Laboratory Testing of a Substrate Mechanism for Decay Inhibition and Exceptional Preservation

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Decay Inhibition and Exceptional Preservation

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Abstract

In general, exceptional fossilization requires the inhibition of organic decay, the promotion of authigenic mineralization, or both. Substrate permeability and chemistry, which have long been hypothesized as important controls on such processes, were experimentally modeled in order to test their effects on organic preservation. Standardized masses of muscle tissue (cod: Gadus morhua) were buried in five different substrates: glass beads of three sizes, illite, and kaolinite. The experiments were regularly monitored for one month with infrared gas analysis (IRGA), which quantified the carbon dioxide emitted by each sample and provided a proxy for decay rate. The resultant data revealed a strong positive association between substrate permeability and decay: samples buried in coarse beads emitted more carbon dioxide and reached a maximum emissions rate earlier than samples buried in fine beads. Samples buried in clay evidenced intermediate decay rates: kaolinite replicates emitted more carbon dioxide than their illite counterparts but achieved a maximum emissions rate simultaneously with them. These results indicate that preservation potential is maximized by low permeability conditions that restrict oxidant diffusion; they also suggest that decay rate is sensitive to different chemical environments. CT scanning of selected samples revealed mineral precipitation, though not soft tissue replication, within the pore spaces of the fine bead, medium bead, and illite replicates; such precipitation, if pervasive enough, may restrict system permeability and thus serve as a further check on oxidant diffusion and decay.

Introduction

Taphonomic decay experiments play crucial roles in deducing the mechanisms behind exceptional fossilization (Behrensmeyer and Kidwell, 1985). By studying taphonomic biases in controlled laboratory settings, paleontologists can better identify their presence in the fossil record and thus shed light on how the twin processes of decay and mineralization regulate preservation (Briggs, 1995). Yet while much work has been done on clarifying each of these factors individually, the exact relationship between them remains elusive. While exceptional fossilization requires the inhibition of decay (Allison, 1988; Briggs and Kear, 1994), it also generally necessitates the promotion of authigenic mineralization, which is encouraged by microbial activity (Briggs, 2003). Thus, decay and mineralization are not strictly inverse processes; rather, they are to some extent directly associated, with an initial amount of the former
needed for the latter (Briggs and Kear, 1993a; Sagemann, 1999). The complete elucidation of their interaction will necessitate the refinement of existing experimental models, which will enable the clarification of more specific taphonomic questions (Briggs, 1995). Sediment will almost certainly feature prominently in any such developments, with its consideration as a key taphonomic parameter promising to spur debate on preservational biases previously left unexplored.

Although substrate conditions likely represent an important control on organic preservation (Butterfield, 1995), their experimental modeling is difficult and has generally been avoided by paleontological investigators. Taphonomic experiments typically measure decay through the assessment of the selected organism’s morphological decomposition; burial within sediments essentially renders such diagnoses impossible, with experiments that attempt to surmount the difficulties of excavation losing much of their precision (Allison, 1988; Briggs and Kear, 1994). The investigation by Plotnick (1986) of arthropod preservation potential represents perhaps the only taphonomic attempt to describe substrate-based decay using entirely qualitative methods, with the results demonstrating the shortcomings of doing so. Having initially buried shrimp carcasses on an open beach, Plotnick (1986) found himself unable to relocate them for excavation and consequently repeated the experiment in a more controlled laboratory setting, sieving his experimental sediments to model ideal substrate conditions. Unfortunately, his sampling regimen—repeatedly excavating, examining, and then reburying the carcasses over the course of four weeks—hastened disarticulation and rendered his morphological observations suspect. This sort of methodological inability to account for sediment has precluded its full investigation and, as Butterfield (1995) notes, thus implicitly validated its characterization as an “inert packing material” unworthy of study. Overcoming such prejudices will require experimental proof that substrate conditions influence decay, which in turn will necessitate the development of innovative sampling methodologies capable of producing such evidence (Briggs, 1995).

Researchers following Plotnick (1986) have almost uniformly attempted to measure substrate-based decay using quantitative techniques, eschewing his morphological observations for more robust analyses. While these have generally been more successful, Allison’s investigations of substrate anoxia (1988; 1990) demonstrate that even quantitative sampling methodologies have sedimentary limitations. Having noted the futility of morphologically
diagnosing excavated carcasses, Allison (1988) chose to measure decay as a function of weight loss by massing discrete tissues before and after burial. Less subjective than traditional techniques, this methodology partially surmounted problems of sediment-related disarticulation and has since been incorporated into both substrate-based and aqueous experiments (Briggs and Kear, 1993b; 1994; Hof and Briggs, 1997; Sagemann et al., 1999). Despite their usefulness, however, weight loss measurements can be biased by osmotic intake (Briggs and Kear, 1994) and authigenic mineralization (Sagemann et al., 1999) of the carcass, both of which can significantly reduce sampling precision. Allison’s definitive attempt (1990) to justify the application of weight-loss measurements to substrate-based decay thus met only partial success: variation between his treatment groups was overshadowed by disparities within them, preventing him from fully describing a relationship between substrate and decay. While Allison (1990) accounted for this disappointment by citing the environmental heterogeneity of his outwardly homogenous sediments, it is entirely possible that his methods were compromised by the experimental error inherent to even the most conscientious excavation. Weight-based methods, after all, require the physical handling of the experimental tissue during analysis and are thus by definition invasive. The struggles of Plotnick (1986) demonstrated that substrate sampling can only preserve experimental integrity if conducted remotely; Allison’s experiments, though well-designed and executed, ultimately serve to validate this maxim.

