may cause air bubbles that prevent the body from sinking during phase one and that clothing may also delay decomposition. Hobischak and Anderson (2002) showed that clothing inhibits invertebrate and scavenging activity while also providing shelter for insects in pond and stream habitats.

Marshall et al. (2009) experimented on the effect clothing has on terrestrial insect succession and animal scavenging in Michigan. They found that clothing inhibits the deposition of Diptera eggs, causing a delay in colonization and a slower rate of decomposition than an unclothed pig cadaver. Also, the presence of clothing deters scavenging from animals. However, Kelly (2006) found clothing to have no influence on terrestrial decomposition or insect colonization on pigs in South Africa. While Kelly recognized that clothing may affect maggot mass distribution and movement, she concluded that it does not significantly affect rates of decomposition.

In an unpublished research paper regarding the decomposition rates of fabric in various water types, Renke (2008) determined that while fabric structure does not change over a four week submersion period, algae will grow on clothing, especially cotton. Janaway (2008) noted that cotton is the predominant natural textile fiber worldwide but did not experiment on its decomposition in a pure aqueous environment.

Keiper and Casamatta (2001) proposed six necessary research areas for algae to be applied to medicolegal situations. First, they say that quantitative documentation of the colonization of algae on a carcass is necessary. Second, algal growth on artificial substrates (such as tiles or pots) needs to be compared to decomposing carcasses. Third, seasonal differences need to be studied. Fourth is the need to study large scavengers, and fifth is to study the effects of severe disruptions such as rain. Lastly, the effect of clothing on algae colonization needs to be examined.

2.5 Impact of Water Scavengers on Decomposition

Sorg et al. (1997) described how carrion decomposing in water act in multiple roles. They are a primary energy source for scavengers, a microhabitat for non-scavengers and a

substrate for grazers. Carrion also are attractive feeding locations for the predators which eat these scavenging fish and insects. These researchers identify fishes, arthropods, mollusks, crustaceans, and echinoderms as primary scavengers which bite, tear, and chew the carrion and its clothing. This ultimately leads to a more rapid rate of decomposition, with disarticulation, dissemination, and bacterial decomposition happening quickly.

Sorg et al. (1997) report that fish, which tear open the skin of carrion thus exposing inner tissues to smaller invertebrates, can begin scavenging within five minutes of submersion. Arthropod macroscavengers, such as crabs and lobsters, are particularly adept at tearing the skin and muscles of carrion settled on the bayou floor. Arthropod microscavengers, such as isopods and small shrimp, are destructive due to their occurrence in large numbers, being able to completely consume 20 squared centimeters of seal flesh in 24 hours. Sorg et al. believe that Neogastropoda are the most forensically-important molluscan scavengers, potentially eating flesh, adipocere, microalgae, and microbes from bone. While Sorg et al. claim that some echinoderms may actively scavenge, others act as indicators of habitat when they become attached to clothing and bones. They conclude that the best indicators of PMSI are the sessile invertebrates, such as barnacles, and bryozoans, which are attached to the body or clothing itself. Examining the swimming behaviour of scavenging amphipods, Ide et al. (2007) found chemoreception to be an important method of carrion detection. While their research was not intended for use in forensic contexts, their findings—and those of the cited literature—could prove potentially useful in investigations of PMSI.

2.6 Louisiana Water

The aforementioned research has been international in scope, with research ranging from oceans to streams in France, Germany, Italy, Macedonia, India, China, Malaysia, France,

Finland, Germany, Chile, the United States, and Canada. Within the American studies, a particular water system is absent from the research: the Mississippi River, even though it is one of the largest in North America and the largest in the United States. Duan and Bianchi (2006) report that the Mississippi River drains forty percent of the United States, is responsible for more than half of the suspended matter and dissolved materials in the Gulf of Mexico, is the world's second largest drainage basin, and ranks seventh in the world for water/sediment discharge.

According to the Louisiana Repository for Missing and Unidentified Persons (2009), forty-seven percent of cases of unidentified individuals found in water are from the Mississippi River. Also, sixty-seven percent of cases of missing persons reported to the Repository last seen near water were from the Mississippi River. While the online Repository only offers the public a limited sample of the state's entire caseload, it still affords some insight into details of actual Louisiana cases. Basset and Manhein (2002) investigated fluvial transport of 233 cases of human remains in the Mississippi River ranging from 1957 to 2001. These cases were from homicides, suicides, recreational drowning, and occupational accidents and involved men, women, and children. Of the cases in which information on the length of time in the Mississippi River was available, Basset and Manhein determined that over ninety percent were discovered within 50 days since death.

The Mississippi River is a forensically-relevant water system in cases of suicide, homicide, and accident investigation. Ideally, its algae should be investigated as a possible means for the determination of PMSI. However, experimentation within the Mississippi River itself is both impractical and unsafe due to its swiftly changing water levels, unpredictable currents, heavy water traffic, and urban locale. Thus, this experiment was conducted within a nearby water system. While the results cannot be directly extrapolated to the Mississippi River,

conducting research in the bayou is a reasonable compromise. Yet, it is still important to consider a few aspects of the Mississippi River for this research.

Rowe (2001) examined hypoxia of the Mississippi River on the Louisiana continental shelf. Hypoxia occurs in deep water when oxygen concentration is less than 62.5 cubed millimoles. Hypoxia is unlikely to occur in shallow water because oxygen is highly available. However, according to Dr. Sibel Bargu Ates, an expert in harmful algae blooms at Louisiana State University, the bacteria present during decomposition will consume the oxygen around the carcass, leading to a similarly hypoxic situation (personal communication). In the early stages of decomposition, nutrients released into the water from the carcass will promote algae growth. Once bacteria from decomposition overtake the carcass and consume the available oxygen, algae will not be able to proliferate. Hynes (1960) also noted that low oxygen environments result in low species diversity. Because clothing inhibits decomposition, the bacteria will not be able to take over the carcass as early, and the algae, hypothetically, should proliferate longer than on an unclothed carcass.

Duan and Bianchi (2006) explained that phytoplankton growth in rivers is controlled by light, temperature, inorganic nutrients, rate of loss from grazing, sedimentation, respiration, and hydraulic flushing. Because rivers are turbulent and well mixed, they have less light availability than standing water, have more suspended solids, and more dissolved organic matter. Additionally, increased nitrogen and phosphorus concentrations from agricultural (fertilizer) and urban (sewage) runoff promote phytoplankton growth. These variables, which are affected by temperature, are all factors of season.

2.7 Neonaticide

Because fetal pigs are the experimental mediums of this research, a brief look at neonaticide is warranted. Neonaticide, defined by Phipps (1999), is the intentional killing of a

newborn child within 24 hours of birth. Experimentation on fetal pigs creates data most relatable to newborn babies. For example, stillborn fetal pigs are used by Archer (2004) to examine seasonality as a factor affecting neonate terrestrial decomposition in Australia. This applicability is due not only to their comparable size, but also the comparable composition of gut microbiota (see Faiver et al. (2002); Gill-King (1997)).

A U.S. Department of Justice (2001) review on child fatality reported that of the approximately 50,000 child deaths in the United States each year, 2,000 are due to abuse and neglect. Of those cases, approximately forty percent of the children are less than a year old. Jason et al. (1983) found that between 1976 and 1979, three percent of all child homicides were of babies younger than one week, and Overpeck et al. (1998) found that between 1983 and 1991, five percent were younger than one day. According to Overpeck et al. (1998), the perpetrator of these homicides was usually the mother.

Jason et al. (1983) reported that women use drowning/falling as the weapon of neonaticide for seven percent of female victims and six percent of male victims. They also found that women perpetrators used drowning and strangulation more than males. However, in an extensive review of the literature, McKee (2006) reported death by drowning could be as high as eighty-four percent. Even in an ethnographic study of Alaskan Innuits and Utes published in 1947, Garber (1947) reported that drowning was one of the primary weapons of infanticide.

Griest and Zummwalt (1989) examined six case studies of child homicide by drowning, finding that most deaths occurred within the home. McKee (2006) also found that most cases of neonaticide occurred in a non-hospital setting, many involving drowning or suffocation. Thus, research into the effect of water on decomposition is directly relevant to the investigation of neonatal death, not only for the cause of death but also for the location of disposal.

2.8 Conclusion

The estimation of PMSI is a crucial step in death investigation. This study recognizes the importance of algae for that estimation, especially as used in cases of neonaticide. While algae have been used successfully in the determination of drowning and crime scene location, few studies exist linking algae to PMSI, and no studies exist examining the algae of Louisiana. Keeping insect and scavenger activity as authentic as possible, this research is intended to realistically examine the use of algae in the estimation of PMSI in a Louisiana bayou.

CHAPTER 3: MATERIALS AND METHODS

3.1 Site Location

Bayou Fountain runs almost the entire southern length of Baton Rouge, Louisiana. It branches south into Elbow Bayou and east into Bayou Manchac. While the word “bayou” conjures thoughts of bald Cypress trees, stagnant water, and alligators, this bayou is more like a stream, having a slow current and stream banks (Figure 3.1).



Figure 3. 1 Site location viewed from the north shore of site 3. Notice that while located in close proximity to a residential zone, the vegetation confers ample privacy to the site. This makes it a realistic body dumping ground.

Bayou Fountain was chosen as the research site for four primary reasons. First, it is located within two miles of the Mississippi River which, as previously noted, is an area of high forensic importance. Second, the particular area of Bayou Fountain chosen for the study

(30°24'2.59"N, 91°10'18.69"W) was accessed only through a private, gated community without public access, allowing for an undisturbed research area. Third, the particular area chosen for the study offers constant depth, current, and shade for all three experimental sites, eliminating environmental variation as much as possible. Last, the bayou meanders between residential areas of Baton Rouge, being both tucked away from the public eye while also readily accessible by foot (Figure 3.2). A desperate individual could easily use Bayou Fountain as a body dumping location, making it a realistic site for forensic research.

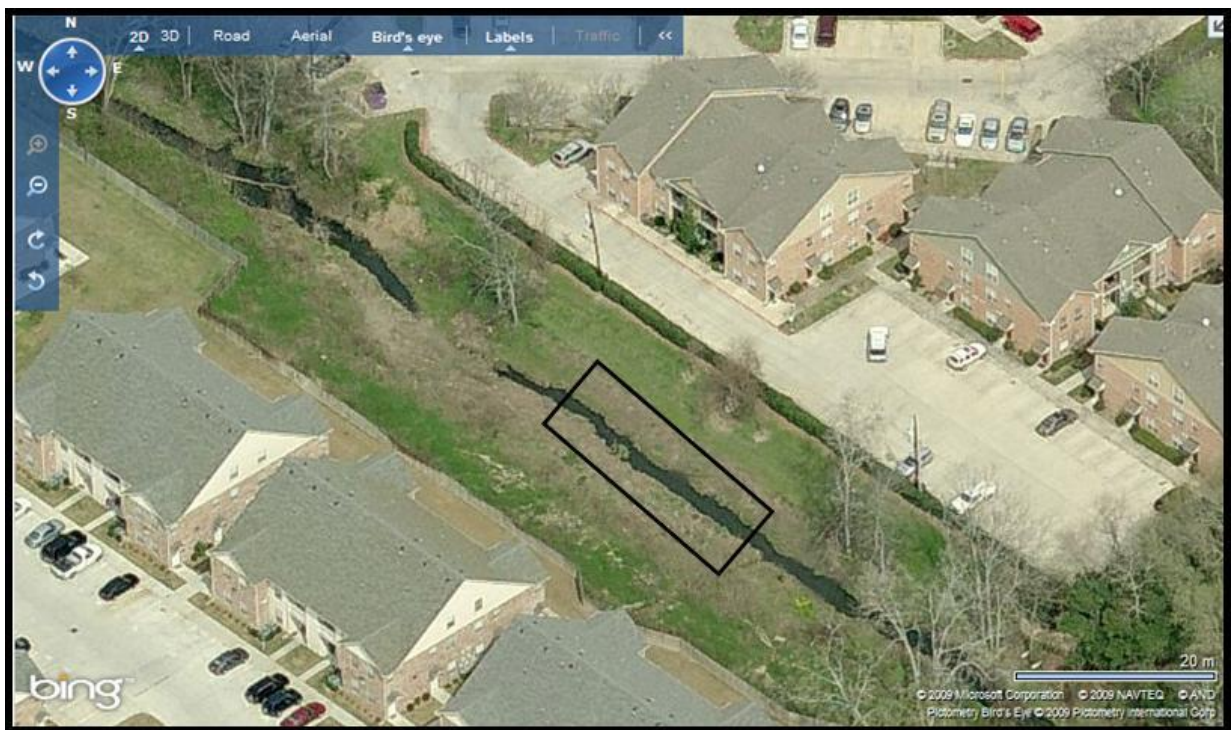


Figure 3. 2 Aerial view of site location. Notice proximity to residential zones. Microsoft product screen shot reprinted with permission from Microsoft Corporation.

3.2 Field Procedures

3.2.1 Preliminary Set-up

Each season, five frozen fetal pigs (*Sus domestica* L.) were obtained from the Swine Unit at Ben Hur Farm, Louisiana State University through Dr. Wayne Kramer of the Department of Entomology. These pigs were not killed for experimental purposes; they were either stillborn or

suffocated newborns through accidental crushing by their mothers and were chosen for similar weight and size. The pigs were slowly thawed on ice in Styrofoam coolers over four days to transition from a state of frozen solid to room temperature. This setup thawed the pigs while preventing the commencement of decomposition.

Three metal fence posts were placed on the Bayou Fountain shore four and one-half meters apart. Two wooden stakes were placed at each shore line two meters from the posts and one and one half meters apart from each other to create three separate experimental sites—clothed, unclothed, and control (Figure 3.3). Tied to the poles with parachute rope (supplied by the United States Air Force) were five Franklin Crab Traps (25.4 x 35.56 centimeter diagonal bottom) reinforced with twelve 20.32 centimeter plastic cable ties. The cages were forced to float by cable-tying small portions of water noodle flotation foam to four sides (Figure 3.4 left).

The cages were placed in Bayou Fountain 24 hours prior to pig deposition. This allowed the site to settle and for minimal disturbance of the aquatic fauna at the commencement of experimentation. The set-up allowed for the pigs to float and sink naturally through the stages of decomposition without becoming washed upon the shore. Because natural conditions were attempted and because algae could not be obstructed by mesh or other light-decreasing materials, scavenging was not prevented. The pigs were completely exposed to the environment, experiencing a full range of natural sunlight, rain, and temperature variation

Pigs A and E were clothed in one hundred percent cotton baby clothing sized zero to three months. This included a white baby sleeper, blue pants, and a blue (spring) or multi-colored (fall) touque (Figure 3.4 right). All metal snaps were removed from the sleeper to prevent contamination from rust, with one hundred percent cotton used to sew the crotch closed.

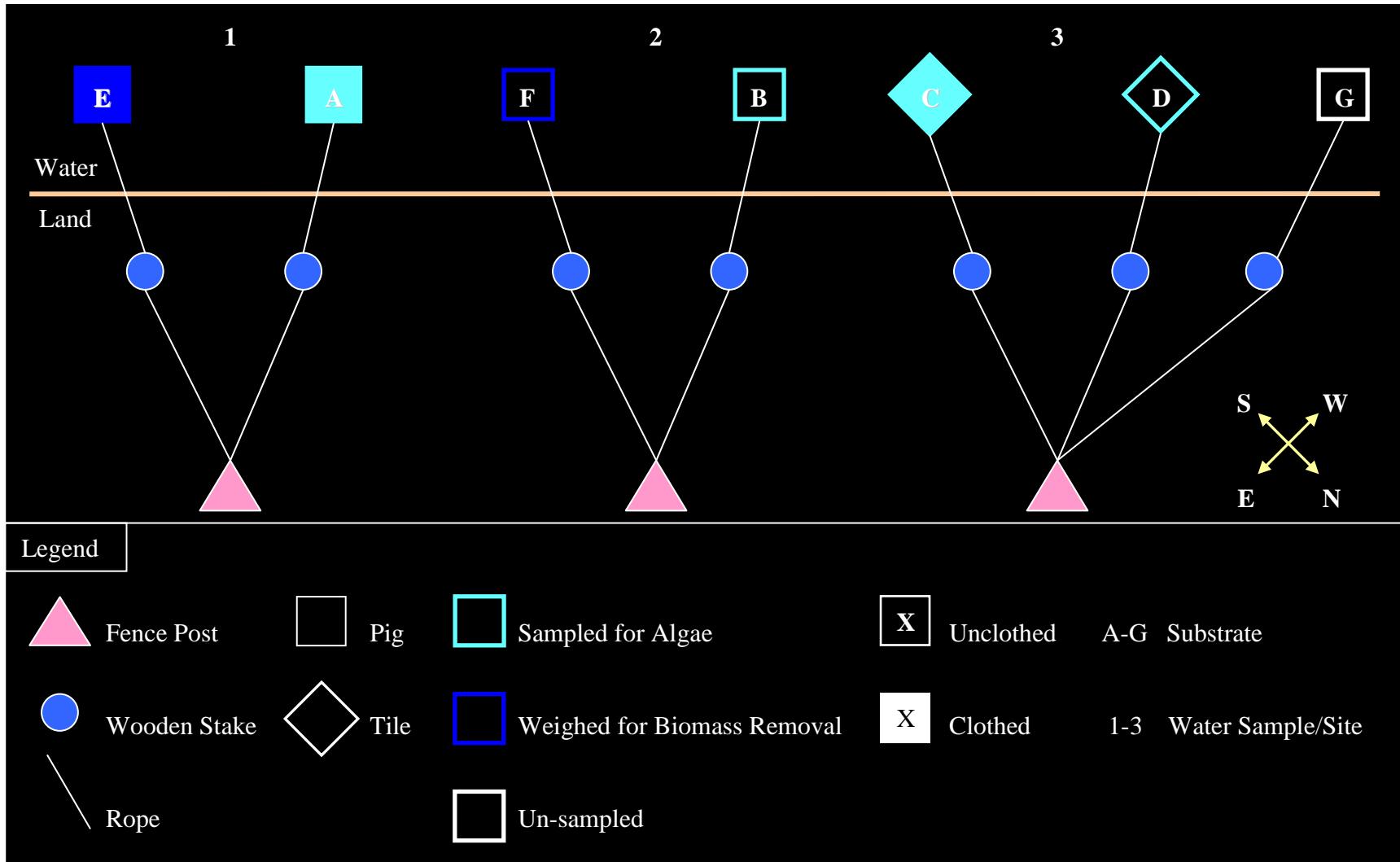


Figure 3. 3 Site map. Site 1 clothed, site 2 unclothed, site 3 control.

The feet of the pants were sewn closed and the touque was sewn to the posterior neck of the sleeper using one hundred percent cotton thread. The fold-over mittens on the sleeper sleeves were used to contain the arms. Pigs B, F, and G were unclothed.

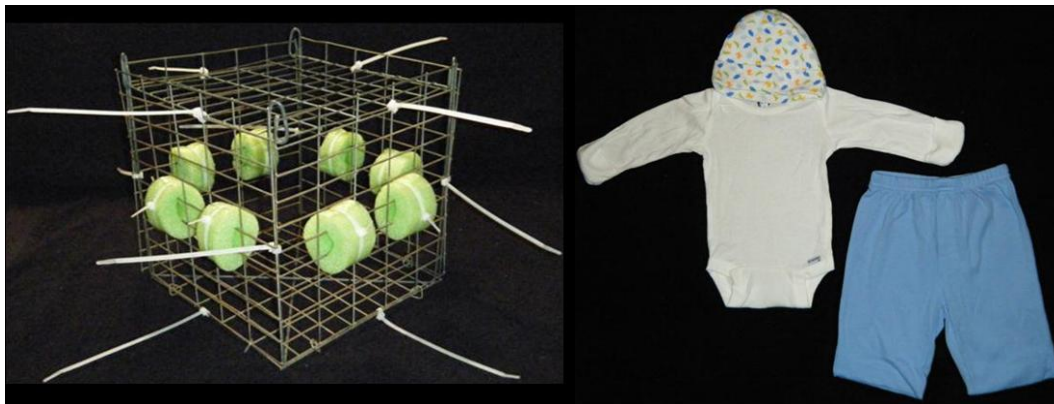


Figure 3. 4 Pig cage (left) and Pig clothing, fall version (right).

The starting and ending weight of each pig was determined using a Bass Pro Shop Fish Scale by subtracting the weight of the empty Franklin Crab Trap cage from the weight of the cage with pig.

Natural slate tiles (40 x 40 centimeter) were placed onto trays of folded chicken wire to allow for lifting with undisturbed algae sampling. Tile C was covered with the same one hundred percent cotton clothing as Pigs A and E, constructed by sewing a portion of sleeper, pants, and touque together with the cotton thread into a large square (Figure 3.5 right). Tile D was unclothed (Figure 3.5 left). It was discovered three days into the spring study that these cages needed to be elevated to prevent excessive sediment accumulation from the bayou floor, so a plastic garbage can was placed in the water underneath each tray so that they remained within two inches of the surface.

The experiment was conducted in two phases: spring and fall. The spring study began May 12, 2009, and ended June 4, 2009. The fall study began November 12, 2009, and ended



Figure 3. 5 Unclothed Tile D (left) and clothed Tile C (right) in wire trays (fall version).

December 15, 2009. The site was visited daily at the same time each morning until the skeletonization stage of decomposition was reached in the spring and until flooding resulted in termination in the fall (24 days in spring, 34 days in fall). Algae samples were taken daily from Pig A, Pig B, Tile C, and Tile D for ten days, then once weekly for the duration of the study (See Appendix A.1 and B.1). Pig E and Pig F were not sampled for algae but rather were weighed every five days for ten days, then once weekly for the duration of the study (See Appendix A.4 and B.5). Pig G was never touched once placed in the water, acting as a control. Water samples were also taken daily for ten days then once weekly for the duration of the study.

In summary, Pig A (clothed) and Pig B (unclothed) were sampled for algae while Pig E (clothed) and Pig F (unclothed) were measured for biomass removal. The tiles were experimental controls, being non-decomposing material. Tile C (clothed) and Tile D (unclothed) were sampled for algae, and Pig G was unsampled.

3.2.2 Water Sampling

Water characteristics were measured at control site three, with turbidity and pH measured daily for ten days then once weekly and dissolved oxygen and salinity measured every five days for ten days then once weekly (See Appendix Table A.2 and B.2). Turbidity was measured using

a turbidity tube and pH was measured using 4.5-10 litmus paper. In addition to the regular sampling schedule, turbidity was also collected during rain events. Water oxygen content was measured using a LaMotte Dissolved Oxygen Kit while salinity was measured using a portable refractometer (See Appendix Table A.2 and B.2). The researcher wore chest waders in order to safely enter the water for sampling, measurement, and photography. As much as possible was done without entering the water, keeping site disturbance to a minimum.

Each sampling day, 500 milliliters of water were collected using a plastic container approximately 45 centimeters (elbow length) below the water surface at site one, two and three. This was done far enough away from the substrates so as not to cause circulation of the water that could potentially affect the algae. This container was wrapped in tinfoil to prevent algae degradation from sunlight. These water samples were placed over a towel in a cooler on ice to prevent algae degradation during transfer to the lab for processing. These samples were examined for chlorophyll *a* concentration of the water. Water Sample Three was also analyzed every five days, then once weekly, for nutrient content (See Appendix Table A.3 and B.3).

3.2.3 Pig and Tile Sample Collection

Maximum Depth of the deepest edge of each pig cage and tile tray was measured each sampling day using a Keson Surveyor's Rope. Additionally, water and air temperature were measured using two methods. An Acurite Wireless Weather Thermometer was used for the air temperature in the shade on the north shore while a Traceable Waterproof Thermometer with Probe/Cable was used for water temperature measured at the depth of the lowest corner of each cage and tray. These measurements were also compared with the temperature data published by the Ben Hur Weather Station (Louisiana Agriclimate Information 2009).

The experimental procedure outlined that all measurements in each site were taken before the algae was sampled (Table 3.1). This limited disruption to the water from sediment kick-up as well as cage placement from disturbed current.

All pigs were attempted to be sampled in a head down manner on the superior side in order to keep sample sites consistent; however, this was at the discretion of the researcher. For example, in the spring study of Pig B, insect activity created a large cavity in the upper torso; so, sampling was conducted on the lower torso. The tiles were sampled in rows, travelling from one side to the other on the superior surface. Most importantly, algae samples were never collected from the same spot twice. This was accomplished by absorbing excess moisture with a cotton ball then marking the sampled area with a black Marvy Fabric Brush Marker on Pig A and Tile C, Krylon Indoor/Outdoor Flat-Black spray paint on Pig B (using a stencil to prevent paint bleeding), and red Crayola crayon on Tile D. Care was taken to leave the unsampled areas unmarked.

3.2.3A Algae Sampling

The algae samplers used in this research were inspired by the Haefner et al. (2004) “modified two-syringe periphyton sampler” and the Aloi (1990) “two-syringe sampler.” Four algae samplers were used—one for each substrate (Pig A, Pig B, Tile C, and Tile D). Each sampler was fashioned using two and six-tenths centimeter diameter PVC pipe cut into approximately seventeen centimeter length tubes. One layer of crafter’s foam was glued with Quick-Grip permanent adhesive to one end of the Pig A, Pig B, and Tile C tubes so that an air-tight seal could be created between the tube and the substrate. Two layers of crafter’s foam were necessary for the Tile D sampler. This modification was made three days into the spring study when, after losing a sample, it was realized that an air-tight seal was not formed with only one

layer. Four Demco Half Inch 1575 brushes were used as scrapers and four 60 cubic centimeter catheter syringes with one centimeter diameter rubber tubing were used as extractors, one brush and one syringe for each sampler (Figure 3.6). Having individual samplers for each substrate prevented contamination between substrates, and thorough daily cleaning with twenty percent Micro-90 soap prevented contamination within each substrate.

Table 3. 1 Site Procedure Outline. Every five days, oxygen content was collected at Site 3 prior to Order #4.

Order	Site	Task
1	All	Overhead Photography, Scene Assessment
2	3	Water Turbidity
3	3	Water Acidity
4	1	Water Sample One
5	1	Pig E: Depth and Temperature
6	1	Pig A: Depth and Temperature
7	1	Pig A: Algae Sample
8	2	Water Sample Two
9	2	Pig F: Depth and Temperature
10	2	Pig B: Depth and Temperature
11	2	Pig B: Algae Sample
12	3	Water Sample Three
13	3	Tile C: Depth and Temperature
14	3	Tile D: Depth and Temperature
15	3	Pig G: Depth and Temperature
16	3	Tile C: Algae Sample
17	3	Tile D: Algae Sample
Throughout	All	Insect Collection, Photography

The cage was placed between the shore line and the thighs of the researcher, keeping the pig underwater for as long as possible. This allowed the cage to be held firmly in place while

leaving both hands of the researcher free, and the algae was disturbed as minimally as possible. The parachute rope was also wound around the wooden stake to keep the cage from drifting. Four of the cable ties were cut using Kobalt wire-cutters so that the top of the Franklin Crab Trap cage could be completely removed. This allowed for unobstructed over-head sampling of the pig.

Algae samples were collected based upon the method outlined by Haefner et al. (2004). First, the substrate was removed from the water. Then, the sampler was placed against the substrate to form an airtight seal. Distilled water was poured into the tube approximately two-thirds full. The brush was inserted into the tube with bristles touching the substrate. The brush was rotated ten rotations right and ten rotations left to dislodge the algae into the distilled water. The brush was then removed and placed aside without the bristles touching any surfaces. The distilled water, now containing algae, was extracted into a syringe then poured into a plastic 250 milliliter container. This container was wrapped in tinfoil to prevent degradation of the algae from sunlight. More distilled water was poured into the tube to collect any remaining algae. This water was then extracted using the syringe and added to the plastic container. The sampler was then removed from the substrate. The brush was rinsed with distilled water over the container to remove any algae trapped within the bristles. The plastic container was placed on a towel in a cooler over ice to prevent algae degradation during transport to the lab for processing. The area was then marked as previously described to prevent duplication of sample locations. This entire procedure was repeated for each substrate, beginning with Pig A, followed by Pig B, Tile C, and then Tile D.

3.2.3B Biomass Removal Measurement

Photographs were taken daily using a FujiFilm Finepix Z33 Waterproof digital camera in addition to detailed field notes of the progress of decomposition on all pigs and tiles. All pigs

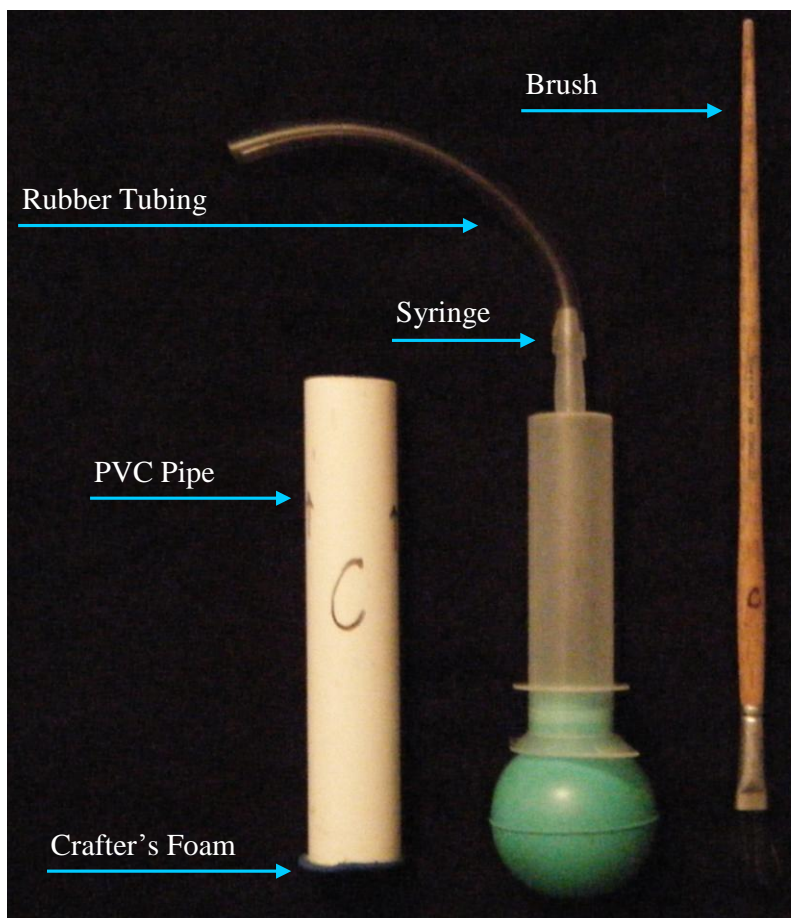


Figure 3. 6 Algae sampler for Tile C.

were visually assessed for rate of decomposition. Pig E and Pig F were not sampled for algae but rather used as controls against the effect of sampling on decomposition. Additionally, Pig E and Pig F were weighed with their cages using a Bass Pro Shop Fish Scale every five days for the first ten days, then weekly for the duration of the study (See Appendix Table A.4 and B.5). This was done as a quantitative measure of biomass removal. Comparisons were made in visual assessments between sampled and unsampled pigs, clothed and unclothed pigs, and biomass removal of Pig E and Pig F.

