

# Chapter 1

## Early Postmortem Changes and Stages of Decomposition

M. Lee Goff

### 1.1 Introduction

When faced with the task of estimating a period of time since death, there are generally two known points existing for the worker: the time at which the body was discovered and the last time the individual was reliably known to be alive. The death occurred between these two points and the aim is to estimate when it most probably took place. This will be an estimate since, it is generally accepted that there is actually no scientific way to precisely determine the exact period of time since death. What is done in the case of entomology is an estimation of the period of insect activity on the body. This period of insect activity will reflect the minimum period of time since death or postmortem interval (PMI) but will not precisely determine the time of death. In most cases, the later point is more accurately known than the former. Individuals tend to recall when they first encountered the dead body with considerable precision. This is typically not in their normal daily routine and it makes an impression, even on those accustomed to dealing with the dead.

Once the body is discovered, those processing the scene make meticulous (at least we hope meticulous) notes including times of arrival, departure, movement of the body and, finally, when the body is placed into the morgue. By contrast, the time at which the individual was last reliably known to be alive is often less precise. This is possibly due to the fact that those having the last contact most probably did not anticipate that this would be their last encounter with the individual and nothing of significance took place at the time. In the absence of something unusual, one rarely notes the time one said “good morning” to a neighbor or passed an acquaintance on the street. The last time the individual was reliably known to be alive may involve statements concerning the last time the individual was seen alive. It may involve hearing the individual or a telephone communication. Some instances may involve the touch or smell of the individual. Obviously there is some latitude possible in this determination and the time frame is often incorrect. For this reason, the precision

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M.L. Goff  
Chaminade University, Forensic Sciences Program, Hawaii, USA

of the time of discovery and collection of specimens become of major significance, as they are the anchor for the estimates. Estimation begins when the insects are collected and preserved, stopping the biological clock.

As the process of estimating the period of insect activity takes place, it must be kept in mind that the parameters of the estimate become progressively wider as the period of time since death increases. The changes to a body that take place immediately following death are often more rapid than those occurring later during the decomposition process. Thus the estimate begins, potentially, with a range of plus or minus minutes, goes to hours, days, weeks, months and finally “its been there a long time.” The last is not the most popular with law enforcement agencies as they had already guessed that. It should also be kept in mind that the estimates presented, by their very nature, are not precise. I have found in my experience that it is typically the more inexperienced investigator who provides the most precise and unchanging estimates of the PMI.

Decomposition is a continuous process, beginning at the point of death and ending when the body has been reduced to a skeleton. Although this process is a continuum, virtually every study presented has divided this process into a series of stages. The number of stages has varied from one to as many as nine, depending on author and geographic region (Goff 1993) (Table 1.1). While the number of stages considered has varied, there does not appear to be a firm relationship between these and the total number of species observed in each study. For example, Cornaby (1974) working in Costa Rica using lizards and toads as animal models noted only 1 stage for

**Table 1.1** Summary of selected decomposition studies giving numbers of recognized stages and taxa listed

Author and Ref.	Date	Locality	Animal model	# Stages	Total # of arthropod taxa
Avila and Goff	2000	Hawaii	Pigs(burnt)	5	68 species
Blacklith and Blacklith	1990	Ireland	Birds, mice	1	27 species
Bornemissza	1957	Australia	Guinea pig	5	45 groups listed
Braack	1986	Africa	Impala	4	227 species
Coe	1978	Africa	Elephants	3	No totals given
Cornaby	Cornaby, 1974	Costa Rica	Lizards, toads	1	172 species
Davis and Goff	2000	Hawaii	Pigs(intertidal)	5	85 species
Early and Goff	1986	Hawaii	Cats	5	133 species
Hewadikaram and Goff	1991	Hawaii	Pigs	5	46 species
Megnin	1894	France	Humans	9	No totals given
Payne	1965	South Carolina	Pigs(surface)	6	522 species
Reed	1958	Tennessee	Dogs	4	240 species
Rodriguez and Bass	1983	Tennessee	Humans	4	10 families listed
Shalaby et al.	2000	Hawaii	Pig(hanging)	5	35 species
Shean et al.	1993	Washington	Pig	–	48 species
Tullis and Goff	1987	Hawaii	Pig	5	45 species

decomposition but recorded 172 different species. By contrast, work in Hawaii by Early and Goff (1986), using domestic cats as the animal model, recognized five stages of decomposition but recorded 133 species. Other studies have recognized other numbers but with no real correlation between stages observed and numbers of taxa reported. To a certain extent, these differences may be related to sampling methods and taxonomic interests of those involved.

