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A Multidisciplinary Validation Study of Nonhuman Animal Models for Forensic Decomposition Research

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Final Report

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Purpose and Objectives of the Project

Over the past century of scientific inquiry into the process of decomposition, nearly every mammal (and other taxa) has been studied. The earliest published studies, dating to the mid-19th century, were ecological studies designed to look at insect succession and development rates or insect behavior. For such studies, the type of carrion used was relatively inconsequential and depended upon what animals were locally available of the desired size. Some of the resulting theories and techniques of these approaches have been applied to forensic science. For instance, ecological entomology studies conducted on a wide range of carrion species has provided a basic scientific framework of insect succession and development that is the foundation of forensic entomology. Similarly, the morphological stages of decomposition were first described from observations of dogs (Reed 1958), pigs (Payne 1965) and guinea pigs (Bornemissza 1957). A potential problem may arise, however, when data derived from animal studies are applied to explain decomposition phenomena in forensic casework of human remains, particularly the postmortem interval.

Our research informs an ongoing important debate about the applicability of nonhuman animal models in forensic research and its probative value in casework. Some argue that pigs are appropriate proxies for human subjects based on size, hair coverage and arthropod succession (e.g., Schoenly et al. 2007). Our project determines if nonhuman animals exhibit the same forensically important characteristics as humans, such as arthropod succession, scavenging behavior, and morphological changes if external environmental factors are held constant.

To date, attempts to address this question has been few and small-scale. Schoenly and colleagues (2007) placed two pigs and one human at the Anthropology Research Facility in Tennessee for 35 days. They found limited differences in anthropod preference between species and concluded that they "confirmed the claim that pig carcasses (of 23–27-kg starting mass) can substitute for human corpses in research and training programs, at least for summer-exposed and unconcealed remains in the first 5 wk

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postmortem (2007:881)." Their oft-cited study to justify pig data as proxies for humans lacked replicates and covered only one season. Our research design is a comparative study of decomposition of 45 pig, rabbit and human subjects to assess if overall decomposition patterns and rates differ between carrion species and quantify any differences in scavenger and insect abundance, diversity and behavior across three seasons.

The specific objectives of this project were to:

1) Apply the Total Body Scoring (TBS) system to make quantitative morphological comparisons between species during decomposition. The TBS system incorporates temperature and time such that individuals and species can be compared on the same scale.
2) Assess insect taxonomic diversity and density differences among individuals and species.
3) Evaluate if scavenger species and behavior differs among subject species and individuals.

Project Design

The project was conducted at the Anthropology Research Facility at the University of Tennessee, Knoxville. This project compares five human, five domestic pig (*Sus scrofa*), and five domestic rabbit (*Oryctolagus cuniculus*) subjects in each of three seasonal trials. Human subjects were enrolled in the study if the weights were under 250 pounds and the bodies were not autopsied or otherwise exhibited external trauma. Pigs and rabbits were supplied by local farms and were euthanized by a veterinarian. Target animal weights were between 100 and 150 pounds for pigs and over six pounds for rabbits (see Appendix 1). The protocols for animal handling and euthanasia were approved by the University of Tennessee Institutional Animal Care and Use Committee (IACUC) Protocol Form. All subjects were placed in a 38ºF morgue cooler for at least 24 hours before placement to equalize body temperature.

The trials occurred during three different seasons (spring, summer and winter) and microenvironments within the ARF. The 15 subjects in each trial were placed in transects within the same microenvironment within the ARF following a randomized block design (Appendix 2). The subjects were placed a minimum of three meters apart. Temperature and humidity data loggers were placed evenly along the transect to collect immediately local climatic conditions and six game cameras

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were established in the area to capture scavenger activity. Rabbits were placed in wire cages to prevent scavengers from removing them from the study area as one of our specific objectives was to examine insect activity variation by species.

Climatological information was obtained from core body temperature, ambient temperature and humidity adjacent to the subjects, and daily mean temperatures from a nearby weather station. Core body temperatures were collected hourly via a rectal probe and data logger, though ultimately these data were not useful due to rapid equilibrium with ambient temperature. Site-specific ambient temperature and humidity were collected one meter above ground level adjacent to the subjects using temperature/humidity data loggers. Daily temperature, rainfall and humidity data were collected from the University of Tennessee Agricultural Campus Weather Station located approximately 0.5 miles from the ARF. All temperature data were converted to Accumulated Degree Hours (ADH) to follow standard practice in entomology (Byrd and Castner 2010) and anthropology (Simmons et al. 2010b). Only temperatures above 0ºC were included in ADD calculations (Vass 2011, Megyesi et al. 2005).

The progression of decomposition and photographic documentation was recorded twice daily (AM and PM) for each subject. The Total Body System (TBS) scoring method, which evaluates tissue coloration, bloating and loss on each body segment independently, was recorded following Megyesi et al. (2005) (see Appendix 3 for the scoring system and data collection form).

Documentation of insect activity was recorded at each observation event. This includes adult insect arrival, presence of fly eggs and immature insects, fly larval migration and emergence of adult flies. Adult flies were collected using aerial sweep nets and then preserved in 70% ethanol. Fly larvae were collected using forceps and then placed in KAA. Other arthropods, including beetles, were preserved in 70% ethanol. Preservation methods from Catts and Haskell (1990) were followed. Adult and larval flies were classified to genera at minimum, and species level where possible. All identifications are made using standard taxonomic keys and under the guidance of an entomologist (Amendt et al., 2011). The results of the taxonomic study will be forthcoming.

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To determine whether scavenger activity differs among the pigs and humans, six infrared, motion sensing Moultrie GAMESPY i60 digital game cameras were strategically placed across each transect to detect motion by a scavenger until the late stages of decomposition. The cameras were moved as physical signs of scavenging were noted during the daily observations.

The relationship between TBS and PMI for each species were calculated in two ways. First, ADD was calculated from TBS using the formula, $ADD = 10^{(0.002*TBS*TBS+1.81)} \pm 388.16$ (Megyesi et al. 2005). This was then plotted against the actual ("real") ADD determined from the average daily high and low temperatures that were added together for the course of the trial. Second, a fuzzy clustering technique was applied to compare decomposition rates across the species. This technique is similar conceptually to discriminant analysis in that it helps to analyze group membership. It is unique in that it allows for the possibility of an observation belonging to more than one group. The variables were TBS and actual ADD. This technique was selected so that data from all three trials could be analyzed simultaneously, even though it represents 45 distinct individuals. The clustering analyses were conducted using NCSS 2007 statistical software. The technique allows the researcher to select how much overlap or how discrete the groups should be kept. For these analyses, groups were allowed to overlap, and observations could have equal probability of belonging to more than one group.

Results

The chart in Appendix 4 demonstrates that the three trials did capture seasonality. Trial 1 took place in the spring and began prior to insect activity, while Trial 2 began and ended (quickly) during peak insect activity in June. Trial 3 was in the winter and, while the average temperature is 0°C, many days dipped below 0°C, including several snowfalls. Data were taken for each trial until 2000 ADH was reached, which in Trial 2 took only 16 days, though photos and insect samples were collected until day 45 when most subjects had skeletonized (400 ADD).

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Decomposition Trends Between Species

The first step of our analysis examined whether the ADD estimated from the Total Body Scoring (TBS) system accurately tracks the real ADD based on the known time of placement of the body. The mean ADD estimated by the Megyesi TBS score was compared to the known ADD beginning with the date of placement at the ARF. The trends for each species were observed by averaging the estimated TBS scores for each individual for each day of the trial and then smoothed.