In this experiment, we test Allison’s (1990) suggestion that variations in substrate permeability control microbial decay through the regulation of oxidant diffusion to buried tissues. Gaines et al. (2005; 2012) would later propose an expanded version of this hypothesis as the central tenet of their model for Burgess Shale-type preservation, arguing that the restricted permeability of such deposits so inhibited bacterial activities that soft tissues survived decay as kerogenized carbon films (Gaines et al., 2008). While this mechanism is theoretically plausible, to our knowledge it has yet to be assessed in an experimental capacity, most likely because investigations of permeability must be conducted in the presence of sediment. In testing a permeability model for decay inhibition and exceptional preservation, then, this experiment seeks to advance the paleontological understanding of substrate-based taphonomy in general, which we do through the exploitation of non-invasive sampling methodologies developed for that purpose. Infrared gas analysis (IRGA) and x-ray computed tomography (CT-scanning) are presented here as effective techniques for assessing substrate-based decay and mineralization,
allowing for its various parameters to be controlled between treatment groups and accurately modeled as potential preservational biases. IRGA sampling in particular opens up new lines of taphonomic investigation, with the results derived from its analyses serving as the foundation for the conclusions drawn from this experiment.

**Methods**

**Experimental Setup**

Fresh Atlantic cod (*gadus mortua*) was selected to serve as the experimental decay tissue and was procured from a local fish market. Unpublished pilot projects previously initiated by these investigators had used whiteleg shrimp (*Penaeus vannamei*) as the decay tissue due to its known taphonomic decay sequence (Allison, 1988; Briggs and Kear, 1993a; Briggs and Kear, 1994; Hof and Briggs, 1997; Plotnick, 1986) and concordance with taxa commonly found in Burgess Shale-type deposits (Caron and Jackson, 2008). Given that this experiment avoided morphological diagnoses and did not seek to replicate Burgess Shale-type conditions, however, alternative decay tissues were acceptable. Cod was chosen because its muscle tissue could be precisely divided into numerous identically-sourced tissue cubes, ensuring the standardization of the organic material decayed in the various experimental units.

Substrate conditions were modeled with five different sediments: silica beads of three sizes (fine = 40-90 µm, medium = 90-135 µm, and coarse = 400-600 µm) and kaolinite and illite clays. Five experimental treatment groups were thus established, with each corresponding to one of the experimental sediments. The fine bead, medium bead, kaolinite, and illite groups each contained four individual replicates, with the coarse bead group only containing three due to supply limitations. Thus, nineteen experimental replicates were created in total. In each of these, a 0.2 gram tissue cube was encased between two 8-milliliter layers of sediment, inoculated with a pinch of Long Island Sound mud, and then flooded with an additional 11.5 milliliters of artificial seawater, bringing the total volume of each sample to 27.5 milliliters (see Figure 1 for schematic representation). The 50-milliliter centrifuge tubes containing the experimental replicates were then sealed and incubated at room temperature. The tissue cubes encased within each replicate were massed before burial in order to ensure that they did not vary by more than
twenty percent from the standard mass; after preparation, they remained undisturbed for the duration of the experiment.

In addition to the five experimental treatment groups, one control group was also established. In these replicates, exposed tissue cubes were inundated without sediment in 27.5 milliliters of artificial seawater. Ten such replicates were created, with their masses varying: four contained 0.20 grams of tissue, three 0.10 grams of tissue, and three 0.30 grams of tissue. An eleventh control replicate was prepared with no tissue at all. Like the experimental replicates, the control replicates were sealed after preparation and left undisturbed; the tissue cubes sank to the bottom and remained there for the duration of the experiment.

The artificial seawater used in the experiment was created by mixing 0.5 cups of artificial calcium powder with 1 gallon of deionized water (proportionally, 0.25 cups calcium powder with 0.5 gallons of water, or 59 milliliters calcium powder with 1893 milliliters of water). This mixture was inoculated with 140 milliliters of seawater taken from the Long Wharf beach in New Haven to ensure the presence of bacteria; 30 milliliters of solid calcium chloride pills were also mixed in to encourage mineral precipitation. Supplementary analysis via ion chromatography (IC) and inductively coupled plasma optical emission spectrometry (ICP-OES) revealed that this solution contained no ionic components uncommon to natural seawater (Pilson, 1988; chapter 4).