## 1.2 Early Postmortem Changes

As death proceeds, there are a series of early changes to the body that result in a definite change in the physical nature and/or appearance of the body prior to the onset of gross, recognizable decompositional changes. These changes have traditionally been used in estimations of the PMI and may be a source of confusion if not recognized. For that reason, they are described here.

### 1.2.1 *Livor Mortis*

One of the early changes observable is livor mortis, also referred to as lividity, postmortem hypostasis, vibices and suggilations. This is a physical process. While the individual is alive, the heart is functioning and circulating the blood. When death occurs, circulation stops and the blood begins to settle, by gravity, to the lowest portions of the body. This results in a discoloration of those lower, dependent parts of the body (Fig. 1.1). Although beginning immediately, the first signs of livor

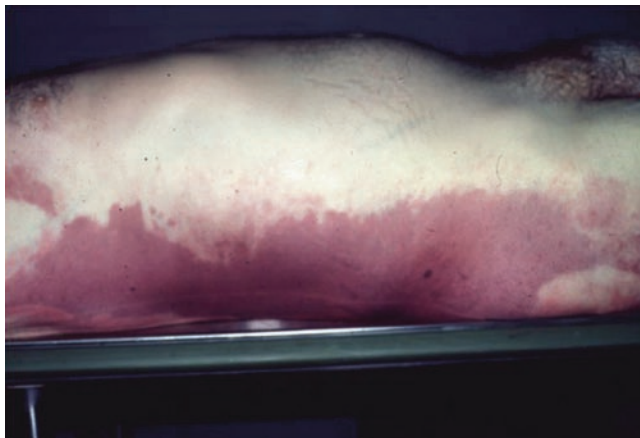


Fig. 1.1 Livor

**Fig. 1.2** Blanching

mortis are typically observed after a period of approximately 1 h following death with full development being observed 3–4 h following death. At this time, the blood is still liquid and pressing on the skin will result in the blood being squeezed out of the area (blanching), only to return once pressure is removed. This situation continues until 9–12 h following death, at which time the pattern will not change and the livor mortis is said to be “fixed.” Any areas of pressure resulting from clothing or continued pressure during this period will not show discoloration (Fig. 1.2).

### ***1.2.2 Rigor Mortis***

This is a chemical change resulting in a stiffening of the body muscles following death due to changes in the myofibrils of the muscle tissues. Immediately following death, the body becomes limp and is easily flexed. As ATP is converted to ADP and lactic acid is produced lowering the cellular pH, locking chemical bridges are formed between actin and myosin resulting in formation of rigor. Typically, the onset of rigor is first observed 2–6 h following death and develops over the first 12 h. The onset begins with the muscles of the face and then spreads to all of the muscles of the body over a period of the next 4–6 h (Gill-King 1996). Rigor typically lasts from 24 to 84 h, after which the muscles begin to once again relax. The onset and duration of rigor mortis is governed by two primary factors: temperature and the metabolic state of the body. Lower ambient temperatures tend to accelerate the onset of rigor and prolong its duration while the opposite is found in warmer temperatures. If the individual has been involved in vigorous activity immediately prior to death, the onset of rigor is more rapid. Body mass and rates of cooling following



**Fig. 1.3** Rigor

death also influence the onset and duration of rigor mortis. As rigor disappears from the body, the pattern is similar to that seen during the onset, with the muscles of the face relaxing first (see Fig. 1.3).