In order to weigh Pig E and Pig F, their cages had to be lifted out of the water. This was done as quickly as possible to limit aggravation of the decomposition process. The effect of this lifting on the algae was of no concern since algae was not sampled from Pig E and Pig F.

3.2.4 Insect Collection

The insects associated with the pig carcasses were collected in tandem with the algae. While forensic entomology was not the aim of the research, the experimental design allowed for the collection of insects without disturbance of the algae communities or cadaver. Aquatic and terrestrial insects were collected from the substrate and within one meter of its immediate surroundings. The cages were never opened or moved to collect insects; all insect collection occurred without disturbing the pigs. This was accomplished with forceps for collection of maggots, an aquarium net for aquatic insects around the cages, and a butterfly net for aerial insects (Tluzak (2009); Parker (2009)).

Soft bodied insects and larvae were killed and preserved in seventy percent ethanol ((Keiper et al. (1997); Hobischak and Anderson (2002)). Flying insects were placed in a kill jar containing ethyl acetate then frozen until pinned. All were collected in 59 milliliter portion cups then transferred to 25 milliliter blood dilution vials, or directly collected in the vials. All hard bodied insects were pinned using BioQuip #2 black enamel pins. All soft bodied insects were preserved in 25 milliliter blood dilution vials in seventy percent ethanol. These insects were identified by Leighanne Lawton with the Louisiana State University Department of Entomology. Because forensic entomology was not the main concern of the research, rearing was not attempted.

3.3 Laboratory Procedure

3.3.1 Chlorophyll Analysis

Once at the lab, algae samples from Pig A, Pig B, Tile C, and Tile D and Water Sample One, Water Sample Two, and Water Sample Three were gently homogenized with ten back-and-fourth hand movements then filtered using 25 millimeter GF/F filters. Any remaining liquid in

the containers was flushed out with distilled water, with the final volume of filtered water recorded (recorded as V_f). The filters were then placed into 15 milliliter plastic centrifuge tubes which were covered with aluminum foil to prevent algae degradation from light. These tubes were kept in the freezer until ready for extraction. The filtrate of Pig A, Pig B, Tile C, and Tile D were discarded. Every five days then once weekly, the salinity and oxygen content of Water Sample Three was measured, the filtrate of Water Sample Three was saved for nutrient analysis (see Section 3.3.2), and one milliliter of all unfiltered substrate samples were collected for microscopic analysis (see Section 3.3.3).

When ready for analysis, the centrifuge tubes containing the filters were removed from the freezer and ten milliliters of ninety percent acetone were added to each then sealed with Parafilm (acetone volume recorded as V_x). The tubes were then returned to the freezer for 24 hours. Once 24 hours passed, the tubes were removed from the freezer and allowed to warm to room temperature in the dark for 30 minutes. They were then centrifuged at 6000 rotations per minute, 3280 g-force for ten minutes in a Damon/IEC Division Clinical Centrifuge. In low light, the supernatant was carefully transferred from the centrifuge tubes into glass 13 x 100 millimeter culture tubes with TainerTop closures.

Once in the culture tubes, the samples were ready for chlorophyll *a* analysis in the Turner Designs 10 AU Flurometer. After being wiped free of fingerprints with a KimWipe, the tube was placed into the flurometer and measured (recorded as F_O). Then, two drops of ten percent HCl acid were added and the tube was placed into the flurometer and measured (recorded as F_A). When dilution was necessary, one milliliter of sample was diluted in nine milliliters of ninety percent acetone in a new culture tube, being a 1:10 ratio (recorded as Dil).

Measurements were then placed into the following equation:

$$\text{Chl } (\mu\text{g}/\text{cm}^2) = ((K \times (F_o - F_a) \times \text{Dil} \times V_x) / V_f) / A$$

Where:

- K = flurometer constant
- F_O = flurometer reading before acidification
- F_A = flurometer reading after acidification
- Dil = dilution factor
- V_x = extraction volume
- V_f = filtered volume
- A = area of sample site

This chlorophyll *a* concentration acted as a quantitative measure of algae biomass. That is, the higher the concentration of chlorophyll *a*, the more algae present on the substrate.

3.3.2 Nutrient Analysis

Forty milliliters of filtrate from Water Sample Three were placed in a 50 milliliter centrifuge tube and frozen until ready for nutrient analysis following the sampling schedule. Dr. John White at the Louisiana State University, Wetland Aquatic Biogeochemistry Laboratory conducted analysis on the water samples for nitrate (Method 353.2, USEPA 1993), ammonium (Method 350.1, USEPA 1993) and dissolved reactive phosphorus (Method 365.1, USEPA 1993) on a Seal Analytical (Mequon, Wisconsin) AQ2+ discrete analyzer using standard colorimetric methods.

3.3.3 Microscopic Analysis

To assess the types of algae present on the substrates and in the water, one milliliter of each homogenized unfiltered Pig and Tile sample was collected in a microtube following the sampling schedule. These samples were then fixed in a few drops of Lugol's Solution (potassium iodine), making it a "tea-like" color. This was also done to 100 milliliters of Water

Sample Three in a 120 milliliter French square. After being allowed to settle for at least 24 hours, the samples were then examined with a Zeiss Observer.A1 AXIO dissecting microscope and photographed with an AXIO Cam MRC.

By identifying the types of algae present using microscopic analysis, qualitative analysis could be conducted into the kinds of algae present on the pigs and tiles. Due to inexperience of the researcher, algae were identified to division only with the intent of recognizing the overall algae abundance.

3.4 Statistical Analysis

Statistical analysis was used to examine three aspects of the research. First, algae growth, measured through chlorophyll *a* concentration, was placed into a linear regression formula to establish if chlorophyll *a* concentration increased over time. The slopes of these regressions were compared to assess the effects of clothing and season on algae growth. One-way analysis of variance (ANOVA) was used to compare the chlorophyll *a* concentrations of the substrates. Second, degree days of decomposition were determined for all pigs to examine the difference in rate of decomposition between sampled and unsampled, clothed and unclothed, and spring and fall. Lastly, the water itself was analyzed with one-way ANOVA to see if significant differences of abiotic factors existed between the two seasons which may have influenced decomposition and algae growth.

3.4.1 Chlorophyll *a* Analysis

As done by Haefner et al. (2004), the results of each algae sample were placed into a linear regression using chlorophyll *a* concentration as a function of time. The line of best fit was used to describe the change of chlorophyll *a* concentration over time. The coefficient of determination (r^2) for each linear regression was examined to assess the formula's viability as a predictor of chlorophyll *a* concentration at a specific time.

Algae growth was quantitatively measured through chlorophyll *a* concentration; chlorophyll *a* concentration was indicative of algae biomass. One-way ANOVA was used to examine the statistical significance of the differences between substrate concentrations and the trend lines of the linear regressions were visually compared to establish similarities and differences. The purpose of these regressions was to see if there was a difference in chlorophyll *a* concentration, and thus algae growth, between pigs and tiles, clothed and unclothed substrates, and spring and fall.

Because chlorophyll *a* concentration measures the amount of algae and not the type, a survey of the microscope algae pictures was additionally conducted to determine the type of algae present in the bayou.

3.4.2 Biomass Analysis

In order to quantify decomposition, weight loss, air temperature, and water temperature were used to establish degree days as outlined by Haefner et al. (2004). Degree days are a thermal unit used to quantify the energy required for the physiological development of an insect (Gennard 2007). Following the example set by Haefner et al., degree days were used not to quantify insect development but rather the progression of decomposition. The mean daily water temperature was determined through linear regression with the daily atmospheric temperatures as recorded at the Ben Hur Weather Station (See Appendix Figure A.6 and Figure B.6). Using this mean daily water temperature, degree days were calculated with negative means classified as zero degree-days (See Appendix Table A.3 and Table B.3). The stages of decomposition were identified for each pig based on macroscopic analysis of morphological change over time and the degree days were compared for the pigs and seasons.

3.4.3 Water Analysis

One-way ANOVA was used to compare the mean pH, mean turbidity, oxygen content, and nutrient content of the experimental site in spring with fall. The purpose of these statistical analyses was to see if water condition was significantly different at opposing times of year.

3.5 Decomposition Analysis

Following the outlines of aquatic decomposition set fourth by both Payne and King (1972) and Boyle et al. (1997), the researcher identified four stages of decomposition with the following criteria to describe aquatic decomposition in Louisiana:

Stage One—Fresh: Initial placement in the water, either floating or sinking. The skin is pink and fresh with no odors or discoloration. Even when carcass sinks, clothing will puff with air and float toward the surface. This stage lasts one to two days in spring, two to four days in fall.

Stage Two—Early Decomposition: The carcass is bloated and rises to the surface of the water. The carcass is firm to the touch, filled with gases and excreting decompositional odors. Bubbles or foam may float on the water, usually around the mouth area. Exposed skin discolors to shades of purple, grey, and blue. Insect activity will be present. A reddish-brown line of tissue splitting may form at the waterline on the torso region. This stage lasts three to eight days in spring, ten to eighteen days in fall.

Stage Three—Advanced Decomposition: Maggots, if present, burrow into the flesh. The carcass is bloated but is no longer firm, being soft and deflated. The carcass will float but has more surface area under than above the water level. Discoloration deepens while facial features and limbs degrade. Adipocere may form. Scavenger activity may occur at any time during decomposition, but it is most predominant and devastating to the carcass during this stage. This stage lasts seven to twelve days in spring, and more than fourteen days in fall.

Stage Four—Sunken Remains: The carcass is no longer bloated and is an indistinguishable piece of pink flesh. This sinks to water bottom and is rendered to bones, sometimes contained within a clump of debris, fabric, and remnants of skin. Skeletonization requires three to four days in spring and an unknown timeframe in fall.

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Spring Study Results

4.1.1 Stages of Decomposition

Figure 4.1 demonstrates the amount of time the spring pigs spent in each phase. To summarize, the clothed pigs (A and E) decomposed slower than the unclothed pigs (B, F, and G). The sampled pigs (A and B) both spent less time in the advanced decomposition stage than their unsampled partner pigs (E and F, respectively), reaching the sunken remains stage sooner. This shows that clothing hampers decomposition while sampling may promote it.

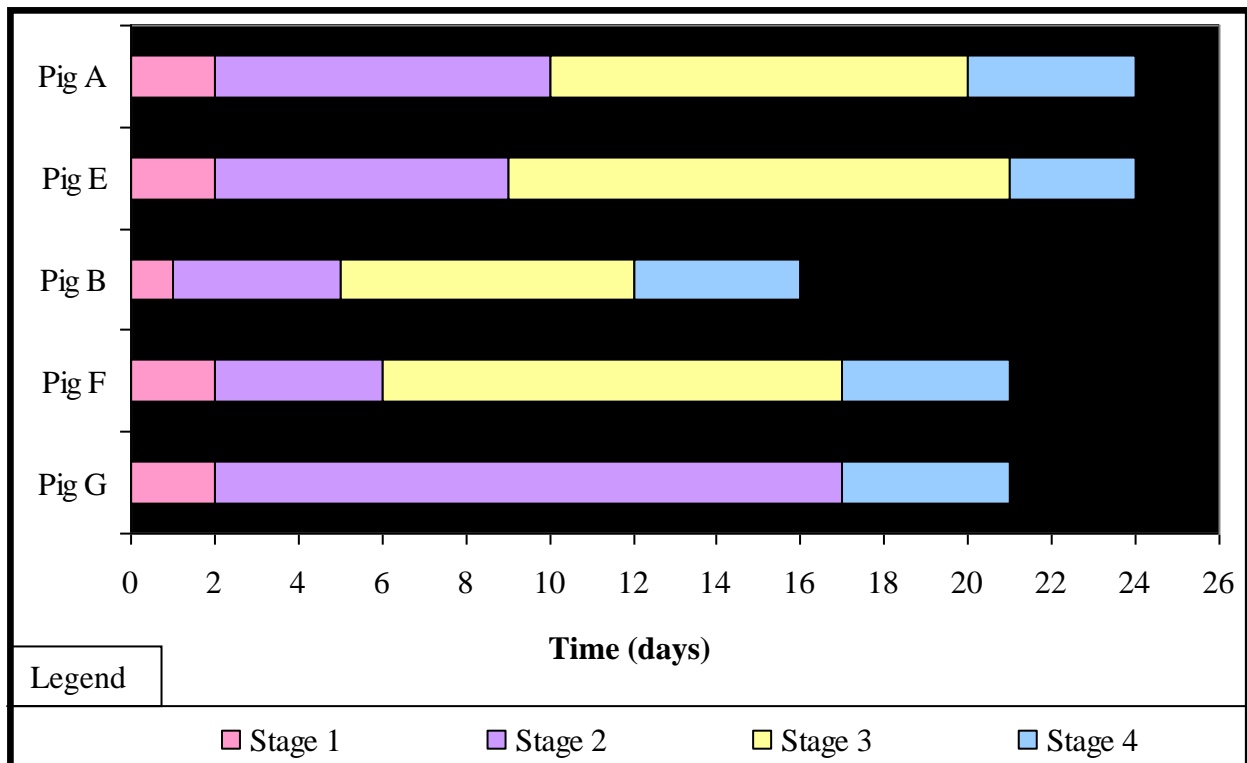


Figure 4. 1 Duration of decomposition stages for each pig, spring.

Algae growth was variable depending on the substrate. For the unclothed pigs (B and F), there was very little growth in stage one. Stage two saw bright green algae growing along the waterline, especially on the submerged areas of the arms, legs and head. No algae grew on the

skin above the water surface. In stage three, the algae became thick and dense, appearing dark and fuzzy. When the carcass sank just below the water surface, algae began to grow on the previously above-water areas. Because algae were only present on the outer surface, it disappeared once scavengers tore into the carcass, with little to no algae present in stage four. Unclothed Tile D had a gradual thickening of algae cover over time, eventually appearing slimy and brownish green (Figure 4.2 right).



Figure 4. 2 Tile C (left) and Tile D (right) at completion of study, spring.

Algae growth was slightly different on the clothed pigs (A and E). In late stage one, the entire carcass was covered with a dusting of brown algae. In stage two, bright green algae grew on the entire carcass, especially along the waterline but also above the water surface. In late stage two and into stage three, algae at and above the waterline became thick and lumpy while underwater algae became more stringy and fuzzy. Late stage three and stage four were the same as for the unclothed pigs. Clothed Tile C had a gradual thickening of algae cover over time. However, it always appeared brown, with only one corner having a green fuzzy appearance (Figure 4.2 left).

Control Pig G was different from all the other substrates. It had the thickest and most abundant algae covering of all pigs. By late stage one, the back was covered with brown algae. This covering became denser during stage two and the back was completely covered with brown

and green algae like a carpet by early stage three. As with the other substrates, once scavenging occurred, the algae disappeared.

The following is a detailed breakdown of the decomposition stages for each spring pig. Though numerous kinds of scavenger activity were apparent on the pigs, only small fish were ever directly observed scavenging the carcasses.

4.1.1A Pig A

Clothed, sampled Pig A took 24 days to become skeletonized. The fabric discouraged scavenger activity and shielded the skin from sunlight. Only discoloration that permeated the fabric could be noted; clothing was not moved to observe the carcass.

In summary, Pig A spent two days in stage one (Day 0-1), eight days in stage two (Day 2-9), ten days in stage three (Day 10-19), and four days in stage four (Day 20-23). Figure 4.3 shows this daily progression of decomposition.

Stage One—Fresh: The clothing puffed with air and floated toward the surface, but the carcass remained underwater in a head-down manner.

Stage Two—Early Decomposition: Bloating caused the carcass to float to the surface with back-up. On the first day of this stage, white foam was present at the mouth region above the water stuck to the cage (Figure 4.4). A brown stain was present on the back that remained throughout the stages and attracted insects (Figure 4.5 left). As the stage progressed, bright green algae grew thicker at the waterline and onto the surface of the back.

Stage Three—Advanced Decomposition: The carcass became soft and submerged just below the water surface. The algae continued to grow on all surfaces, being very lumpy and thick (Figure 4.5 right). Heavy scavenger activity on day 17 resulted in tremendous loss of biomass; its carcass shape was no longer recognizable by day 18.



Figure 4. 3 Progression of decomposition daily overview: Pig A, spring. Fresh Stage (Day 0-1); Early Decomposition (Day 2-9); Advanced Decomposition (Day 10-19); Sunken Remains (Day 20-23).



Figure 4. 4 Foam at mouth region of Pig A: Day 2, spring. This foam only appeared on this pig on this day, not seen again in spring. Notice the previous day's sample spot marked with fabric marker (arrow).

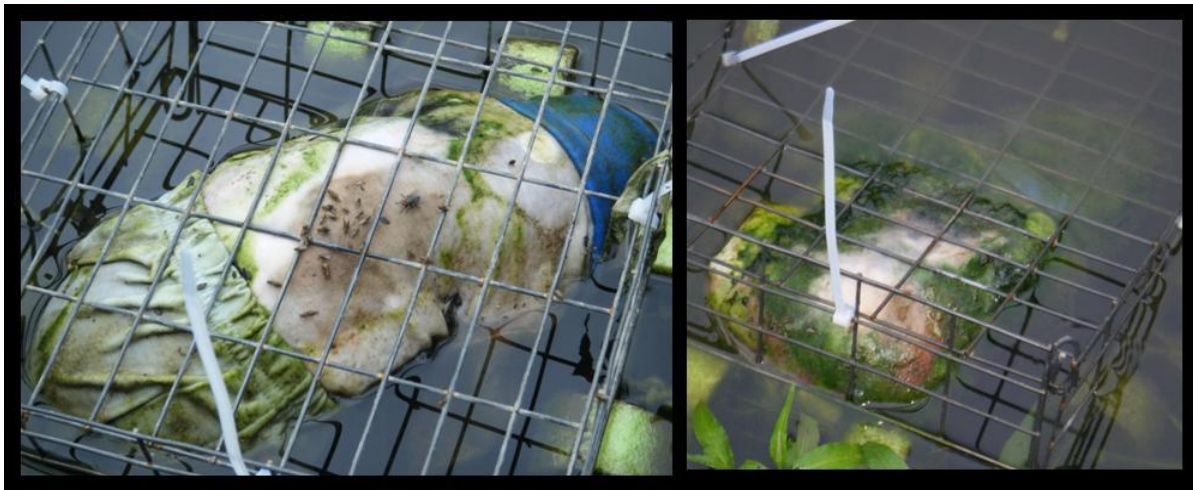


Figure 4. 5 Brown stain on back of Pig A: Day 5 (left) and Day 13 (right), spring. Note that this spot was never completely covered with algae. Note also the thickened algae on the deflated Day 13 carcass.

Stage Four—Sunken Remains: The cage sank to the bayou floor with very little flesh visible from the surface. Fish were still active and remnants of clothing were puffed with air. When lifted, bones and just a few remnants of skin were observed to be contained within clothing at the bottom of the cage (Figure 4.6).



Figure 4. 6 Skeletonized remains of Pig A: completion of spring session.

4.1.1B Pig E

Clothed, unsampled Pig E took 24 days to become skeletonized. The fabric helped to discourage scavenger activity and to shield the skin from sunlight. Only discoloration that permeated the fabric could be noted; clothing was not moved to observe the carcass.

In summary, Pig E spent two days in stage one (Day 0-1), seven days in stage two (Day 2-8), 12 days in stage three (Day 9-20), and three days in stage four (Day 21-23). Figure 4.7 shows this daily progression of decomposition.

Stage One—Fresh: The sleeves and pant legs puffed with air, causing the extremities to rise above the water level. The rest of the carcass was submerged left side up.

Stage Two—Early Decomposition: The carcass was in a sideways, head-down position with lots of green algae growth. The torso was bloated firm and the clothing still had pockets of air bubbles keeping the limbs upwards. Only the rump and lower back region of the carcass floated above the water surface. From the shore, the carcass was unrecognizable, appearing only as dirty, algae covered clothing. A reddish-brown line of split tissues showed through the fabric of the sleeper at the torso (Figure 4.8).

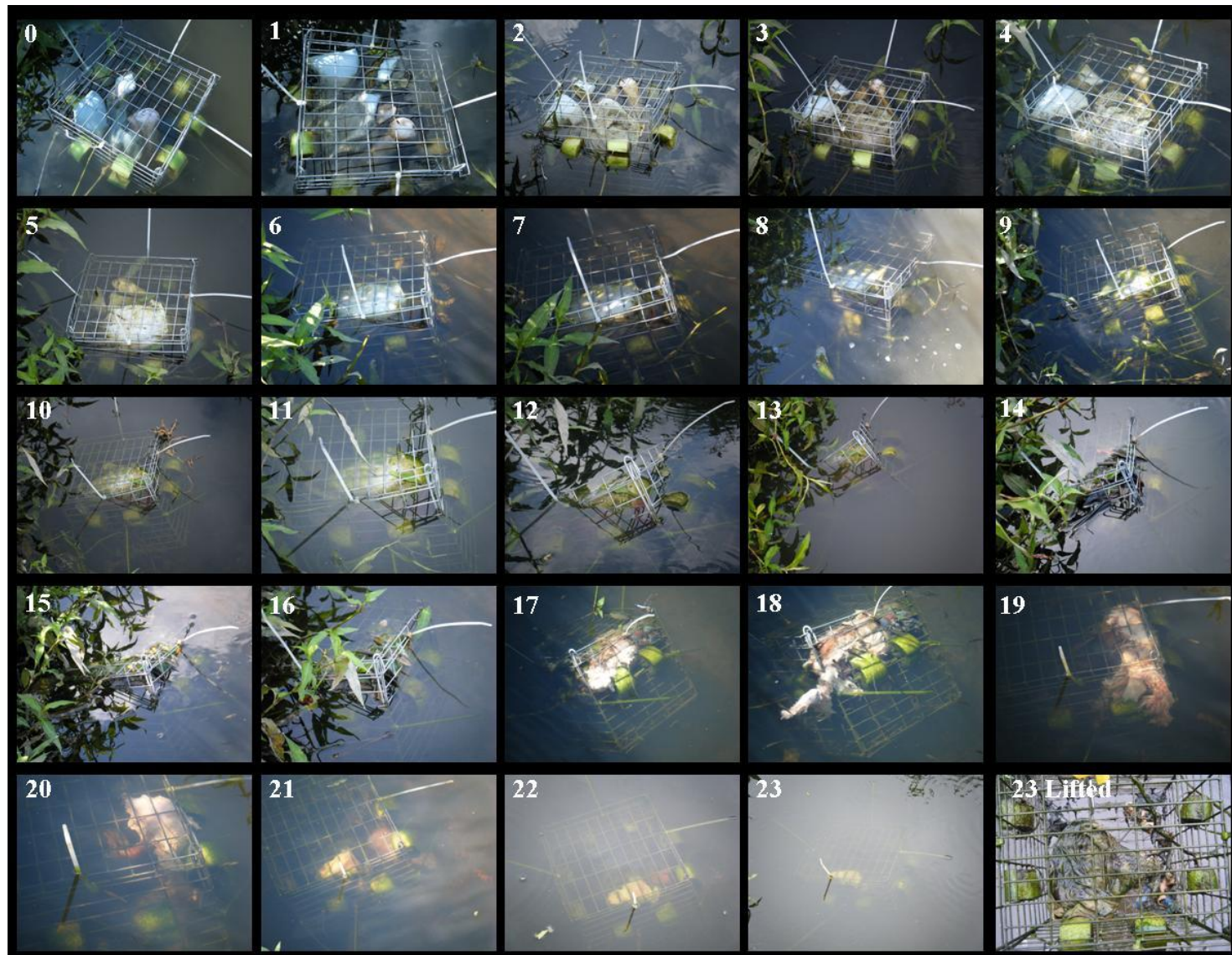


Figure 4. 7 Progression of decomposition daily overview: Pig E, spring. Fresh Stage (Day 0-1); Early Decomposition (Day 2-8); Advanced Decomposition (Day 9-20); Sunken Remains (Day 21-23).



Figure 4. 8 Split tissues observed through fabric, Pig E: Day 7, spring. Arrow points to area of reddish-brown discoloration from tissue splitting.

Stage Three—Advanced Decomposition: The carcass became more submerged and heavily covered with algae until Day 17. On this day, heavy scavenger activity resulted in previously unexposed flesh being uncovered and the clothing being torn away. While the carcass remained floating and unclothed, bones were exposed and limbs were torn out of the cage boundary. On Day 20, the carcass was completely vertical, with the head just under the surface of the water.

Stage Four—Sunken Remains: Some clothing remained floating at the surface while pieces of tissue and bone were visible outside the cage on the bayou floor. Bones were contained by clothing and debris at the bottom of the cage (Figure 4.9).



Figure 4. 9 Ribs within a clump of fabric and skin, Pig E: Day 23, spring.

4.1.1C Pig B

Unclothed, sampled Pig B was the fastest of all spring session pigs to decompose, taking only 16 days to become skeletonized. Having no fabric to cover its skin, scavenger activity was high early on—an entire leg was missing by the fourth day of submersion. Also, the flesh was completely exposed to the sun and heat, with intense insect activity. Maggots caused a great amount of damage to the torso; Pig B was the only substrate to be affected by maggots burrowing into its flesh. Discoloration was easily noted from direct observation.

In summary, Pig B spent one day in stage one (Day 0), four days in stage two (Day 1-4), seven days in stage three (Day 5-11), and four days in stage four (Day 12-15). Figure 4.10 shows this daily progression of decomposition.

Stage One—Fresh: The carcass floated belly up with the left limbs crossed over the carcass to the right. The chin was up with the back of the head submerged.

Stage Two—Early Decomposition: Intense scavenger activity to the rump area caused significant damage, with the left leg completely removed and the anus area shredded. The torso became dark purple while a reddish-brown line of splitting tissue formed on the back at the waterline (see Figure 4.11 left). The umbilicus region became black with putrefaction while a few small, white, moist lumps appeared on the back.

Stage Three—Advanced Decomposition: The torso became deflated and the carcass sunk below the waterline. A bright green ring of algae outlined the entire carcass along with the reddish-brown line of tissue splitting. The face became black with putrefaction (Figure 4.11 right). The small lumps on the back appeared as a moist, hard ridge (Figure 4.11 left) and the intestines protruded from the anus. A strong, putrid odor was present and maggots were burrowing into the armpit. Scavenger activity was severe.

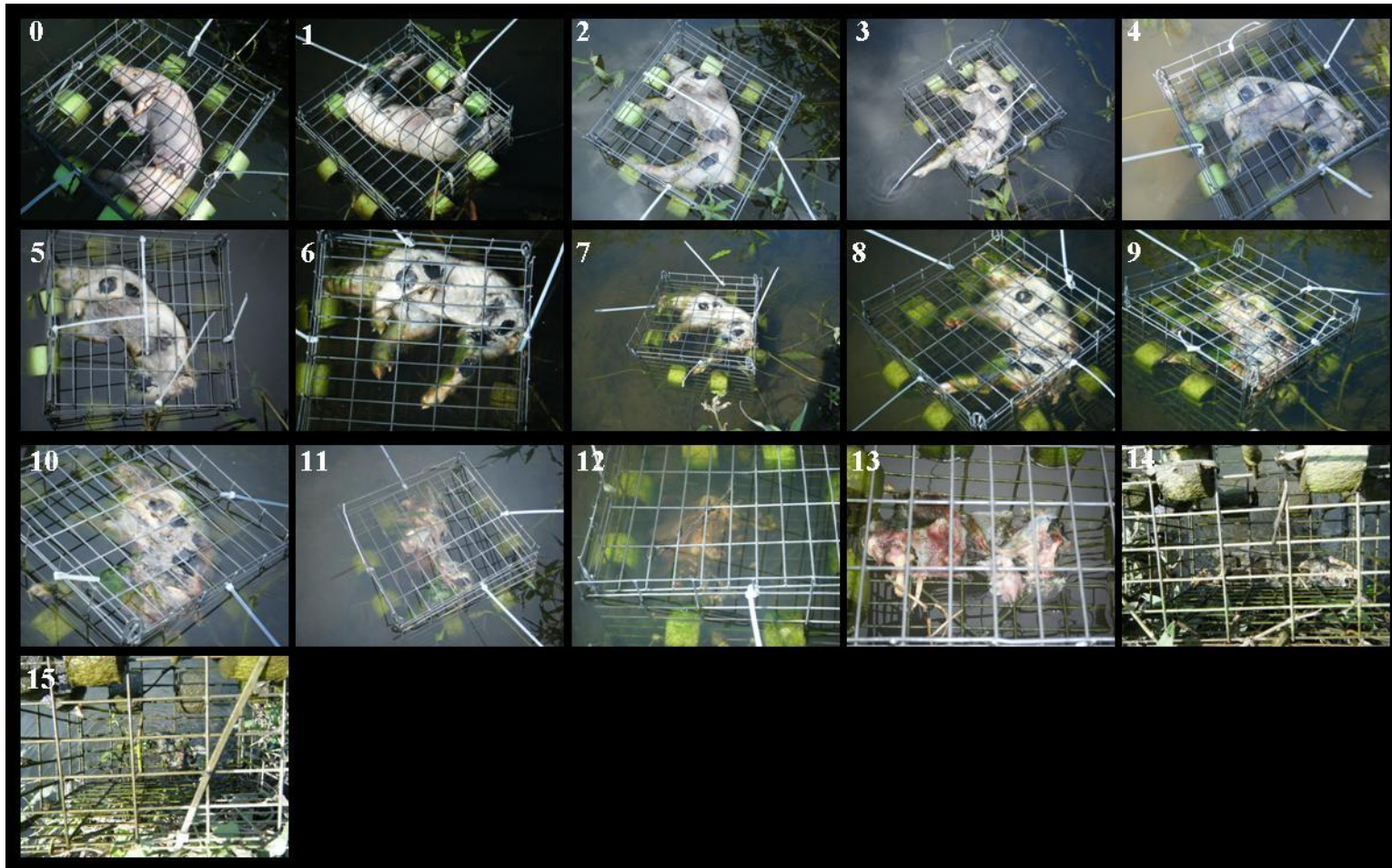


Figure 4. 10 Progression of decomposition daily overview: Pig B, spring. Fresh Stage (Day 0); Early Decomposition (Day 1-4); Advanced Decomposition (Day 5-11); Sunken Remains (Day 12-15).