None of the estimated ADD of any species had a strong correlation with real ADD in Trial 1, but the patterns between the species are also distinct (Appendix 5). The rabbits had an early decomposition rate that was much faster than that of either the pigs or humans. The pigs and humans tracked together well until insect activity began in earnest about 25 days into the trial and the pigs began to skeletonize faster than the humans. The human subjects had already mummified such that when insect activity did begin the flies were largely uninterested in non-fresh tissues and large maggot masses did not form. The pigs did not mummify, exhibited body-encompassing maggot masses, and therefore skeletonized more rapidly. This trend is best represented by the lower graph in Appendix 5. The estimated ADD of the humans largely underestimated the real ADD while the opposite is true for the rabbits early and most of the pigs later in decomposition.

Trial 2 was in the summer and the graph in Appendix 6 illustrates that pig decomposition occurred more rapidly than that of the rabbits and humans. Insect oviposition was immediate and the pigs skeletonized faster than the other species. While the human remains were affected by insects, the progression towards skeletonization was slower than in the pigs and the soft tissue of the humans often mummified.

A much different pattern emerged in Trial 3 (Appendix 7). Human decomposition occurred at a much faster rate than that of the nonhuman species. Moreover, one human subject in particular skeletonized more quickly than any of the other subjects. Trial 3 began on December 1 and no insect

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activity occurred for over 100 days so the faster human decomposition rate is due solely to scavenging (see below).

The results from fuzzy clustering analysis offer more fine-grained insight into these general trends. In order to have a finer scale for the analyses, the data were broken into subsets: ADD less than 100, 100-300 ADD and 300-400 ADD. 400 ADD was used as the cutoff because the second trial ended at 400 ADD.

The best solution for the early decomposition rates (under 100 ADD) was a two cluster option (Appendix 8). The pigs and humans made up the majority of the first cluster, with the second cluster being composed mainly of rabbits. The best solution was identified by three goodness of fit statistics; an average silhouette, Dunn's coefficient, and Dc(U). The first two statistics are maximized, while the third is minimized when the solution is consistent. For 100-300 ADD, the best solution was again two clusters (Appendix 9). Similar to the previous analysis, the pigs and humans showed much more similarity than either group to the rabbits. This pattern is also true for ADD 300-400 (Appendix 10) in that pigs and humans remain more similar to each other throughout the decomposition process than either species does to the rabbits.

Separating the data into each trial begins to show the seasonal variation more clearly, and the separation between species as well. Pigs and humans demonstrate a much more similar pattern to each other than to rabbits in Trial 1 between 100 and 300 ADD. This can be clearly seen when looking at photographs from this period; both pigs and humans display obvious color changes while the rabbit shows no outward indicator of decomposition progress (Appendix 11). Examining Trial 2 between 300 and 400 ADD, a separation between all three species is observed. Morphologically, the humans had more visible mummification while the pigs showed higher amounts of bone exposure, and the rabbits showed changes that did not neatly correspond to any of the TBS categories (Appendix 12). Trial 3 showed a considerable amount of variability between the three species. The rabbits progressed very slowly, while the pigs

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demonstrated only minimal discoloration and desiccation. The humans had a significant amount of intraspecific variation due to scavenging (Appendix 13).

Differential Insect and Scavenging Activity

Within each trial, the reason for the distinction between pigs and humans varied. For example, in Trial 2, the insect activity was more localized on the human subjects, while the entire body of the pigs were covered in larvae (Appendix 14). In contrast, species differences in Trial 3 was due to differential scavenging activity.

Maggot activity occurred more rapidly and consumed the pigs and rabbits more completely than the humans. Notably, observing decomposition in rabbits in the same manner as humans is extremely difficult as changes cannot be visualized so the TBS scores may be misleading for the rabbits in the first and second trials. For instance, Appendix 15 shows Rabbit 4 four days after placement in Trial 2 and demonstrates significant maggot activity in the lower half of the body and skeletal elements are visible. However, less than 24 hours earlier this same rabbit showed no external signs of decomposition. There were no maggot masses on the face or anal region or anywhere externally observable until significant internal consumption had occurred. Thus, a systematic morphological observation system cannot be applied to rabbits.

A total of four scavenger species were documented at the study areas but their activities varied by season and by subject species. Raccoons were the most commonly observed scavenger, followed by birds, opossum and skunk. Only human subjects were scavenged in Trials 1 and 2 (two humans in Trial 1 and four in Trial 2) and the overall impact of scavenging was relatively minimal given the amount of consumption by the insects. However, all five human subjects were scavenged in Trial 3, which occurred before significant scavenging of other species. Appendix 16 shows the number of days after placement in which scavenging is first observed on each scavenged subject. This indicates that all three species were initially scavenged with some additional human subjects added before most of the pigs. However,

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scavenging of the two earliest pigs were either the tongue pulled out (but not actually consumed) from one pig and a small penetration of the abdomen (nipple removal) on the other. The fur was also extracted from the rabbits early on (and raccoons could have easily accessed the remains in the cage), yet this graph of the second date of scavenging demonstrates that the scavengers did not return to the pigs or any of the rabbits until after all five of the humans had been scavenged. This strongly indicates a preference for the humans over the other species.

All of the humans and eventually all of the pigs were scavenged in Trial 3, but the timing and anatomical distribution of scavenging greatly differed. With one notable exception in which scavenging began on the face, human scavenging began on the limbs while the snout, abdomen, neck and back were the initial preferred scavenging sites on pigs given the anatomical distribution of muscle in the pig. In the future we will create heat maps of the scavenging distribution, timing and number of scavenging events for each species.

The TBS method did not estimate accumulated degree days reliably for any of the species in any trial. The method does not take into account scavenging, which can result in rapid skeletonization in different regions of the body, nor differential decomposition within an anatomical region. Though the total body scores are supposed to be unidirectional and sequential, scavenging of the face may result in a score jumping from 1 (fresh) to 10 or 11 in a single day. Moreover, differential scavenging of the limbs is ignored by the TBS because only the lower score is used. Improvement of the total body score method should include a separate category for scavenging and a larger number of body regions.

Implications for Criminal Justice Policy and Practice in the U.S.

The results of this study clearly demonstrate differential decomposition among humans and nonhuman animals. Rabbits do not make a suitable proxy for humans; they are consistently different in their decomposition rates and patterns and do not permit the application of any morphological scoring system that can be applied to humans. Pig decomposition is closer to humans but still differ in overall

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decomposition trends. Namely, pigs show less intraspecific variation (perhaps due to same diet, weight, age, etc). This means that pigs could be useful for certain baseline decomposition data (e.g., determining what carrion insects are present in a certain environment) but lack important variability seen in the human decomposition patterns. In particular, the intensity and pattern of scavenging observed on the human subjects is not captured by the nonhuman study subjects while insect activity may not be as impactful for humans as it is for pigs. Seasonality also plays a role in differential decomposition as pigs and rabbits decompose faster than humans when insects are present and scavenging is more likely to occur in the winter. In sum, our data indicates that human data is best for determining human patterns of decomposition in forensic cases.

Scholarly Products

Conference Papers Presented:

2016 Steadman DW, Dautartas AM, Jantz LM, Mundorff AZ, Vidoli GM. A Multidisciplinary Validation Study of Nonhuman Animal Models for Human Decomposition Research. Paper presented at the National Institute of Justice Forensic Science Research and Development Symposium, Las Vegas, NV. February 23, 2016.

2016 Steadman DW, Dautartas AM, Mundorff AZ, Vidoli GM, Jantz LM. Differential Raccoon Scavenging Among Pig, Rabbit, and Human Subjects. Paper presented at the American Academy of Forensic Sciences, Las Vegas, NV. February 27, 2016.