### ***1.2.3 Algor Mortis***

Once death has occurred, the body ceases to regulate its internal temperature and the internal temperature begins to approximate the ambient temperature. In most instances this involves a cooling of the body until ambient temperature is reached, most often in a period of 18–20 h (Fisher 2000). Although there are several different approaches, the rate of cooling is most often expressed by the equation:

$$PMI (hours) = \frac{98.6 \text{ Body Temperature } (^{\circ}F)}{1.5} \quad (1)$$

Any estimate of the postmortem interval obtained using this technique should be limited to the very early stages of death (18 h or less) and treated with care. There are several obvious factors involved in the cooling of the body that may easily influence the rate at which this occurs. The size of the individual is a major factor. A smaller individual will cool more rapidly than a larger individual in the same set of conditions. Exposure to sunlight or heating may also influence the rate of cooling as may clothing and a number of other factors. The most commonly used temperature in these calculations are from the liver although rectal temperature may also be employed.

**Fig. 1.4** Glove

### ***1.2.4 Tache Noir***

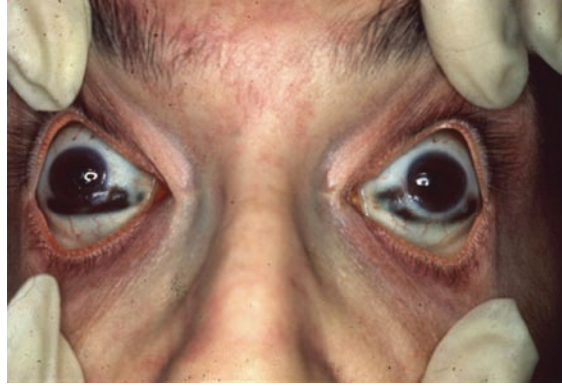
Following death, the eyes may remain open and the exposed part of the cornea will dry, leaving a re-orange to black discoloration. This is termed tache noire (French for “black line”) and may be misinterpreted as hemorrhage. Unlike hemorrhage, this will have symmetrical distribution, corresponding to the position of the eyelids (see Fig. 1.5).

### ***1.2.5 Greenish Discoloration***

As the body decomposes, gasses are produced in the abdomen and other parts of the body. While the exact composition of the gasses may vary from body to body, a significant component of these gasses is hydrogen sulfide ( $H_2S$ ). This gas is a small molecule and readily diffuses through the body. Hydrogen sulfide will react with the hemoglobin in blood to form sulfhemoglobin. This pigment is greenish and may be seen in blood vessels and in other areas of the body, particularly where livor mortis has formed.

### ***1.2.6 Marbling***

As the anaerobic bacteria from the abdomen spread via the blood vessels, the subcutaneous vessels take on a purple to greenish discoloration, presenting a mosaic appearance, similar to what is seen in cracking of old marble statuary. Typically this is seen on the trunk and extremities (see Fig. 1.6).

**Fig. 1.5** Tache noir**Fig. 1.6** Marbling

### ***1.2.7 Skin Slippage***

Upon death, in moist or wet habitats, epidermis begins to separate from the underlying dermis due to production of hydrolytic enzymes from cells at the junction between the epidermis and the underlying dermis. This results in the separation of the epidermis which can be easily removed from the body. Slippage may first be observed as the formation of vesicle formation in dependent portions of the body. In some instances, the skin from the hand may separate from the underlying dermis as a complete or relatively complete unit. This is termed glove formation and can be removed from the hand as an intact unit. This skin can be used for finger printing, often with better results than if the skin remains on the hand (see Fig. 1.4).

### ***1.2.8 Mummification***

In a dry climate, a body will desiccate. The low level of humidity will serve to inhibit bacterial action and typically there will be some exclusion of insects and other scavengers from the body. The temperatures will be either very hot or very cold in this type of situation. The desiccated tissues and skin will have a leathery appearance and will survive for long periods of time with minimal change. In hot, dry climates, mummification can occur within a period of several weeks (see Fig. 1.7).

### ***1.2.9 Saponification***

This is the process of hydrolysis of fatty tissues in wet, anaerobic situations, such as submersion or in flooded burials. The tissues take on a waxy appearance and consistency. This process requires a period of several months to complete (see Fig. 1.8).

### ***1.2.10 Putrefaction***

Putrefaction is nature's recycling process. It is the result of the combined activities of all organisms involved in decomposition, reducing the body to a skeletal state.



