Egyptian Journal of Aquatic Biology & Fisheries Zoology Department, Faculty of Science, Ain Shams University, Cairo, Egypt. ISSN 1110 – 6131 Vol. 28(5): 2051 – 2071 (2024) www.ejabf.journals.ekb.eg



Forensic Importance of Aquatic Arthropods and Molecular Biomarkers in Criminal Investigations on Floating Cadavers: A Narrative Review

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ARTICLE INFO

Article History: Received: Sept. 21, 2024 Accepted: Oct. 25, 2024 Online: Oct. 30, 2024

Keywords:

Aquatic arthropods, Floating cadavers, Aquatic insects, Molecular biomarkers

ABSTRACT

In an aquatic environment, determining the cause and manner of death for bodies recovered from water can be quite challenging. Postmortem modifications add another layer of complexity to this process. First, it must be established that the recovery site is the primary death scene; for example, a death may occur on land, with the body subsequently disposed of by being submerged in water, and vice versa. Therefore, understanding how environmental conditions and variables affect the distribution of arthropods, including insects, on carcasses is essential for estimating the time interval after death. However, research and references focusing on the relationship between arthropods and corpses from a forensic perspective are sparse and often disconnected, particularly regarding the aquatic environment and aquatic arthropods. These organisms play a significant role in corpse analysis and detection during investigations, especially in criminal cases involving the flotation of bodies infested with fly larvae. The presence of these larvae is influenced by the aquatic environment, which is affected by various factors such as water type, chemicals present, physical characteristics, microbes in the surrounding water, and seasonal variations at the time of death. While many larvae have been identified at different developmental stages, their overall quantity tends to be lower compared to those typically found on terrestrial carcasses. Nonetheless, the presence of aquatic insects on corpses can serve as a valuable indicator of the location of death in relation to the aquatic environment. Thus, arthropod analysis is an important tool in investigations involving cadavers discovered in aquatic settings.

INTRODUCTION

Indexed in Scopus

Forensic arthropodology is the study of arthropods and other invertebrates found at crime scenes (Catts & Goff, 1992). Arthropods, including insects, are commonly associated with decomposing vertebrate corpses (carrion) (Amendt *et al.*, 2004). The post-mortem interval (PMI) means that arthropods, including insects, reach the corpses.

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We can use it to determine the time after death, for example: the time distance between death and the discovery of the corpse, the movement of the corpse, the manner of death, and the causes of death. The suspects are at the scene of death (Sukontason *et al.*, 2007).

In a liquid medium, decomposition proceeds quite differently than it does in an airborne one. Like the typical decomposition process in a dry environment, clothes, bacteria, temperature, and animal predation; all have an impact on postmortem alterations in water. A body in water will have a different look depending on other factors like current and the physical changes caused by tissue saturation. Postmortem decomposition happens quickly when the body is taken out of the watery environment, as the author and others have noticed. Because of this, it is advised that, as soon as a corpse is recovered from the water, the postmortem examination not to be postponed for an extended period of time (**Dolinak** *et al.*, **2005**).

✤ Significance of forensic microbiology in legal investigations

The contamination of dairy products by bacteria is of emerging concern to human health. Forensic dairy microbiology represents a vital role in criminal investigations by offering crucial insights into dairy products' origin, handling, and potential adulteration. Microbial analysis can help set up timelines, find the source of contamination, and decide the cause of spoilage or illness. For instance, the presence of specific microorganisms can show whether a product has been improperly stored, transported, or processed. Furthermore, the analysis of microbial profiles can aid in finding potential perpetrators, setting up links between crime scenes and suspects, and corroborating witness testimonies (Schmedes et al., 2016). By using time since death decomposition analysis, forensic experts may also examine the era and the progressive changes that happened at the same time. Usually, the evolution may be broken down into many discrete stages: 1. Fresh; 2. Bleated; 3. Decay; 4. Post-decay; 5. Dry skeleton; Every step is also linked to an approximate time frame in which it can happen, taking into account any additives that can change such time frames. A unique species of insect and bacterium is also known to have arrived at each of these stages. DNA sequencing is a time-tested technique used for research purposes in forensic situations. Furthermore, this approach includes a variety of jobs and initiatives that were previously disregarded. Thus, the more modern technique in microbial analysis is DNA sequencing. Many additional details regarding the environment, how it changes over time, and how it interacts with and alters the ecology of its larger surrounds might be quite useful in the field of forensic research. For example, it may result in new, more accurate methods for determining the time of death and locating corpses that have been hidden (Patnaik & Jana, 2005).