Analyses I: Decay Rate Measurements

Infrared gas analysis (IRGA) was used to measure the rate at which carbon dioxide was emitted from each sample. IRGA measures the interference by gas samples on projected infrared wavebands; because carbon dioxide readily absorbs such wavebands, discrepancies between the IRGA beam projector and detector reflect its concentration (Mulkey and Smith, 2008). The
integral values that result can be correlated with a standard gas solution to calculate the parts per million concentration and total flux volume of any carbon dioxide present. The IRGA model used, an LI-7000 CO₂/H₂O analyzer from Li-COR Biosciences, can measure carbon dioxide concentrations to the individual micromole and is therefore a powerful tool for quantifying gas fluctuations within closed environments. While many IRGA-based experiments have studied photosynthetic gas exchange (Long et al., 1996), some have focused on microbial-induced soil respiration (Bekku et al., 1995), a phenomenon similar to the experiments conducted here.

IRGA analysis was conducted over a period of thirty-three days, with Day 1 sampling initiated twenty-four hours after sample preparation. Because high carbon dioxide concentrations reduce IRGA accuracy, sampling was performed as often as possible, daily during peak carbon dioxide production and generally at least three times per week. To prevent the buildup of excess carbon dioxide, the centrifuge headspaces were flushed for three minutes with carbon dioxide-free gas following every sampling event. Therefore, carbon dioxide concentrations were interpreted as rates rather than total volumes, standardized as milliliters of carbon dioxide produced per hour per gram of fish. Replicates remained sealed for the duration of IRGA analysis, with invasive sampling not initiated until SEM analysis.

Analyses II: Mineral Precipitation Measurements

Immediately following IRGA sampling, selected replicates were analyzed by x-ray computed tomography (CT-scanning) in order to identify any mineral precipitation that had occurred during decay. One sample from each of the five experimental groups and one sample from the 0.2 gram control group were imaged using an eXplore CT 120 micro-CT scanner from General Electric, which captured image slices at resolutions of 40 micrometers throughout the entirety of each replicate. The resultant frames were used to select promising samples for supplemental analysis by scanning electron microscopy (SEM), which was undertaken with the goal of capturing images of bacterial pitting and authigenic mineralization similar to those produced by Briggs and Kear (1994) and Hof and Briggs (1997).

Structures of interest revealed by SEM were further investigated by energy dispersive x-ray spectroscopy (EDS), which conducted rudimentary diagnoses of elemental presence or absence. Although EDS could not quantitatively measure elemental concentrations, the relative data it provided was used to infer the chemical composition of selected regions. Similar
techniques were previously exploited by Gaines et al. (2005) and Lin et al. (2011) to infer the compositions of mineralized fossil tissues. Unlike CT-scanning, SEM and EDS analysis required the physical excavation and manipulation of the sample sediments, which was carried out by hand with the use of precise picking tools and micrometer-scale sieves.

Results

Results I: Decay Rate Data

Summary

Initial permeabilities of the silica bead replicates were calculated using the Rumpf and Gupte (1975) model, \( k = \left( \frac{\varepsilon}{5.6} \right) d^2 \), where \( k \) is the permeability, \( \varepsilon \) the porosity, and \( d \) the diameter of the spherical particle. Bead porosity was estimated as 0.383, the reference value given by Dullien (1992) for poured, randomly packed spherical particles; grain diameters were averaged to 500 µm for the coarse beads, 112.5 µm for the medium beads, and 65 µm for the fine beads. Thus, the bead permeabilities were estimated as 227.69 µm² for the coarse beads, 11.53 µm² for the medium beads, and 3.85 µm² for the fine beads. As Andersen and Kristensen (1992) demonstrate, sediment porosity can be more exactly calculated by capturing the evaporation of infiltrated water; given the aims of this experiment, however, scientific estimates were sufficient.

Individual replicates within each experimental treatment group emitted similar amounts of carbon dioxide at the same rates and were thus averaged together to produce trendlines indicative of their general decay progressions (complete sampling records can be viewed in Appendix I). The peaked nature of the carbon dioxide emission rates observed in nearly all samples corroborates other charted accounts of quantitatively-measured decay (Allison, 1990; Andersen and Kristensen, 1992) and suggests that, under idealized conditions, organic matter can survive indefinitely. No tissue masses were fully dissipated over the course of the experiment; outside the laboratory, of course, natural processes recycle organic matter on a time scale far quicker than the one here (Plotnick, 1986).

Silica Bead Replicates

The silica bead replicates evidenced two general decay progressions (Figure 2). While the coarse bead samples produced more total carbon dioxide than the medium bead samples, their graphs mirrored each other in slope and peaked simultaneously between Days 14 and 15, at 110
microliters of carbon dioxide per hour per gram of fish each. These results contrasted sharply with the fine bead samples, which produced far less carbon dioxide throughout the course of the experiment and never reached a conclusive emissions peak. The greatest carbon dioxide production rate achieved by the fine bead samples, reached on Day 31, was only 19 microliters of carbon dioxide per hour per gram of fish; given their group’s overall emission trend and the cumulative amount of carbon dioxide emitted by the other samples, this value likely represents a localized peak rather than a true maximum in decay.

Figure 2. Silica bead carbon dioxide emissions, with decay rates of fine bead, medium bead, and coarse bead samples.


*Clay Replicates*

In general, the kaolinite samples produced more carbon dioxide than the illite samples, with the discrepancy most pronounced during the first half of the experiment (Figure 3). However, the emissions rates for both clays mirrored each other closely in terms of their general trend. Both groups peaked in production on Day 15, with the kaolinite samples averaging 83 microliters of carbon dioxide per hour per gram of fish and the illite samples averaging 53 microliters per hour per gram of fish.