**Fig. 1.7** Mummification



**Fig. 1.8** Saponification

## 1.3 Decomposers

In order to consider the process of decomposition and the stages involved, it helps to have some understanding of what organisms will be involved in the process. Some of these have already been mentioned with respect to the early changes to the body mentioned above. There are four primary categories of organisms involved in decomposition.

### 1.3.1 *Bacteria*

There are bacteria associated with both the external and internal aspects of the human body. While alive, the body defends against these organisms and, in fact, many are actually beneficial. There is a large component of anaerobic bacteria associated with the human digestive system. Some of these exist normally in our intestines, such as *E. coli*, and, as long as they remain in place do no damage and may assist in breakdown of food and materials. By contrast, the same organism in the wrong place, such as the kidneys, etc., will result in a serious disease condition. Once the individual dies, there are few barriers to keep them in any particular place and human tissues are excellent growth media. Shortly after death, these bacteria begin to digest the body from the inside out. This activity is particularly evident in the areas of the head and abdomen. The metabolic activities of these bacteria are major components of the decomposition processes.

### **1.3.2 Fungi/Molds**

As noted earlier, the outer surface of the human body is comprised of dead material. This dead outer layer is necessary to assist in the survival of the human body. As a normal process, as new tissues are produced below, the outer *stratum corneum* is shed as dander. As it is shed, any attached spores of molds or fungi are also shed from the body. Following death, the outer layer is no longer shed and the mold and fungi spores with begin to colonize the external surface of the body, often forming significant mats on the body.

### **1.3.3 Insects**

Insects and other arthropods are the primary organisms involved in the major decomposition of the body. They arrive at exposed remains shortly after death, often in less than 10 min, and quickly begin their activities. As the rest of this work is devoted to their activities, no more needs to be said here.

### **1.3.4 Vertebrate Scavengers**

A dead human is a potential food resource for a number of vertebrate scavengers. Carnivores of all sizes can rapidly alter a dead body. Even small rodents can cause significant damage to a body in a relatively short period of time. In the wild it may take less than a week for scavengers to completely skeletonize a body. In addition to non-domesticated animals, common domestic animals and rodents will also feed on the body in the absence of their normal food. Pet dogs and particularly cats will feed on their deceased owners, most often attacking the face and exposed limbs first.

## **1.4 Factors Delaying Decomposition**

While there are a number of different organisms involved in the process of decomposition, there are also several types of factors that serve to stop or retard the rate at which the process continues. These barriers to decomposition fall into three broad categories.

### **1.4.1 Physical Barriers**

Physical barriers to decomposition are those that prevent access of the body by physical means. A body buried in the soil does not decompose as quickly as one exposed on the surface.

In a similar manner, a body enclosed in a sealed casket or placed into some form of sealed container will also exhibit a delayed decomposition.

### ***1.4.2 Chemical Barriers***

The embalming process is specifically designed to prevent the decomposition of the body, with natural body fluids being drained and replaced with various preservative fluids. As the body is then typically placed into a casket, the process should, if done properly, delay decomposition for an extended period of time. The presence of insecticides on, in or in the vicinity of the body may also serve to delay the onset of insect activity for a period of time. It should be noted that insecticides will not permanently delay the colonization of the body by insects. In many cases, immature insects are able to survive on a body with concentrations of an insecticide that would prove fatal to the adults of the same species (Gunatilake and Goff 1989)

### ***1.4.3 Climatic Factors***

Temperature can serve as a major factor delaying decomposition. At lower temperatures, bacterial growth and insect activity can be retarded or even arrested. At temperatures below 6°C most insect activity ceases but may resume once temperatures rise above this threshold. In a similar manner, high temperatures will also result in cessation of insect activity, and, if in a dry habitat, result in mummification of the body. Wind also serves to inhibit insect flight and thus colonization of the body. Many texts will indicate a wind speed in excess of 16 km/h will inhibit insect flight. This should not be accepted as a firm wind speed as in many tropical and island areas, tradewinds typically blow at a speed greater than this and there is significant insect activity. Rainfall may also serve as a temporary barrier but, once the rain ceases, the insects again become quite active.