2016 Dautartas AM, Steadman DW, Mundorff AZ, Jantz LM, Vidoli GM. A Comparison of Seasonal Decomposition Patterns Between Human and Nonhuman Animal Models. Paper presented at the American Academy of Forensic Sciences, Las Vegas, NV. February 26, 2016.

2015 Dautartas AM, Jantz LM, Vidoli GM, Steadman DW. A Multidisciplinary Validation Study of Nonhuman Animal Models for Forensic Decomposition Research: A Time Series Approach. Paper presented at the American Academy of Forensic Sciences, Orlando, Florida. February 19, 2015.

Publications in Preparation:

Dautartas AM, Jantz LM, Vidoli GM, Steadman DW. Differential insect activity and taxonomy across human and nonhuman subjects. A Comparison of insect activity and taxonomy between humans and nonhuman animal models across three seasons. Paper to be submitted to *Forensic Science International*.

Steadman DW, Dautartas AM, Mundorff AZ, Vidoli GM, Jantz LM. Differential Raccoon Scavenging Among Pig, Rabbit, and Human Subjects. Paper to be submitted to the *Journal of Forensic Sciences*.

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Dautartas AM, Steadman DW, Mundorff AZ, Jantz LM, Vidoli GM. A Comparison of Seasonal Decomposition Patterns between Human and Nonhuman Animal Models using Fuzzy Clustering. Paper to be submitted to the *Journal of Forensic Sciences*.

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Appendices

Appendix 1. Weight and sex data for human subjects and weight data for pig and human subjects in all three trials.

Subject	Trial 1 Sex/Weight	Trial 2 Sex/Weight	Trial 3 Sex/Weight
Human 1	Male/179	Female/123	Female/126
Human 2	Female/159	Male/147	Female/173
Human 3	Male/186	Female/116	Male/188
Human 4	Male/167	Male/191	Female/186
Human 5	Male/167	Female/235	Male/161
Pig 1	140	113	103
Pig 2	133	104	106
Pig 3	140	106	126
Pig 4	148	130	117
Pig 5	150	88	106
Rabbit 1	8	9	7
Rabbit 2	8	8	8
Rabbit 3	6	7	5
Rabbit 4	7	8	7
Rabbit 5	8	8	9

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Appendix 2. Photograph of the transect of subject placement in Trial 1.

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Appendix 3. Total Body Score data collection form and TBS scoring categories.

Recorder:

Date:

Megyesi scoring data collection sheet

Human:

Donation	Head & Neck		Trunk		Limbs		TBS	Est. ADD	Comments
	AM	PM	AM	PM	AM	PM			

Pig:

Individual	Head & Neck		Trunk		Limbs		TBS	Est. ADD	Comments
	AM	PM	AM	PM	AM	PM			
1									
2									
3									
4									
5									

Rabbit:

Individual	Head & Neck		Trunk		Limbs		TBS	Est. ADD	Comments
	AM	PM	AM	PM	AM	PM			
1									
2									
3									
4									
5									

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Categories and stages of decomposition for the head and neck.

A.	**Fresh**	
1.	Fresh, no discoloration.	1 pt
B.	**Early Decomposition**	
1.	Pink-White appearance with skin slippage and some hair loss.	2 pts
2.	Gray to green discoloration: some flesh still relatively fresh.	3 pts
3.	Discoloration and /or brownish shades particularly at edges, drying of nose, ears, and lips.	4 pts
4.	Purging of decompositional fluids out of eyes, ears, nose, mouth, some bloating of neck and face may be present.	5 pts
5.	Brown to black discoloration of flesh.	6 pts
C.	**Advanced decomposition**	
	1. Caving in of the flesh and tissues of eyes and throat.	7 pts
	2. Moist decomposition with bone exposure less than one half that of the area being scored.	8 pts
	3. Mummification with bone exposure less than one half that of the area being scored.	9 pts
D.	**Skeletonization**	
	1. Bone exposure of more than half of the area being scored with greasy substances and decomposed tissue.	10 pts
	2. Bone exposure of more than half of the area being scored with dessicated or mummified tissue.	11 pts
	3. Bones largely dry, but retaining some grease.	12 pts
	4. Dry bone.	13 pts

Categories for the Trunk

	A. Fresh	
	1. Fresh, no discoloration.	1 pt
	B. Early Decomposition	
1.	Pink-white appearance with skin slippage and marbling present.	2 pts
2.	Gray to green discoloration: some flesh still relatively fresh.	3 pts
3.	Bloating with green discoloration and purging of decompositional fluids.	4 pts
4.	Postbloating following release of the abdominal gases, with discoloration changing from green to black.	5 pts
	C. Advanced Decomposition	
1.	Decomposition of tissue producing sagging of flesh; caving in of the abdominal cavity	6 pts
2.	Moist decomposition with bone exposure less than one half that of the area being scored.	7 pts
3.	Mummification with bone exposure less than one half that of the area being scored.	8 pts
	Skeletonization	
1.	Bones with decomposed tissue, sometimes with body fluids and grease still present.	9 pts
2.	Bones with dessicated or mummified tissue covering less than one half of the area being scored.	10 pts
3.	Bones largely dry, but retaining some grease.	11 pts
4.	Dry bone.	12 pts

Limbs

A.	**Fresh**	
1.	Fresh, no discoloration.	1 pt
B.	**Early Decomposition**	
1.	Pink-white appearance with skin slippage on hands and/or feet.	2 pts
2.	Gray to green discoloration: marbling; some flesh still relatively fresh.	3 pts
3.	Discoloration and/or brownish shades, particularly at edges, drying of fingers, toes, and other extremities.	4 pts
4.	Brown to black discoloration, skin having a leathery appearance.	5 pts
C.	**Advanced Decomposition**	
1.	Moist decomposition with bone exposure less than one half that of the area being scored.	6 pts
2.	Mummification with bone exposure less than one half that of the area being scored.	7 pts
D.	**Skeletonization**	
1.	Bones exposure over one half of the area being scored, some decomposed tissue and body fluids remaining.	8 pts
2.	Bones largely dry, but retaining some grease.	9 pts
3.	Dry bone.	10 pts

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Appendix 4. Season, dates, temperatures and duration of Trials 1-3.

Trial	Season	Placement Date	Max Low (°C)	Max High (°C)	Avg Temp (°C)	Study Duration
1	Spring	March 14, 2014	2.6	24.2	16.2	75 days
2	Summer	June 13, 2014	19.5	28.7	24.2	45 days
3	Winter	December 1, 2014	0	17.8	5.1	123 days