![Figure 3. Clay carbon dioxide emissions, with decay rates of illite and kaolinite samples.](image-url)
Control Replicates

The control samples evidenced differential carbon dioxide emissions based on their masses and were graphed accordingly (Figure 4). The 0.1 and 0.2 gram samples followed each other’s emission trends closely, with the 0.1 gram samples producing slightly more carbon dioxide before peak production but the 0.2 gram samples producing more afterwards. Both groups peaked on Day 9, at 216 and 217 microliters of carbon dioxide per hour per gram of fish respectively. In contrast, the 0.3 gram samples peaked on Day 15 at 145 microliters of carbon dioxide per hour per gram of fish. Until this point, its carbon dioxide emission rates had been substantially less than those of the 0.1 and 0.2 gram groups; however, its trendline surpassed their graphs on Day 16 due to its less pronounced post-peak decline. As expected, the control replicate that contained no tissue produced negligible amounts of carbon dioxide compared to the others, and was consequently not graphed.

Figure 4. Control carbon dioxide emissions, with decay rates of 0.1, 0.2, and 0.3 gram tissue samples.
Results II: Mineral Precipitation Data

CT-Scanning Evidence of Mineral Precipitation

CT-scan images were defined by a grayscale density gradient in which regions of higher density were shaded lighter in color than regions of lower density. Consequently, empty air pockets appeared darkest, followed by the slightly lighter fish tissue (approximately 1.05 g/cm³ (Alexander, 1958)) and the various experimental sediments (silica beads approximately 2.5 g/cm³ according to their manufacturers; kaolinite clay approximately 2.65 g/cm³ and illite clay approximately 2.75 g/cm³ (Totten et al., 2002)). The brighter flakes and specks that appeared in some of the samples were of a different hue entirely and were consequently interpreted as evidence of mineral precipitation. Two lines of evidence supported this conclusion. First, the densities of many authigenic minerals commonly produced in decay experiments exceed those of the experimental sediments and would thus have a lighter grayscale shading. Second, authigenic minerals reported in the literature have a size consistent with the high-density regions observed here, which at 40 to 135 micrometers in diameter fell within the general range reported by Briggs and Kear (1994) and Hof and Briggs (1997). SEM imaging would subsequently indicate that the individual mineral crystals present in the samples rarely exceeded lengths of 10 micrometers, suggesting that the high-density regions evidenced by CT-scanning represented hotspots of crystallization rather than specific mineral grains themselves.

CT-scanning did not reveal mineral precipitation in all of the experimental treatment groups. While prevalent in the fine bead, medium bead, and illite samples (Figures 5-7), high-density regions were wholly absent from the coarse bead and kaolinite ones (Figures 8-9), resulting in those samples’ being studied more cursorily during SEM analyses. The fish tissue itself was never observed to be mineralized in any of the treatment groups and was consequently excluded from all SEM analyses; instead, clumped and discolored sediments were preferentially excavated due to their potential to represent mineral cementation. The replicates that provided these sediments had been allowed to incubate for roughly 120 days following CT-scanning; it was hoped that this delay would allow for the growth and further proliferation of any precipitated mineral crystals.
Figure 5. CT-scan of fine bead replicate. Transverse orientation. Scale bar approximately 1 centimeter. Lighter regions of high gravity suggest potential sites of mineral precipitation. Central structure represents fish tissue surrounded by air pockets.

Figure 6. CT-scan of medium bead replicate. Transverse orientation. Scale bar approximately 1 centimeter. Lighter regions of high gravity suggest potential sites of mineral precipitation. Darker regions represent fish tissue.
Figure 7. CT-scan of illite replicate. Transverse orientation. Scale bar approximately 1 centimeter. Lighter regions of high gravity suggest potential sites of mineral precipitation. Darker regions alternately represent fish tissue or air pockets.

Figure 8. CT-scan of coarse bead replicate. Transverse orientation. Scale bar approximately 1 centimeter. No evidence of mineral precipitation. Darker regions represent fish tissue.
SEM and EDS Identification of Mineral Precipitation

Although SEM analysis failed to reveal any mineral precipitation within the illite, kaolinite, or coarse bead samples, numerous mineralized structures were observed throughout the medium and especially fine bead samples. These were classified as representing three different crystal morphologies and were subsequently recognized as carbonate minerals. EDS analysis was helpful in validating mineral identifications but suffered interference from the platinum SEM coating, silica glass beads, and evaporative salts that pervaded the sediments.

The first crystal morphology, mineralized spars normal to or flushed against the bead surfaces (Figure 10), had been previously reported in taphonomic experimentation by Briggs and Kear (1994) and was identified as acicular aragonite. These minerals formed radiating bundles but were also manifested as independent crystal needles whose terminal ends were occasionally squared off rather than pointed (Figure 11), a characteristic described by Flügel (2010; pg. 292) as diagnostic. EDS analysis supported this interpretation by returning peaks for both calcium and oxygen (Figure 12).
Figure 10. Low resolution SEM image of fine bead sample, with sediment surfaces evidencing pervasive acicular mineral precipitation.