## **1.5 Relationships of Insects to a Body**

There are several distinct relationships between an insect and a decomposing body. The population of insects and other arthropods encountered in any given habitat will contain elements unique to that habitat and components having a wider distribution. Within this population there will be species having some type of relationship to the decomposing body. This relationship will vary with taxon and not all relationships will be of equal value to the investigation. All must be considered as, under different circumstances, there will be different values for the relationship. There have been four basic relationships between a decomposing body and insects and other arthropods (after Goff 1993).

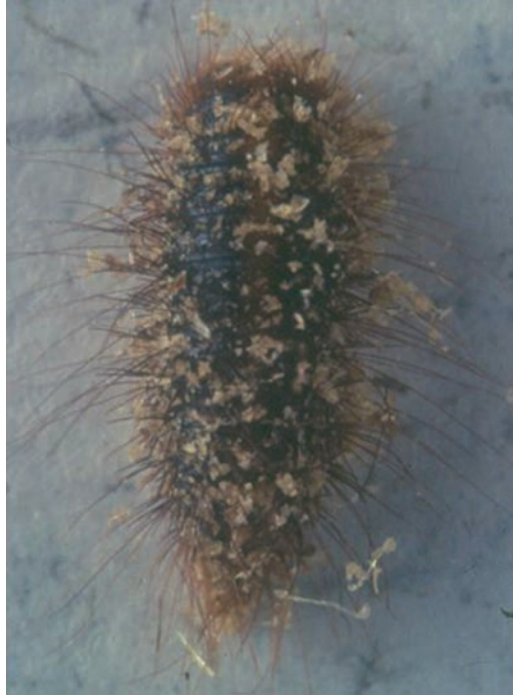
### 1.5.1 *Necrophagous Species*

Those taxa actually feeding on the corpse. This group includes many of the true flies (Diptera) particularly the blow flies (Calliphoridae) and flesh flies (Sarcophagidae) who are early invaders and Beetles (Coleoptera: Silphidae and Dermestidae) . This group includes species that may be the most significant isolatable taxa for use in estimating a minimum period of insect activity on the body during the early stages of decomposition (days 1–14) (see Figs. 1.9–1.11).

**Fig. 1.9** *Chrysomya* Lv II



**Fig. 1.10** Piophilidae

**Fig. 1.11** Dermestes

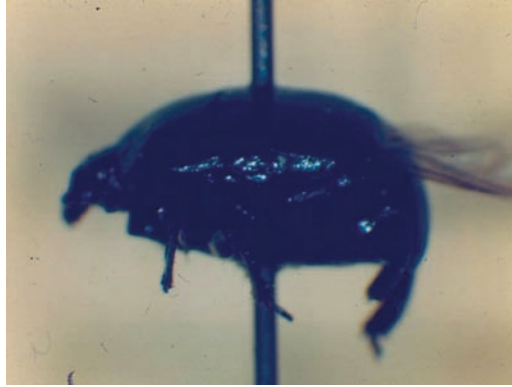
### ***1.5.2 Predators and Parasites of Necrophagous Species***

The predators and parasites of the necrophagous species comprise the second most significant group of carrion-frequenting taxa. Many of the beetles (Coleoptera: Silphidae, Staphylinidae, and Histeridae), true flies (Diptera: Calliphoridae, Muscidae and Stratiomyidae), and Wasps (Hymenoptera) parasitic on fly larvae and puparia are included (Figs. 1.12 and 1.13). In some species, fly larvae (maggots) that are necrophages during the early portions of their development become predators on other larvae during the later states of their development, as is the case for *Chrysomya rufifacies* and *Hydrotaea aenescens*.

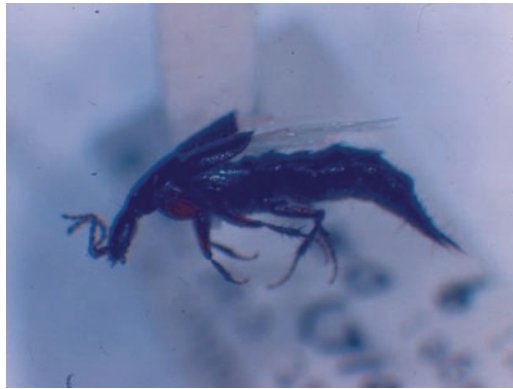
### ***1.5.3 Omnivorous Species***

Included in this category are the taxa such as wasps, ants and some beetles, that feed on both the corpse and associated arthropods. Early and Goff (1986) observed that large populations of these species may actually retard the rate of carcass removal by depleting the populations of necrophagous species (see Fig. 1.14).