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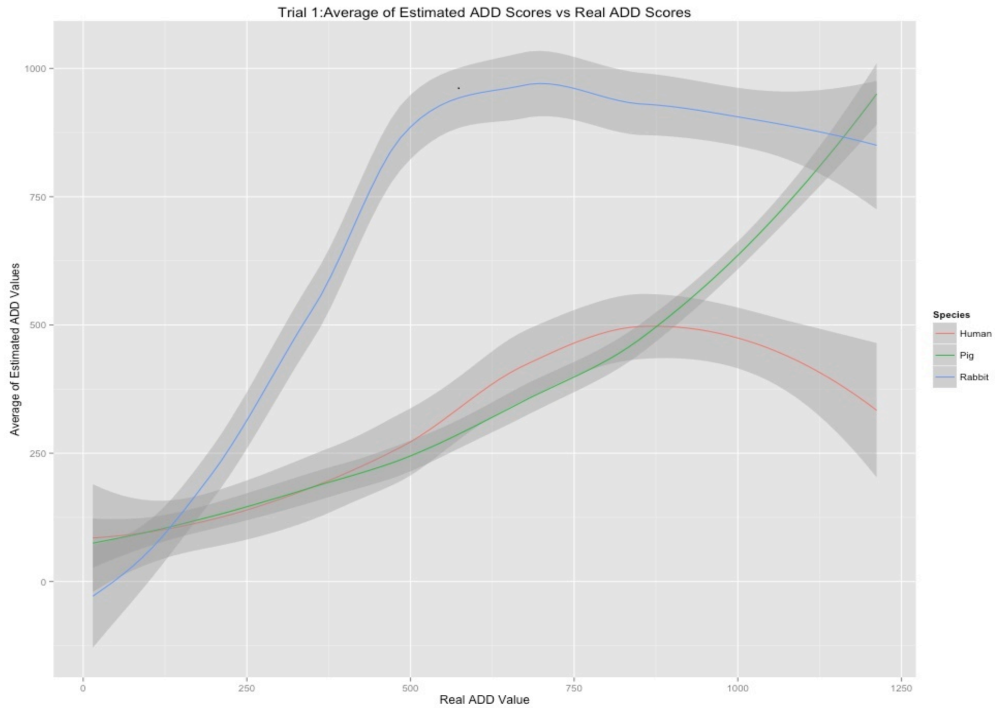
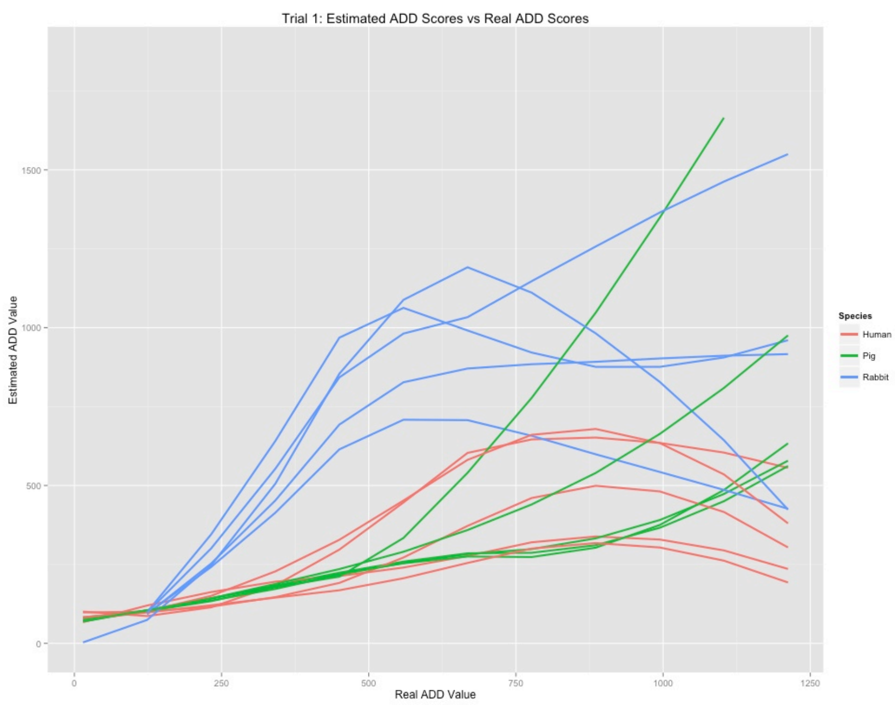
Appendix 5. Plots of estimated ADD calculated by TBS scores and known ADD by species (top) and data by individual (bottom) for Trial 1 (spring). The rabbit decomposition rate is initially faster than the other species but pig decomposition accelerated when insect activity began. Human decomposition slowed despite insect availability due to mummification.





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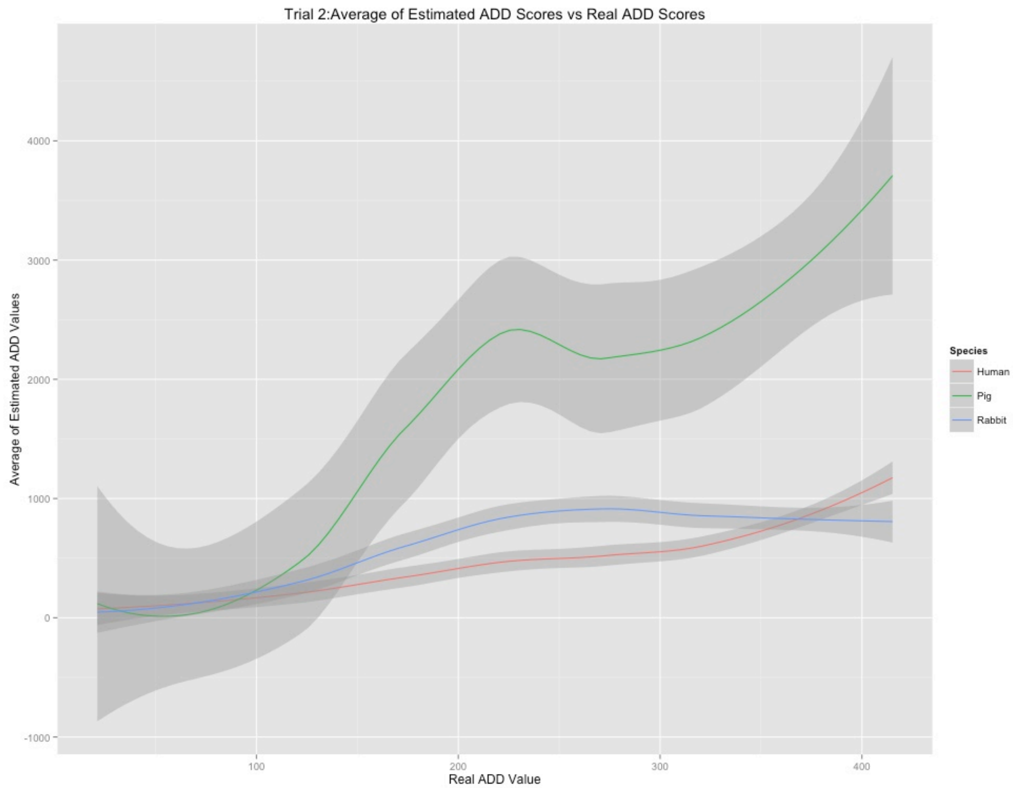
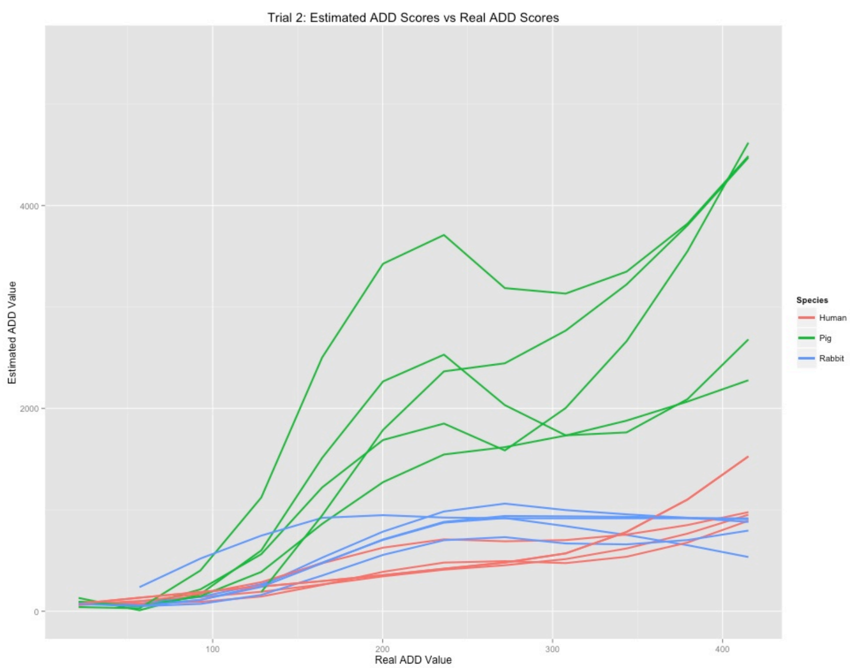
Appendix 6. Plots of estimated ADD calculated by TBS scores and known ADD by species (top) and data by individual (bottom) for Trial 2 (summer). Pig decomposition was more rapid due to rapid and intense insect activity.