Stage Four—Sunken Remains: The carcass sunk to the bayou floor, with all soft tissue completely consumed by scavengers. Fish were seen actively removing chunks of flesh from the remains. Early in the stage, the vertebral column was completely exposed. By the end of the stage, no bones remained within the cage, with only a single, small piece of skin remaining wrapped around the cage wires.

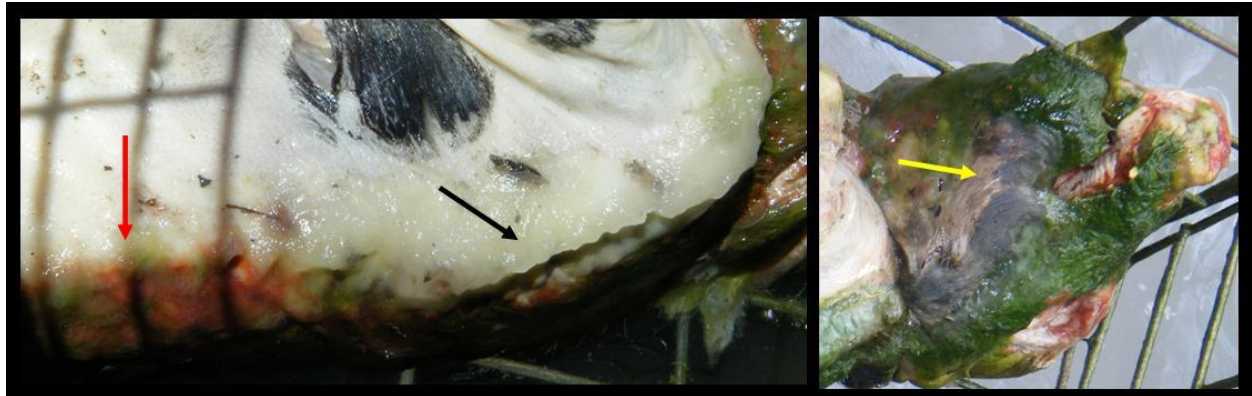


Figure 4.11 Tissue splitting and moist ridge on back of Pig B: Day 7 (left) and black putrefaction on head Day 10 (right), spring. Red arrow points to split tissue; black arrow points to ridge; yellow arrow points to black putrefaction.

4.1.1D Pig F

Unclothed, unsampled Pig F took 21 days to become skeletonized. Like Pig B, it was completely exposed to scavengers and the elements, becoming completely skeletonized sooner than the clothed pigs.

In summary, Pig F spent two days in stage one (Day 0-1), four days in stage two (Day 2-5), 11 days in stage three (Day 6-16), and four days in stage four (Day 17-20). Figure 4.12 shows this daily progression of decomposition.

Stage One—Fresh Remains: The carcass floated left side up horizontally, with the left side of the head above water. The umbilicus became very dry, beginning to mummify. The carcass emitted a sour smell.



Figure 4. 12 Progression of decomposition daily overview: Pig F, spring. Fresh Stage (Day 0-1); Early Decomposition (Day 2-5); Advanced Decomposition (Day 6-16); Sunken Remains (Day 17-20).

Stage Two—Early Decomposition: The skin of the torso above the water surface progressed in color from purple to bluish-grey. Substantial green algae growth was present along the waterline, with the underwater portion of the limbs especially covered. Fly activity was also present particularly at the rump, though their eggs were washed away. Intense scavenging tore the stomach, allowing the intestines to fall out. Additionally, scavenging resulted in the complete removal of the face. A pinkish-brown line of splitting tissue was present along the back.

Stage Three—Advanced Decomposition: The carcass became deflated with a prominent ring of thick, bright, fuzzy green algae around the whole carcass at the waterline. The line of tissue splitting became darker, more pronounced, and present also in a ring around the carcass at the waterline. The carcass became completely submerged just under the water level, with new algae growing on the previously above-water areas (Figure 4.13). The ring of algae became dark and thick. Scavenger activity shredded the anus region and completely exposed the ribs, legs, and articulated vertebrae.

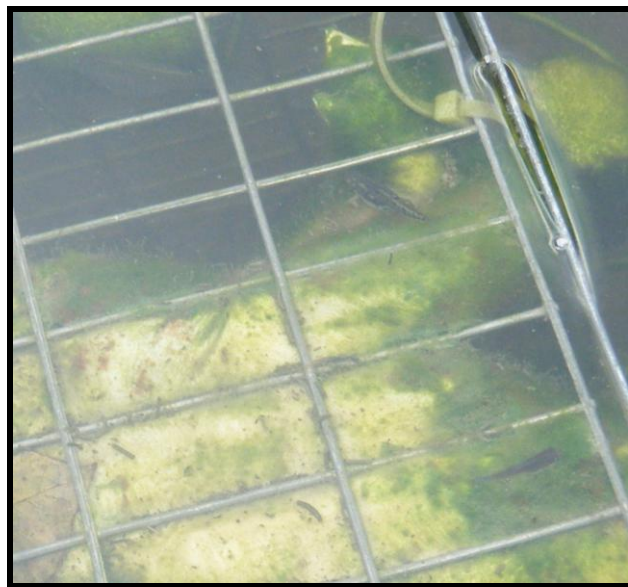


Figure 4. 13 Algae growth on newly submerged areas of skin on Pig F: Day 12, spring. Notice the ring of dark algae on the outer edge with lighter algae on the interior.

Stage Four—Sunken Remains: The highly scavenged flesh appeared pink and sank to the bottom of the bayou floor. High fish activity was apparent with no visible algae. A pile of bones was collected at the bottom of the cage, trapped within some skin (Figure 4.14).



Figure 4. 14 Bones within a clump of skin, Pig F: Day 23, spring.

4.1.1E Pig G

Unclothed, unsampled control Pig G spent the least amount of time above the water surface. Once scavengers removed the hind legs, the pig was not buoyant enough to offset the weight of the cage; being completely bloated allowed the pig to float to the top of the cage but not to bring the top of the cage over the surface of the water. Pig G experienced a similar pattern of decomposition as the other pigs but was not exposed to direct sunlight or fly activity. However, it also had the most surface area available for water scavengers, which resulted in the pig skipping stage three, going directly from early decomposition to sunken remains.

In summary, Pig G spent two days in stage one (Day 0-1), 16 days in stage two (Day 2-17), and four days in stage four (Day 18-21). Figure 4.15 shows this daily progression of decomposition.

Stage One—Fresh Remains: The carcass sank to the bottom of the bayou floor, covered in brown dirt and algae.

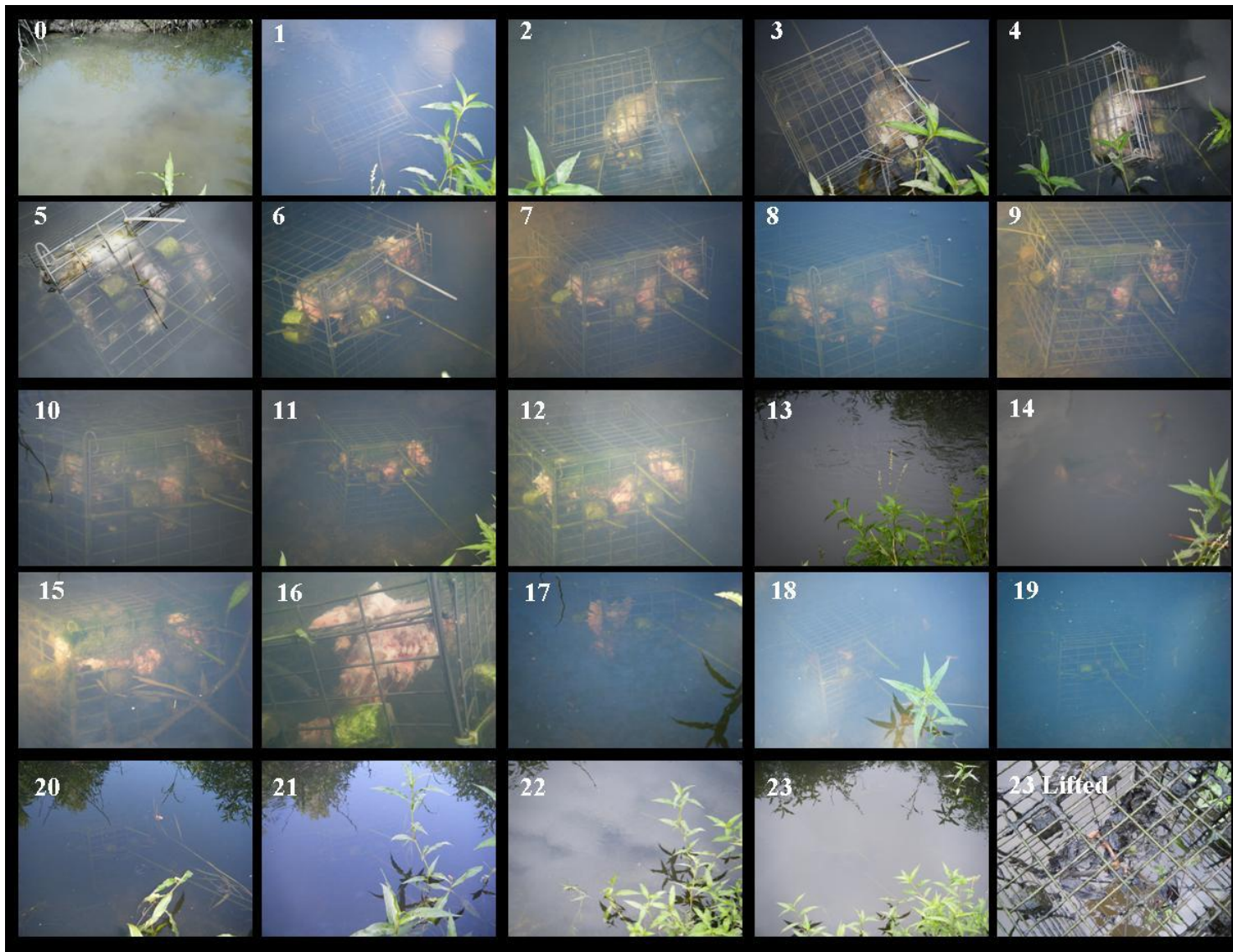


Figure 4. 15 Progression of decomposition daily overview: Pig G, spring. Fresh Stage (Day 0-1); Early Decomposition (Day 2-17); Sunken Remains (Day 18-23).

Stage Two—Early Decomposition: The bloated carcass floated rump up, its surface covered in clumps of brownish-green algae. On Day 6, the hind legs were gone and the anus area was shredded from scavenging, resulting in the pig no longer floating above the surface but rather horizontally underwater. Maroon and pink discoloration of the face was visible initially, but the entire surface quickly became blanketed with a thick covering of algae (Figure 4.16). Scavenging resulted in the loss of both legs and ears and deformation of the mouth, eventually rendering the carcass to an unrecognizable piece of flesh (Figure 4.17).



Figure 4. 16 Thick algae growth on the posterior of Pig G: Day 15, spring.



Figure 4. 17 Intense scavenger activity exposing the ribs of Pig G: Day 16, spring.

Stage Four—Sunken Remains: Never reaching the advanced decomposition stage, the intense scavenging rapidly rendered the pig to bones, some left piled in a clump of leaves, dirt, and skin at the bottom of the cage (Figure 4.18).



Figure 4. 18 Bones within a pile of debris, Pig G: Day 23, spring.

4.1.2 Calculation of Degree Days

Table 4.1 shows the initial and final weights of all pigs. Unsampled Pig E and Pig F were weighed for biomass removal throughout the study. The first weight measurement recorded for Pig E was more than its initial weight because the clothing absorbed moisture once placed in the water. This did not occur with unclothed Pig F.

Table 4. 1 Initial Weight, Final Weight and Weight Loss of All Pigs, Spring.

Pig	Initial Weight (kg)	Final Weight (kg)	Weight Loss (kg)
A	1.75	0.5	1.25
B	1	0	1
E	1.75	0.5	1.25
F	1.25	0	1.25
G	1.75	0.25	1.5

Weight loss was positively correlated with time for both Pig E ($r^2 = 0.80$) and Pig F ($r^2 = 0.99$). Overlaying the stages of decomposition onto the graph of biomass removal (Figure 4.19) shows that weight loss was steady and gradual for Pig F throughout all the stages. Pig E did not experience any weight loss until stage three, after which point biomass was removed quickly. These graphs suggest that, in spring, clothing retards biomass removal in the early stages of decomposition by protecting the carcass from scavengers and the elements.

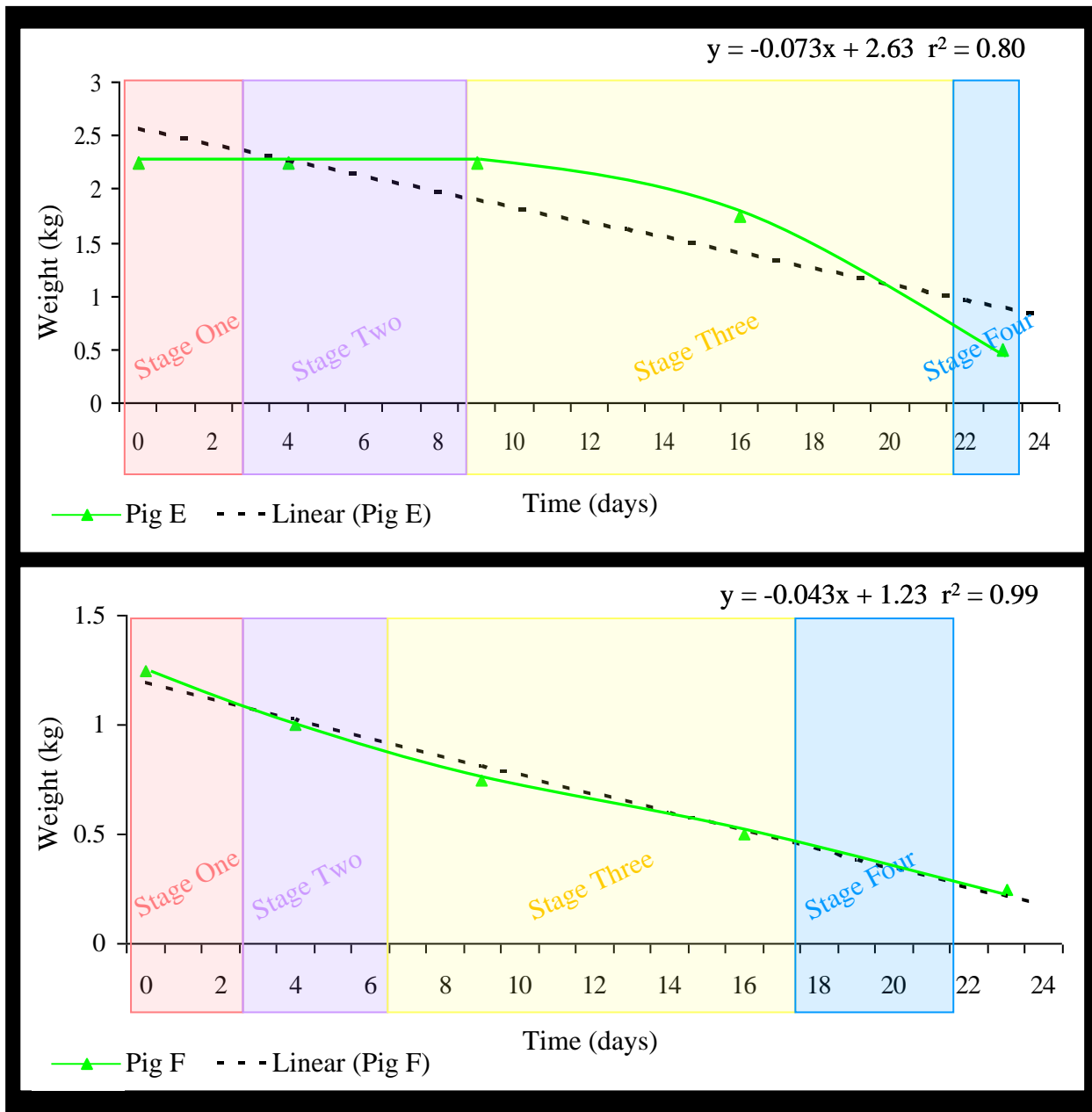


Figure 4. 19 Weight loss for Pig E (top) and Pig F (bottom) by stage of decomposition, spring.

To confirm that clothing delays decomposition, the accumulated degree days of each stage of decomposition were determined using the method outlined by Gennard (2007) (Table 4.2). A base temperature of 0°C was used, below which a body would freeze. This follows the example set by Haefner et al. (2004) in their examination of decomposition.

Table 4.2 Accumulated Degree Days for the Stages of Decomposition for Each Pig, Spring.

Stage of Decomposition	Total Accumulated Degree Days				
	Unclothed: LA			Unclothed: DE	
	Pig B	Pig F	Pig G	Pond 1 Pig	Pond 2 Pig
1: Fresh Remains	25.04	50.53	50.53	70.86	51.33
2: Early Decomposition*	100.90	99.84	357.60	279.24	243.09
3: Advanced Decomposition	160.51	257.76	0	208.40	130.77
4: Sunken Remains	96.65	95.73	95.73	148.44	130.77
Total	383.10	503.86	503.86	706.94	555.96

While sampling affected the duration of the stages for the clothed pigs, the pigs did not have a different total amount of degree days necessary for complete skeletonization. Sampling affected unclothed Pig B, with both unsampled unclothed Pig F and Pig G requiring more total accumulated degree days. Both clothed pigs required more time than all unclothed pigs to become completely skeletonized. The two clothed pigs were very similar to each other, the two unsampled unclothed pigs were very similar to each other, and the unclothed sampled pig was different from them all. The clothed pigs required more time to decompose than the unclothed.

In summary, by examining biomass removal and accumulated degree days, data indicate that a clothed pig will decompose slower than an unclothed pig and that sampling affects the total decomposition time of an unclothed pig in the spring. The clothed pig was not particularly affected by sampling compared to the clothed unsampled pig. However, these conclusions do not take into account insect and scavenger activity. While this activity was present on all pigs, it was the most prevalent on Pig B, the only pig colonized by maggots. This insect and scavenger activity requires further examination to enrich the analysis of decomposition in Bayou Fountain.

4.1.3 Insect and Scavenger Activity

Fish and tadpoles were witnessed eating algae from all the pigs and tiles. Additionally, fish were seen eating the flesh of the pigs and tearing through clothing and tissue. While the fish

actively ate algae off the carcasses throughout the research period, the eating of flesh by fish occurred once soft tissue was exposed by larger scavengers. While never witnessed, turtles and crawfish may have been responsible for the initial heavy scavenging; a crawfish was seen within the folds of fabric on Pig A and a turtle was seen resting upon Pig A's cage (Figure 4.20).



Figure 4. 20 Turtle upon Pig A: Day 12, spring.

This heightened fish activity also attracted snakes, but these snakes did not scavenge the pigs. Rather, the snakes ate the fish which were scavenging the pigs. Three different kinds of snakes were seen repeatedly. While the exact number of snakes and their precise taxonomic identity are unknown, a broad banded water snake, an eastern ribbon snake, and either a diamondback or a cottonmouth snake was observed (Figure 4.21).

In addition to the fish, the snakes likely preyed upon the numerous frogs encountered at the site. These frogs likely fed upon the insects attracted to the decomposing pigs. Many birds were seen in the area, but none was witnessed directly on the pigs.

Clothing shielded Pig A and Pig E from scavenging; both did not experience substantial tissue loss during the first week. Pig A had visible evidence of scavenging on Day 17. Pig E was scavenged sometime after being lifted for weighing on Day 9, being visibly scavenged when lifted on Day 16. The unclothed pigs were scavenged very early, all having noticeable tissue loss before the end of the first week. The leg of Pig B was gone on Day 2, the face of Pig F was gone

and the stomach was open on Day 4, and the hind legs of Pig G were both gone on Day 6. This tissue loss allowed for easy scavenging by the fish which contributed to the fast decomposition.



Figure 4.21 Broad-banded water snakes on Pig E: Day 2 (top left) and Pig A: Day 8 (top right); Diamondback or Cottonmouth at Site 3: Day 9 (bottom right); Eastern Ribbon Snake at Site 1: Day 12 (bottom left), spring.

Insect activity also contributed to the durations of stage length. Pig F and Pig G did not experience substantial insect activity. For Pig G, this was due to the lack of surface area available above the water level, and for Pig F, it was also due to the clothing inhibiting odor and access to flesh. Clothed Pig A had insect activity primarily at the spot of the brown stain, likely because this area was saturated with secretions and odors (Figure 4.22). Maggots were present on Day 6 and Day 7 but were washed away before causing any damage. Pig F had insect activity early in the decomposition process, but the eggs were washed away before maggots hatched. Pig B had the most intense insect activity of all the pigs. Blowfly eggs were present on the upper torso on Day 4 and were hatched and burrowing with spiracles above the water for the next five days. By Day 10, the entire chest was open and the ribs were exposed (Figure 4.23).



Figure 4. 22 Insect activity at area of brown stain on Pig A: Day 5 (left), Day 7 (center), Day 12 (right), spring.

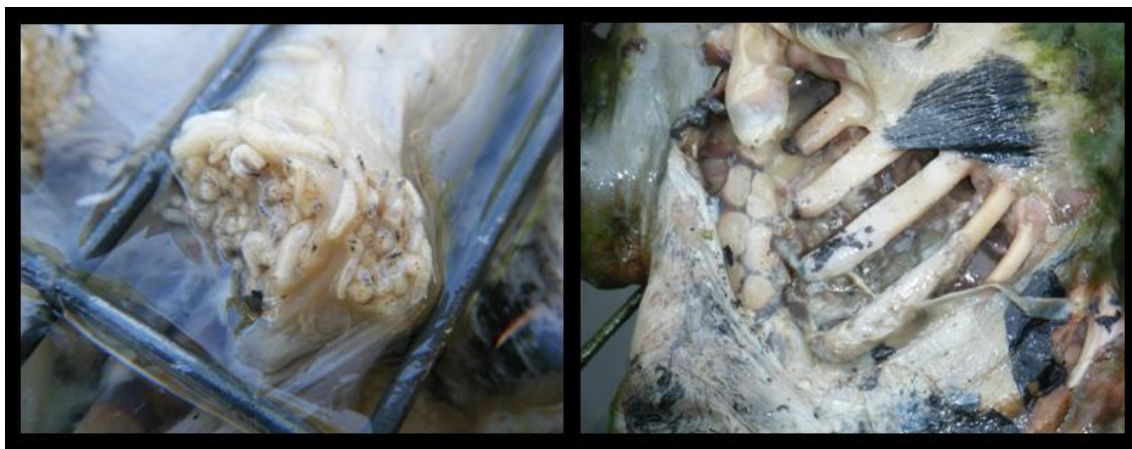


Figure 4. 23 Maggot activity on torso of Pig B: Day 7 (left) and Day 10 (right), spring.

Most of the insect activity was from terrestrial flies; only a few aquatic insects were collected. It is possible that aquatic insects were present within the folds of fabric and underneath the carcass, but because the carcass was not repositioned for their collection, they were not seen. Figure 4.24 shows the collected insects from all pigs.

Air and water temperature played a large role in this scavenger and insect activity. The average temperature throughout the duration of the study was high, with air temperature at the time of collection (approximately 9 am) ranging from 18.89°C to 28.22°C and average water temperature at the time of collection ranging from 20.45°C to 26.62°C. These high temperatures gave insects and scavengers ample opportunities to attack and colonize the pigs (Figure 4.25; for

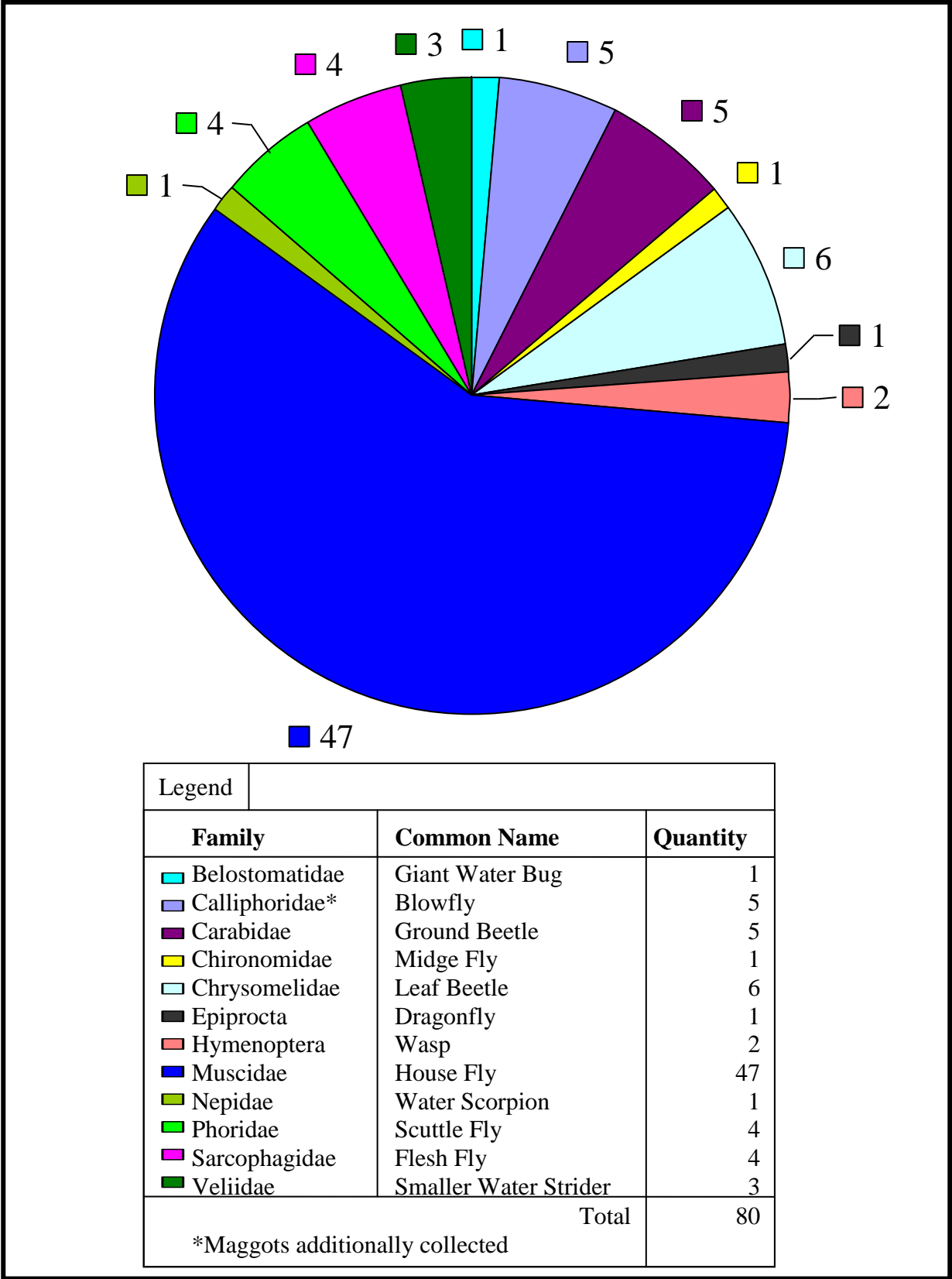


Figure 4. 24 Quantity of insects collected from all Pigs, spring.

regression calculation see Appendix Figure A.6). Adipocere was not seen on any of the pigs, most likely because of this heavy scavenging. Considering O'Brien and Kuehner's (2007) statement that adipocere formation tends to occur between 21-45°C, the average spring temperatures may have been ideal for adipocere formation had intense scavenging not occurred.