This review aimed at providing general direction to forensic arthropologists on the possibility of analyzing forensic data according to aquatic arthropods and their morphologcal characteristics of larvae, growth, and distribution of species and describing the aquatic environment in which the body was found floating in criminal investigation (Fig. 1).

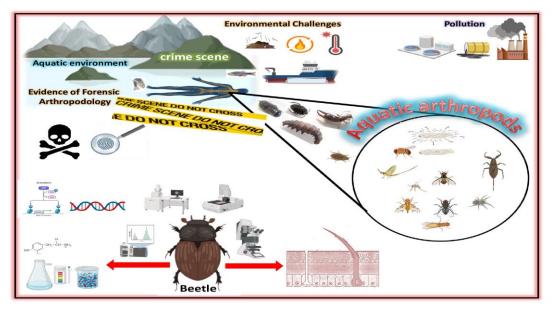


Fig. 1. Diagram showing the review items.

* Aquatic arthropods associated with postmortem changes of the carrion's body

Aquatic arthropods can be extremely helpful in forensic investigations when bodies are discovered in or close to water (Elango et al., 2021). These bodies may be the result of accidents, homicides, suicides, or natural disasters (Magni et al., 2015). Despite the complexity of decomposition in freshwater or marine environments—where numerous factors can interact, such as the presence of scavengers, the type of water body, wounds, biodiversity, and clothing—the body typically disarticulates slowly over time (Magni et al., 2015). In particular freshwater habitats, insects can make up more than 95% of all macroinvertebrate individuals or species (Merritt & Wallace, 2009). Arthropoda use the dry or skeletonized remains as living quarters, a food resource, or a surface for attaching to, and algae can offer food for other arthropods to feed on. The identification of these organisms can be useful in determining the origin and movement of the remains during their presence in the water body, as well as the length of time the remains have been floating (Magni et al., 2015; Abd El-Aziz et al., 2023b). Identification of aquatic macroinvertebrates for understanding the part of these organisms shown in crime scene investigations will be discussed in the following parts.

Aquatic arthropods, e.g., Ephemeroptera, Plecoptera, Diptera, Trichoptera, Simuliidae, and Chironomidae use the body remains as a shelter, then they utilize determining postmortem interval (PMI) (Pirtle *et al.*, 2019). Necrophagous insects

(mostly Diptera: Sarcophagidae, Calliphoridae, Muscidae) are often used to estimate min PMI in terrestrial environments (Zimmerman & Wallace, 2008). The greatest predominant arthropods of forensic utility in saltwater environments are crustaceans, including crabs, crayfish, and barnacles (including acorn and gooseneck barnacles). While barnacles are sessile species that permanently adhere to substrates and use the carcass solely as shelter, crabs and crayfish are motile animals that feed on the carcass (Pirtle *et al.*, 2019).

***** The carcass decomposition stages in aquatic environments

Megnin (1894) was the first to describe the stages of decay in aquatic environments. He talked about the relationships between different terrestrial entomological fauna and each of the eight decomposition stages that he identified for these post-mortem alterations. These stages eventually served as synopses for five phases (Payne, 1965). There are five decomposition stages of cadvers. Numerous writers observed similar observations. The process by which the intricate molecules and organs of human and animal bodies gradually transform into simpler organic stuff is known as decomposition. Five phases of decomposition are often identified in vertebrates: fresh, bloat, active decay, advanced decay, and dry or skeletonized. Determining the postmortem interval (PMI) can be aided by investigators having knowledge of the various phases of decomposition. Environmental conditions and other variables can affect how quickly human remains decompose. Temperature, burning, humidity, and oxygen availability are examples of environmental parameters. Additional elements include attire, body size, and the reason for death (Valdes-Perezgasga et al.m 2010; Aly et al., 2013; Abd El-Aziz & El Shehaby, 2019; Abd El-Aziz et al., 2019, 2022a, b, c, d), with minor modifications (Fig. 2).