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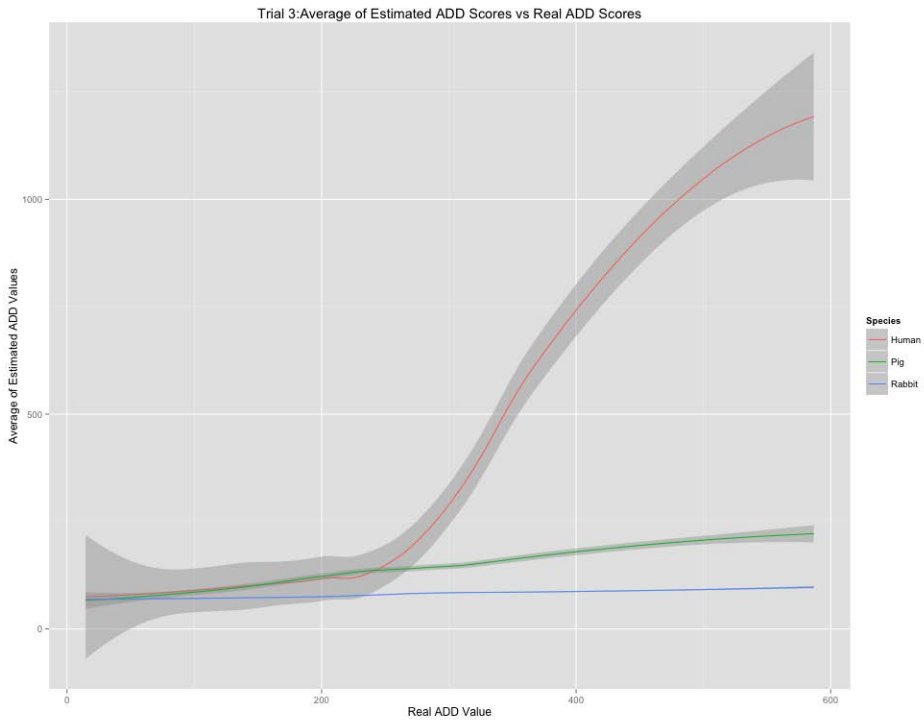
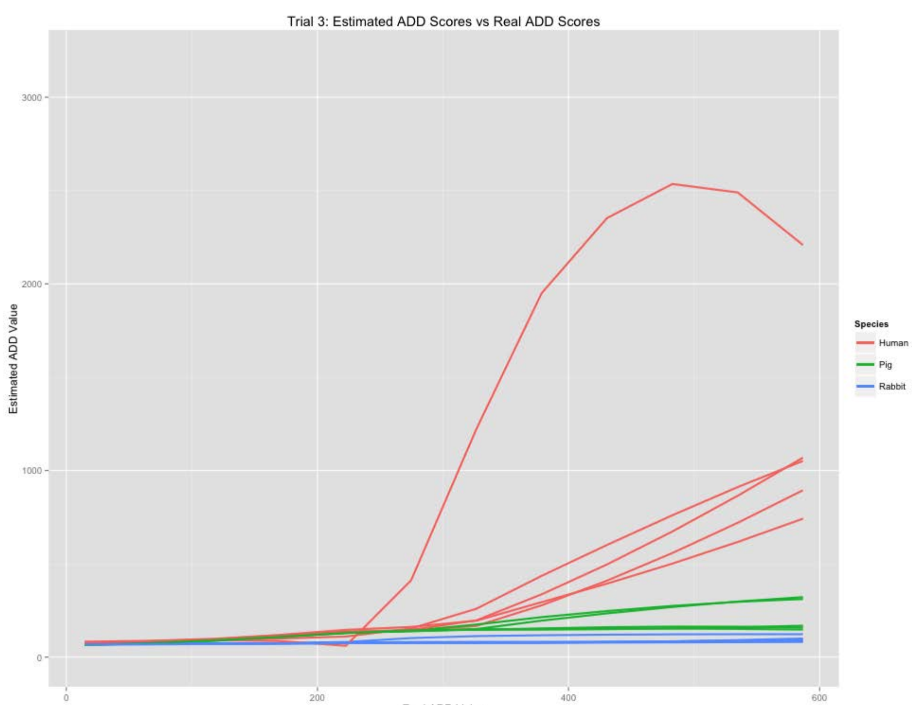
Appendix 7. Plots of estimated ADD calculated by TBS scores and known ADD by species (top) and data by individual (bottom) for Trial 3 (winter). Human decomposition was much faster due to scavenging. The scavengers preferred humans overall but especially one subject.





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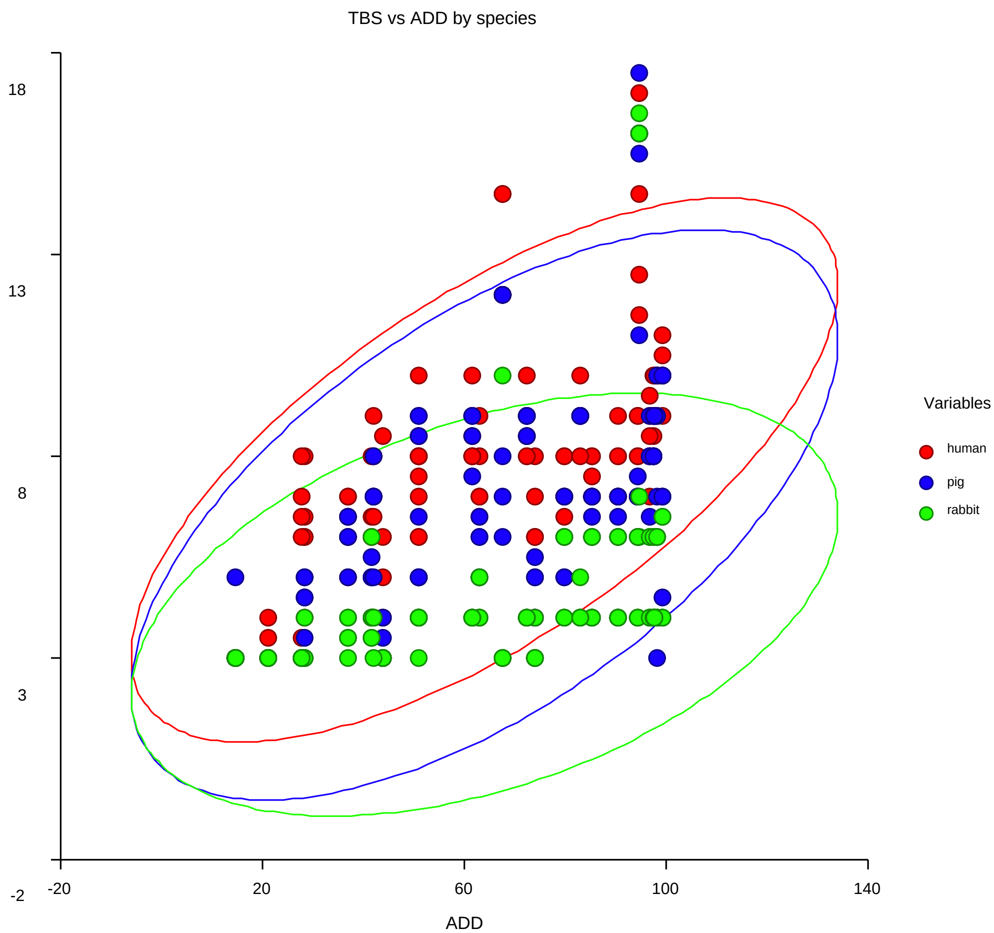
Appendix 8. Fuzzy clustering analysis of known ADD and TBS score for all species across all trials for the first 100 ADD. The ellipses represent 95% of the variation. There are clear outliers from all three species. The pigs and humans cluster together in the first cluster and rabbits form the majority of the second cluster. Average silhouette: .4, Dunn's coefficient: .58, Dc(U): .42.