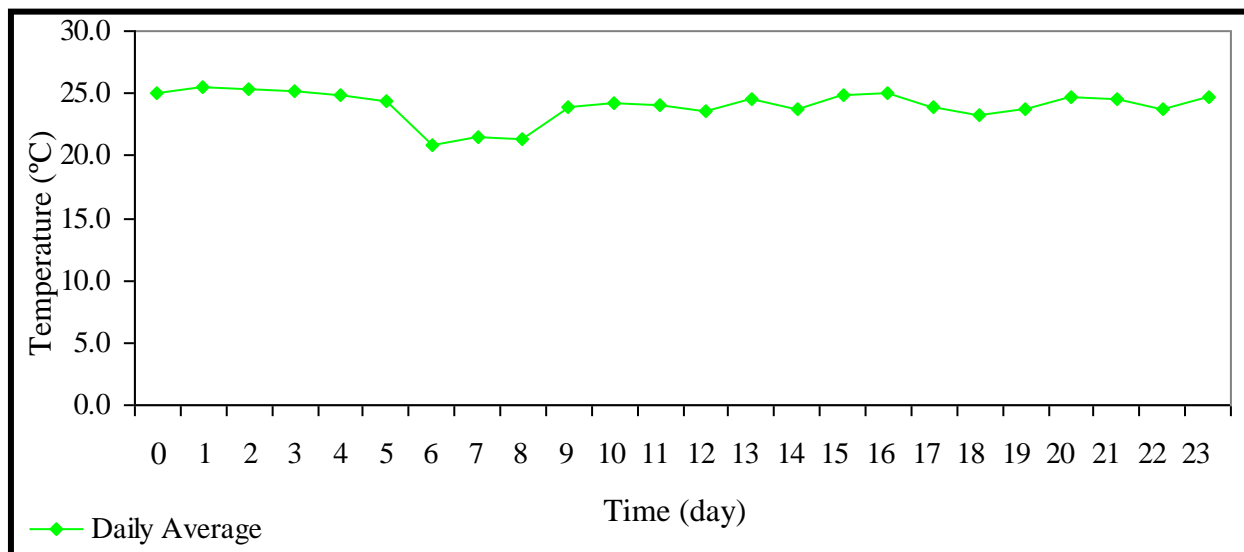


Figure 4. 25 Average daily water temperature from regression with Ben Hur weather data, spring. Original data retrieved from: <http://www2.lsuagcenter.com/weather/tabledata.asp?stationId=3>

The unclothed pigs were scavenged early in the study, spending less time in the water intact than the clothed pigs. Because of the differences in decomposition stage duration, one could expect that algae growth would be different between the clothed and unclothed pigs because of differences in nutrient excretion, differing amounts of surface area available for algae attachment and differing time periods available for growth. The few rain events had no visible effect on decomposition or algae growth, with the pigs always staying within one half meters of the surface (Figure 4.26).

4.1.4 Algae Analysis

Chlorophyll *a* concentrations were measured for each sampling date (Table 4.3). One-way ANOVA was then used to compare the chlorophyll *a* concentrations between the substrates

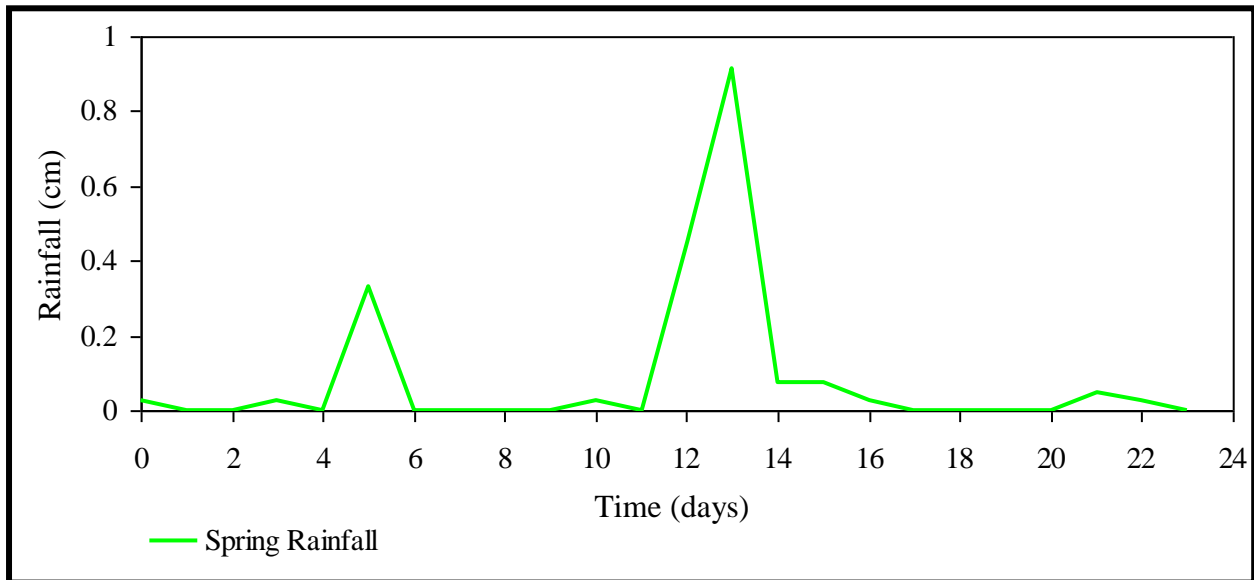


Figure 4. 26 Rain events, spring. Data retrieved from:
<http://www2.lsuagcenter.com/weather/tabledata.asp?stationId=3>

Table 4. 3 Chlorophyll *a* Concentrations of all Substrates, Spring.

Chlorophyll <i>a</i> Concentration, Spring ($\mu\text{g}/\text{cm}^2$)				
Day	Pig A	Pig B	Tile C	Tile D
0	0.40	0.20	0.83	0.07
1	2.31	0.03	5.35	0.53
2	1.09	0.06	13.89	
3	2.09	2.96	0.74	2.08
4	9.62	2.60	3.61	5.24
5	3.80	0.07	8.28	6.29
6	0.65	1.57	7.78	9.64
7	3.60	4.13	10.20	11.71
8	5.67	0.13	14.44	23.36
9	20.95	0.84	10.14	9.82
16	53.82		42.99	15.72
23	74.67		55.04	14.01
* Sample lost				
.. No sample collected due to lack of biomass				

at both the $\alpha = 0.10$ and $\alpha = 0.05$ levels (See Appendix Table A.5). Linear regressions were created relating Chlorophyll *a* concentration to time for each substrate. These regressions were graphed (see Appendix Figure A.1 to A.5) and their trends compared (Figure 4.27).

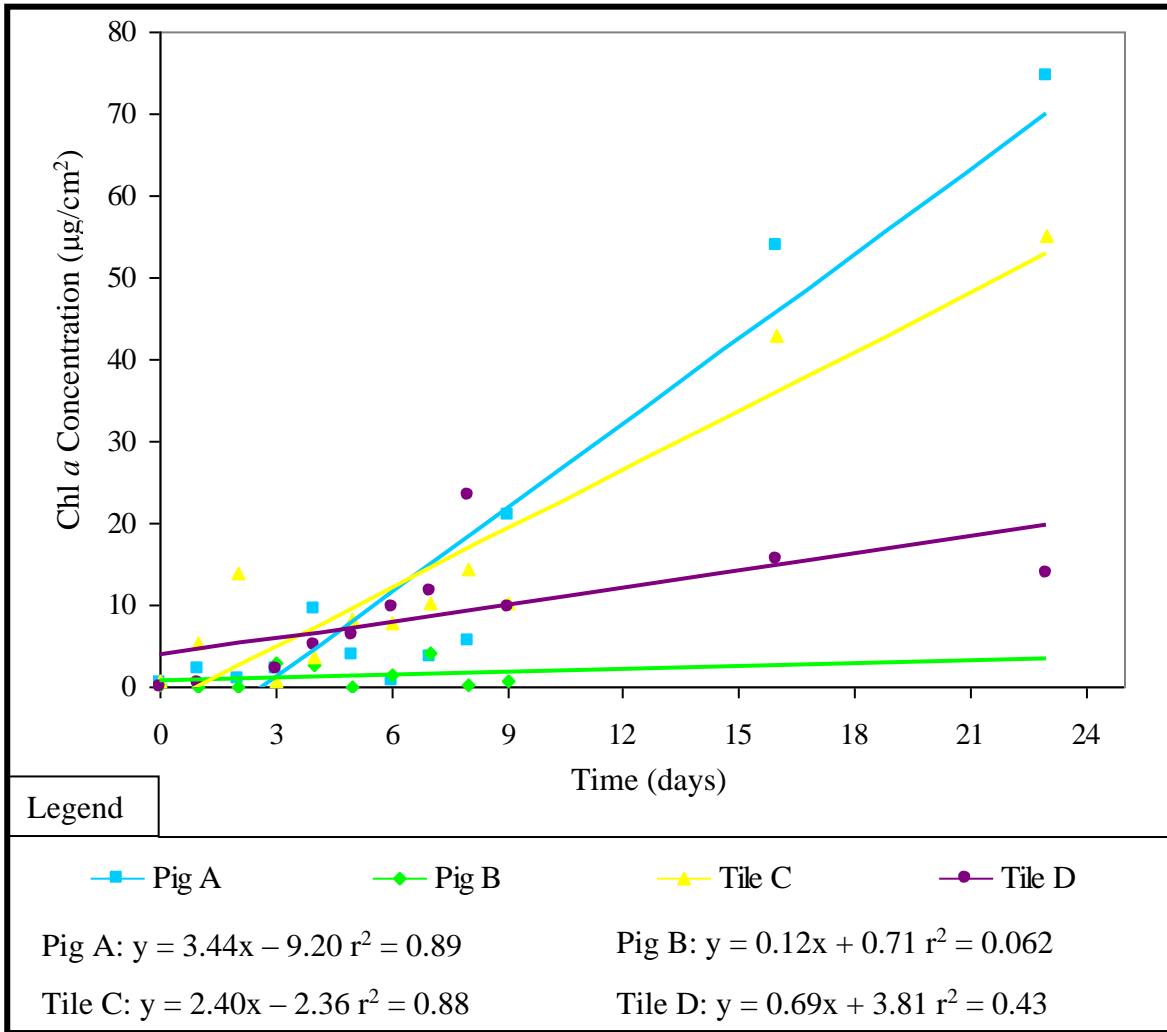


Figure 4. 27 Chlorophyll *a* (Chl *a*) concentrations over time for all Pigs and Tiles, spring. Note that Pig B has 10 samples ($n = 10$) while all others have 12 samples ($n = 12$).

The amounts of chlorophyll *a* collected from Pig A, Tile C, and Tile D were not significantly different from each other at $\alpha = 0.05$ or $\alpha = 0.10$. However, the amount collected from Pig B was significantly different than all the other substrates at $\alpha = 0.10$ (with Pig A: $p = 0.091$) as well as at $\alpha = 0.05$ with the tiles (with Tile C: $p = 0.024$ and Tile D: $p = 0.0034$).

All four substrates demonstrated a positive relationship between chlorophyll *a* concentration and time. However, the r^2 value was very weak for Pig B ($r^2 = 0.062$), meaning that a poor linear relationship existed between chlorophyll *a* concentration and time. This was likely due to the sample size and complications resulting from heavy insect and scavenger activity. The slopes of Pig A ($r^2 = 0.89$), Tile C ($r^2 = 0.88$) and Tile D ($r^2 = 0.43$) were much steeper than Pig B, meaning that chlorophyll *a* concentration increased the slowest on the unclothed pig. Clothed Pig A and Tile C had the steepest slopes, suggesting that spring algae grow best on fabric regardless of the presence of a decomposing body. The chlorophyll *a* concentration in the water samples had negligible change throughout the entire study period; thus, the amount of algae present on the substrates was different than in the water (See Appendix Figure A.5). In the water, phosphorus levels decreased ($r^2 = 0.27$), nitrate levels remained stable ($r^2 = 0.0083$), and ammonium levels decreased ($r^2 = 0.43$) throughout the study period (see Appendix Table A.2). Additionally, pH remained between 7 and 7.5, turbidity remained greater than 60 cm except during one rain event, oxygen content ranged from 0.2 to 4.0 ppm, and salinity remained at zero ppt throughout the study period (see Appendix Table A.1).

One must consider two important points when interpreting these results. First is the availability of algae to be sampled on a clothed versus unclothed substrate. Cotton absorbs water, diffusing moisture to areas of clothing that are not submerged, allowing algae to grow above the water level. Unclothed substrates, on the other hand, can only foster algae growth at or below the water surface. Because the location of sampling was consistent on the substrates, clothing offered more surface area for algae growth than the unclothed substrates. Second, one must consider that, due to scavenging, samples were collected for only ten days from the

unclothed pig, whereas 23 days from the others. It is possible that a stronger positive relationship may have occurred for Pig B had there been more time and samples.

In summary, all substrates demonstrated a positive relationship between chlorophyll *a* and time, with unclothed Pig B likely having a poor r^2 value due to complications from scavenging. Thus, algae may be used in estimations of PMSI. Additionally, the results show that a clothed substrate is more conducive to algae growth than an unclothed substrate

When examined microscopically, pennate diatoms appeared to be the dominant algae of the spring samples (Figure 4.28). Pennate diatoms, which can attach to surfaces more easily than centric diatoms, were likely supported by the shallow bayou environment.

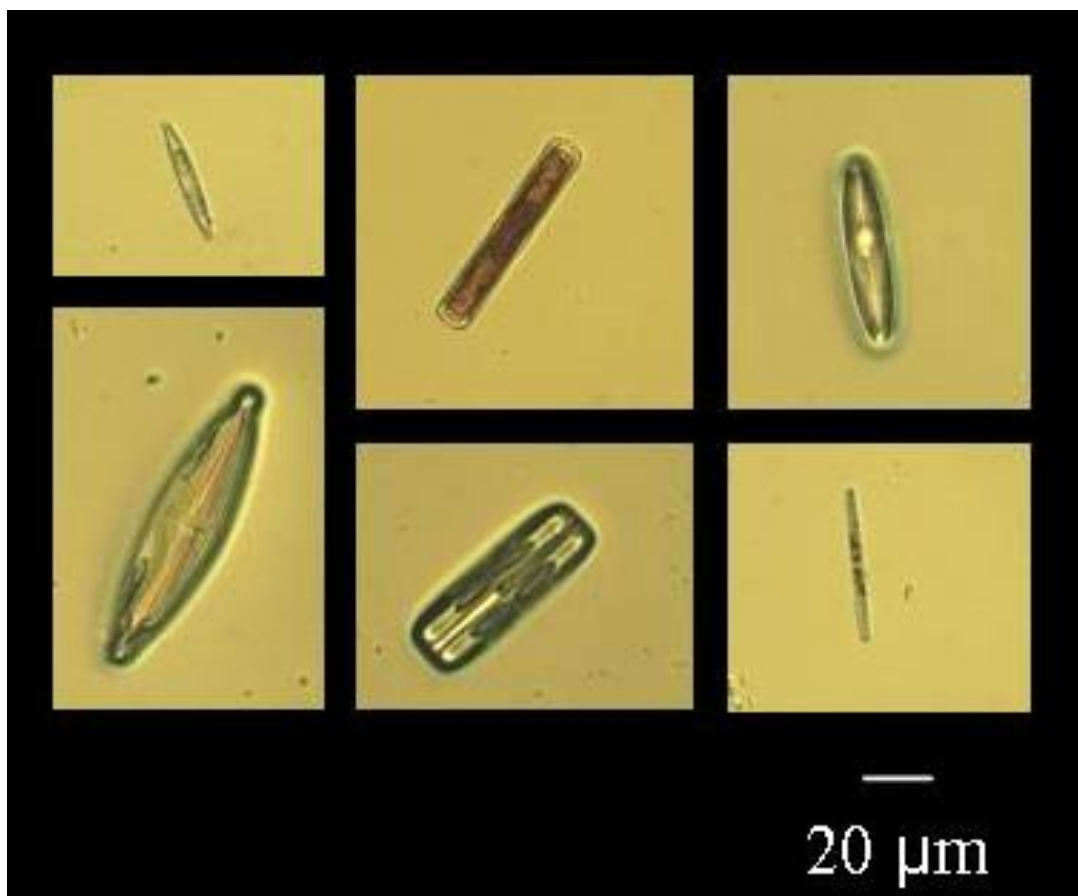


Figure 4. 28 Pennate diatoms collected from Tile C: Day 4, spring.

4.1.5 Spring Study Summary

The results show that a clothed pig will decompose more slowly than an unclothed pig. This is because clothing shields the carcass from the elements, insects, and scavengers. Mechanical damage from algae-sampling did not have a major effect on the rate of decomposition of the clothed pig but may have contributed to the quick rate of decomposition of the unclothed pig. Scavenger and insect activity occurred first on the unclothed pigs, significantly affecting their total number of decomposition degree days.

Algae accumulated on all pigs and tiles as seen through an increase of chlorophyll *a* concentration with time. However, both clothed substrates had higher concentrations and stronger linear relationships than both unclothed substrates. Throughout the entire length of the spring study, all pigs and tiles remained within one meter of the water surface and turbidity was greater than 60 centimeters for all but the single rain event. Therefore, sunlight availability was adequate for optimum growth. The sunlight, warm temperatures, and nutrients from decomposition provided an ideal environment for algae growth on all substrates, especially those that were clothed.

The results of the spring study suggest that algae can be used for estimations of PMSI. While scavenger and insect activity speed up decomposition and make algae collection difficult, clothing slows decomposition and provides greater surface area for algae collection.

4.2 Fall Study Results

4.2.1 Stages of Decomposition

Figure 4.29 demonstrates the amount of time the fall pigs spent in each phase. To summarize, the clothed pigs (A and E) decomposed slower than the unclothed pigs (B, F, and G). The sampled pigs (A and B) did not experience a different duration of decomposition stage

length than their unsampled partner pigs (E and F, respectively), though Pig A had a slightly shorter period of early decomposition than Pig E. Pig B and Pig F shared identical stage length durations. This shows that clothing hampers decomposition while sampling has little effect.

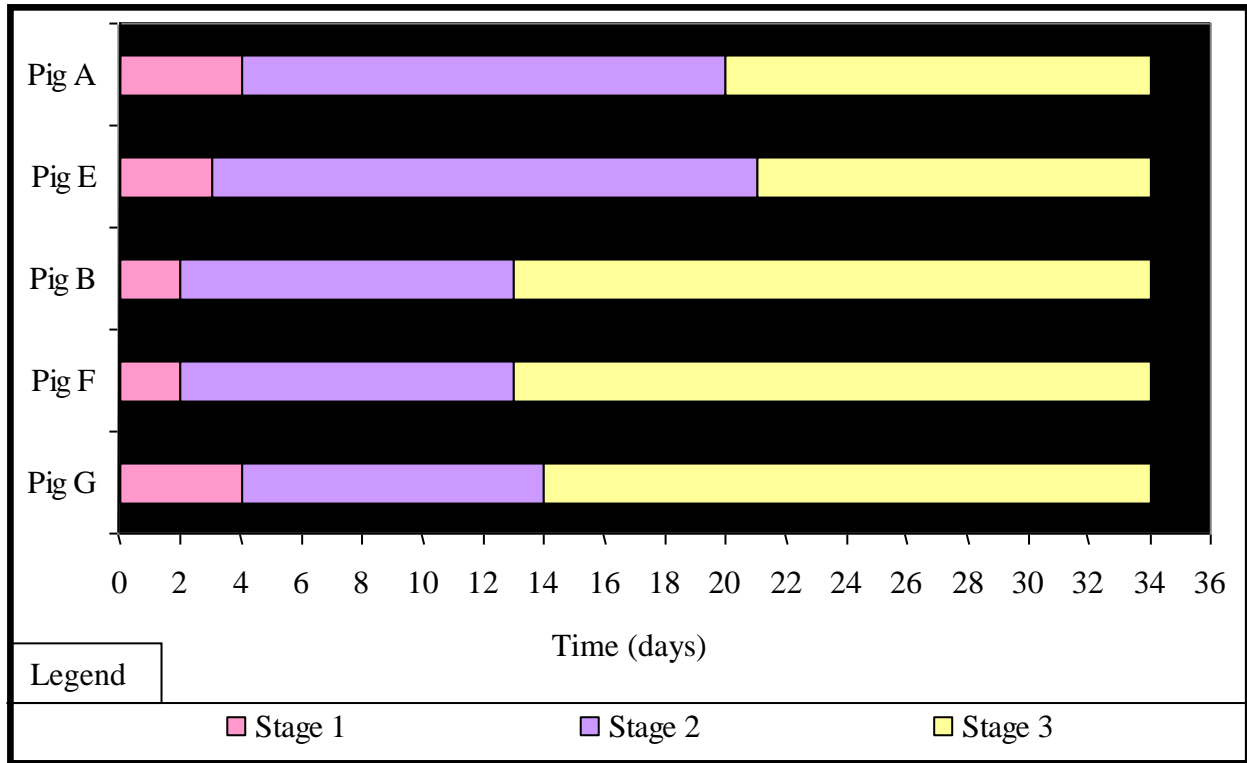


Figure 4. 29 Duration of decomposition stages for each pig, fall.

Algae growth was variable depending on the substrate. For the unclothed pigs (B and F), no growth was present in stage one. Stage two saw brown algae growing on underwater surfaces, especially on the limbs and head. Green algae grew upon the waterline of Pig F. No algae were present on the skin above the water surface of either pig. In stage three, the algae continued to accumulate on submerged areas of the carcass. When the carcass sank just below the water surface, algae grew on previously above-water areas. Green algae appeared on the waterline of Pig B for one day only, after which point the pig was completely submerged and became covered in sediment. Pig G was covered in mud for the duration of stage one and early

stage two. Submerged areas, especially the limbs, became covered in brown algae during stage two and three as did Pig B and Pig F. Unclothed Tile D had a gradual thickening of algae cover over three-fourths of its surface, eventually appearing brown and slimy (Figure 4.30 right). One corner never fostered algae growth, and by the completion of the study much had washed off.

Algae growth was slightly different on the clothed pigs (A and E). In stage one, submerged areas of the carcass were covered in a dusting of brown colored algae. Bright green algae first grew along the waterline of both pigs in stage two eventually thickening and spreading to non-submerged areas. Clothed Tile C had a gradual thickening of algae cover over time, becoming dark brown. This continued until rain activity dislodged the fabric and removed the algae from the tile by the end of the study (Figure 4.30 left).



Figure 4. 30 Tile C (left) and Tile D (right) at completion of study, fall.

The fall session can be characterized by cold temperatures, storm activity, and heavy rain. The rain was so constant and plentiful that the research site severely flooded, restricting all access to the substrates (Figure 4.31). On Day 30, professional scuba diver Marc Massom accessed the site, at which point it was decided to end the fall session on Day 33 due to extreme

weather. Upon completion, none of the pigs had reached the full skeletonization stage though Pigs A, E, and G had some skeletal elements exposed. Thus, the true length of the advanced decomposition and skeletonization stages is unknown.



Figure 4. 31 Research site pre-flood: Day 12 (left) and flooded: Day 26 (right), fall.

The following is a detailed breakdown of the decomposition stages for each fall pig.

4.2.1A Pig A

Clothed, sampled Pig A took 19 days to reach advanced decomposition. The fabric shielded the skin from the sun and acted as insulation from the cold. Only discoloration that permeated the fabric could be noted; clothing was not moved to observe the carcass.

In summary, Pig A spent four days in stage one (Day 0-3), 15 days in stage two (Day 4-19), and 14+ days in stage three (Day 20-33). Figure 4.32 shows this daily progression of decomposition.

Stage One—Fresh: Blood from the pig saturated the clothing. The carcass sank to the bottom head-down while the left sleeve puffed with air and floated toward the surface.

Stage Two—Early Decomposition: Bloated and stiff from decompositional gases, the pig floated to the surface with the left side facing up. A few loose bubbles were seen on Day 14 and the carcass remained firm.



Figure 4. 32a Progression of decomposition daily overview: Pig A, fall. Fresh Stage (Day 0-3); Early Decomposition (Day 4-19); Advanced Decomposition (Day 20-33+). Part (a) shows Day 0-24, Part (b) shows Day 25-33.



Figure 4. 32b (cont'd) Progression of decomposition daily overview: Pig A, fall. Fresh Stage (Day 0-3); Early Decomposition (Day 4-19); Advanced Decomposition (Day 20-33+). Part (a) shows Day 0-24, Part (b) shows Day 25-33.

Stage Three—Advanced Decomposition: Primarily submerged, only a portion of the sleeper was above the water level. After Day 25 the pig was no longer visible due to flooding. When lifted on Day 33, there was no discoloration but skin slippage was apparent, intestines were exposed, and bile poured onto the lower carcass (Figure 4.33).

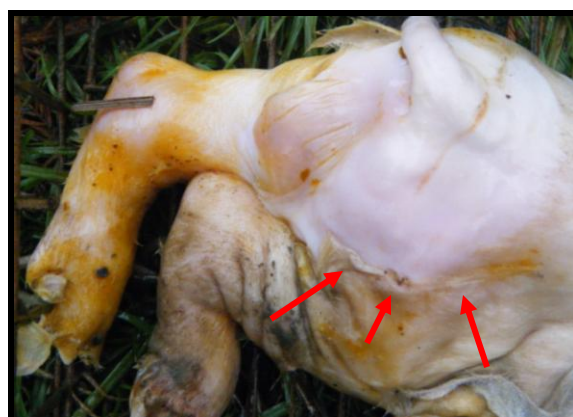


Figure 4. 33 Skin slippage on tail region with bile excretion of Pig A: Day 33, fall. Arrows indicate area with skin slippage.

4.2.1B Pig E

Clothed, unsampled Pig E took 21 days to reach advanced decomposition. The fabric shielded the skin from the sun and acted as insulation from the cold. Only discoloration that permeated the fabric could be noted; clothing was not moved to observe the carcass.

In summary, Pig E spent three days in stage one (Day 0-2), 18 days in stage two (Day 3-20), and 13+ days in stage three (Day 21-33). Figure 4.34 shows this daily progression of decomposition.



Figure 4. 34a Progression of decomposition daily overview: Pig E, fall. Fresh Stage (Day 0-2); Early Decomposition (Day 3-20); Advanced Decomposition (Day 21-33+). Part (a) shows Day 0-24, Part (b) shows Day 25-33.



Figure 4.34b (cont'd) Progression of decomposition daily overview: Pig E, fall. Fresh Stage (Day 0-2); Early Decomposition (Day 3-20); Advanced Decomposition (Day 21-33+). Part (a) shows Day 0-24, Part (b) shows Day 25-33.

Stage One—Fresh: The carcass floated at the surface left side up with the left arm across the torso.

Stage Two—Early Decomposition: Stiffness from decompositional gases caused the carcass to remain suspended between the cage floaties when lifted on Day 4 (Figure 4.35).

Bubbles were present near the mouth on Day 3 with the carcass in a firm bloat. The pig floated belly down throughout the stage.



Figure 4.35 Stiffness from decompositional gases causing suspension between the floaties of Pig E: Day 4, fall.

Stage Three—Advanced Decomposition: Appearing deflated and flattened, the carcass was mostly submerged. After a period of flooding the cage was beached on Day 22. It was no longer seen after Day 25 due to complete submergence in the deep flood water. Upon removal

on Day 33, the pants were no longer being worn, the intestines were completely detached, and some skull elements were exposed (Figure 4.36).

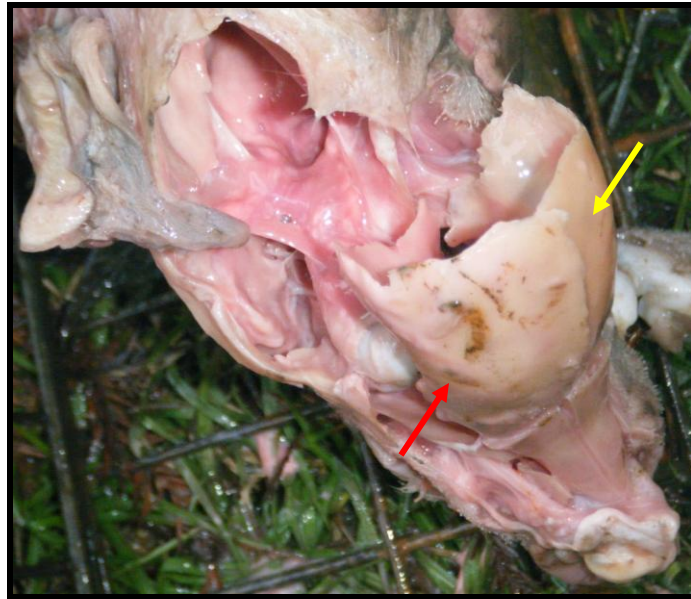


Figure 4. 36 Exposed skull elements of Pig E: Day 33, fall. Red arrow points to right parietal bone, yellow arrow points to left parietal bone.

4.2.1C Pig B

Unclothed, sampled Pig B took 13 days to reach advanced decomposition. In summary, Pig B spent two days in stage one (Day 0-1), 11 days in stage two (Day 2-12), and 21+ days in stage three (Day 13-33). Figure 4.37 shows this daily progression of decomposition.

Stage One—Fresh: The carcass floated at the surface with left side up. The umbilicus region appeared darker than the rest of the carcass.

Stage Two—Early Decomposition: The carcass was bloated firm with skin slippage especially on the limbs and rump. Small white lumps with a moist appearance formed on the back region above the water (Figure 4.38 left), eventually conglomerating into a hard, pink, callus-like mass (Figure 4.38 right). The back above water discolored slightly purple while the belly underwater discolored grey-blue. Adipocere began to appear as small white spots on the rump region below the callus-like mass.