**Fig. 1.12** Histeridae



**Fig. 1.13** Philonthus



**Fig. 1.14** Anopolepsis



### ***1.5.4 Adventive Species***

This category includes those taxa that simply use the corpse as an extension of their own normal habitat, as is the case for the springtails (Collembola), spiders, centipedes, and millipedes.

### ***1.5.5 Accidentals***

Another category that is not always recognized but may still be of significance is what might be termed “accidentals.” These are species that have no real relationship to the corpse but still are found on the body. These insects may have fallen onto the body from surrounding vegetation, thus possibly supplying some information on postmortem movement of a body. On the other hand, when an insect stops flying, it has to land on something and that “something” might happen to be the body. This is a fact all too often ignored, even by entomologists.

## **1.6 Stages of Decomposition**

### ***1.6.1 Numbers of Stages***

As noted earlier, there have been a number of different stages proposed for the decomposition process. Keep in mind that the process is a continuum and discrete stages, characterized by physical features and distinctive assemblages of insects, do not exist in nature. Regardless, virtually every study conducted has attempted to divide the process into stages. While artificial, these stages have definite utility. First, they allow for easy organization of research reports and discussion. There is also a utility in court proceedings. Typically in the United States, juries are composed of individuals with little if any background in the biological sciences. They often confused and repulsed by the process of decomposition they are being asked to consider. Under these circumstances, use of stages gives them something to use for reference and makes their task somewhat easier, if not more pleasant.

In studies conducted in Hawaii, five stages have been recognized and these appear to be easily applied to studies conducted in temperate areas (Lord and Goff 2003). These stages are: Fresh, Bloated, Decay, Postdecay and Skeletal or Remains. The most common modification of this set is to subdivide the Decay Stage into Active Decay and Advanced Decay stages. Given the subjective nature of these stages, the Decay Stage is treated here as a single stage. As detailed discussions of insect and arthropod succession are presented elsewhere in this book, the treatment here will be primarily an overview and details of specific insect activity left to the more detailed discussions.

### 1.6.2 *Fresh Stage*

The Fresh Stage begins at the moment of death and continues until bloating of the body becomes evident. There are few distinctive, gross decompositional changes associated with the body during this stage although greenish discoloration of the abdomen, livor, skin cracking, tache noir may be observed. The insect invasion of the body generally begins with the natural body openings of the head (eyes, nose, mouth and ears), anus and genitals, and wounds present on the body. The first insects to arrive under most circumstances are the Calliphoridae (blow flies) and Sarcophagidae (flesh flies). Female flies will arrive and begin to explore the potential sites for oviposition or larviposition. These flies will often crawl deep into the openings and either deposit eggs of first instar larvae or maggots (Fig. 1.10). While the openings associated with the head are uniformly attractive to flies, the attractiveness of the anus and genital areas may depend on their being exposed or clothed. Wounds inflicted prior to death have been observed to be more attractive to flies for colonization if inflicted prior to death, when blood is flowing, than wounds inflicted postmortem and lacking a blood flow. During this stage, the eggs laid in the body begin to hatch and there is internal feeding activity, although there may be little evidence of this on the surface (see Fig. 1.15).



**Fig. 1.15** Fresh stage



### ***1.6.3 Bloated Stage***

The principal component of decomposition, putrefaction, begins during the Bloated Stage. The anaerobic bacteria present in the gut and other parts of the body begin to digest the tissues. Their metabolic processes result in the production of gasses that first cause a slight inflation of the abdomen. When this is noted, the Bloated Stage is considered to begin. As this progresses, the body may assume a fully inflated, balloon-like appearance. The combined processes of putrefaction and the metabolic activities of the maggots begin to cause an increase in the internal temperatures of the body. These temperatures can be significantly above ambient temperature (50°C+) and the body becomes a distinct habitat, in many ways independent of the surrounding environment. The adult Calliphoridae are strongly attracted to the body during this stage in decomposition and significant masses of maggots are observed associated with the head and other primary invasion sites. While these populations are visible externally, there are larger populations present internally. Internal pressures caused by production of gasses result in the seeping of fluids from the natural body openings during this stage and the strong smell of ammonia is noted. These fluids seep into the substrate beneath the body and this becomes alkaline. The normal soil fauna will leave the area under the body as a result in this change in the pH and the invasion of a set of organisms more closely associated with decomposition begins (see Figs. 1.16 and 1.17).