Figure 11. High resolution SEM image of fine bead sample. Acicular mineral precipitation interpreted as aragonite needles and bundles. Central glue-like structure likely represents evaporative salt, as suggested by EDS.
Figure 12. EDS analysis conducted on region imaged in Figure 11. Calcium and oxygen peaks suggest the presence of a carbonate mineral, interpreted as aragonite.
The second crystal morphology, a flat, pitted pavement that coated the bead surfaces (Figure 13), was identified as sheeted calcite. This conclusion was validated by the amorphous crystallization and porous texture of the mineralization, which matched descriptions for calcite bundles previously provided by Briggs and Kear (1994). EDS analysis supported this interpretation by returning peaks for both calcium and oxygen (Figure 14).

Figure 13. High resolution SEM image of fine bead sample. Pavement-like mineral precipitation interpreted as sheeted calcite.

Figure 14. EDS analysis conducted on region imaged in Figure 13. Calcium and oxygen peaks suggest the presence of a carbonate mineral, interpreted as calcite.
The third crystal morphology, a closely packed pavement of stubby crystals with triangular cross-sections (Figures 15-16), compared favorably with the high-magnesium calcite cement described by Flügel (2010; pg. 290-291). EDS analysis conducted on this morphology returned unique magnesium peaks in addition to oxygen and calcium ones (Figure 17), strongly suggesting the validity of the identification.

Figure 15. Low resolution SEM image of fine bead sample, with sediment surfaces evidencing pervasive pavement-like mineral precipitation.
Figure 16. High resolution SEM image of fine bead sample. Dense, trigonally-structured mineral precipitation interpreted as magnesium calcite.

Figure 17. EDS analysis conducted on region imaged in Figure 16. Calcium, oxygen, and magnesium peaks suggest the presence of a carbonate mineral, interpreted as magnesium calcite.
Discussion

Decay and Substrate Permeability

Data derived from this experiment was interpreted with the assumption that the production of carbon dioxide can be used to model decay (as postulated by Andersen and Kristensen (1992) in a more biologically-oriented study). Our control replicates, designed to test this statement, at first glance appear to invalidate it: the heavier tissue masses, expected to undergo the most decay and emit an accordingly proportioned amount of carbon dioxide, were in general outperformed by the lighter ones (Figure 4). This contradiction, however, can be explained by the surface to volume ratios of the cube-shaped tissue masses. Because of their size, the smaller tissues would have been more fully penetrated by bacteria earlier than the larger ones; consequently, they initially emitted more carbon dioxide. By the end of the experiment, bacteria had thoroughly infiltrated all the tissue samples, resulting in the manifestation of the expected carbon dioxide trends. Ultimately, then, the control replicates indicate that carbon dioxide emission rates can indeed be accepted as a relative proxy for decay, legitimizing the conclusions subsequently drawn from IRGA data.

Our experiments validate the hypothesis that permeability and decay are related by a strongly positive association (Gaines et al., 2005; 2012). The relatively impermeable fine bead replicates clearly emitted less carbon dioxide than the medium bead replicates, which in turn emitted less carbon dioxide than the coarse bead replicates (Figure 2). Because all other experimental variables (tissue mass, water composition, bacterial load, sunlight exposure, etc) were standardized across the various experimental treatment groups, this trend indicates the potential of permeability to serve as a major control on the preservation of organic matter (as initially predicted by Allison (1990)). That the control replicates underwent by far the most decay (Figure 4) only further validates this conclusion: with their tissue masses exposed to infinitely permeable conditions, these samples allowed for unrestricted oxidant diffusion and consequently decomposed to the furthest extent.

Despite the overall strength of the IRGA data, the inconsistent correlation between the specific permeabilities and decay rates of the various treatment groups is somewhat problematic. While the fine and medium bead replicates were far closer to each other in permeability than either was to the coarse bead replicates, their carbon dioxide emission rates are surprisingly
divergent, with those of the medium beads mimicking the coarse beads (Figure 2). Partially accounting for this discrepancy is the possibility that some of the carbon dioxide emitted by the fine bead samples remained trapped in the sediment and thus escaped IRGA measurement, as indicated by CT-scan evidence of subterranean air pockets (Figure 5). A more intriguing explanation, however, is that there exists some minimum level of permeability above which oxidant diffusion (and thus decay) proceeds relatively uninhibited. Though ostensibly unlikely, a threshold of this sort would not necessarily need to fall directly between the medium and fine bead permeabilities: in theory, it could lie beneath both of them, with the otherwise disproportional decay inhibition evidenced by the fine bead samples facilitated by mineral precipitation.