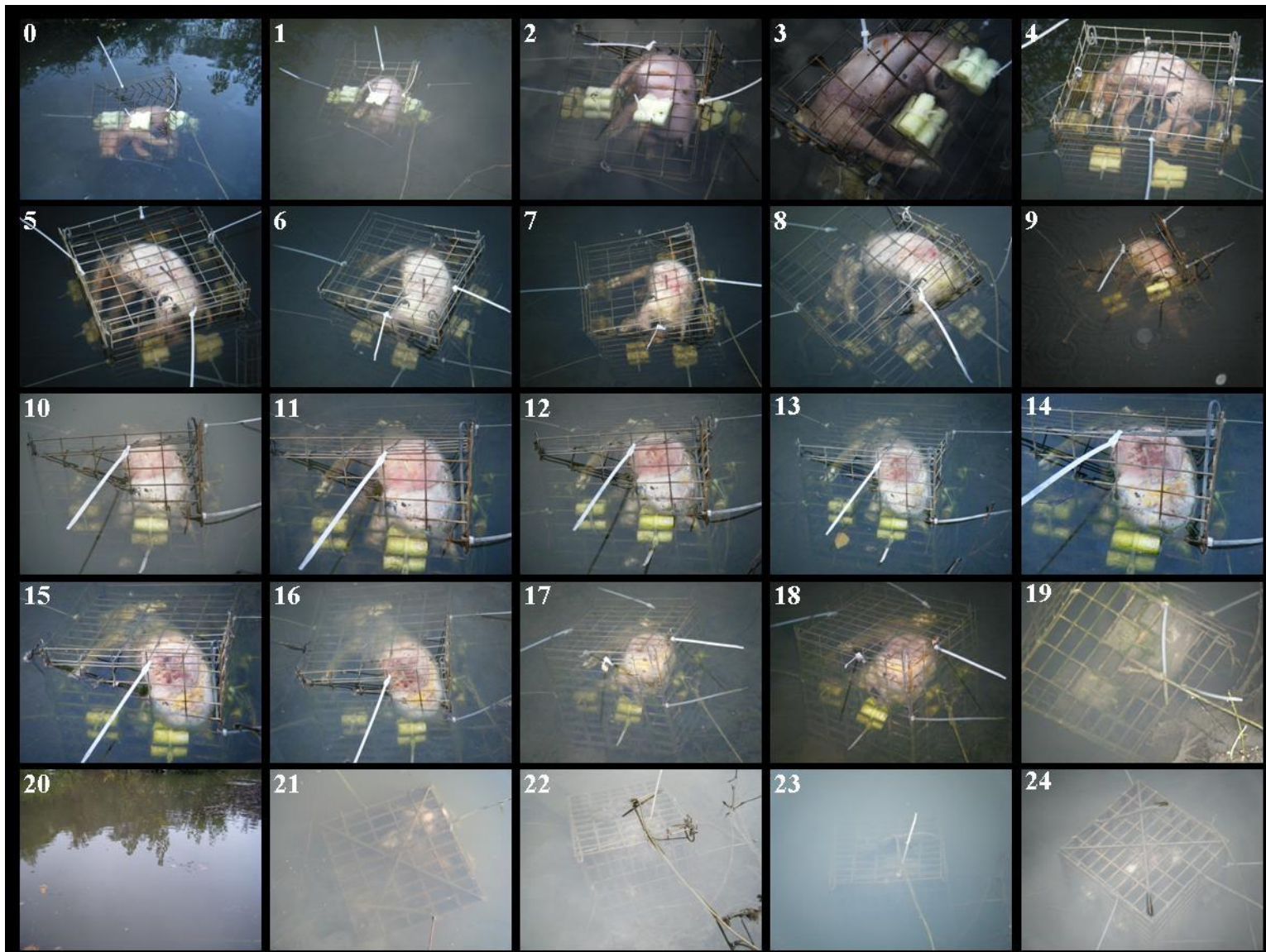


Figure 4. 37a Progression of decomposition daily overview: Pig B, fall. Fresh Stage (Day 0-1); Early Decomposition (Day 2-12); Advanced Decomposition (Day 13-33+). Part (a) shows Day 0-24, Part (b) shows Day 25-33.



Figure 4. 37b (cont'd) Progression of decomposition daily overview: Pig B, fall. Fresh Stage (Day 0-1); Early Decomposition (Day 2-12); Advanced Decomposition (Day 13-33+). Part (a) shows Day 0-24, Part (b) shows Day 25-33.



Figure 4. 38 Small white lumps, Day 4 (left) which conglomerated into a callus-like pink mass, Day 12 (right) of Pig B, fall.

Stage Three—Advanced Decomposition: The pig floated left side and rump up until Day 18 after which point it was completely submerged. The adipocere changed from the white spots (Figure 4.39 left) into thick, bright yellow lumps (Figure 4.39 right). The callus-like mass mummified on top, turning brown. The pig was not seen after Day 25 due to intense flooding. Upon lifting on Day 33, the lower left ribs were exposed and the intestines were exposed.



Figure 4. 39 Adipocere formation from small white dots, Day 13 (left) into bright yellow lumps, Day 15 (right) on Pig B, fall.

4.2.1D Pig F

Like Pig B, unclothed, unsampled Pig F took 13 days to reach advanced decomposition. In summary, Pig F spent two days in stage one (Day 0-1), 11 days in stage two (Day 2-12), and 21+ days in stage three (Day 13-33). Figure 4.40 shows this daily progression of decomposition.

Stage One—Fresh: The carcass floated at the surface with right side up, having the head, limbs and rump underwater.

Stage Two—Early Decomposition: The skin progressed from dark pink to greenish-blue to grayish purple. Skin slippage occurred on the limbs and carcass with loose bubbles present near the mouth on Day 4. The carcass was stiff from decompositional gases, staying suspended between the floaties when lifted for weighing on Day 4 and Day 9. A thick ridge of callus-like skin formed around the outline of the carcass at the waterline, creating a large, pinkish-brown mass on the rump. Adipocere began to appear as small white spots.

Stage Three—Advanced Decomposition: Slight maggot activity was present near the ear, but these maggots remained relatively inactive before being washed away, causing only minor damage. The torso became spotted brown with areas of bright pink, eventually discoloring to a brownish grey-purple. Adipocere changed from white to bright, thick orange, especially present along the waterline (Figure 4.41) (this atypical coloration is discussed in Section 5.1). The pig was beached after a flooding period on Day 22 but continued to float with right side up. After Day 25 the pig could not be viewed due to intense flooding; however, it could be seen floating on the water surface from the shore. The carcass was completely submerged from Day 31 to Day 33 and was not visible. Upon lifting on Day 33, the pig was completely intact with neither bones nor internal organs exposed

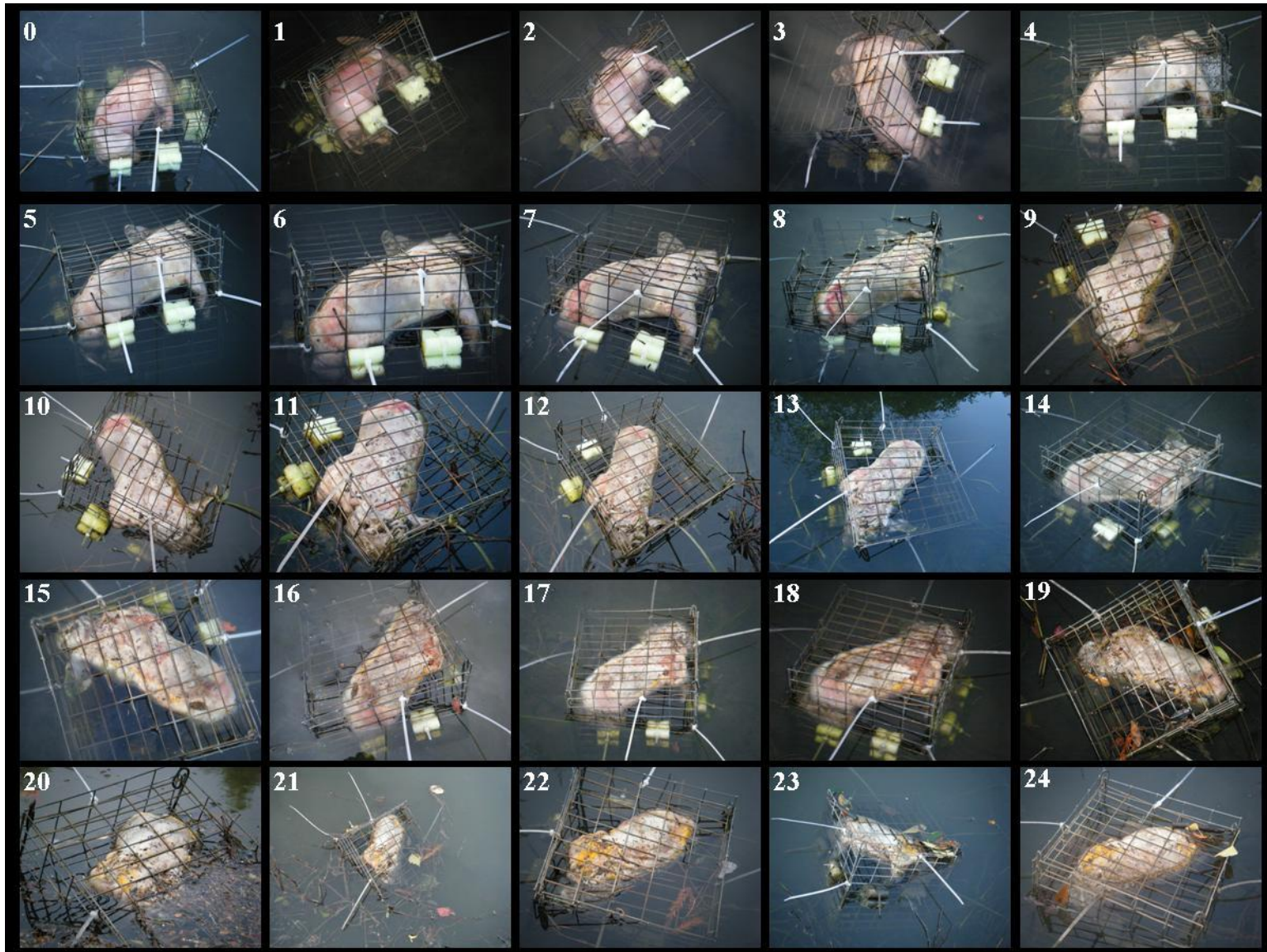


Figure 4. 40a Progression of decomposition daily overview: Pig F, fall. Fresh Stage (Day 0-1); Early Decomposition (Day 2-12); Advanced Decomposition (Day 13-33+). Part (a) shows Day 0-24, Part (b) shows Day 25-33.



Figure 4. 40b (cont'd) Progression of decomposition daily overview: Pig F, fall. Fresh Stage (Day 0-1); Early Decomposition (Day 2-12); Advanced Decomposition (Day 13-33+). Part (a) shows Day 0-24, Part (b) shows Day 25-33.

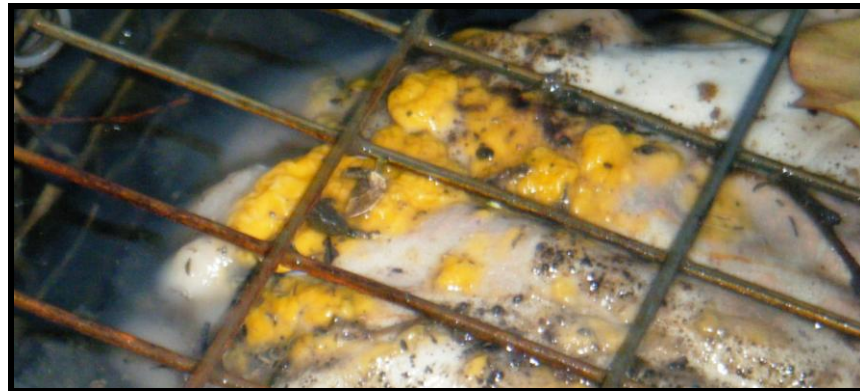


Figure 4. 41 Adipocere taking on a bright orange hue on Pig F: Day 25, fall.

4.2.1E Pig G

Unclothed, unsampled Control Pig G took 14 days to reach advanced decomposition. In summary, Pig G spent four days in stage one (Day 0-3), ten days in stage two (Day 4-13), and 20+ days in stage three (Day 14-33). Figure 4.42 shows this daily progression of decomposition.

Stage One—Fresh: The pig sunk to the bottom of the floor, becoming completely covered with mud.

Stage Two—Early Decomposition: Floating in a firm bloat with belly down, the pig experienced skin slippage, especially on the limbs and belly region, discoloring to a greenish-blue grey. On Day 5, the carcass was stiff from decompositional gases and showed Livor mortis (Figure 4.43). Thick foam was expelled from the carcass through a small hole in the back. A large quantity of fly eggs was present upon the back, and callus-like skin formed on the belly.

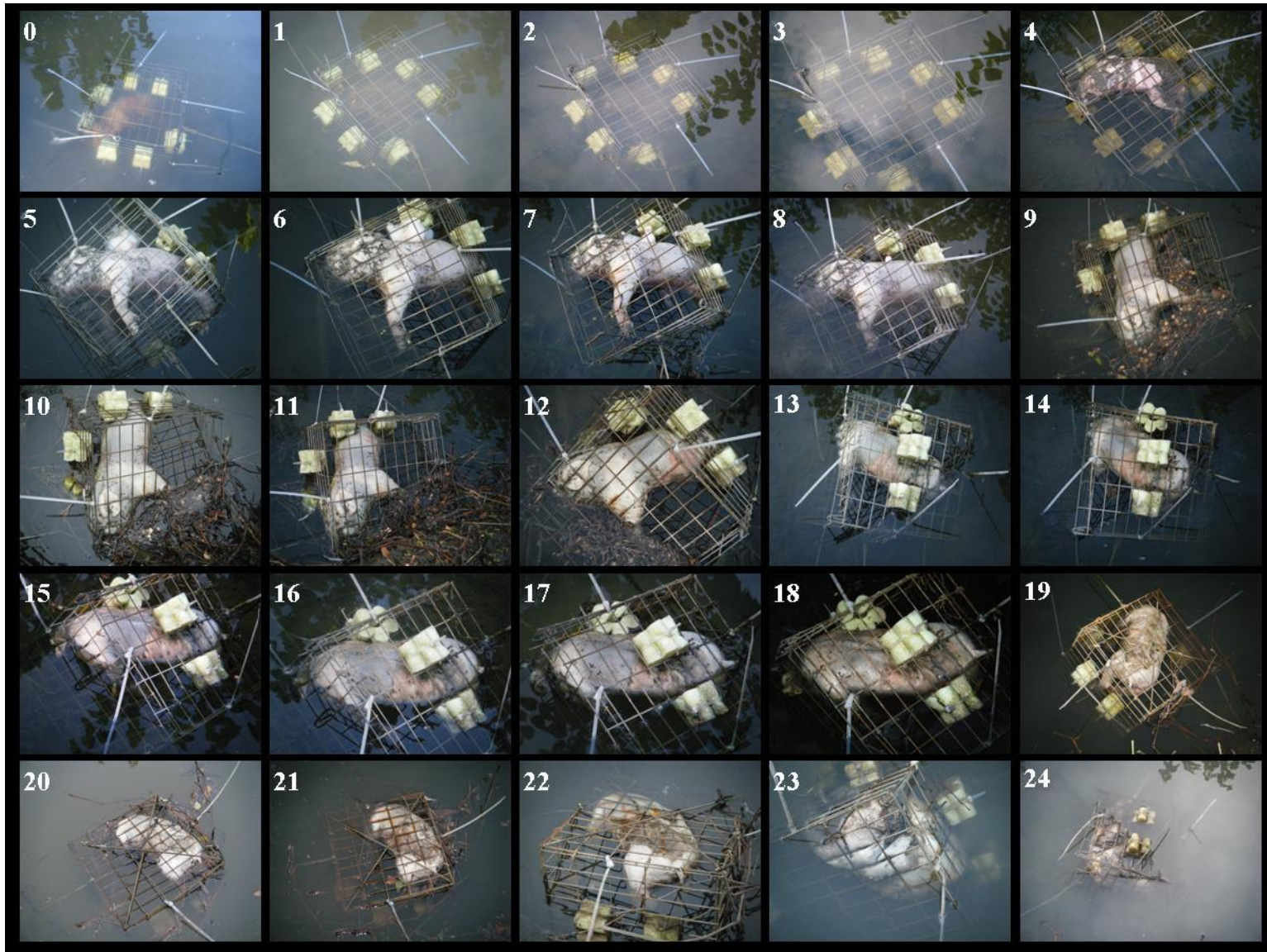


Figure 4. 42a Progression of Decomposition daily overview: Pig G, fall. Fresh Stage (Day 0-3); Early Decomposition (Day 4-13); Advanced Decomposition (Day 14-33). Part (a) shows Day 0-24, Part (b) shows Day 25-33.



Figure 4.42b (cont'd) Progression of decomposition daily overview: Pig G, fall. Fresh Stage (Day 0-3); Early Decomposition (Day 4-13); Advanced Decomposition (Day 14-33). Part (a) shows Day 0-24, Part (b) shows Day 25-33.



Figure 4. 43 Livor mortis on the underside of the torso and stiffness from decompositional gases of Pig G: Day 5, fall.

Stage Three—Advanced Decomposition: The carcass discolored dark blue-grey and deflated; the vertebrae were visible as a ridge under the skin (Figure 4.44). Black putrefaction appeared on the left shoulder. The carcass was beached on Day 18 after flooding, found resting on the shore. The pig was submerged and not seen after Day 25 due to flooding. Upon lifting on Day 33, the skin appeared blue-toned and the skull bones were exposed (Figure 4.45).



Figure 4. 44 Ridge running the length of the back from torso deflation of Pig G: Day 17, fall.

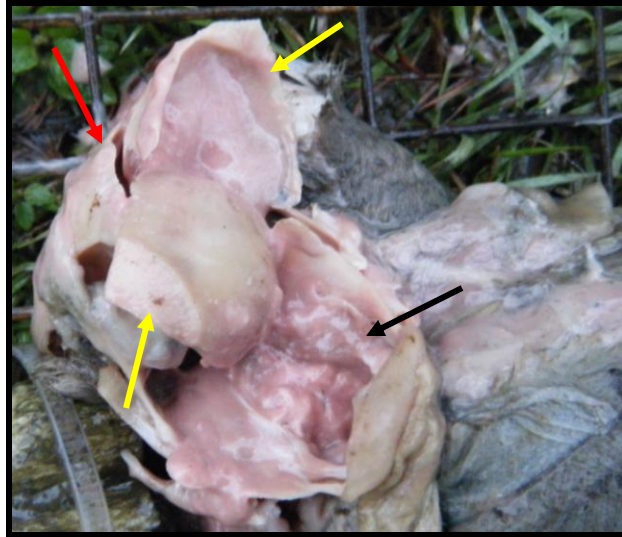


Figure 4. 45 Exposed skull elements of Pig G: Day 33, fall. Red arrow points to frontal bone, yellow arrows point to both parietal bones, black arrow points to occipital bone.

4.2.2 Calculation of Degree Days

Table 4.4 shows the initial and final weights of all pigs. Unsampled Pig E and Pig F were weighed for biomass removal throughout the study. Pig E's final weight was more than its initial weight, most likely because the clothing and tissues absorbed water.

Table 4. 4 Initial Weight, Final Weight and Weight Loss of All Pigs, Fall.

Pig	Initial Weight (kg)	Final Weight (kg)	Weight Loss (kg)
A	1.25	0.75	0.5
B	1.5	0.75	0.75
E	1	1.5	-0.5
F	1.25	0.88	0.37
G	1.5	0.75	0.75

Weight loss was negatively correlated with time for Pig E ($r^2 = 0.06$) and positively correlated with time for Pig F ($r^2 = 0.16$), though very weakly. Overlaying the stages of decomposition onto the graph of biomass removal (Figure 4.46) shows that Pig E gained weight,

likely from water absorbance. At the end of stage three, biomass loss had occurred. Pig F also gained weight throughout stage two, only slightly losing biomass at the end of stage three. Ultimately, both pigs lost very little weight by the termination of the study. These graphs suggest that biomass removal does not occur in the fall for clothed or unclothed pigs until later into the Advanced Decomposition Stage.

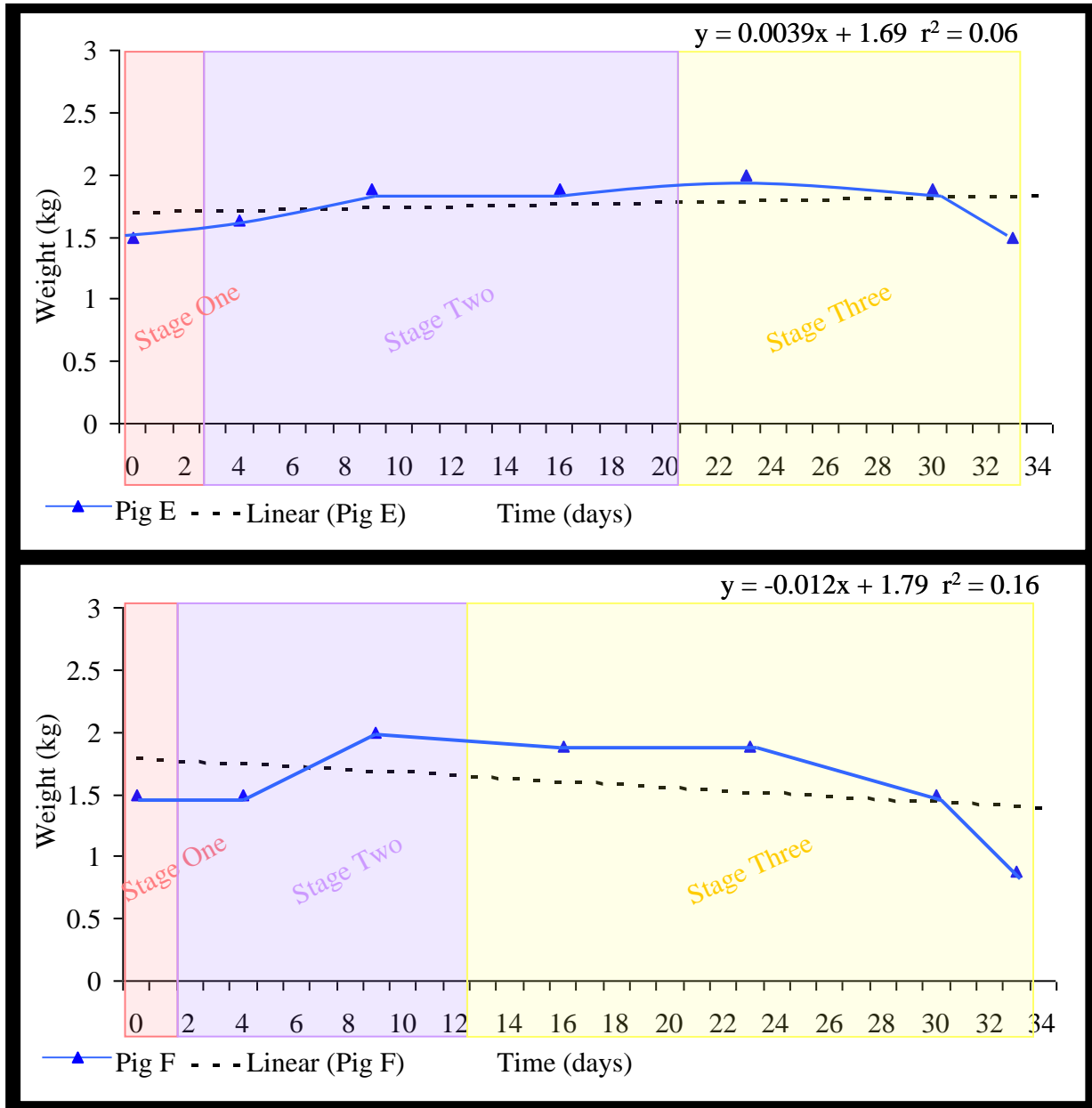


Figure 4. 46 Weight loss for Pig E (top) and Pig F (bottom) by stage of decomposition, fall

The accumulated degree days of each stage of decomposition were calculated as in section 4.1.3, shown in Table 4.5.

Table 4.5 Accumulated Degree Days for the Stages of Decomposition for Each Pig, Fall.

Stage of Decomposition	Total Accumulated Degree Days				
	Clothed: LA		Unclothed: LA		
	Pig A	Pig E	Pig B	Pig F	Pig G
1: Fresh Remains	66.32	49.67	33.16	33.16	66.32
2: Early Decomposition	262.85	295.70	181.54	181.54	164.92
3: Advanced Decomposition	227.49	211.28	341.96	341.96	325.42
4: Sunken Remains
Total	556.66	556.65	556.66	556.66	556.66

Sampling had no effect on the rate of decomposition of the unclothed pig, with Pig B and Pig F requiring identical accumulated degree days for all stages. Sampling did not have a substantial affect on the clothed pig, with Pig A requiring more degree days than Pig E for stage one and three but less for stage 2. Control Pig G was intermediate, spending more time in stage one than the other unclothed pigs, the shortest time of all pigs in stage two, and more time than the clothed pigs in stage three. It would appear that clothing hampers decomposition, with the clothed pigs requiring more accumulated degree days to reach advanced decomposition than the unclothed.

In summary, by examining biomass removal and accumulated degree days, this research has shown that a clothed pig will decompose slower than an unclothed pig and that sampling does not substantially affect decomposition in the fall. However, these conclusions do not take into account insect activity and flooding. Insects were present on all pigs but maggots were only present on Pig B and Pig F. Flooding affected all pigs, but they reacted differently to the current and rainfall. This insect and flooding activity requires further examination to enrich the analysis of decomposition in Bayou Fountain.

4.2.3 Insect Activity and Effects of Rain

No scavenger activity occurred during the fall study. Only a small black snake (likely a broad banded water snake) was seen on two occasions on Day 4, first near Pig G then again at site one. Fish were present early on in the study but were not seen damaging the pigs. Large egrets were seen on numerous occasions in the vicinity of the pigs but never directly upon the cages. Frogs were also seen in the area and directly upon the pigs but were not witnessed causing damage.

Insects were present on all pigs, but most active on unclothed Pig G, Pig B, and Pig F. Eggs were present on Pig G at the floatie on the back, with some scattered by water to other areas of the back (Figure 4.47 left). Only very slight maggot movement was seen from Day 13-15, never with burrowing. Pig F also had some maggot activity, with movement on the rump and burrowing into the ear area (Figure 4.47 right). However, this damage was minor and movement only lasted from Day 13-17. Pig F and Pig G had the most insect activity because they were unclothed and had accessible surface area above the water for prolonged periods. Pig B had some flies present on the carcass but no eggs were laid. Clothed Pig A and Pig E had very few insects present on the carcass. Figure 4.48 shows the insects collected from all pigs.



Figure 4. 47 Blowfly eggs below the floatie on the back of Pig G: Day 13 (left) and behind the ear of Pig F: Day 17 (right), fall.

Rain was present throughout the study, with flooding events occurring on Day 19 through Day 21 and again from Day 26 through Day 33 (Figure 4.49). Only Pig F remained floating

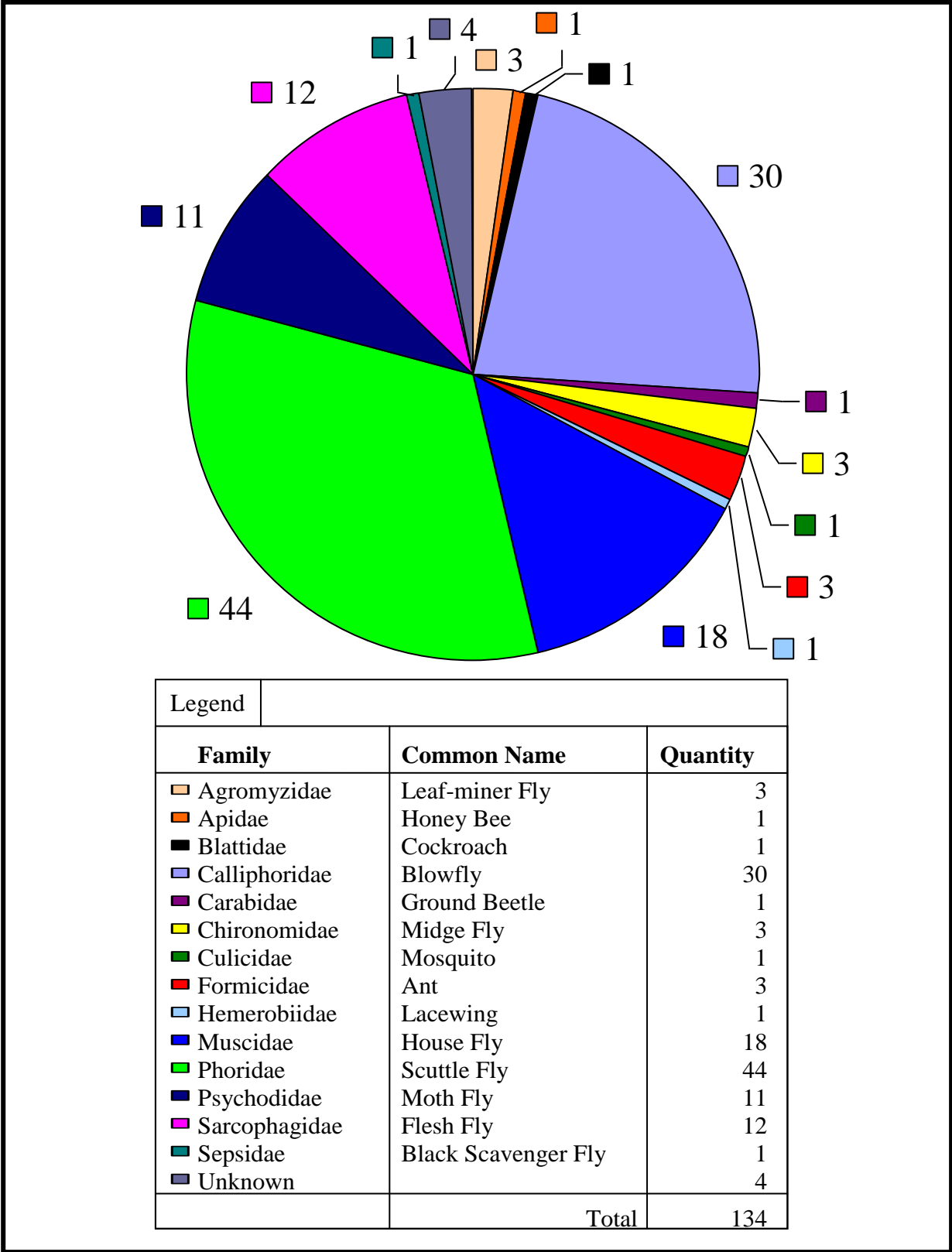


Figure 4. 48 Quantity of insects Collected from all Pigs, fall

during the latter flood event, with all others being completely submerged. Intense rain on the morning of Day 18 resulted in an increase in the water level. When this rain stopped and the water level lowered that afternoon, Pig E and Pig F had been pushed to shore and caught in debris while Pig G was entirely beached upon the south shore.