### ***1.6.4 Decay Stage***

While the start and termination points for the stages of decomposition are largely subjective, there is a definite physical event marking the start of the Decay Stage.



**Fig. 1.16** Nose



**Fig. 1.17** Bloated stage

This is when the combined activities of the maggot feeding and bacterial putrefaction result in the breaking of the outer layer of the skin and the escape of the gasses from the abdomen. At this point, the body deflates and the Decay Stage is considered to begin. During this stage, strong odors of decomposition are present. The predominant feature of this stage is the presence of large feeding masses of Diptera larvae. These are present internally, externally and often spilling onto the ground beside the body. While some Coleoptera have been arriving during earlier stages of decomposition, they increase in numbers during the Decay Stage and are often quite evident. Some predators, such as the Staphylinidae, are seen during the Bloated Stage and they become more evident now, along with others, such as the Histeridae. In addition to the predators, necrophages are also evident, increasing in numbers as the process continues. By the end of this stage, most of the Calliphoridae and Sarcophagidae will have completed their development and left the remains to pupariate in the surrounding soil. By the end of the Decay Stage, Diptera larvae will have removed most of the flesh from the body, leaving only skin and cartilage (see Fig. 1.18).

### ***1.6.5 Postdecay Stage***

As the body is reduced to skin, cartilage and bone, the Diptera cease to be the predominant feature. In xerophytic and mesophytic habitats, various groups of Coleoptera will replace them, with the most commonly seen being the species in



**Fig. 1.18** Decay stage

the family Dermestidae. These arrive as adults during the later stages of the Decay Stage but become predominant as adults and larvae during the Postdecay Stage. Their feeding activities remove the remaining dried flesh and cartilage from the bones and the scraping of their mandibles leave the bones with a cleaned, polished appearance. In wet habitats (swamps, rainforests, etc.), the Coleoptera typically are not successful. They are replaced in the process by other groups, including several Diptera families, such as the Psychodidae, along with their respective predator/parasite complexes. Associated with this stage in both types of habitats is an increase in the numbers and diversity of the predators and parasites present. The soil-dwelling taxa increase in number and diversity during this stage (see Fig. 1.19).

### ***1.6.6 Skeletal/Remains Stage***

This stage is reached when only bones and hair remain. Typically, there are no obviously carrion-frequenting taxa seen during this stage. During the earlier portions of the Skeletal Stage, there are a number of soil-dwelling taxa, including mites and Collembola, that can be used in estimating the period of time since death. As time passes, the pH of the soil begins to return to the original level and there is a gradual return of components of the normal soil fauna during this stage. There is no definite



**Fig. 1.19** Postdecay



**Fig. 1.20** Skeletal

end point to this stage and there may be differences in the soil fauna detectable for a period of months or sometimes years, indicating that a body was there at some point in time (see Fig. 1.20).

## 1.7 Protocol for Decomposition Studies

In order to have adequate data for use in estimations of the period of insect activity, it is necessary to conduct baseline studies of the decomposition process. While there has been a general similarity among studies, there have also been significant differences at the species level in taxa. This is particularly true for those species arriving later in the decomposition process. Many of the Diptera and Coleoptera species involved have somewhat cosmopolitan distributions but other groups tend to be localized. In order to assure the most accurate estimates, it is essential that studies used for estimations be from similar habitats and geographic regions to those in which the body is discovered. For example, work by Early and Goff (1986) and Tullis and Goff (1987) were both conducted on the island of Oahu, Hawaii, and separated by a distance of only 5 miles. The Diptera species were basically the same for both studies but the Coleoptera were markedly different. This was due to the study by Early and Goff being conducted in a xerophytic habitat while that by Tullis and Goff was located in a rainforest. Subsequent studies conducted on a beach some 11 miles away by Davis and Goff (2000) yielded still other species. Other perceived differences may be related to the aims of the study or the taxonomic interests of the investigator. For these reasons, it is important to have data available from baseline studies conducted following a standardized protocol. The protocol presented below is what has been used in studies conducted in the Hawaiian Islands. While some modifications may be needed for particular habitats, it can serve as a general model for conducting decomposition studies.