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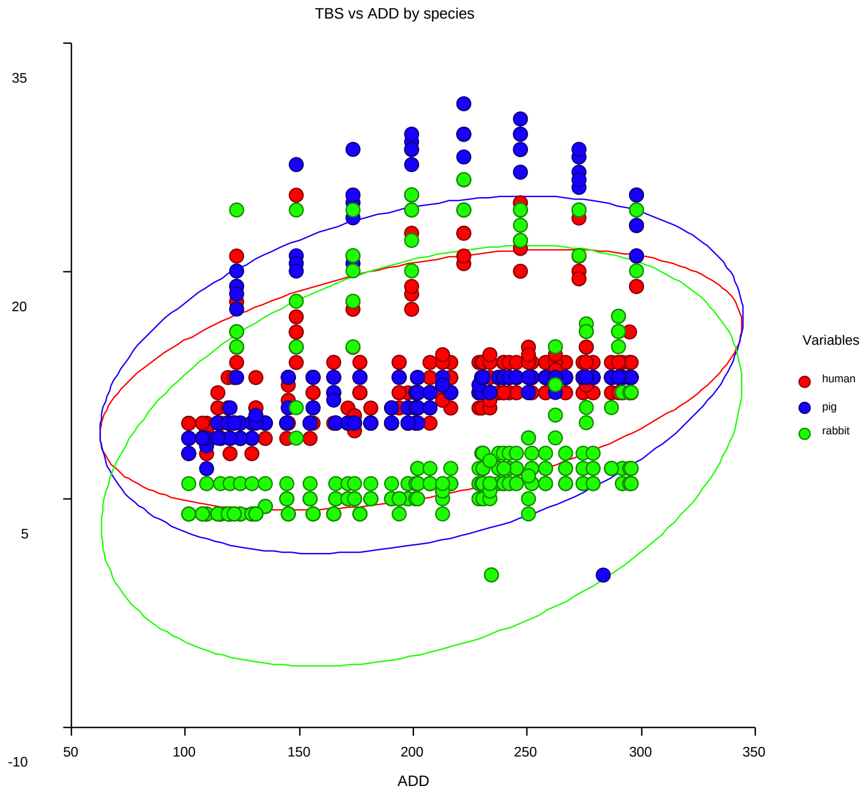
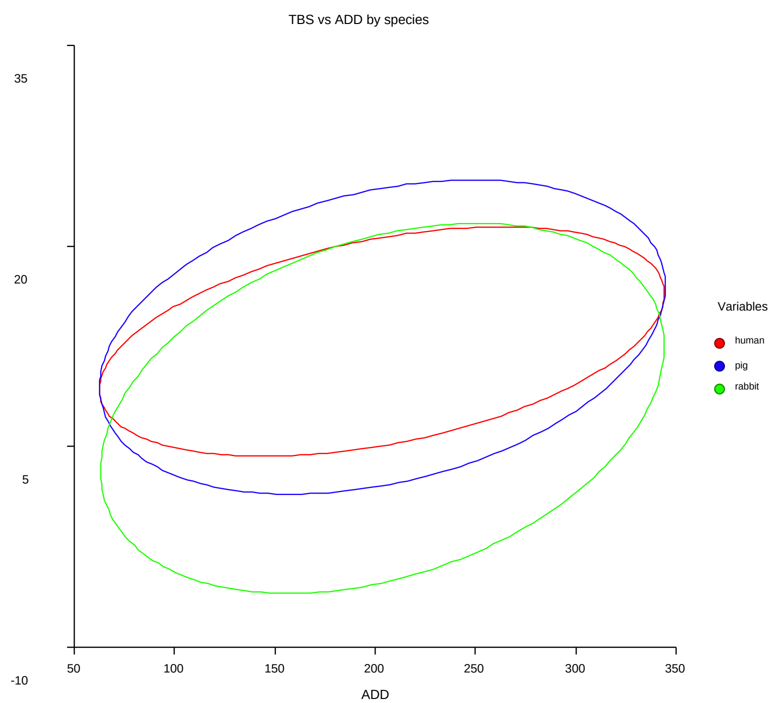
Appendix 9. Fuzzy clustering analysis of known ADD and TBS score for all species across all trials between 100 and 300 ADD with all data points (top) and without (bottom). Average silhouette: .95, Dunn's coefficient: .98, Dc(U): .01.





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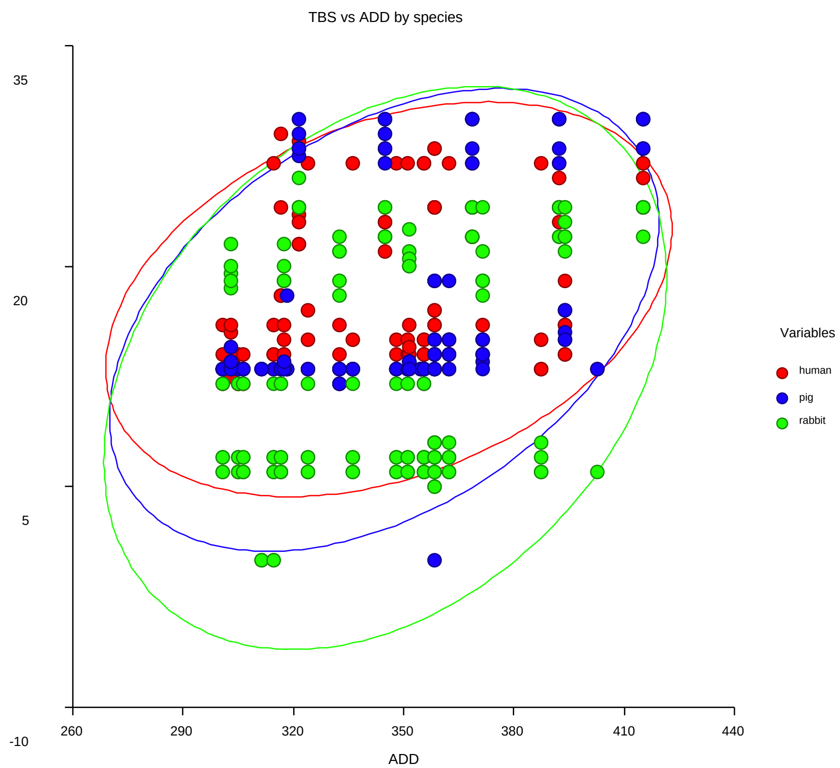
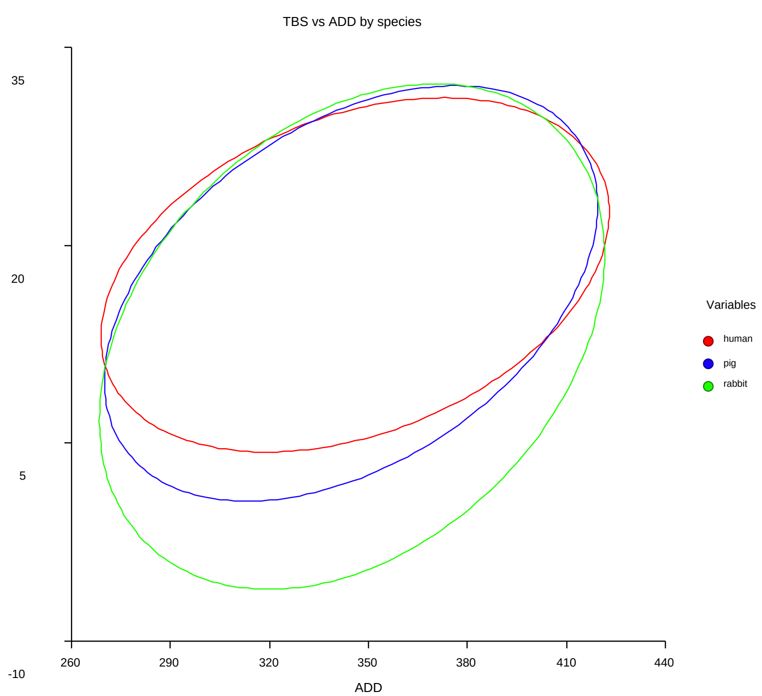
Appendix 10. Fuzzy clustering analysis of known ADD and TBS score for all species across all trials between 300 and 400 ADD with all data points (top) and without (bottom). Average silouhette: .57, Dunn's coefficient: .79, Dc(U): .21.