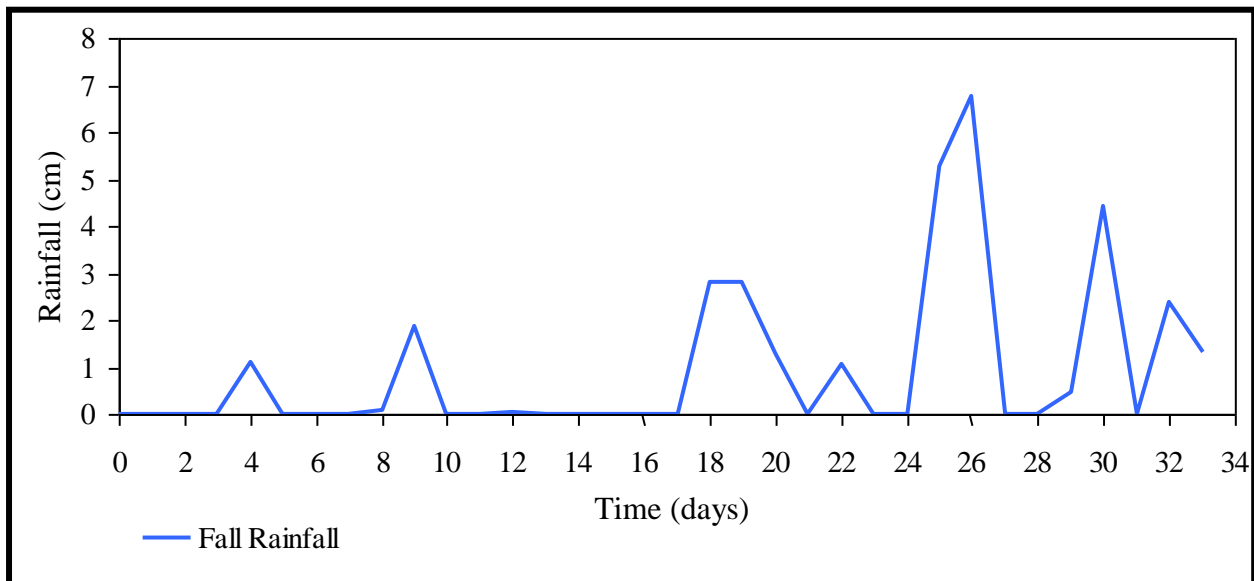


Figure 4. 49 Rain events, fall. Retrieved from:
<http://www2.lsuagcenter.com/weather/tabledata.asp?stationId=3>

This rain and flood activity had a tremendous impact on the fall study. First, high water levels made the site inaccessible to the researcher, with samples unable to be collected and morphological changes un-viewable. Attempting to access the site was potentially hazardous; thus, observations could only be made from the shore. Second, the addition of large quantities of water created more depth for the submerged pigs. Before flooding, the pigs had remained within one-half meter of the surface; after flooding, they were estimated to be up to one and one-half meters deep. Because algae growth is dependant on light availability, the increased turbidity and depth resulted in algae stress. This effect on algae, coupled with the safety of the researcher, resulted in the termination of the study.

Cool fall temperatures also impacted the study (Figure 4.50). The average temperature throughout the duration of the study was low, with air temperature at the time of collection (approximately 7 am) ranging from 0.39°C to 19.11°C and average water temperature at the time of collection ranging from 12.45°C to 19.45°C. The cool temperatures acted to refrigerate the pigs, delaying decomposition and possibly hindering scavenger activity.

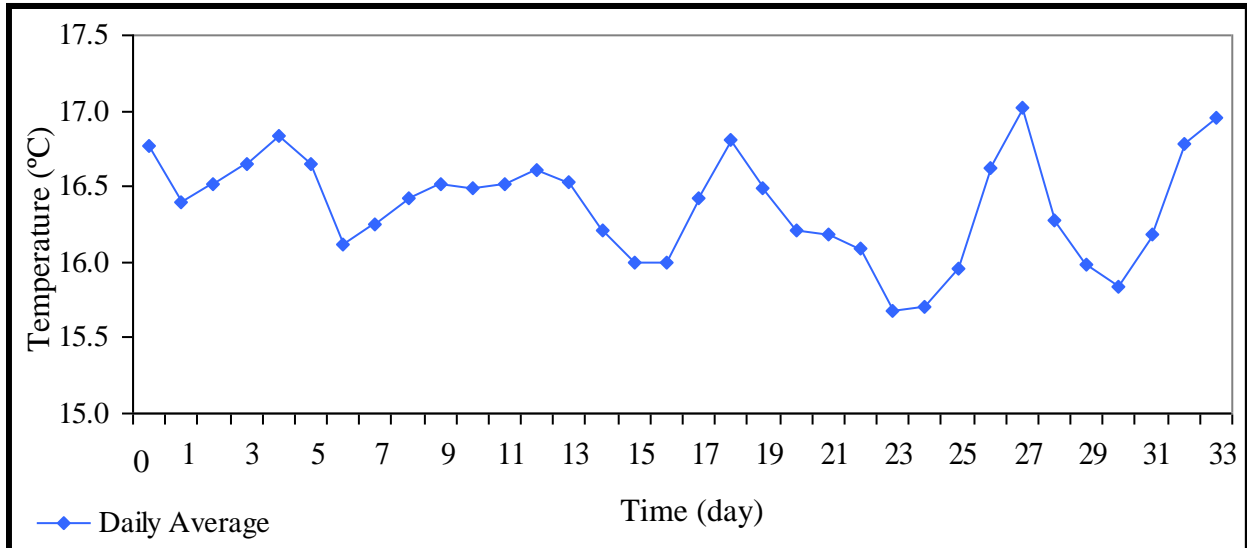


Figure 4. 50 Average daily water temperature from regression with Ben Hur weather data, fall. Original data retrieved from: <http://www2.lsuagcenter.com/weather/tabledata.asp?stationId=3>

4.2.4 Algae Analysis

Chlorophyll *a* concentrations were measured for each sampling date (Table 4.6). One-way ANOVA was then used to compare the chlorophyll *a* concentrations between the substrates at both the $\alpha = 0.10$ and $\alpha = 0.05$ levels (See Appendix Table B.5). Linear regressions were created relating Chlorophyll *a* concentration to time for each substrate. These regressions were graphed (see Appendix Figure B.1 to B.5) and their trends compared (Figure 4.51).

As shown in Table 4.8, the amounts of chlorophyll *a* collected from Pig A, Pig B, Tile C, and Tile D were not significantly different from each other at $\alpha = 0.05$ or $\alpha = 0.10$. All four substrates demonstrated a positive relationship between chlorophyll *a* concentration and time,

Table 4. 6 Chlorophyll *a* Concentrations of all Substrates, Fall.

Chlorophyll <i>a</i> Concentration, Fall ($\mu\text{g}/\text{cm}^2$)				
Day	Pig A	Tile C	Pig B	Tile D
0	0.16	0.42	0.13	0.22
1	0.85	0.56	0.38	0.21
2	0.97	0.09	0.28	0.50
3	1.63	0.47	0.42	0.32
4	1.61	0.45	0.91	0.45
5	1.60	1.47	1.18	0.71
6	0.84	2.33	1.59	0.91
7	2.22	2.14	0.85	3.80
8	3.33	2.53	0.72	4.05
9	0.73	5.59	1.15	7.04
16	4.12	69.41	7.06	7.76
23	4.20	1.27	3.73	1.99
30
33	3.04	0.54	2.81	1.37
.. No samples collected due to flooding				

though with weak r^2 values. These poor linear relationships are likely due to disturbed algae growth from frequent and heavy rainfall. The slope of Tile C ($r^2 = 0.049$) was much steeper than that of Pig A ($r^2 = 0.52$), Pig B ($r^2 = 0.42$) and Tile D ($r^2 = 0.083$), meaning chlorophyll *a* concentration increased the fastest over time. This suggests that fall algae grow best on fabric regardless of the presence of a decomposing body (the poor r^2 value was also a result of an extreme outlier, explained below). The chlorophyll *a* concentration in the water samples had negligible change throughout the entire study period; thus, the amount of algae present on the substrates was different than in the water (see Appendix Figure B.5). In the water, phosphorus levels decreased ($r^2 = 0.91$) and nitrate levels decreased ($r^2 = 0.19$) most likely due to biological assimilation, while ammonium levels remained stable ($r^2 = 0.057$) throughout the study period (see appendix Table B.3). Additionally, pH remained between 7 and 7.5, oxygen content ranged from zero to 8.0 ppm, and salinity remained at zero ppt throughout the study period. Turbidity

remained greater than 60 cm except during rain events, where it ranged from 22.6 cm to 51.6 cm (see Appendix Table B.1).

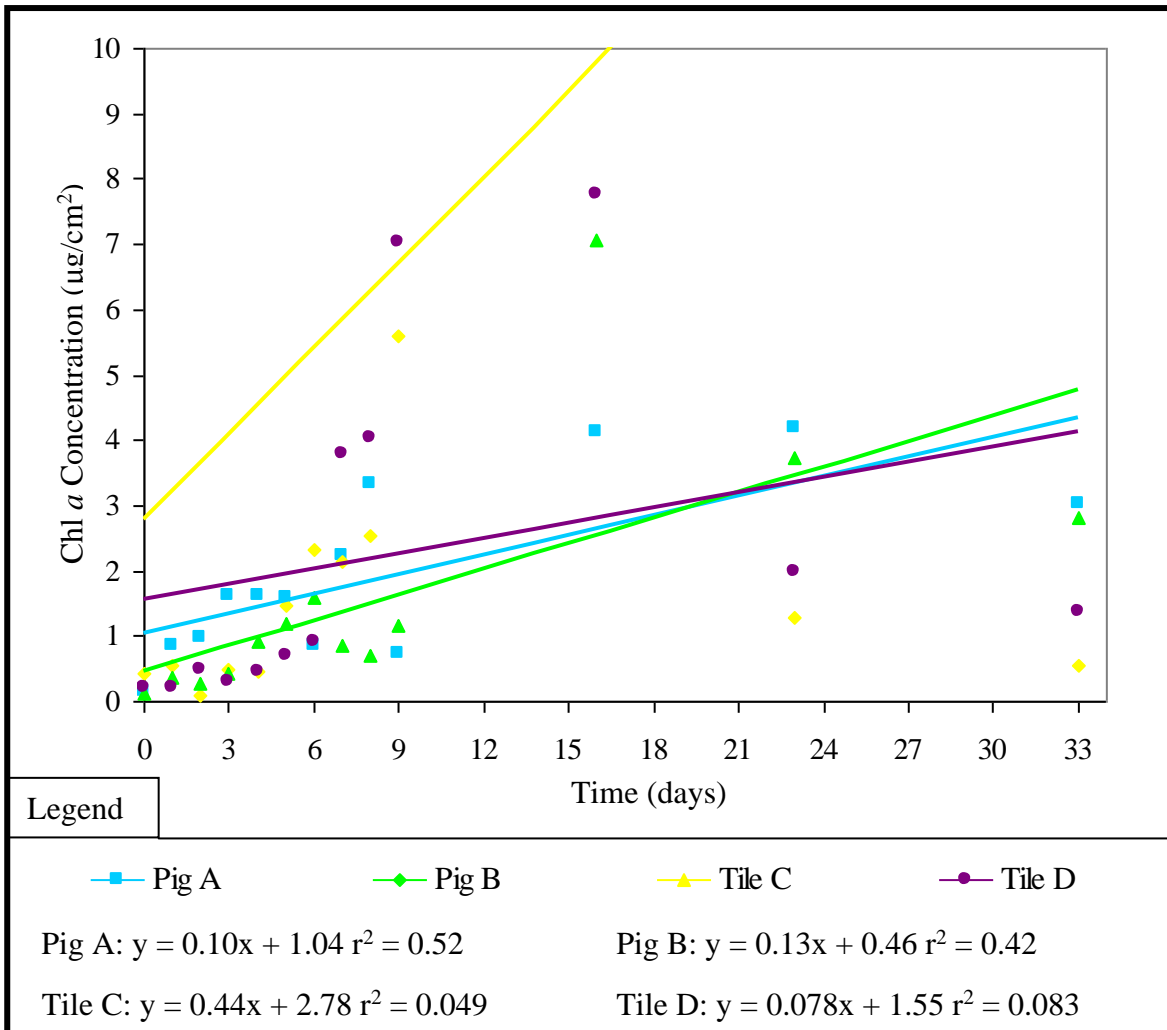


Figure 4. 51 Chlorophyll *a* (Chl *a*) concentrations over time for all Pigs and Tiles, fall.

The trend lines show that Tile C was substantially different than the other substrates. This is because a very high chlorophyll *a* concentration was recorded on Day 16; when this outlier is removed, the trend line of Tile C slopes very weakly. However, more than likely this trend of very high chlorophyll *a* concentration would have continued on Tile C had it not been for rain. Nearly all substrates reached their highest chlorophyll *a* value on Day 16, the last

sample date before heavy flooding. Thus, Tile C's outlier is not removed from the examination and Tile C is considered the steepest slope despite having the lowest r^2 value.

The effects of rain must be considered when interpreting these results. Significant rainfall events occurred after Day 16. This rain had four effects on the algae collected from all substrates. First, the mechanical damage of heavy rain droplets on the pig and tile surfaces may have washed off existing algae and made it difficult for new algae to attach. Second, rain caused significant turbidity in the water, decreasing light penetration to the algae, causing cell stress. Third, heavy rains caused flooding which resulted in faster current. This current jostled the substrates and caused many to become washed upon the shore; Tile C was tipped upside down on Day 19 and the fabric was completely pushed aside when removed on Day 33. This would have caused cell stress and prevented new growth. Fourth, the increased depth from flooding decreased the algae's access to light, again causing cell stress and the prevention of new growth.

Perhaps chlorophyll *a* concentration would have been greater after Day 16 had this rain not occurred, with stronger linear relationships and steeper slopes a result. Additionally, Tile C likely would have continued its very strong concentration increase, with the Day 16 sample not being an outlier at all.

The cold fall temperatures must also be considered when interpreting these results. While the daily averages were above 10°C, the mornings were very cool, sometimes less than 0°C. These cold temperatures possibly caused algae stress and potentially lowered their growth rates.

In summary, all substrates demonstrated a positive relationship between chlorophyll *a* and time; thus, algae can be used as an indicator of PMSI. Clothing had an impact on algae growth on the tiles, but less effect on the pigs.

When examined microscopically, pennate diatoms appeared to be the dominant algae of the fall samples, just as was seen in spring (Figure 4.52). Again, this is because pennate diatoms, which can attach to surfaces more easily than centric diatoms, were likely supported by the shallow bayou environment.



Figure 4. 52 Pennate diatoms collected from Tile C: Day 16, fall.

4.2.5 Fall Study Summary

The results of this case study show that a clothed pig will decompose slower than an unclothed pig. This is likely because clothing shields the carcass from the elements. The mechanical damage caused by the act of algae-sampling did not have a significant effect on either pig's rate of decomposition. Insect activity was present on all pigs but most prevalent on those which were unclothed; however, because maggots were not viable, insect activity had little effect on total decomposition degree days.

Algae accumulated on all pigs and tiles as seen through an increase of chlorophyll *a* concentration with time. Pig A, Pig B, and unclothed Tile D had very similar concentrations, with clothed Tile C having the highest recorded concentration. The pigs had the strongest linear relationships between chlorophyll *a* concentration and time but did not have the steepest slope.

Substrate depth was strongly affected by flooding events, with increased turbidity and current caused from heavy rainfall. Thus, sunlight was not always available and mechanical damage was present to cause cell stress and prevent algae growth. The lack of sunlight, cold temperatures, and heavy rain provided inhospitable conditions for algae growth on any substrate.

The results of the fall study suggest that algae can potentially be used to estimate PMSI on a decomposing body. Climate activity of the fall season makes algae growth and collection difficult and slows the rate of decomposition

4.3 Seasonal Comparison

While there was no significant difference between the mean initial weight of the spring and fall pigs using one-way ANOVA at $\alpha = 0.05$, a significant difference existed between their mean weight loss (at $\alpha = 0.05$, $p = 0.007$). Also, fall pigs required more decompositional degree days than spring pigs, while clothed pigs required more decompositional degree days than unclothed pigs.

In both study sessions, chlorophyll *a* concentration increased over time on all substrates. Figure 4.53 is a combination of Figure 4.27 and Figure 4.51 and shows all chlorophyll *a* concentration regressions. One-way ANOVA comparing chlorophyll *a* concentrations of each substrate between seasons at both the $\alpha = 0.10$ and $\alpha = 0.05$ levels was conducted (See Appendix Table B.6). The results show that clothed Tile C had steep slopes in both spring and fall while unclothed Pig B had gentle slopes in both spring and fall. Season did not result in significantly different chlorophyll *a* concentrations for either Tile C or Pig B at $\alpha = 0.10$ or $\alpha = 0.05$. Clothed Pig A and unclothed Tile D both had steep slopes in spring and a gentle slope in fall. Season resulted in significantly different chlorophyll *a* concentrations for Pig A (at $\alpha = 0.10$, $p = 0.066$) and Tile D (at $\alpha = 0.05$, $p = 0.0045$).

The spring session resulted in steeper slopes than the fall session, with the exception of Tile C which was steep in both seasons and Pig B which was gentle in both seasons. In general, algae grew faster in the spring than in the fall. Algae grew quickly on a clothed tile and slowly on a naked pig regardless of season. The fastest growth rate occurred on clothed spring Pig A and the slowest on unclothed fall Tile D. In summary, algae grow faster in the spring than in the fall and the presence of clothing has more impact on the growth of algae than the presence of decomposing matter.

Algae grew differently in spring than in fall because of seasonal differences in climate, nutrient, and water conditions. Compared using one-way ANOVA, spring temperatures were significantly warmer than fall (at $\alpha = 0.05$, $p < 0.00001$) while fall rainfall was significantly heavier than spring (at $\alpha = 0.05$, $p = 0.019$). Though there was no significant difference in turbidity or oxygen content at $\alpha = 0.05$, the spring water was significantly more acidic (at $\alpha = 0.05$, $p = 0.02$). There was no significant difference between the levels of nitrate and ammonium between the seasons at $\alpha = 0.05$, but there was significantly more phosphorus in the spring (at $\alpha = 0.05$, $p = 0.00039$). Salinity was measured throughout both studies but, being a freshwater system, remained at zero ppt. These results are summarized in Table 4.7.

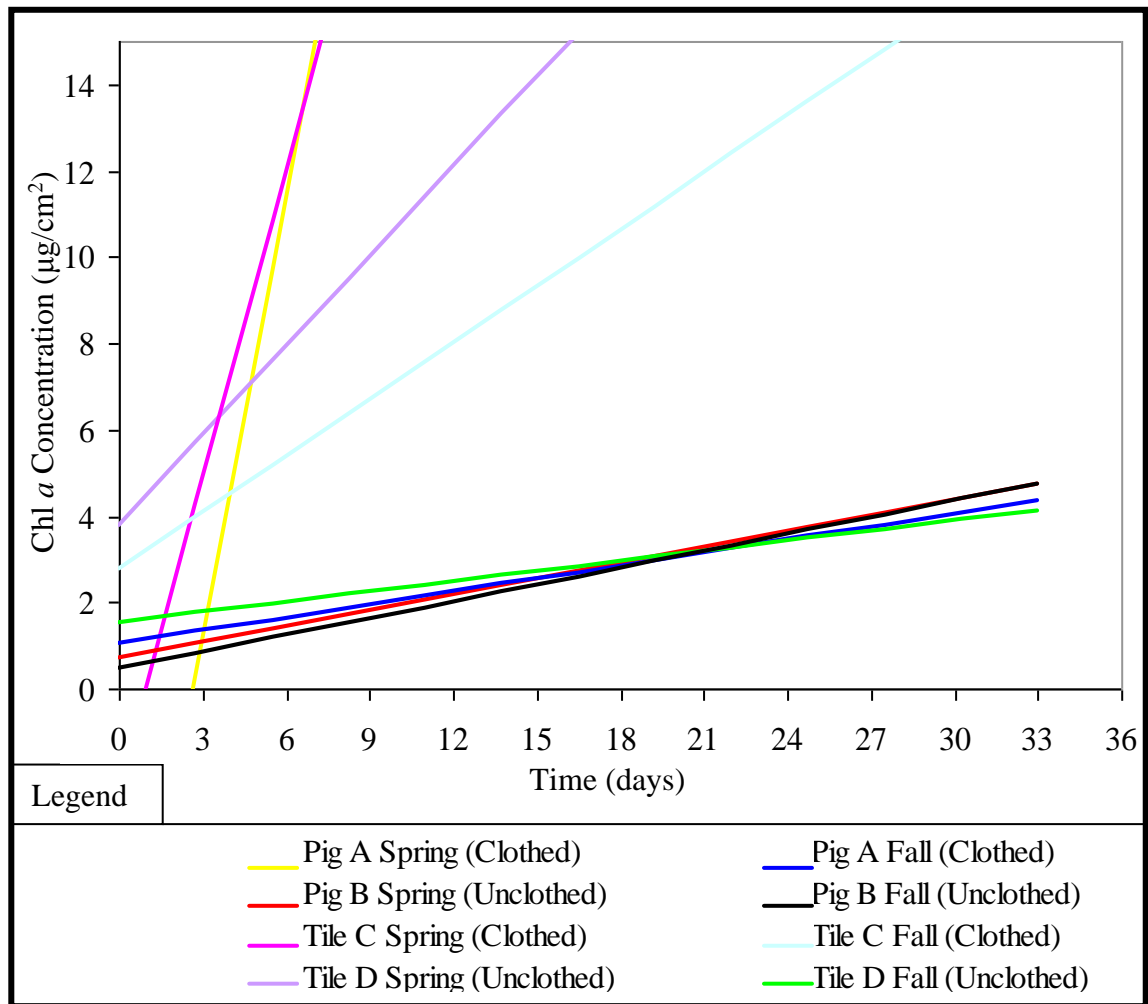


Figure 4. 53 Chlorophyll *a* (Chl *a*) concentrations over time for all Pigs and Tiles, spring and fall. See Chapter 4 for linear equations.

Table 4. 7 Summary of Seasonal Differences Influencing Algae Growth. P-values calculated using One-way ANOVA.

	Seasonal Difference at $\alpha = 0.05$	P-value	Description
Temperature	Yes	<0.00001	Warmer temperatures in Spring
Rainfall	Yes	0.019	More rain in Fall
Turbidity	No	..	No significant difference
Oxygen Content	No	..	No significant difference
pH	Yes	0.02	More acidic in Spring
Nitrate	No	..	No significant difference
Ammonium	No	..	No significant difference
Phosphorus	Yes	0.00039	More phosphorous in Spring
Salinity	No	..	No significant difference

CHAPTER 5: DISCUSSION

The results of this research show that a cadaver will decompose faster in spring than in fall and slower when a cadaver is clothed than unclothed. While the act of sampling does not significantly affect the rate of decomposition, scavenger and insect activity can significantly reduce the amount of time necessary to reach skeletonization.

5.1 Decomposition

Generalizations about aquatic decomposition can be made through comparisons with the broader literature on PMSI. Like Haefner et al. (2004), who studied PMSI in Pennsylvania streams from November to December using fetal pigs, this research shows that a fetal pig in water requires more than 200 accumulated degree days (ADD) to reach the advanced decomposition stage in cool temperatures, ranging from 214.7 ADD in the bayou to 392.1 ADD in the Pennsylvania stream pool (Table 5.1). The comparison also shows that a fetal pig will decompose faster in a Louisiana bayou than in a Pennsylvania stream in fall/winter. (Comparisons cannot be made for later stages because this study's fall session was stopped due to flooding.) Similarly, like Zimmerman and Wallace (2008), who studied PMSI in Delaware brackish ponds from May to July using fetal pigs, this research shows that a fetal pig in water requires an average of 530 ADD to become skeletonized in warm temperatures, with skeletonization occurring as soon as 383 ADD in the bayou to 706.92 ADD in the pond (Table 5.2). The comparison also shows that a fetal pig will decompose faster in a Louisiana bayou than in a Delaware brackish pond in spring/summer. However, the bayou pigs experienced major scavenging which significantly decreased their total ADD.

The brightly colored adipocere seen in Figures 4.39 and 4.41 requires discussion. Adipocere, described in Section 2.3, is a waxy, greyish-white substance. Knight (1991) explains that while adipocere itself is off-white, it can be stained green or red through contact with

Table 5. 1 Accumulated Degree Days of Unclothed Pigs in Winter, Louisiana and Pennsylvania. Louisiana fetal pigs (Pig B, Pig F, Pig G) from this study, November to December; Pennsylvania fetal pigs (Riffle Pigs, Pool, Pigs) adapted from Haefner et al. (2004), November to December.

Stage of Decomposition	Total Accumulated Degree Days				
	Unclothed: LA			Unclothed: PA	
	Pig B	Pig F	Pig G	Riffle Pigs	Pool Pigs
1: Fresh Remains	33.16	33.16	66.32	76.80	151.20
2: Early Decomposition*	181.54	181.54	164.92	188.10	240.90
Total	214.70	214.70	231.24	264.90	392.10

*Haefner et al.'s (2004) separate "Early Floating" and "Early Floating Decay" stages are combined into a single "Early Decomposition" for purposes of comparison.

Table 5. 2 Accumulated Degree Days of Unclothed Pigs in Summer, Louisiana and Delaware. Louisiana fetal pigs (Pig B, Pig F, Pig G) from this study, May to June; Delaware fetal pigs (Pond 1 Pigs, Pond 2 Pigs) adapted from Zimmerman and Wallace (2008), May to July.

Stage of Decomposition	Total Accumulated Degree Days				
	Unclothed: LA			Unclothed: DE	
	Pig B	Pig F	Pig G	Pond 1 Pigs	Pond 2 Pigs
1: Fresh Remains	25.04	50.53	50.53	70.86	51.33
2: Early Decomposition*	100.90	99.84	357.60	279.24	243.09
3: Advanced Decomposition	160.51	257.76	0	208.40	130.77
4: Sunken Remains	96.65	95.73	95.73	148.44	130.77
Total	383.10	503.86	503.86	706.94	555.96

*Zimmerman and Wallace's (2008) separate "Early Floating" and "Early Floating Decay" stages are combined into a single "Early Decomposition" for purposes of comparison.

decompositional fluids or blood. Knight (1997) also explains that initially adipocere resembles greasy, rancid butter, but over time becomes grey-white and brittle. Thus, adipocere can exist in a small range of colors depending on the environment and time span. However, no publications were found describing adipocere as brightly colored or yellowish-orange. I suggest two possible solutions to this discrepancy. First, the adipocere on Pig B and Pig F in the fall may have been stained through contact with the bayou environment, perhaps through enriched particles present in the water. Second, the adipocere may have been contaminated by bacteria or fungus which resulted in the bright color. Micozzi (1991) explains that a decomposing body provides an ideal

habitat for fungal organisms; perhaps the heavy rainfall and cool temperatures promoted such growth. Further study should be conducted into adipocere formation in Louisiana water.

5.2 Chlorophyll *a* Concentration

Generalizations can also be made about algae growth through comparisons with the broader literature. Like Casamatta and Verb (2000), this research identified diatoms, specifically pennate diatoms, as the dominant algae growing on the cadaver. Just as found in Haefner et al. (2004), the results of this research show that increases in chlorophyll *a* concentration over time can potentially be used to estimate PMSI. Figure 5.1 shows the fall results of this research alongside those published in Haefner et al. (the Haefner et al. values are estimated from their Figure 6). While this research examined clothed and unclothed pigs and tiles in a single water environment, Haefner et al. examined unclothed pigs and tiles in two separate environments: pool and riffle areas of two streams. Also, this research allowed the pigs to sink and float naturally through the stages of decomposition while Haefner et al. secured their cages to the stream floor, forcing their pigs to remain submerged. Figure 5.1 shows that in both the Louisiana bayou and the Pennsylvania streams, chlorophyll *a* concentration increased over time, with very similar growth on both Louisiana pigs and the riffle stream pigs. The pigs in the Pennsylvania pool stream had a slightly different growth rate than all the other pigs, though still within a close range. The Louisiana tiles had higher growth rates than the Pennsylvania tiles, especially Louisiana clothed Tile C. Also, rain events in both environments resulted in decreased amounts of chlorophyll *a*. Differences in chlorophyll *a* values between the two studies could be due to differences of geography, water system, nutrient levels, current, and temperature, or, more importantly, specimen mobility. Haefner et al.'s pigs were able to sink and float within the confines of the cages but were never able to reach the water surface, being protected from

insect activity and drying of tissue. Because they were held in place, Haefner et al.'s pigs likely experienced less rotation and movement than the Louisiana pigs, allowing algae to accumulate undisturbed with constant light availability. Thus, while Figure 5.1 shows that both studies had very similar chlorophyll *a* concentrations, the conditions of the experiments were different. By allowing natural body movement through the process of decomposition, this thesis offers a different approach to the use of algae for estimations of PMSI than Haefner et al.