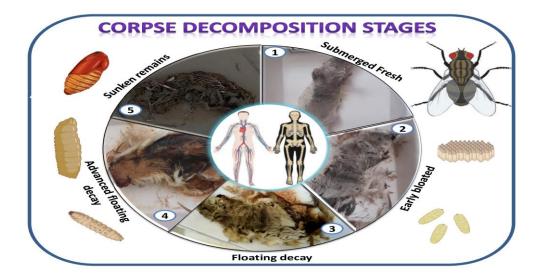


Fig. 2. The decaying stages in aquatic environments

1. Submerged fresh stage

Fresh (Autolysis) carrion: In this stage, there is no association with insects. The corpse undergoes algor mortis, rigor mortis, and livor mortis. Duration: 1-9 days. This stage ends when the body floats to the surface, with no outward signs of decomposition.

2. Early floating stage

The carrion rises to the surface. Terrestrial insect species are drawn to carcasses that protrude above the water. The smell of decay is strong and noticeable. The carcass shows indentations from wire. Time frame: two to ten days.

3. Floating decay stage

Both the fore and rear limbs began to shed their skin, and the corpses took on a blackish hue. Gas release caused the abdomen to contract, but the corpse remained floating. Impressions of wires were still visible. Time frame: five to eighteen days.

4. Advanced floating decay stage

This stage is characterized by liquefied tissues and an intact intestine protruding from the abdomen, along with a ruptured intestinal pelvis. There is severe larval infestation and putrefaction, leading to skin and fur separation, while the entire body surface remains largely intact. Fumes escape from the body as it continues to float. A large portion of exposed body parts disappears due to the feeding habits of blowfly larvae, which grow and begin to move. Separation of toe and arm bones occurs during this time. Time frame: eight to twenty-two days.

5. Sunken remains stage

In this stage, parts of bones and fragments of skin turn black. The smell of decay becomes barely noticeable, and many young water bugs are discovered. The time frame for this stage ranges from nine to nineteen days.

According to **Welch** (1952), fluctuations in aquatic benthos associated with benthic communities are influenced by various factors, including chemical, physical, and biological elements. The substrate conditions of the bottom sediments are also crucial.

Late-arriving beetles and flies often rely on the actions of early colonists to prepare the excavation for them. For instance, the activity of early successional dung beetles can significantly increase the bacterial content in manure (Lussenhop *et al.*, **1980**). These bacteria often serve as a food source for subsequent colonists (Landin, **1961**). Most insect activity occurs in the first one or two days following excrement deposition; however, insects arriving in cattle dung in tropical regions also exhibit regular colonization patterns (Horgan, 2002; Marchiori *et al.*, **2003**).

Earthworms provide numerous benefits to soil, animals, and humans during their lives and even after death (Abd El-Aziz & Bashandy, 2019; Abd Ellah *et al.*, 2019; Abd El-Aziz & Ali, 2021; Abd El-Aziz *et al.*, 2022e; Salem *et al.*, 2022; Abd El-Aziz *et al.*, 2023a). Earthworms often appear in manure later in the decomposition sequence. Their consumption and mixing of dung are essential for effective decomposition, especially in colder regions (Holter, 1977; Putman, 1983; Abd El-Aziz & Abdelhamid, 2024; Abd El-Aziz *et al.*, 2024). For instance, Denholm-Young (1978) calculated that in temperate climates, earthworms consume 30 to 40 percent of the dry mass of dung in winter and 50 to 60 percent in summer. In tropical areas with prolonged dry seasons, dried manure often accumulates on the soil surface until rain arrives. In dry tropical pastures, termites help break down dry dung (Freymann *et al.*, 2008). Additionally, birds and animals may facilitate dung breakdown and erosion by mechanically breaking up dung pats (Horgan & Berrow, 2004; Dawood *et al.*, 2024) (Fig. 3).