Decay and Mineral Precipitation

Decay has long been associated with the precipitation of calcium carbonate and has more recently been recognized as a key controlling factor in its formation (Berner et al., 1970; Chafetz and Buczynski, 1992; Briggs and Kear, 1994). Bacterial decay reactions (see Berner, 1981) initially lower and then subsequently elevate pH levels, resulting in an early buildup of carbonate saturation that subsequently leads to precipitation (Briggs and Kear, 1994; Hof and Briggs, 1997). Mineral cations can be derived either from the environment or from the decaying organism itself, with the magnesium and calcium observed in this experiment perhaps originating in the artificial seawater (Briggs, 2003). That calcium phosphate precipitation was not observed is somewhat surprising, given that fish tissue can provide a steady source of phosphate ions (Briggs and Kear, 1993a). However, Sagemann et al. (1999) reported that calcium phosphate stabilizes at a lower pH than calcium carbonate, suggesting that any precipitation may have been recrystallized as calcium carbonate during the ensuing pH rebound, before sampling occurred. pH meters are commonly applied to taphonomic experiments to monitor changes in sample alkalinity (Briggs and Kear, 1994; Sagemann et al., 1999); their use here, however, would have been invasive and was thus rejected as non-essential.

While numerous taphonomic experiments have induced the mineralization of soft tissue decayed in aqueous conditions (Briggs and Kear, 1993a; 1994; Hof and Briggs, 1997), this experiment is unique in that it evidences how mineral precipitation within the substrate can also affect decay. CT-scanning and SEM analysis of the fine bead replicates evidenced no
mineralization of the decaying tissue itself; rather, mineral precipitation occurred on the surfaces of the silica beads in the surrounding sediment pores. Though these were never fully occluded, the pervasive deposition of carbonate within them (Figures 10-11; 13; 15-16) would likely have increased the tortuosity of the decay microenvironment and thus restricted its permeability, perhaps depressing it below some critical threshold required for efficient oxidant flow. Such a scenario would explain why the fine bead replicates underwent far less decay than the medium bead replicates (Figure 2): though the two treatment groups had similar initial permeabilities, mineral precipitation would have reduced the overall porosity of the fine beads to a greater proportional extent and thus altered their decay progression more significantly. The mechanism for Burgess Shale-type preservation proposed by Gaines et al. (2005; 2012) envisioned similar conditions in which low original porosity was exacerbated by the precipitation of micron-scale cements during the early stages of decay. This experiment thus demonstrates the potential validity of their model and sheds light on factors that may clarify other examples of exceptional preservation, most notably concretions.

Carbonate concretions are formed by the precipitation of carbonate cements around a central nucleus, the decay of which may provide the super-saturation of ions necessary for localized mineralization (Raiswell and Fisher, 2000). McCoy et al. (in press) argue that such mineralization can decrease the permeability of the decay microenvironment and thus restrict oxidant flux, initiating a positive feedback cycle that further inhibits decay and increases localized preservation potential (Figure 18). Viewed in tandem with the carbon dioxide data, the mineral precipitation observed here supports this hypothesis. Three-dimensional reconstructions of the experimental CT-scans evidence that mineralization hotspots (when present in the fine bead, medium bead, and illite replicates, respectively Figures 5-7) surrounded the decaying tissue in all dimensions, developing in both proximal and more distant vicinities (Figure 19). Because the small size of the experimental vessels imposes an artificial boundary on the decay microenvironment, it is difficult to extrapolate how far such mineral precipitation might have extended had it been spatially unchecked. Nonetheless, this globular pattern of mineral precipitation evokes comparison to concretionary growth via pervasive or (depending on the ultimate size of the decay microenvironment) outwardly-radiating concentric growth (Raiswell and Fisher, 2000). True concretions have never been successfully induced in laboratory conditions (Berner, 1968), but most attempts have not incorporated sediment into their
experimental setups. The results presented here indicate that restricted permeability may be a crucial prerequisite for concretionary preservation, with substrate a necessary component of its modeling.

Figure 18. Illustration of positive feedback between sediment permeability and concretionary preservation. Figure from McCoy et al. (in press).
Clays

Decay and Substrate Chemistry

Unlike the silica bead replicates, the clay samples could not be controlled so as to only vary in permeability. Their unpredictable grain shapes, bacterial loads, and elemental compositions made them far more heterogeneous than the other experimental treatment groups and thus difficult to compare to them directly. Though their permeabilities were never explicitly measured, clay particles are smaller than even the fine bead replicates, which approximated very fine sand grains (Wentworth, 1922); nonetheless, both the illite and kaolinite replicates produced more carbon dioxide and peaked earlier than them. While the clay carbon dioxide emissions may have been slightly elevated by the presence of organic matter in their dry sediments (as evidenced by auxiliary controls), their decay rates were still greater than would be expected given their assumedly low permeabilities and therefore merited segregation from the silica bead data. Clay treatment groups were introduced to the experiment in order to model substrate chemistry, not permeability; thus, their decay was interpreted independently.
Butterfield (1995) argued that clay minerals contribute to organic preservation by virtue of their cation exchange capacities, which have been proven effective in absorbing and neutralizing degradative enzymes that might otherwise enhance decay (Mortland and Gieseking, 1951; Butterfield, 1990). Clays would therefore be considered an ideal substrate for exceptional preservation, with their presence at numerous Burgess Shale-type deposits corroborating this hypothesis (Butterfield, 1990; Gaines et al., 2008). This experiment investigated the strength of such claims by comparing the decay of organic tissue in illite and kaolinite; both clays have similarly low cation exchange capacities, respectively 10-40 and 3-5 milliequivalents per 100 grams (Carroll, 1959). Mortland and Gieseking (1951) had previously demonstrated illite’s greater potential to inhibit specific enzymatic reactions; IRGA analysis validates this trend with regards to decay in general (Figure 3). Though numerous uncontrolled factors likely influenced the clay data, the apparent agreement between these experimental results and the literary representation of clay cation exchange indicates that sediment-organic interactions may indeed play important roles in decay inhibition. If nothing else, the illite-kaolinite dichotomy conclusively disproves the musings of Allison (1990) that compositionally-different substrates might foster essentially similar decay environments. Despite the probable inconsistencies of their respective permeabilities, the illite and kaolinite decay rates were clearly differentiated (Figure 3), indicating that the imprecision of Allison’s weight-based measurements likely produced more experimental error than his uncontrolled sediments.