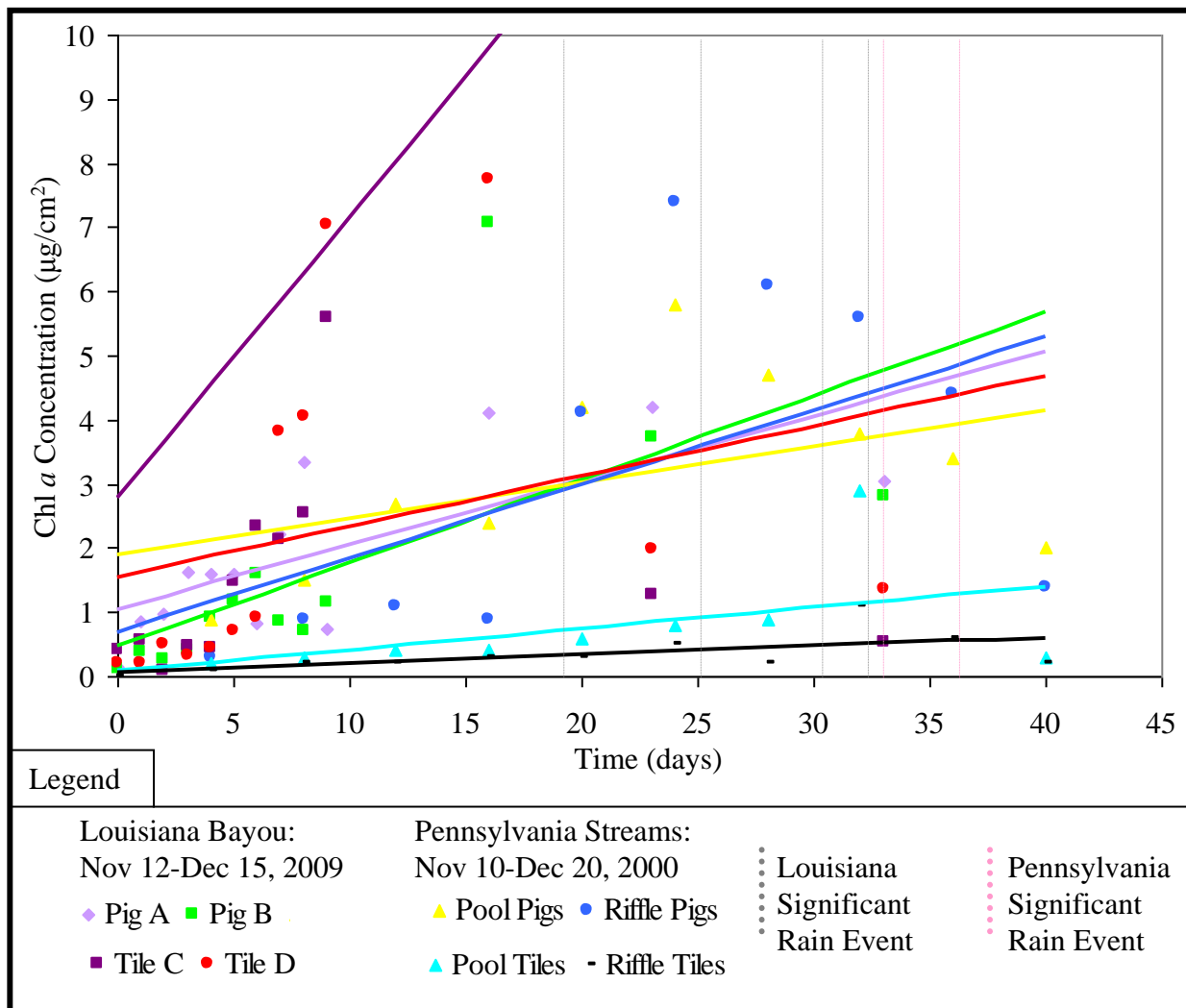


Figure 5. 1 Chlorophyll *a* (Chl *a*) concentration over time in a Louisiana bayou for clothed fall Pig A, unclothed fall Pig B, clothed Tile C, and unclothed Tile D compared with Haefner et al. (2004)'s Pennsylvania stream for unclothed winter pool pigs and tiles and unclothed riffle pigs and tiles. Pennsylvania data adapted from Haefner et al.'s Figure 6.

Unlike Haefner et al. (2004), this research shows that a decomposing body does not necessarily produce higher chlorophyll *a* values than a non-decomposing body; rather, the presence of cotton fabric is more significant than the presence of decomposing matter for algae growth. Table 5.3 shows the average chlorophyll *a* concentrations collected off the pigs and tiles of the Louisiana fall study and the Haefner et al. winter study. In the first five days, Louisiana clothed Pig A had a higher chlorophyll *a* average than all other substrates. From Day 10 to Day 30, clothed Tile C had a higher chlorophyll *a* average than all other substrates. From Day 5 to Day 9, only unclothed Tile D had a higher average than either clothed Pig A or clothed Tile D. Both Louisiana tiles had higher averages than the Pennsylvania tiles in all time frames, with clothed Tile C having the highest average of all substrates in both locations. Thus, presence of fabric influences the amount of algae growth on a cadaver.

Table 5. 3 Chlorophyll *a* Concentration Average/Range in Winter, Louisiana and Pennsylvania. Louisiana fetal pigs (Pig B, Pig F, Pig G) and tiles (Tile C, Tile D) from this study, November to December; Pennsylvania fetal pigs (Riffle Pigs, Pool, Pigs) and Tiles (Riffle Tiles, Pool Tiles) adapted from Haefner et al. (2004), November to December.

Substrate	Day 0-4		Day 5-9		Day 10-Completion (Fall: 33) (Haefner: 40)	
	Chlorophyll <i>a</i> ($\mu\text{g}/\text{cm}^2$)		Chlorophyll <i>a</i> ($\mu\text{g}/\text{cm}^2$)		Chlorophyll <i>a</i> ($\mu\text{g}/\text{cm}^2$)	
	Avg.	Range	Avg.	Range	Avg.	Range
FALL						
Clothed Pig A	1.04	(0.16-1.63)	1.74	(0.73-3.33)	3.79	(3.04-4.20)
Unclothed Pig B	0.42	(0.13-0.91)	1.10	(0.72-1.59)	4.54	(2.81-7.06)
Clothed Tile C	0.40	(0.09-0.56)	2.81	(1.47-5.59)	23.74	(0.54-69.41)
Unclothed Tile D	0.34	(0.21-0.50)	3.30	(0.71-7.04)	3.71	(1.37-7.76)
HAEFNER ET AL.						
Unclothed Pool Pigs*	0.90	n/a	1.50	n/a	3.63	(2.00-5.80)
Unclothed Riffle Pigs*	0.30	n/a	0.90	n/a	3.88	(0.90-7.40)
Unclothed Pool Tiles*	0.20	n/a	0.30	n/a	0.90	(0.3-2.9)
Unclothed Riffle Tiles†	0.10	n/a	0.20	n/a	0.43	(0.2-1.1)

*Values are estimated from Haefner et al. (2004) Figure 6.

5.3 Practical Considerations

The range of recorded chlorophyll *a* concentrations is also shown in Table 5.3 for the clothed and unclothed pigs in each season (averages and ranges are not calculated after the first ten days in five day intervals because this research did not take multiple samples during that time frame). In general, the ranges show that higher values of chlorophyll *a* can be expected with increased time, though low values can occur in any time frame. Because there is considerable overlap between these ranges, an investigator taking a single algae sample off a cadaver to estimate PMSI needs to consider many interacting variables affecting algae growth before using any previously calculated linear regression formulas. For this reason, the following considerations are suggested for the practical use of algae in estimations of PMSI:

1. Geographic location of the water system: The geography of the water system will determine what types and quantities of algae are present in the water. If a particular location does not have an adequate amount of fast growing algae present in the water column, particularly diatoms, it may not have abundant algae growth on a cadaver. If a body is discovered in water with low levels of algae in the water column, the amount of chlorophyll *a* collected may be less than expected if the water had high amounts.

2. Type of water system: Algae require specific nutrient, salinity, pH, and sunlight levels to survive in a particular habitat. Even within a small geographic area, different water systems will have different levels of these requirements and will thus have different types and quantities of algae. For example, the environmental conditions present in a Louisiana bayou will be different than the conditions present in a Louisiana lake, river, or stream. If a body is discovered in a Louisiana stream, the amount of chlorophyll *a* collected may be different than expected for a body discovered in a Louisiana bayou.

3. Season of collection: As this research shows, season is a determining factor for the amount of algae in a water system. In Louisiana, more algae were present on a cadaver in spring than in fall, due to many interacting factors such as temperature, rainfall and water nutrient levels. If a body is discovered in fall or winter, the amount of chlorophyll *a* collected may be less than expected for a body found in spring or summer.

4. Types of algae present in the water: Different kinds of algae grow at different rates according to their specific requirements and characteristics. If a body of water contains fast growing algae, a higher amount of chlorophyll *a* concentration may be collected than expected for a body of water with slower growing algae.

5. Amount of turbulence and light availability: As evident in Figure 5.1, rain acts to remove algae from the surface of the cadavers, decreasing the collected chlorophyll *a* concentrations. Also, rain will dilute the chlorophyll *a* present in the water itself due to increased water volume. In Louisiana, the bayou water experienced decreased amounts of chlorophyll *a* after heavy rain events, due to substantial increases in water volume from the rain and flooding (see Appendix Figure B.5). High turbulence could have a similar effect, causing movement of the cadaver and removal of algae. If a body is discovered in water with high turbulence or during a period of heavy rainfall, the amount of chlorophyll *a* collected may be less than expected if the water was calm.

Algae growth is dependant upon access to sunlight; algae growth on the superior surface of a submerged cadaver will be greater than on the inferior surface, where shading from sunlight will hinder growth. Therefore, algae sampled from the top of a body will result in more chlorophyll *a* than algae sampled from the underside of a body. However, if the body turns and rotates while submerged, there may not be an obvious top or bottom, with algae growth spread

more evenly on all surfaces. If a sample is collected from the area of a cadaver most densely covered by algae, the amount of chlorophyll *a* collected may be more than expected from a less densely covered area of a cadaver. Currently no standard guidelines exist for where an algae sample should be collected from a body.

6. Presence of Clothing: If an unclothed cadaver is floating at the water surface, algae will only be able to grow at and below the water level. If clothing is present, algae not only have a strong binding surface, they can grow above the water level due to moisture diffusion through the fabric. If a clothed body is discovered, the amount of chlorophyll *a* collected may be more than expected for an unclothed body.

7. Evidence of Scavenger/Insect Activity: Insect and scavenger activity can significantly decrease the amount of time necessary for a cadaver to become skeletonized. When tissues are removed, less surface area is available for algae growth upon a cadaver. Additionally, scavenger and insect activity can remove algae present on tissues, exposing internal flesh that does not have any algae growth. If a body is discovered with heavy insect or scavenger activity, the amount of chlorophyll *a* collected may be less than expected for an undisturbed cadaver.

These seven considerations must be taken into account when chlorophyll *a* is used to estimate PMSI in a forensic case. Much more research is necessary to develop a standard protocol of how and where samples are taken, and more experimentation is necessary in different geographic locations and water systems to produce more linear regression formulas.

5.4 Future Concerns

While the results of this study indicate that there is tremendous promise in the use of algae for the estimation of PMSI, the design of the experiment proved to be problematic both for statistical analysis and for sampling purposes.

The sample size of chlorophyll *a* measurements was small, and because the algae grew on top of itself over time, the samples were not truly independent. This prevents the data from conforming to the Normal distribution, making statistical analysis difficult. Additionally, true replication is not possible for two reasons. First, taking numerous samples from a single pig does not take into account differential algae growth based on body part; it is unknown whether algae grow differently on one part of the body over another, and access to light must be considered. Second, taking algae samples from two separate pigs would not be true replication because of the immense variability in the process of decomposition—it would be impossible to have two pigs decompose identically due to variations in buoyancy, position, scavenging, and insect activity. Additionally, numerous pigs in one location could cause eutrophication which would alter algae growth. Thus, unless the pigs are in the same position, have the same insect and scavenging activity, are at the same depth, and are sampled from the exact same spot, samples collected in a natural setting are not truly comparable. More research is necessary to determine the best algae sampling strategy on a cadaver.

Finally, there is the problem with lack of control. Doing an outdoor experiment on taphonomy naturally includes environmental variability which adds realism to the study, especially in the forensic context. During the fall study, the pigs were beached due to current action from heavy rainfall. To protect the integrity of the experiment, the pigs were put back into the water; in real life, a beached body would likely be discovered by a passerby or would have continued to decompose on land. However, many uncontrolled variables acted on the experiment which were never witnessed and were un-quantified, such as large spring scavengers. Louisiana's rich biological diversity and highly variable climate reduced experimental control.

The precise results of this experiment can only be applied to fetal bodies recovered from Bayou Fountain; the formulae cannot be projected onto other geographical locations, climates, or

body masses. However, the concept of using algae to estimate PMSI is universal and can be applied to any water system. If more research were conducted in numerous geographic locations and on different sizes of pigs, a collection of data could be created to affirm the utility of algae in the estimation of PMSI.

CHAPTER 6: SUMMARY AND CONCLUSIONS

While algae are often used in forensic science for the determination and confirmation of death by drowning, their utility for the estimation of PMSI has been greatly underutilized. Algae are present in all water systems and will grow upon decomposing matter; yet, very little published literature exists on their use in PMSI estimation. Because PMSI is difficult to predict due to the variable nature of water, the reaction of the submerged body within water, and the lack of truly sarcophagous aquatic insects, algae are a potentially invaluable tool for the forensic anthropologist. This research investigates the utility of algae as an indicator of PMSI in a Louisiana bayou while simultaneously studying stages of decomposition, considering both seasonality and clothing as factors.

In this study, season plays the most important role in the process of decomposition of a body in water. Temperature, rainfall, and biological organisms act upon a cadaver, affecting its progression from a fresh to a skeletonized state. Clothing also plays a crucial role, slowing the rate of decomposition and providing ample surface for algae growth. By examining the decomposition of ten pigs, five in the spring and five in the fall, results indicate that a clothed pig will decompose slower than an unclothed pig, and a pig in cool fall temperatures will decompose slower than a pig in warm spring temperatures. Also, algae growth is fastest in the spring and on clothed substrates due to more ideal growing conditions and available surface area for growth. In both seasons and on all substrates, algae growth conformed to a positive linear relationship with time.

This study has shown that algae on a decomposing body can be used to estimate PMSI by measurement of chlorophyll *a* concentration. While the precise results of the study are applicable only to fetal bodies recovered from Bayou Fountain, Baton Rouge, Louisiana, the concept of using algae to estimate PMSI could be applied to any geographic location. More

studies are needed to create a collection of data on the types and concentrations of algae growth on different body masses in various water systems, and more investigation is required into the ideal location of sampling upon a decomposing substrate.

The results of this research are not wholly academic in nature. They apply to the forensic professional investigating water death, the forensic anthropologist studying water decomposition, and the algologist examining algae growth. The intent of this study is not only to enrich the academic knowledge of the utility of algae for estimation of PMSI, but also to bring attention to the growing need for collaboration between scientists investigating forensic cases.

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APPENDIX A: SPRING DATA

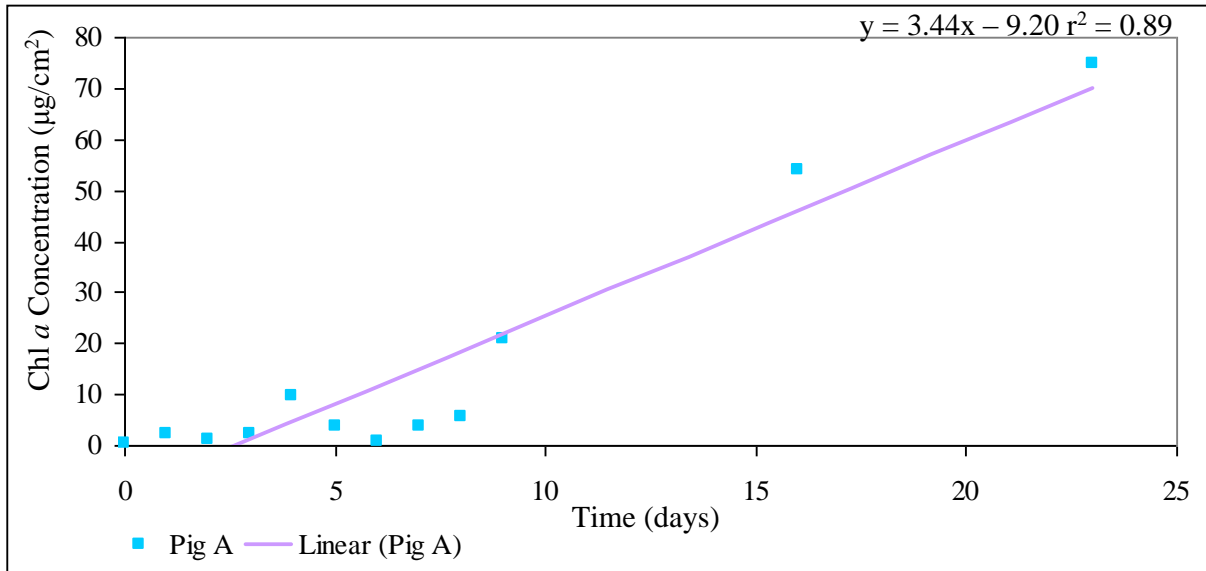


Figure A.1 Chlorophyll *a* (Chl *a*) concentrations over time for clothed Pig A, spring (n = 12).

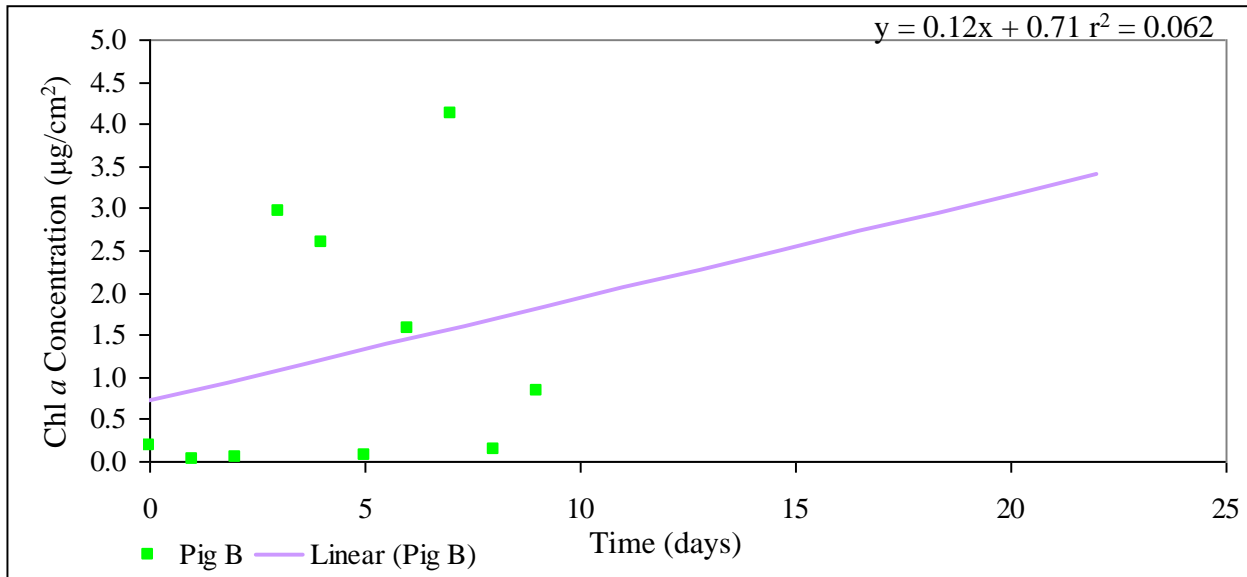


Figure A.2 Chlorophyll *a* (Chl *a*) concentrations over time for unclothed Pig B, spring (n = 10).

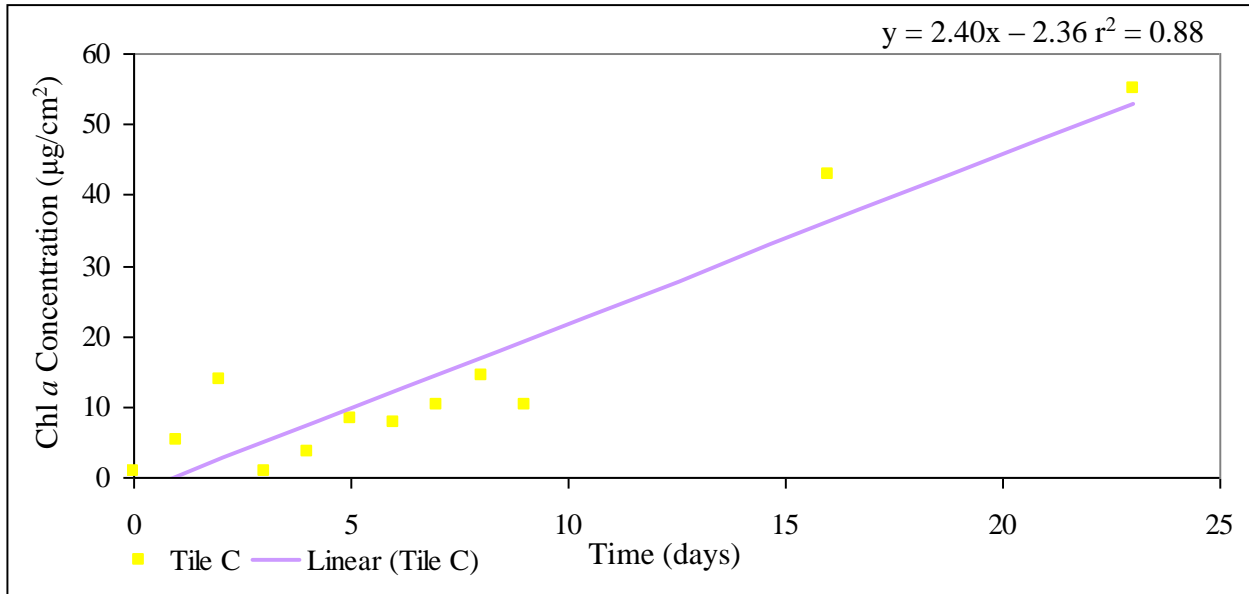


Figure A.3 Chlorophyll *a* (Chl *a*) concentrations over time for clothed Tile C, spring (n = 12).

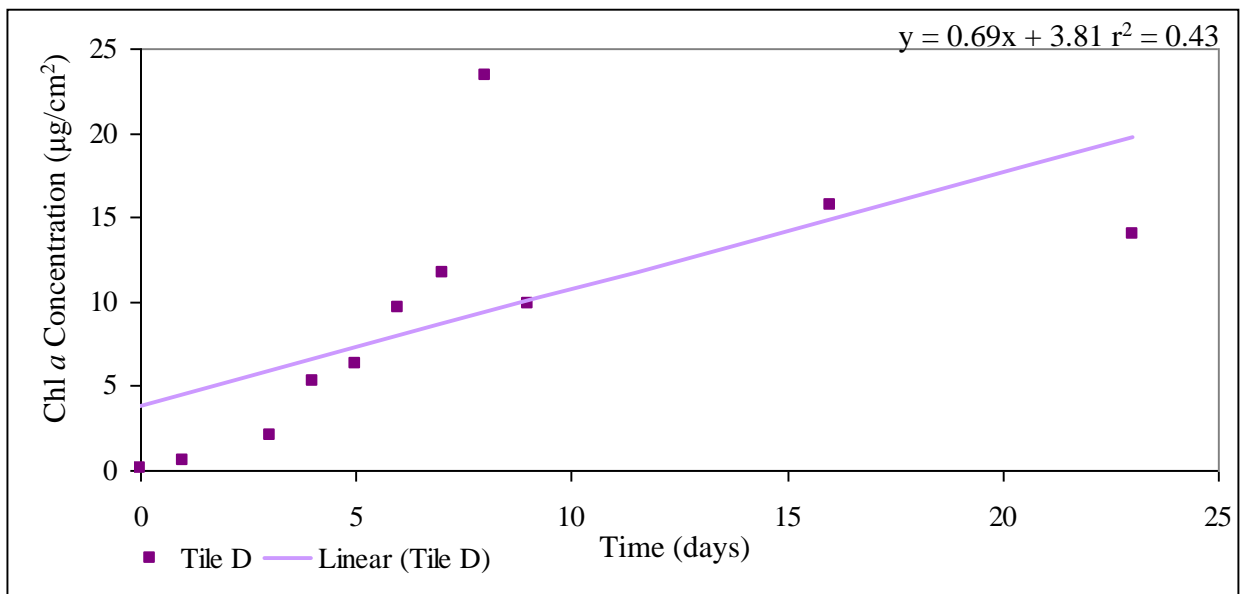


Figure A.4 Chlorophyll *a* (Chl *a*) concentrations over time for unclothed Tile D, spring (n = 11).

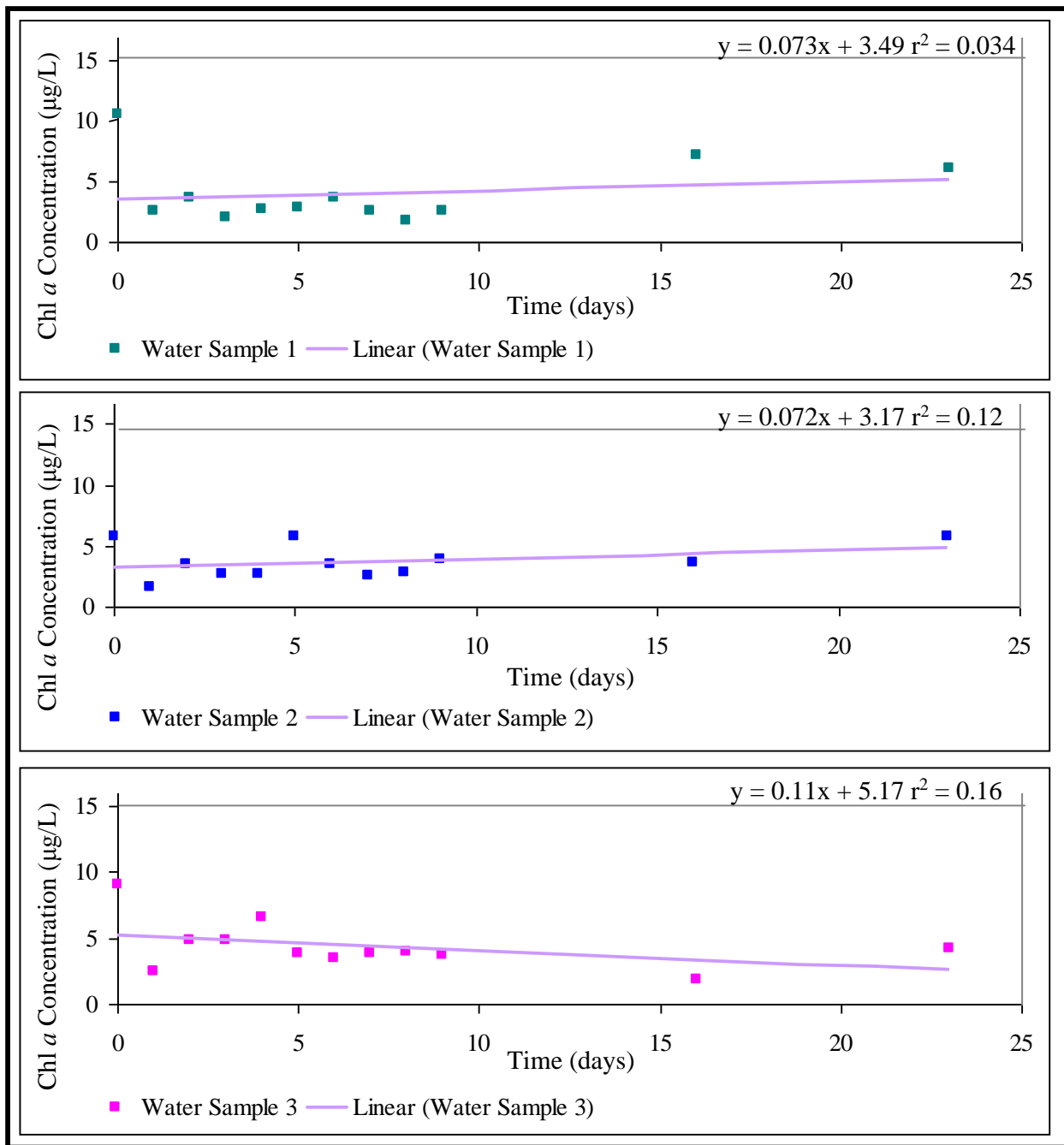


Figure A.5 Chlorophyll *a* (Chl *a*) concentrations over time for Water Sample 1 (top), Water Sample 2 (middle) and Water Sample 3 (bottom), spring (n = 12).

Table A. 1 Scene Water Data, Spring.

Scene Data, Spring				
Day	pH	Turbidity	Oxygen ppm	Salinity ppt
0	7	20.2	2	0
1	7.5	>60
2	7	>60
3	7.5	>60*
4	7.5	>60	4	0
5	7.5	>60*
6	7	>60
7	7.5	>60
8	7.5	>60
9	7.5	>60	1.2	0
13	..	29.4*
16	7.5	>60	0.2	0
23	7.5	>60	0.4	0

*raining

Table A.2 Water Nutrient Content, Spring.

Water Nutrient Levels: Spring			
Day	Dissolved Reactive Phosphorus (mg P L⁻¹)	Nitrate (mg N L⁻¹)	Ammonium (mg N L⁻¹)
0	0.51	0.058	0.55
4	0.70	0.043	0.48
9	0.50	0.053	0.31
16	0.48	0.030	0.46
23	0.47	0.066	0.33

Table A.3 Biomass Loss of Pig E and Pig F, Spring.

Biomass Loss, Spring		
Day	Pig E (kg)	Pig F (kg)
0	2.25	1.25
4	2.25	1
9	2.25	0.75
16	1.75	0.5
23	0.5	0.25

Calculation of Regression Formula, Spring				
Day	Scene Average Water Temp (°F)	Ben Hur Air Temp (°F)	Scene Average Water Temp (°C)	Ben Hur Air Temp (°C)
0	77.94	78	25.52	25.56
1	79.29	78	26.27	25.56
2	79.92	79	26.62	26.11
3	78.11	78	25.62	25.56
4	77.46	79	25.25	26.11
5	76.17	73	24.54	22.78
6	69.15	62	20.64	16.67
7	69.43	67	20.79	19.44
8	68.81	68	20.45	20.00
9	73.31	78	22.95	25.56
10	72.60	74	22.56	23.33
16	76.63	81	24.80	27.22
23	76.20	70	24.56	21.11

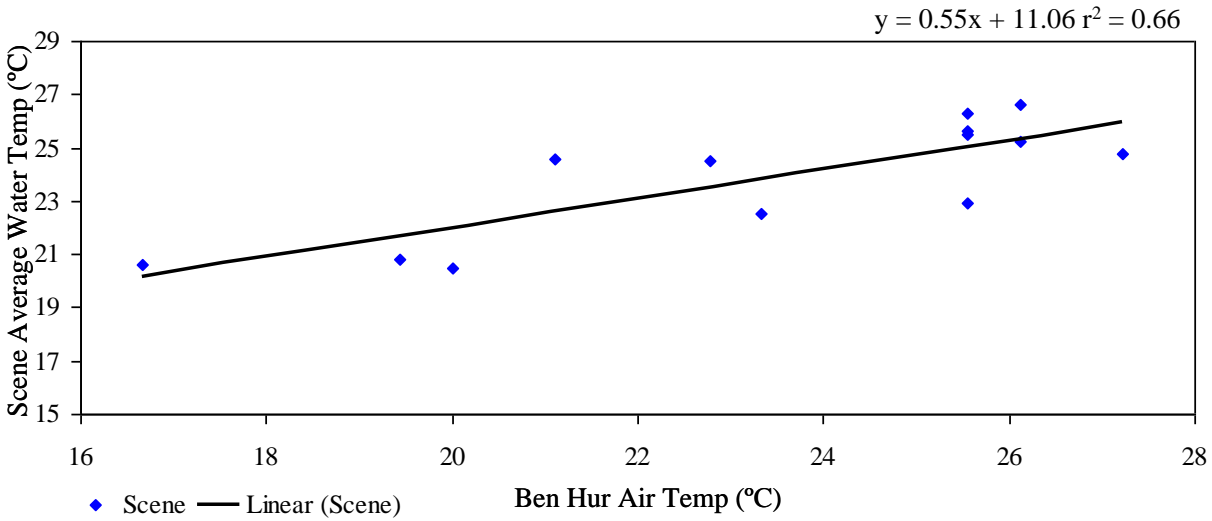


Figure A. 6 Calculation of temperature regression formula, spring. Scene average water temperature was the average of all 7 temperature readings each day.