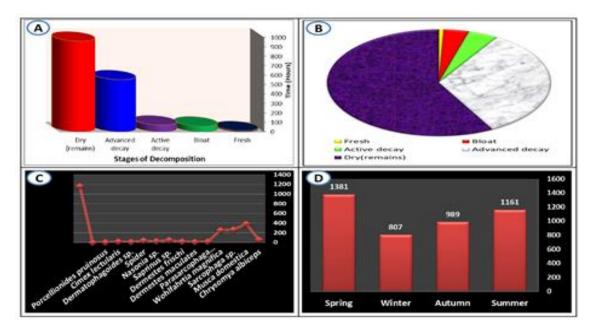


Fig. 3. (**A&B**) Length of time of colonization estimate by stages of decomposition. (**C**) Number of arthropods from rat carrions. (**D**) Number of arthropods from rat carrions in four seasons

✤ Collection of aquatic arthropological evidence

The evidence collected from arthropods is conceded to be the procedure of evidence collection built on arthropod signs found in crime investigations. Doubt evidence is not sensibly conserved at a crime scene postmortem; it could be problematic for an arthropodologist to make an accurate identification and classification of species; e.g., wholly features morphology are not conserved (**Joseph** *et al.*, **2011**).

• Factors affecting the decomposition stages of floating cadavers

A. Environmental factors

In a liquid medium, decomposition proceeds quite differently than it does in an airborne one. Like the typical decomposition process in a dry environment, clothes, bacteria, temperature, and animal predation all have an impact on postmortem alterations in water. A body in water will have a different look depending on other factors like current and the physical changes caused by tissue saturation. Postmortem decomposition happens quickly when the body is taken out of the watery environment, as the author and others have noticed. Because of this, it is advised that, as soon as a corpse is recovered from the water, the postmortem examination not to be postponed for an extended period of time (**Dolinak** *et al.*, **2005**) (Fig. 4).

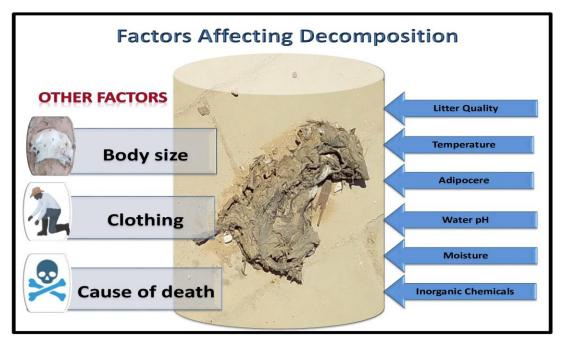


Fig. 4. Factors affecting on carrions decomposition

i. Temperature and water current

Regarding the seasonal abundance of total numbers of arthropod successions, the highest peaks were observed during spring, while the lowest was in winter. The increased numbers of arthropods through spring might be owing to suitable conditions of environmental issues through this season during the year, which contains chemical, physical, and biological features. Ngqulana (2012) described an aquatic ecosystem and mentioned that the seasonal variations play a main role in structuring the aquatic insect community. According to Adedeji *et al.* (2012), water temperature affects biological tolerances and chemical reaction rates; vertical variations can control a water column's total stratification through water density. Cold water temperatures help decelerate the decomposition procedure of carcasses. But hot containers and tropical builds of aquatic do the opposite. The current has mainly an automatic influence on objects in the water (Caruso, 2016).

ii. Litter quality, inorganic chemicals and plants

The abundance of plant species in the Nile creates a mosaic of communities, which is one of its defining features (Zahran, 2009). Decomposing plant material provides food for aquatic detritivores and creates refuges for midges, mayflies, and other aquatic insects, facilitating the prevention of predation during insect development. This occurs most abundantly in vegetated areas, which are environments created by emergent plants and open water (Magee *et al.*, 1999). Vegetation appears to have a significant impact on macroinvertebrate communities (Battle *et al.*, 2001). Insects such as Ephemeroptera, Odonata, Hemiptera, Coleoptera, Trichoptera, Lepidoptera, and Diptera are abundant in Egypt's aquatic environments (Agami, 1989).

iii. Adipocere and humidity

The pace of decomposition of carrion is dependent on the moisture content of the surrounding environment. Grease production is impacted by humidity, and the rate of degradation is accelerated. More arid settings, on the other hand, may dry out quicker but will typically break down more slowly (Schoenen & Schoenen, 2013).