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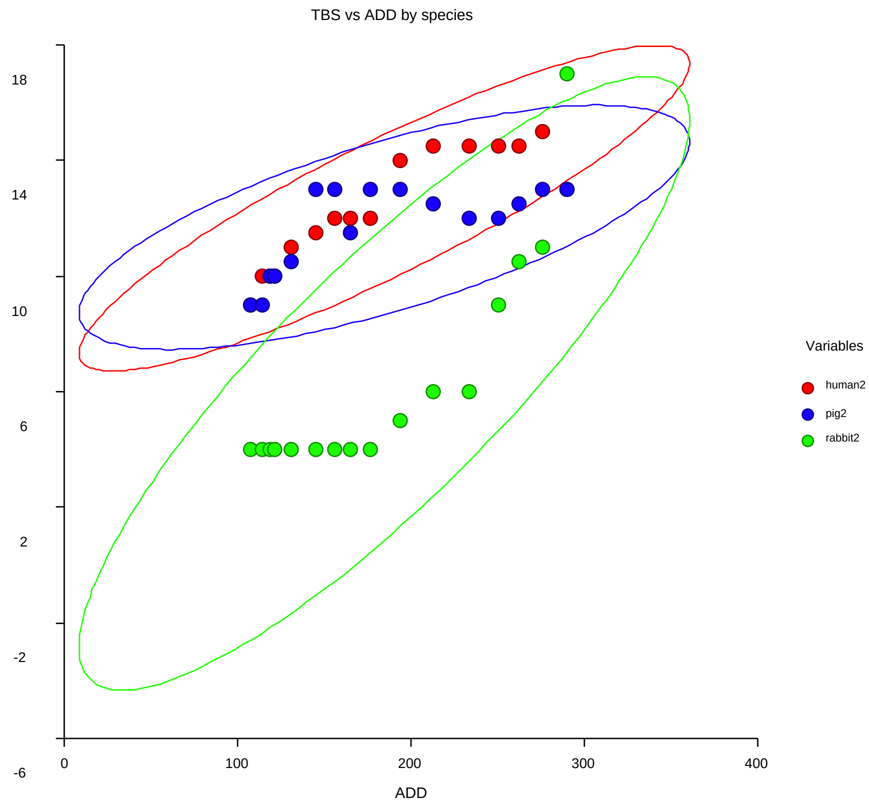
Appendix 11. Fuzzy cluster of all three species in Trial 1 between 100 and 300 AD demonstrating tighter clustering of the humans and pigs. The pigs and humans demonstrate color changes, though none are observable on the rabbits (bottom).



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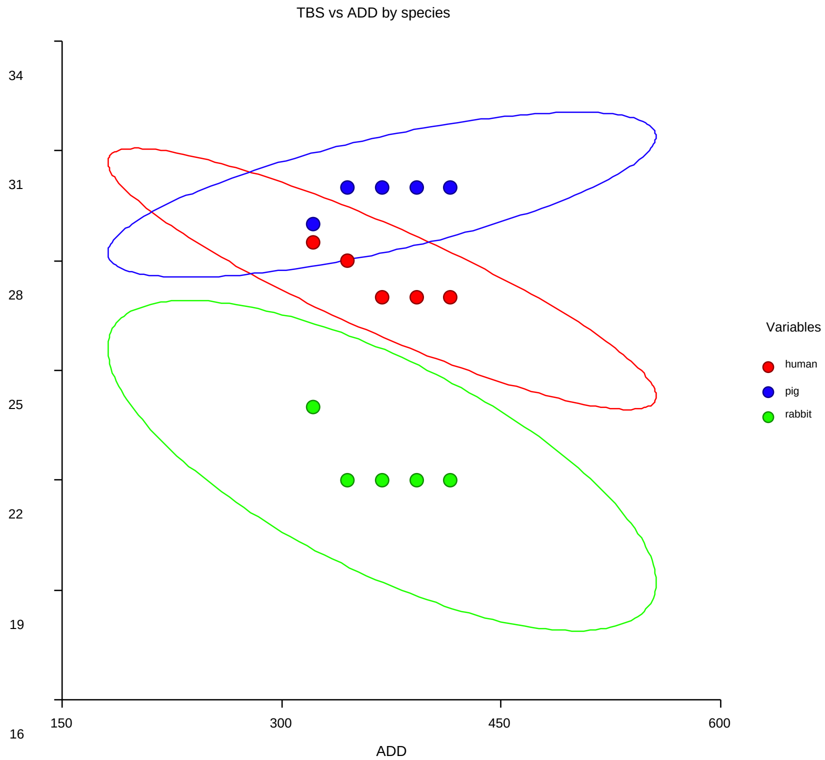
Appendix 12. Fuzzy cluster of all three species in Trial 2 between 300 and 400 AD shows differentiation of decomposition rates among the species and complete separation of the rabbit pattern from the pig and humans. Note skeletonization of the pig due to insect activity but mummification of the human.



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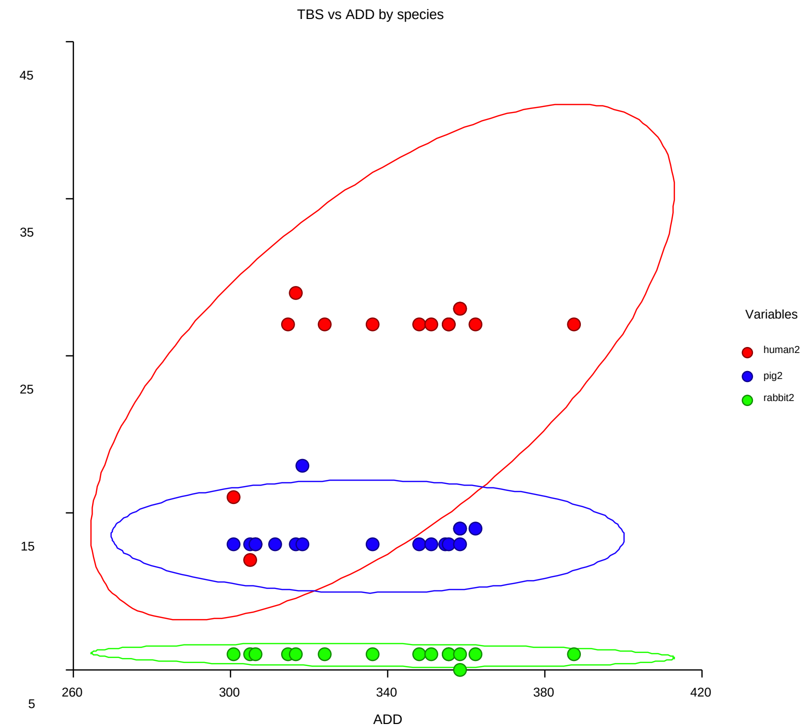
Appendix 13. Fuzzy cluster of all three species in Trial 3 between 300 and 400 AD shows much higher TBS scores for humans due to scavenging. Note nearly complete skeletonization of the human subject while the pig and rabbit are still in the fresh stage (below).