Table A. 4 Accumulated Degree Days, Spring

Calculation of Accumulated Degree Days, Spring.										
Day	Ben Hur Avg (°C)	Scene Avg (°C)	Base (°C)	ADD	ΣADD	Stage A ADD	Stage E ADD	Stage B ADD	Stage F ADD	Stage G ADD
0	25.56	25.03	0	25.03		1		1 25.04	1	1
1	26.39	25.49	0	25.49	50.53	1 50.53	1 50.53	2	1 50.53	1 50.53
2	26.11	25.34	0	25.34	75.86	2		2	2	2
3	25.83	25.19	0	25.19	101.05	2		2	2	2
4	25.28	24.88	0	24.88	125.93	2		2 100.90	2	2
5	24.44	24.43	0	24.43	150.36	2		3	2 99.84	2
6	17.78	20.78	0	20.78	171.14	2		3	3	2
7	19.17	21.54	0	21.54	192.68	2		3	3	2
8	18.89	21.39	0	21.39	214.07	2	163.55	3	3	2
9	23.61	23.97	0	23.97	238.04	2 187.52	3	3	3	2
10	24.17	24.28	0	24.28	262.32	3		3	3	2
11	23.89	24.12	0	24.12	286.44	3		3 160.51	3	2
12	22.78	23.52	0	23.52	309.96	3		4	3	2
13	24.72	24.58	0	24.58	334.54	3		4	3	2
14	23.06	23.67	0	23.67	358.21	3		4	3	2
15	25.28	24.88	0	24.88	383.09	3		4 96.65	3	2
16	25.56	25.03	0	25.03	408.12	3			3 257.76	2 357.60
17	23.61	23.97	0	23.97	432.10	3			4	4
18	22.22	23.21	0	23.21	455.31	3			4	4
19	23.33	23.82	0	23.82	479.13	3 241.08	3		4	4
20	25.00	24.73	0	24.73	503.86	4	289.79		4 95.73	4 95.73
21	24.72	24.58	0	24.58	528.44	4				
22	23.33	23.82	0	23.82	552.26	4				
23	25.00	24.73	0	24.73	576.99	4 97.86	4 73.13			
Total:						576.99	576.99	383.09	503.86	503.86

Table A. 5 One-Way ANOVA Calculations for all Substrates, Spring.

Comparison	Source of Variation	SS	df	MS	F	P-value	F critical at $\alpha = 0.10$		F critical at $\alpha = 0.05$	
							F critical	Reject?	F critical	Reject?
A = B	BG	1013.45	1	1013.45	3.1501	0.091	2.97	Yes	4.35	No
	WG	6434.50	20	321.73						
	Total	7447.96	21							
A = C	BG	1.21	1	1.21	0.0028	0.96	2.95	No	4.30	No
	WG	9569.67	22	434.98						
	Total	9570.88	23							
A = D	BG	202.24	1	202.24	0.61	0.44	2.96	No	4.32	No
	WG	6920.26	21	829.54						
	Total	7122.50	22							
B = C	BG	947.80	1	947.80	5.9702	0.024	2.97	Yes	4.35	Yes
	WG	3175.10	20	158.75						
	Total	4122.90	21							
B = D	BG	310.13	1	310.13	11.209	0.0034	2.99	Yes	4.38	Yes
	WG	525.69	19	27.67						
	Total	845.82	20							
C = D	BG	172.81	1	172.81	0.99	0.33	2.96	No	4.32	No
	WG	3660.85	21	174.33						
	Total	3833.66	22							
Legend										
BG: Between Group			df: Degrees of Freedom							
WG: Within Group			MS: Mean Square							
SS: Sum of Squares			F: F-Calculated							

APPENDIX B: FALL DATA

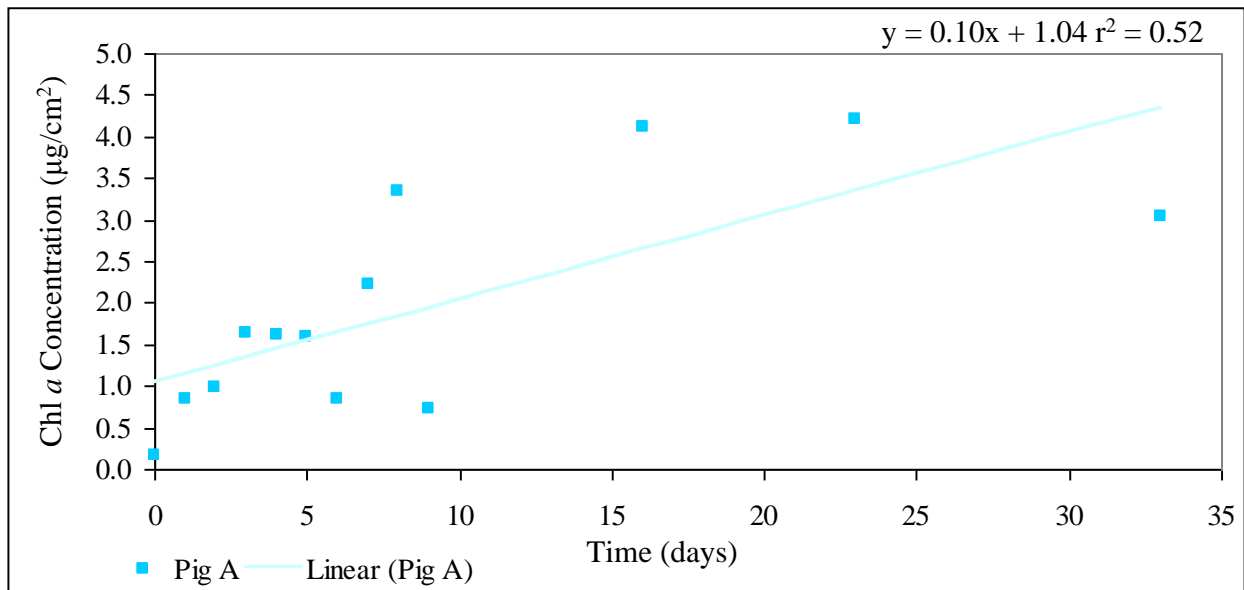


Figure B.1 Chlorophyll *a* (Chl *a*) concentrations over time for clothed Pig A, fall (n = 13).

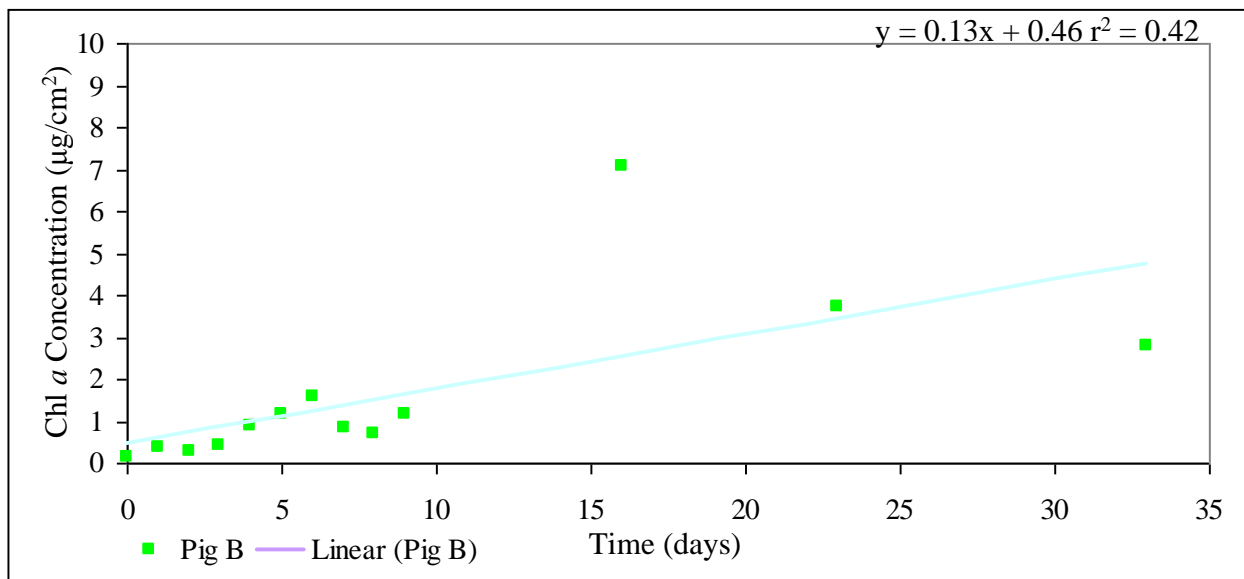


Figure B.2 Chlorophyll *a* (Chl *a*) concentrations over time for unclothed Pig B, fall (n = 13).

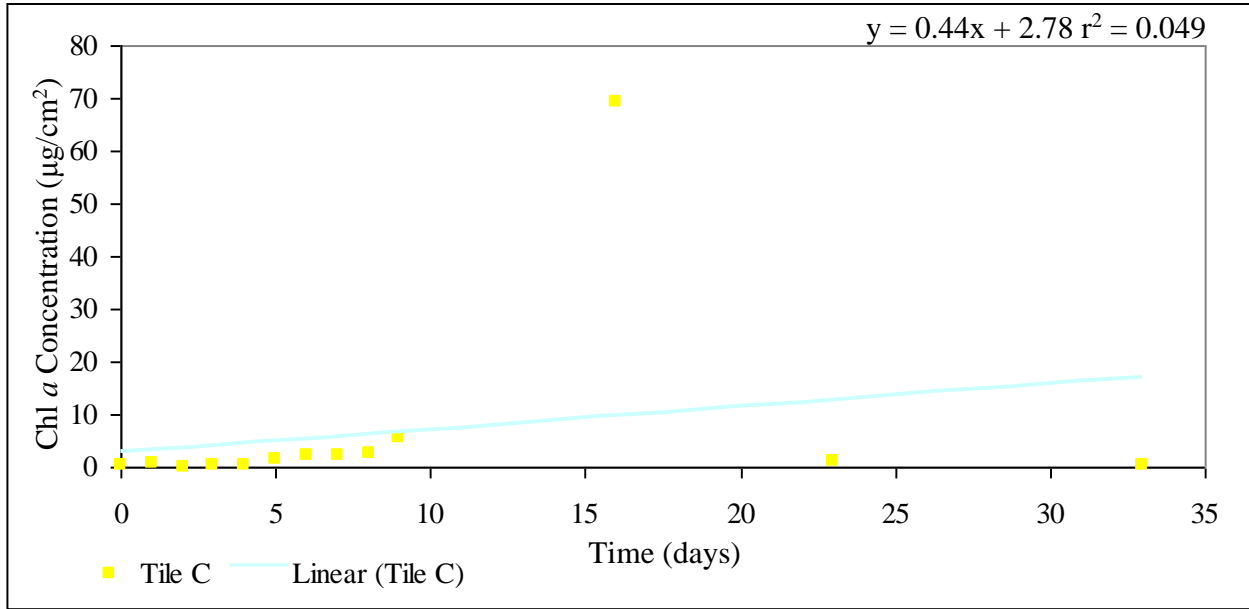


Figure B.3 Chlorophyll *a* (Chl *a*) concentrations over time for clothed Tile C, fall (n = 13).

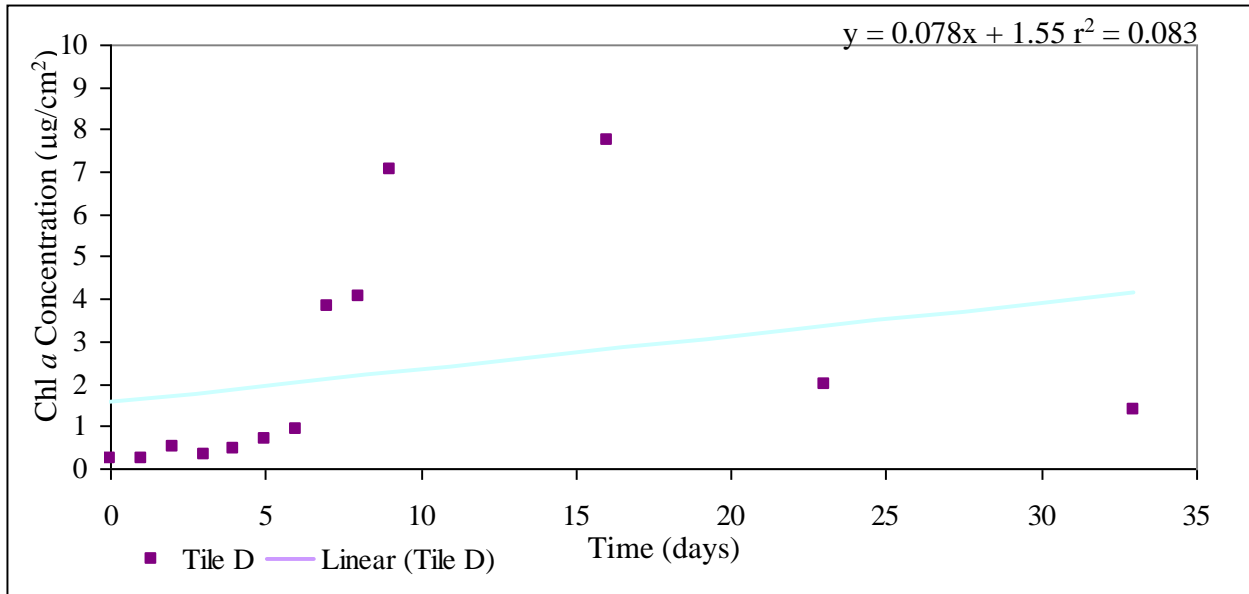


Figure B.4 Chlorophyll *a* (Chl *a*) concentrations over time for unclothed Tile D, fall (n = 13).

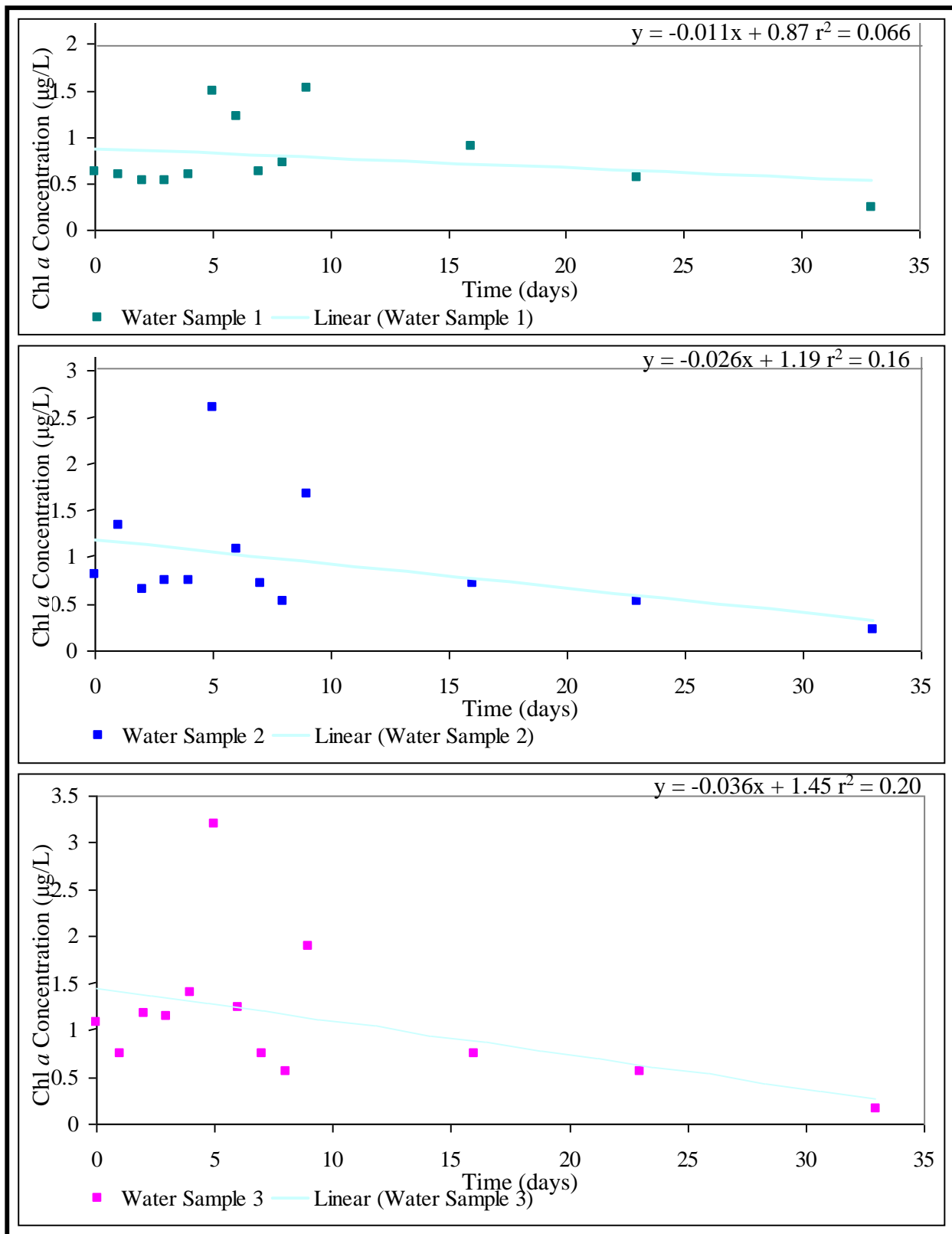


Figure B.5 Chlorophyll *a* (Chl *a*) concentrations over time for Water Sample 1 (top), Water Sample 2 (middle) and Water Sample 3 (bottom), fall (n = 13).

Table B. 1 Scene Water Data, Fall.

Scene Data, Fall				
Day	pH	Turbidity cm	Oxygen ppm	Salinity ppt
0	7.5	>60	0	0
1	7	>60
2	7	>60
3	7	>60
4	7.5	>60*	1	0
5	7	>60
6	7	>60
7	7	>60
8	7	>60
9	7.5	34.8*	3.6	0
16	7	>60	0.6	0
20	..	22.6*
21	..	51.6
22	..	>60*
23	7	33.6	4.8	0
25	..	>60*
26	..	24.4*
27	..	35
28	..	45
30	7.5	28.2*	8	0
31	..	38.2
33	7.5	36*	4.8	0
*rain				

Table B.2 Water Nutrient Content, Fall.

Water Nutrient Levels: Fall			
Day	Dissolved Reactive Phosphorus (mg P L ⁻¹)	Nitrate (mg N L ⁻¹)	Ammonium (mg N L ⁻¹)
0	0.40	1.40	0.35
4	0.33	0.09	0.31
9	0.28	0.40	0.45
16	0.26	0.16	0.65
23	0.19	0.50	0.24
30	0.18	0.15	0.02
33	0.19	0.13	0.03

Calculation of Regression Formula, Fall				
Day	Scene Average Water Temp (°F)	Ben Hur Air Temp (°F)	Scene Average Water Temp (°C)	Ben Hur Air Temp (°C)
0	63.61	50.00	17.56	10.00
1	58.71	39.00	14.84	3.89
3	61.93	44.00	16.63	6.67
4	63.81	52.00	17.67	11.11
5	63.80	40.00	17.67	4.44
6	67.07	38.00	19.48	3.33
7	56.40	40.00	13.56	4.44
8	56.70	45.00	13.72	7.22
9	64.17	53.00	17.87	11.67
16	99.25	47.00	37.36	8.33
23	60.77	30.00	15.98	-1.11
30	53.87	43.00	12.15	6.11

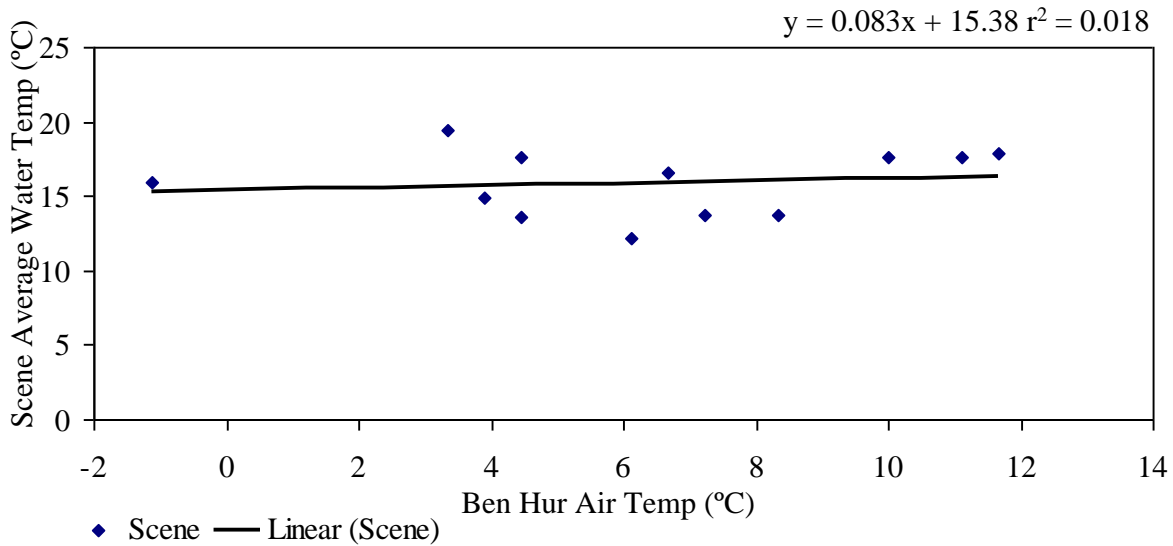


Figure B.6 Calculation of temperature regression formula, fall. Scene average water temperature was the average of all 7 temperature readings each day

Table B.3 Accumulated Degree Days, Fall

Calculation of Accumulated Degree Days, Fall															
Day	Ben Hur Avg (°C)	Scene Avg (°C)	Base (°C)	ADD	ΣADD	A Stage	A ADD	E Stage	E ADD	B Stage	B ADD	F Stage	F ADD	G Stage	G ADD
0	16.67	16.76	0	16.76		1		1		1		1		1	
1	12.22	16.39	0	16.39	33.16	1		1		1	33.16	1	33.16	1	
2	13.61	16.51	0	16.51	49.67	1		1	49.67	2		2		1	
3	15.28	16.65	0	16.65	66.32	1	66.32	2		2		2		1	66.32
4	17.50	16.83	0	16.83	83.15	2		2		2		2		2	
5	15.28	16.65	0	16.65	99.80	2		2		2		2		2	
6	8.89	16.12	0	16.12	115.92	2		2		2		2		2	
7	10.56	16.26	0	16.26	132.17	2		2		2		2		2	
8	12.50	16.42	0	16.42	148.59	2		2		2		2		2	
9	13.61	16.51	0	16.51	165.10	2		2		2		2		2	
10	13.33	16.49	0	16.49	181.59	2		2		2		2		2	
11	13.61	16.51	0	16.51	198.10	2		2		2		2		2	
12	14.72	16.60	0	16.60	214.70	2		2		2	181.54	2	181.54	2	
13	13.89	16.53	0	16.53	231.23	2		2		3		3		2	164.92
14	10.00	16.21	0	16.21	247.44	2		2		3		3		3	
15	7.50	16.00	0	16.00	263.45	2		2		3		3		3	
16	7.50	16.00	0	16.00	279.45	2		2		3		3		3	
17	12.50	16.42	0	16.42	295.87	2		2		3		3		3	
18	17.22	16.81	0	16.81	312.68	2		2		3		3		3	
19	13.33	16.49	0	16.49	329.16	2	262.85	2		3		3		3	
20	10.00	16.21	0	16.21	345.37	3		2	295.70	3		3		3	
21	9.72	16.19	0	16.19	361.56	3		3		3		3		3	
22	8.61	16.09	0	16.09	377.65	3		3		3		3		3	
23	3.61	15.68	0	15.68	393.33	3		3		3		3		3	
24	3.89	15.70	0	15.70	409.04	3		3		3		3		3	
25	6.94	15.96	0	15.96	424.99	3		3		3		3		3	

Table B.3 Cont.

26	15.00	16.63	0	16.63	441.62	3	3	3	3	3
27	19.72	17.02	0	17.02	458.64	3	3	3	3	3
28	10.83	16.28	0	16.28	474.91	3	3	3	3	3
29	7.22	15.98	0	15.98	490.89	3	3	3	3	3
30	5.56	15.84	0	15.84	506.73	3	3	3	3	3
31	9.72	16.19	0	16.19	522.92	3	3	3	3	3
32	16.94	16.79	0	16.79	539.71	3	3	3	3	3
33	18.89	16.95	0	16.95	556.66	3 227.49	3 211.28	3 341.96	3 341.96	3 325.42
Total:						556.66	556.66	556.66	556.66	556.66

Table B. 4 Biomass Loss of Pig E and Pig F, Fall.

Biomass Loss, Fall		
Day	Pig E (kg)	Pig F (kg)
0	1.5	1.5
4	1.63	1.5
9	1.88	2
16	1.88	1.88
23	2	1.88
30	1.88	1.5
33	1.5	0.88

Table B.5 One-Way ANOVA Calculations for all Substrates, Fall.

Comparison	Source of Variation	SS	df	MS	F	P-value	F critical at $\alpha = 0.10$		F critical at $\alpha = 0.05$	
							Reject?	Reject?	Reject?	Reject?
A = B	BG	0.64	1	0.64	0.23	0.63	2.93	No	4.26	No
	WG	66.22	24	2.76						
	Total	66.86	25							
A = C	BG	147.69	1	147.69	0.82	0.37	2.93	No	4.26	No
	WG	4306.05	24	179.42						
	Total	4453.74	25							
A = D	BG	0.62	1	0.62	0.14	0.71	2.93	No	4.26	No
	WG	103.71	24	4.32						
	Total	104.34	25							
B = C	BG	167.85	1	167.85	0.93	0.34	2.93	No	4.26	No
	WG	4329.51	24	180.40						
	Total	4497.36	25							
B = D	BG	2.54	1	2.54	0.48	0.50	2.93	No	4.26	No
	WG	127.18	24	5.30						
	Total	129.71	25							
C = D	BG	129.10	1	129.10	0.71	0.41	2.93	No	4.26	No
	WG	4367.01	24	181.96						
	Total	4496.11	25							
Legend										
BG: Between Group			df: Degrees of Freedom							
WG: Within Group			MS: Mean Square							
SS: Sum of Squares			F: F-Calculated							

Table B.6 One-Way ANOVA Calculations for each Substrate, Spring and Fall.

Comparison	Source of Variation	SS	df	MS	F	P-value	F critical at $\alpha = 0.10$		F critical at $\alpha = 0.05$	
							F critical	Reject?	F critical	Reject?
A_{spring} = A_{fall}	BG	1045.48	1	1045.48	3.74	0.07	2.94	Yes	4.28	No
	WG	6435.91	23	279.82						
	Total	7481.40	24							
B_{spring} = B_{fall}	BG	0.78	1	0.78	0.25	0.62	2.96	No	4.32	No
	WG	64.80	21	3.09						
	Total	165.59	22							
C_{spring} = C_{fall}	BG	372.70	1	372.70	1.15	0.29	2.94	No	4.28	No
	WG	7439.80	23	323.47						
	Total	7812.50	24							
D_{spring} = D_{fall}	BG	267.28	1	267.28	10.00	0.0045	2.95	Yes	4.30	Yes
	WG	588.06	22	26.73						
	Total	855.34	23							
Legend										
BG: Between Group			df: Degrees of Freedom							
WG: Within Group			MS: Mean Square							
SS: Sum of Squares			F: F-Calculated							

VITA

Sophia Renke was born in Fort Saskatchewan, Alberta, Canada, in 1985 to Wayne and Brygeda, both lawyers. Always having the value of law instilled within her, Sophia became interested in forensic science at an early age. Inspired by a documentary on the Anastasia Romanov investigation, she began researching anthropology by reading Dr. Maples' "Dead Men Do Tell Tales" (Maples and Browning 1994). By grade 12, Sophia's dedication to forensics resulted in a work placement position at the Office of the Chief Medical Examiner of Alberta which transformed into a position as a forensic pathology technician. After trips to Europe to visit archaeological sites and to participate in an ancient grave excavation in Greece, Sophia knew she wanted to pursue archaeology, anthropology, and forensics as a career.

Attending the University of Alberta under advisor Owen Beattie, Sophia completed an Honors Bachelor of Arts in 2007, writing her honor's thesis on human dentition. Sophia was then admitted into a master's program at Louisiana State University on a graduate assistantship. Under advisor Mary H. Manhein, Sophia worked for two years as a graduate research assistant in the F.A.C.E.S. Laboratory and completed her master's thesis in 2010 on using algae to estimate postmortem submersion interval.

In August of 2010, Sophia will begin a joint Juris Doctor and Legum Baccalaureus program at the University of Colorado, USA, and the University of Alberta, Canada. Through the study of law, Sophia plans to use her strong forensic training to have an impact on forensic science in the United States, Canada and abroad.