iv. Arthropods and animal predation

Insect activity and other arthropod and animal predators fluctuate greatly between the watery and terrestrial environments. The body will frequently rise to the surface, where it will be accessible to common arthropod predators like blowflies and carrion beetles. The body portion that is submerged will be open to attack from different predators. The remnants' condition and appearance can be changed by aquatic insects. Huge fish, large crabs, and turtles are examples of creatures that may injure tissue in ways that resemble bodily trauma. Soft tissue is the food source for small fish, crabs, shrimp, and other

invertebrates. If given the chance, these animals can totally deform exposed bodily portions (**Duband** *et al.*, **2011**).

B. Other factors

✓ Cadaver body mass

The body size of the carcass is a vital factor because it likewise affects the decomposition rat. The greater body weight and fatter, the faster it decomposes. This is for after the postmortem, the fat will liquefy and constitute a great part of the decomposition. Humans with less obesity will have the slowest breakdown. This is for older adults and the little ones (**Mann et al., 1990**).

✓ Cadaver clothing

The decomposition of corpses is affected by clothing and other types of covering because they limit the carrion's contact to outside features like weather factors, water, and insect access. It reduces decomposition by slowing the collection of arthropods around the carcass. Nevertheless, arthropod activity will rise because the cover will provide additional heat and defense from the sun, provided that an idyllic environment for larvae to grow simplifies organic decomposition (**Haglund** *et al.*, **2001**).

✓ Manner of death

The cause of the postmortem can likewise have an impact on the pace of decomposition, mostly through acceleration. Dead wounds, such as knife wounds or other body abrasions, draw insects because they offer a suitable location for them to deposit their ova, which can speed up the decomposition process (Smith, 2014; Abd El-Aziz, 2024).

• Fly larvae of forensic significance found on floating corpses

Getting a valid estimate of the time since death, or the minimum post-mortem interval (min PMI), is a basic problem for medico-legal death investigators when analyzing human remains. The most trustworthy source of time measurement throughout the body's decomposition process may be the carrion fauna that colonizes it, particularly insects like blowflies (Diptera: Calliphoridae) (**Rognum** *et al.*, **2016**). When forensic entomologists are called upon to estimate post-mortem periods during a death investigation, they rely on knowledge of the factors that impact the growth and development of blowflies (Diptera: Calliphoridae), which are the primary colonizers of decaying remains. In terrestrial settings, a large portion of this effort has been classified and measured, but in aquatic contexts, it is scarce. The longest immature life stage in the life cycle of a blowfly involves metamorphosis and lasts from the creation of the puparium to adult emergence. A body in an aquatic environment might be fully immersed, floating on the surface, or

connected to water but not submerged or floating (such as washing up on a beach or beached on a seashore after a flood). The two species of blowflies—*Lucilia sericata* and *Calliphora vomitoria*—as well as their survival in tap, river, or saline water for up to three days are examined, along with the impact of the age of the intra-puparial forms, or "pupal age." Before being submerged, the entire puparia of both species—*L. sericata* and *C. vomitoria*—were split into four age cohorts: "white," "young," "middle," and "old." In every one of the three types of water, *L. sericata* intrapuparial forms had a threefold higher survival rate than *C. vomitoria* intrapuparial forms. In tap water, both species had the best survival rate. When submerged, younger and older intra-puparial forms had a higher and statistically significant survival rate than medium intra-puparial forms (**Paola** *et al.*, **2021**).