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Appendix 14. Differential insect activity on the human on Day 7 of Trial 2 in which maggot activity is localized (top) and pig (bottom) on Day 6 of the same trial.

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Appendix 15. Rabbit 4 in Trial 2 demonstrating maggot activity and skeletonization of the lower limbs four days after placement (top). However, there is no evidence of insect activity 24 hours earlier, indicating that insect activity and overall morphological changes during decomposition is more difficult to observe in rabbits due to fur.

Rabbit 4 on June 17 am

Rabbit 4 on June 16 am

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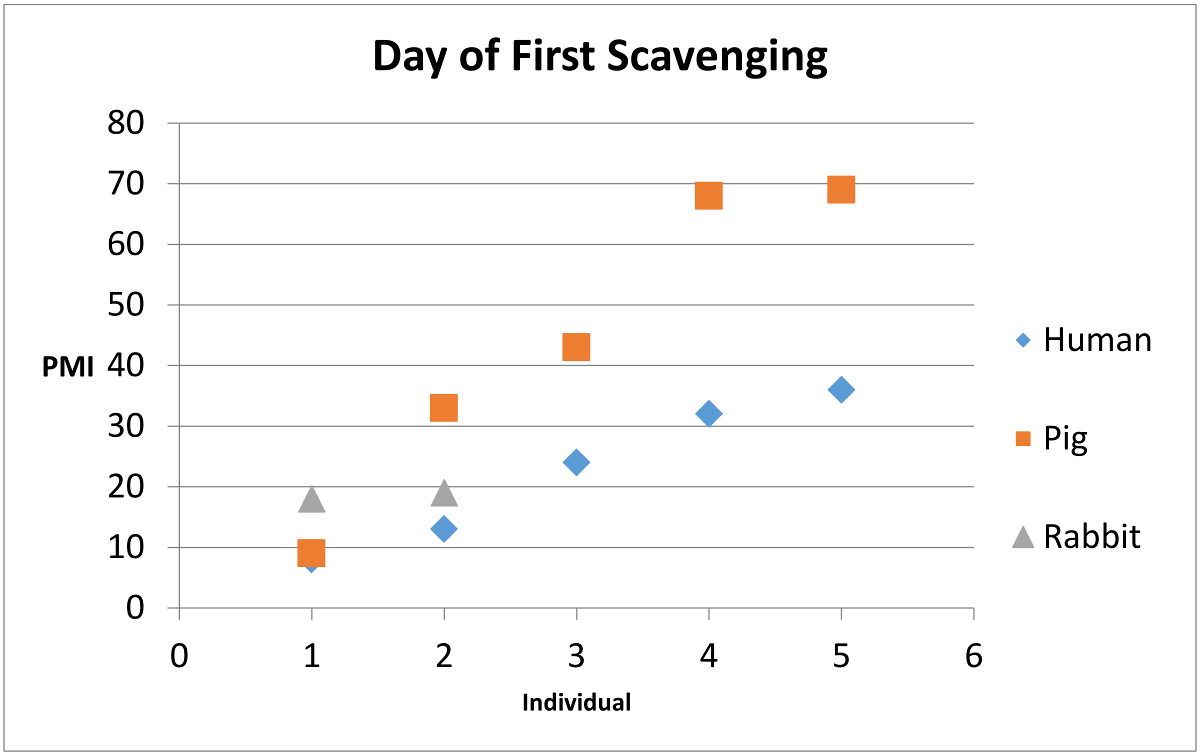
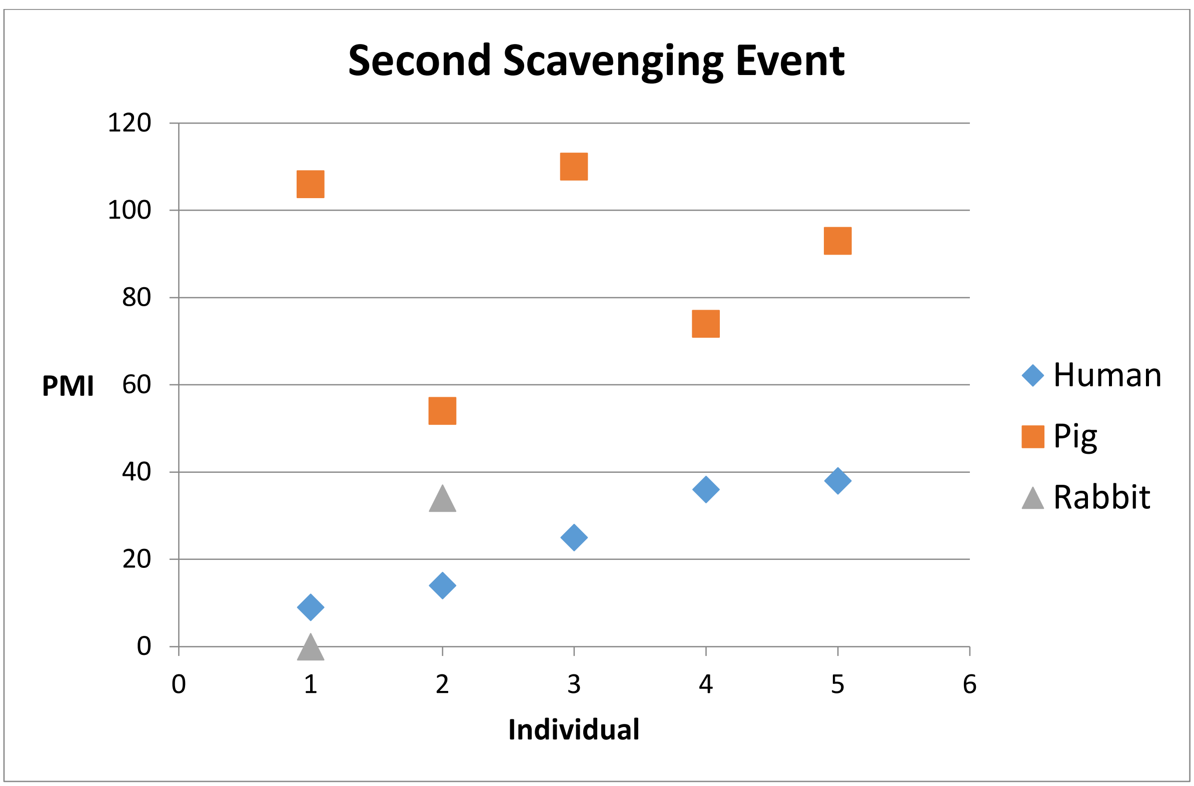
Appendix 16. Time of first (top) and second (bottom) scavenging on the 15 subjects in Trial 3. While scavengers visited some pit and rabbit cadavers early, they did not return to the bodies until all of the human subjects had been scavenged.





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