Bacterial Analyses

Visual observations were made on the various replicates during IRGA sampling; these were non-invasive and rather involved diagnosing sediment conditions via the centrifuge tubes’ translucent walls. While the silica bead sediments and control sample tissues changed little in appearance, the clay substrates underwent notable color changes throughout the course of the experiment. In several of the illite and kaolinite replicates, the sediment surrounding the decaying tissue had blackened by Day 10, prior to peak carbon dioxide production (Figure 20). Several of the kaolinite samples also developed orange and yellow discolorations that permeated the sediment-water interface and clogged the water column itself (Figure 21). Though the blackened sediment can likely be attributed to anoxia (Allison, 1988), these results were sufficiently intriguing as to merit invasive investigation, which was conducted simultaneously with SEM preparation. Bacterial DNA was extracted from the water columns and sediments of a
single illite and kaolinite replicate; DNA was also extracted from a dry sample of each clay for control purposes. These samples were analyzed by an outside laboratory, which performed microbial diversity analyses on them using tagged pyrosequencing. The data returned percentage and abundance counts for all six samples, revealing their bacterial diversities from the kingdom to species levels.

Preliminary analysis of the microbial data was conducted at the order level for purposes of expediency. Sixty-two bacterial orders were identified in the various samples; however, nine of these composed more than seventy percent of the diversity present in each individual sample (Appendix II). The Bray-Curtis dissimilarity (Figure 22) and Jaccard Index (Figure 23) tests were applied to the entire dataset in order to identify any cluster patterns present; both found concordances between water and sediment samples taken from the same experimental replicate, with the dry control samples identified as closer to each other than either was to its experimental counterpart. This information demonstrates that different clay sediments— even those with similar
initial bacterial loads—foster different bacterial communities, which presumably reflect unique
decay microenvironments and sediment chemistries (Butterfield, 1995). Future analyses will
more fully explore the significance of this data, which has the potential to unite both taphonomic
and bioenvironmental research.
Conclusion

Having quantitatively assessed substrate-based decay in a controlled laboratory setting, this experiment endorses the permeability model for exceptional preservation proposed by Gaines et al. (2005; 2012) and furthermore suggests that sediment chemistry may play a supplementary role in decay inhibition (Butterfield, 1995). The data presented here also helps clarify the “critical balance” between decay and mineralization (Briggs and Kear, 1993a) by indicating that substrate conditions may regulate both. IRGA data evidences that organic decay is at least partially controlled by substrate permeability, but also suggests that permeability itself may be influenced by decay-induced mineral precipitation. A more complete understanding of this relationship will likely require analyses of mineralization more quantitative than the ones performed here, which were limited to presence/absence-type investigations and distributional interpretations. Correlating experimental decay with real-time fluctuations in substrate porosity and permeability would be useful and perhaps possible with the continued development of non-invasive scanning technology. Advances in sampling will thus prove crucial as taphonomic models increase in complexity through the incorporation of sediment and other unexplored variables.

To the best of our knowledge, this experiment represents what may be the most conclusive attempt (rivaled only by Allison (1990)) to characterize substrate-based decay using actualistic methodologies. In providing a permeability-based legitimization for Butterfield’s (1995) general assertion that substrate inhibits decay, its findings have significant ramifications for taphonomic experimentation in general. Unpublished pilot projects previously initiated by these investigators revealed that shrimp carcasses decayed in aqueous systems underwent a different morphological progression than those buried in substrate: the water samples evidenced
more bloating and rupturing but remained intact far longer than the sedimentary ones, superficially resisting fragmentation but disaggregating totally if disturbed (Figures 24-25). Plotnick (1986) interpreted similar results as proof that sediment expedited decay while water prolonged structural integrity. IRGA analysis indicates the opposite, insinuating that aqueous systems may actually obscure rather than elucidate certain aspects of morphological taphonomy. While aqueous setups are convenient for the modeling of preservational biases (of which permeability is just one), their use in the delimitation of taphonomic thresholds specific to individual taxa may inadvertently produce experimental artifacts rather than true representations of natural decay (as noted by Briggs and Kear (1993b)). Laboratory results must be applied to the fossil record with great care; the data presented here underscores the need to recognize experimental parameters as simplified and interpret their results accordingly (Allison, 1990).
Substrate conditions aside, this experiment demonstrates the potential of IRGA for future taphonomic application, either as a supplement to more established methodologies or as a primary analysis on its own. Less subjective than morphological diagnosis and more robust than weight-based measurement, IRGA is most importantly non-invasive, with its analyses supporting the repeated sampling of individual replicates yet still preserving experimental integrity. Furthermore, IRGA sampling is straightforward and easy to conduct, allowing for the rapid testing of numerous samples and thus increasing the statistical power of experiments while minimizing human error. IRGA does have shortcomings; most notably, it cannot distinguish experimentally relevant carbon dioxide from that emitted by more extraneous processes (in this experiment, the decomposition of organic matter likely embedded within the unsterilized clays). Thus, IRGA is probably best employed as a relative rather than absolute measure of decay, with the use of controls ensuring its accuracy. Nonetheless, its relevancy to the measurement of sediment-based decay would appear to be unparalleled. The rise of non-invasive sampling methodologies has the potential to change the way paleontologists think about (or at least
measure) decay— and as this experiment demonstrates, their results can elucidate both sediment-based experimentation and taphonomic investigation in general.