Molecular biomarkers for determination of postmortem interval in drowning cadavers

• Investigating gene activity in submerged bodies

Molecular biology techniques play a significant role in forensic medicine, particularly in the areas of identification, paternity testing, immigration proceedings, and the examination of biological stains in criminal cases in addition to identifying the reason and cause of death, particularly in cases of violent and unexpectedly sudden deaths, which raise serious social and medicolegal implications for both the public and the individual (Maeda et al., 2010). Identifying victims and biological materials is just one of the numerous ways that forensic molecular pathology helps explain human death and evaluates each individual's death based on biological molecular evidence. It also helps visualize dynamic functional changes occurring during the dying process but are not visible through morphology. The genetic background (genomics), metabolic deterioration (metabolomics), vital phenomena involving degenerative products (proteomics) and activated biological mediators, as well as dynamics of gene expression (up-/downregulation: transcriptomics), can be detected using real-time reverse transcription-PCR (RT-PCR), DNA analysis, immunohisto-/immunocytochemistry in combination with biochemistry, and relative quantification of mRNA transcripts, as well (Maeda et al., **2014**). Since decomposition in a wet environment differs from that in other environments in terms of both the changes that occur and the rate at which they occur, recovering bodies from water is a regular task for any medical examiner or coroner office. In a wet environment, normal breakdown processes happen more slowly because of the anaerobic environment and lower temperatures (Caruso, 2016). In addition to various abiotic and biological factors that affect the decomposition of carrion in water, insect evidence (such as blowflies), which is frequently used to estimate the PMI, is not present earlier to the carcasses floating on water (Merritt & Wallace, 2009). Wang et al. (2022) conducted a study about the target genes (β -actin, 18S, and GAPDH) in the rat's brain tissue to estimate the PMI of a carcass in water. The results show changes in expressions of afterdeath GAPDH, β -actin, and 18S within 7 days using 5S as the reference gene. The GAPDH gene increased in gene expression relative to the 5S gene, and then its expression was gradually down-regulated on day 2. The gene expression of β -actin was up-regulated on days 0–6, which positively correlated with the decomposition time.

The forensic application of miRNA analysis has attracted a lot of interest in the last 10 years. The main aim of the forensic application of miRNA analysis has been the identification of bodily fluids in order to offer a universal study that validates the identity of biological stains that remain unknown after being taken from crime scenes or evidence objects. Mammalian blood contains miRNAs, which are very biostable and easily circulate. They have a high degree of sensitivity and specificity when detected in human serum and plasma (Alzahrani *et al.*, 2023). Mohamed *et al.* (2023) reported that microRNA-23b-3p and microRNA-381-3p levels showed a significant difference in lung and brain tissues in drowned rats at different postmortem intervals. Another study by Yu *et al.* (2015) compares saltwater and freshwater in mice drowning models. They found that a high expression of miR-706 was found to be a potent biomarker for the identification of drowning patterns in brain tissue in the freshwater drowning group compared to the control and saltwater drowning groups.

Barranco *et al.* (2020) focused on immunohistochemical expression of the renal aquaporin 2, V2R, AVP, and renin in cases of drowning in order to understand the distinctions between drowning in freshwater and drowning in saltwater compared to death by gunshot to the head. Renal tissue samples were collected from different females and males of different ages. The samples were taken from the left and right kidney, including the medulla and cortex. The result showed a higher expression of AVP and AQP2 in cases of saltwater drowning than in freshwater drowning and control cases. V2R shows no statistically significant differences between the studied groups. The renin tubular expression was increased in both saltwater drowning and freshwater drowning compared to controls (Fig. 5).

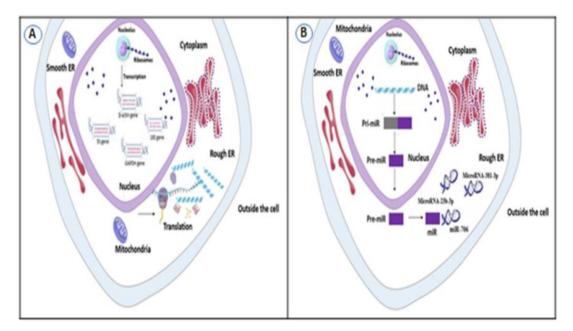


Fig. 5. (A) β -actin, GAPDH, and 5S genes in the rat's brain tissue were associated with the PMI increased at the beginning of death. (B) High expressions of miR-706, MicroRNA-23b-3p and microRNA-381-3p levels in rat's brain tissue at the beginning of death