### 1.7.1 *Animal Model*

Animal model should be a domestic pig, ca. 20–30 kg in weight. For each study, three animals will be required. Animals will be dispatched with a single shot from a 38 cal. firearm at 0600 on day 1 of the study. The bullet is to traverse the head laterally, ear to ear. Alternately, the animal will be obtained from a commercial piggery and killed by commercial methods. In this case, the animal will be double bagged and transported to the site immediately following death.

### 1.7.2 *Arrangement of the Animals at the Site*

Placement of the animals for the study should be no less than 50 m apart. One animal will be placed directly on the ground and a thermocouple probe inserted into the anus; one animal placed on a wire mesh weight platform (constructed of 2.5 cm<sup>2</sup> welded wire mesh, reinforced with 2.5 cm diameter wooden dowels. Nylon rope should be attached to each corner of the weight platform to allow for weighing with the hand-held scale); and one animal placed on a welded wire mesh screen. There should not be any significant elevation from the ground for either of

the animals placed on wire mesh. Each animal should be protected by an enclosure cage constructed from 2.5 cm<sup>2</sup> welded wire mesh. Dimensions of this enclosure cage should be 1 m × 1 m × 0.5 m. Each corner of the enclosure cage should be secured using plastic tent pegs and nylon ropes.

### ***1.7.3 Climatic Data***

A hygrothermograph or equivalent instrument along with a high/low thermometer will be placed at the site to record temperature and relative humidity. These will be placed inside a weather station to prevent direct sunlight from altering the temperature data. A rain gauge should also be placed at the site.

### ***1.7.4 Sampling***

Sites should be visited at least twice daily for the first 14 days of the study. One visit should be at 1 h past solar zenith. The other visit will be at a time determined by the conditions of the site, but should be constant. Additional visits to the site are desirable, as time allows. Following the first period, the site should be visited on a daily basis for the next 14–21 days. There may be some variations to this schedule, based on differences between sites.

During each visit, weight of the animal on the weight platform will be recorded using a scale. It may prove valuable to construct a tripod over the enclosure cage to hold the scale during the weighing process. This may easily be assembled using 2 × 4's. Rainfall, maximum–minimum temperatures noted. The internal temperature of the animal with the thermocouple probe should be recorded. Temperatures should be recorded for the upper surface of the animal, areas of obvious arthropod activity (maggot masses, etc.) and the soil immediately adjacent to the animal. Observations should be made of the physical condition of each animal. Photographs will be taken of each animal on a daily basis. Observations of arthropod activity should be made for the animals on the weight platform and directly on the ground. No sampling should occur from either of these animals. The animal on the wire mesh screen will be sampled at each visit for arthropods.

Representative samples of immature arthropods collected from the animal on the wire mesh screen should be split into two portions. One part to be fixed in KAA for 1–3 h (depending on the taxon involved) and then transferred into ETOH for identification, and the other placed into rearing chambers to obtain adults. Adult insects should be preserved appropriately.

Soil samples should be taken every 3 days from beneath the sample animal. These samples should be 10 cm in diameter and ca. 0.5 cm deep. The samples should be taken from soil under areas of obvious arthropod activity. Samples should be processed using a Berlese funnel.

### 1.7.5 *Identifications*

Identifications should be as detailed as possible and confirmed by systematists familiar with the groups. This should be accomplished for all taxa, even those considered to be “common”. Voucher specimens should be deposited in an appropriate institution for future reference.

Each study site will be slightly different, and the above steps may be modified for each situation. The aim is to collect as much data from each study as possible. It is better to have a surplus of data as opposed to missing recording of data.

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