Acknowledgements

Funding for this project was provided by research grants from the Geological Society of America (GSA) and the Paleontological Society. Travel arrangements for the presentation of these results at the GSA Annual Meeting in Denver were jointly covered by a GSA travel grant and the Saybrook College Babcock Travel Research Fellowship. Thanks are due to all for their generosity and commitment to undergraduate research.

While this project was conceptualized within the Derek Briggs laboratory, numerous other researchers provided material and human support crucial for its realization. Most notably, the Mark Bradford laboratory provided IRGA access and training, with Emily Oldfield guiding us through the sampling process. Dr. Zhen Zhuang kindly facilitated the use of a CT-scanner at the Yale Medical School, which was supplemented by work performed in the Gerald Conlogue laboratory at Quinnipiac University. Dr. Ruth Blake and Dr. Jordan Peccia offered consulting on bacterial investigation, with the latter assisting us in DNA extraction. Bacterial analysis was conducted by the Research and Testing Laboratory (Lubbock, Texas), facilitated by Dr. John Hanson. Thanks are also due to Dr. Zhenting Jiang for assisting us with SEM analysis, Dr. Susan
Butts for managing our logistical expenditures, Rachel Racicot for modeling our CT-scan reconstructions, and Dr. Pincelli Hull for offering advice on sampling and serving as the second reader for this paper. Additionally, I would like to convey my appreciation to the Yale University Department of Geology and Geophysics, which has consistently supported my work on this project and offered me a community through which to focus my intellectual development.

As a final note, my two collaborators on this project merit especial mention. I am deeply grateful to my advisor, Professor Derek Briggs, for welcoming me into his laboratory ever since I joined it in 2012 as a summer research assistant. His mentorship has provided me with a wealth of paleontological knowledge and has played a major role in shaping the direction of this project. Above all, I am indebted to Victoria McCoy, who has served as my lab partner and scientific mentor ever since we initiated our first decay experiments last fall. By giving me the chance to participate in her research, Tory has allowed me to gain first-hand experience with the scientific method, shaping my Yale education as much as any of my professors. This project would not have taken place without her expertise, and I am proud to consider myself her “first student.”
Literature Cited


**Appendix I: IRGA Data Sheets**

All values represent milliliters of carbon dioxide produced per hour per gram of fish.

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Appendix II: Clay Bacteria Order Diversity
Values represent percentage abundances of each order within each sample, as measured by Research and Testing Laboratory (Lubbock, Texas).

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Appendix III: Poster Presentation
Presented at the GSA Annual Meeting, October 2013.

Laboratory testing of the influence of substrate on decay inhibition and exceptional preservation
Victoria E. McCoy¹, Robert T. Young¹, and Derek E.G. Briggs²
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1. Introduction
Exceptional fossilization requires the inhibition of decay and/or the promotion of mineralization. Substrate permeability (Gaines et al., 2005; 2012) and chemistry (Butterfield, 1995) are hypothesized as controlling these factors, and were experimentally modeled here in order to test their effects on organic preservation.

2. Methods
1. Cod tissue in five substrates: three sizes of glass beads; illite; kaolinite.
2. Infrared gas analysis (IRGA): measured carbon dioxide emission; proxy for decay.

3a. IRGA Results
Sediment permeability: higher permeability replicates (larger grain size) emitted more carbon dioxide than lower permeability replicates (smaller grain size), peaking earlier.
Sediment chemistry: kaolinite replicates emitted more carbon dioxide than illite replicates, peaking simultaneously.

3b. Bacteria Results
Different colors of bacterial growth in kaolinite and illite suggests the development of different microfaunas.
Microbial diversity analyses pending.

3c. Micro-CT Scanning Results
No soft tissue mineralization.
Mineral precipitation within sediment in the fine beads, medium beads and illite.
No mineral precipitation in coarse beads or kaolinite.

4. Conclusions
Preservation potential maximized by low permeability sediments.
Different chemical environments foster specific decay microenvironments.
No soft tissue mineralization, but negative association between organic decay and mineral precipitation suggests general trend.

5. Acknowledgements
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