• The impact of death on gene expression

After death, the body undergoes a series of several physiological processes, including alterations in molecular and genetic evidence (Javan et al., 2024). These postmortem alterations are influenced by a diverse range of factors, including the corpse's fat content, intoxication, infections or injuries, the presence of clothing or other protective coverings, the temperature, and the geographical location at which the body is discovered, etc. (Shedge et al., 2023). Studies analyzed how lipids, particularly triglycerides and fatty acids, change during the decomposition of bodies, and considered how these compounds might be used as markers to determine PMI and identify burial sites (Caballero-Moreno et al., 2024). Postmortem toxicological investigations are used to determine if foreign substances caused impairment, contributed to death, or were a cause of death. A toxicological investigation is performed in all cases involving young adults and children; it may also be desirable in cases of occupational accidents, traffic accidents, homicides, etc. (Skopp, 2010; Omran, 2022). Reactive oxygen species (ROS) and antioxidant stress indicators in living cells are affected by tissue injury in favor of ROS. The ROS level also exhibits a time-dependent increase after death (Clarkson & Thompson, 2000). Bonadio et al. (2021) listed a number of biomarkers linked to the buried and submerged groups, suggesting that each of these environments can have a unique impact on the post-mortem RNA abundance.

Recent biochemical, molecular, and metabolic profiles of different bodily fluids correlated with the postmortem period more accurately than the conventional physical examination (Welson et al., 2020). Postmortem gene expression patterns have been used in several investigations to determine the cause and time of death (Scott et al., 2020). Welson et al. (2020) evaluated PMI in relation to HMGB1 molecular gene expression in the male albino rat's main organs, as in the kidney, heart, and testis. The result shows an increase in HMGB1 gene expression from 48h until 120h postmortem in the heart and kidney rather than the testis, which showed a weak correlation. While De-Giorgio et al. (2023) determined HMGB1 proteins and related components (RAGE and Beclin1) in the skin tissue of adult male mice in order to determine post-mortem immunohistochemical, the result shows that cytoplasmic HMGB1 negativization indicates that more than 48 hours have elapsed since death, nuclear HMGB1 negativization with high cytoplasmic HMGB1 intensity indicates that death occurred between 12 and 36 hours prior, and nuclear HMGB1 overexpression indicates that death occurred within the last 12 hours. Over time, the cytoplasm's levels of Beclin1 and RAGE also decreased. The negativization of the latter proteins may suggest that more than 24 and 36 hours had elapsed from the moment of death, respectively. When employing immunohistochemistry to determine PMI in forensic practice, these markers may prove useful.

Noshy (2020) selected four genes related to apoptosis as transformation-related protein 53 (Trp53), caspase 3 (Casp3), BCL2-associated X protein (Bax), and B cell leukemia/lymphoma 2 (Bcl2). According to the findings, postmortem Casp3 expression overexpressed in a time-dependent manner; Bax expression increased from 3 to 18 hours after death before declining 24 hours later; Bcl2 expression dropped in a time-dependent manner after death, and Trp53 expression increased from 3 to 6 hours before it started declining from 9 to 24 hours after death. Hanna et al. (2021) determined the postmortem interval by using immunohistochemical markers (P53 and caspase-3) and a histological analysis of the rats' skeletal muscles at different times. The findings showed that histological alterations developed gradually throughout the period after death. The results of p53 and caspase-3 immunohistochemistry revealed a significantly negative correlation with PMI. It was determined that skeletal muscle immunohistochemistry of apoptotic markers (p53 and caspase-3) and postmortem histological alterations may be useful in determining a postmortem interval. Halawa et al. (2019) examined the expression of Bcl2 and Caspase-3 in the brain tissues of rats in three groups: the control group, the room temperature group, and the postmortem heat stress group. The result determined that Bcl-2 in the room temperature group and heat stress group significantly decreased at 6h of PMI compared with the control group. While caspase-3 in the room temperature group and heat stress group decrease from 1h until 6h compared with the control group.

CONCLUSION

After death, the body undergoes several physiological changes, including alterations in genetic and molecular evidence. Numerous factors can influence these postmortem changes, such as the body's fat content, intoxication, pre-existing diseases or injuries, the presence of clothing or protective coverings, temperature, and the location where the body is found. Determining the time interval following death is essential, and understanding how environmental factors and conditions affect the distribution of arthropods, particularly insects, on carcasses is crucial.

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