Faculty of Science & Technology

Alteration of Bloodstain Patterns by Diptera: Objective Analysis Based on Wonder's 2001 Criteria

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Abstract

The blood feeding activity of flies at crime scenes can be confounding. Where a high number are present at a crime scene, a forensic investigator could mistakenly sample a fly artefact with the assumption that it is blood spatter, thus, making errors in the reconstruction of the event, or even recovering DNA from an individual who has not attended the scene.

Three experiments were conducted to investigate the blood feeding activity, and blood artefact patterns created by flies following a blood meal. The respective trials were undertaken in a staged environment that can be considered operationally relevant to the field of bloodstain pattern analysis (BPA); in which approximately 500 flies per experiment were exposed to tray of 500ml of horse blood in a sealed 3m x 3m gazebo for a period of approximately 72 hours.

The resulting patterns, consisting of a total of 539,507 individual fly blood artefacts, were objectively compared to recreated impact and expirated bloodstain patterns. These comparisons focused on overall pattern shape, total artefact/stain numbers, artefact/stain density per cm^2 and the distance from the blood source in which they were deposited. By utilising the bloodstain size classification proposed by Laber in 1985 as a model, individual artefact/stain size was also examined. Informal observations and recordings were also made regarding similarities in individual artefact/stain colour, artefact/stain alignment and artefact/stain morphology but these were not quantified. Lastly, the present-day suitability of Wonders 2001 objective criteria for BPA was assessed against its suitability to include the feeding/deposition activity of flies, which is currently not accounted for.

Results found highly significant differences between genera in the zones which their artefacts were deposited, the size of these artefacts ($\chi^2_{0.05,\ 4} = 314093$, $p < 0.01$), their count ($F_{0.05,\ 4,\ 476025} = 137082$, $p < 0.01$) and their densities ($F_{0.05,\ 2,\ 538278} = 77680$, $p < 0.01$). Furthermore, these results indicate that Diptera have a high affinity for window areas. From a total of 170,898 within this area, where fly artefacts were considerably more abundant than in other areas, *Calliphora*

deposited 33.9% of its total artefacts, *Hydrotaea* 31.1% and *Lucilia* 29.1%, suggesting that artefact deposition /bloodstain pattern alteration by Diptera may occur significantly more in areas of light ingress.

Moreover, in the tested window area, all five artefact categories (*Calliphora*, *Lucilia*, *Hydrotaea*, impact spatter and expirated spatter) displayed significantly different count totals ($F_{0.05,\ 4,\ 310417}$ = 364774, p <0.01) thus, significantly different respective densities ($F_{0.05,\ 4,\ 284227\ =}$ 360217, p <0.01). Additionally, impact spatter, expirated spatter and the fly artefacts from the three genera of flies exhibited significant differences in their respective zonal distribution (A-E) in both artefact count ($F_{0.05,\ 4,\ 120369}$ = 38787, p < 0.01) and artefact density ($F_{0.05,\ 4,\ 107509\ =}$ 4597, p < 0.01). These findings suggest that within fly artefact deposition patterns, and generated impact and expirated bloodstain patterns, distinctive areas in which artefacts/bloodstains will be of higher or lower count and density are present. As shown here, this could be confounding for a BPA analyst when fly artefact patterns are overlaid on top of these spatter patterns, creating a uniformly dense mixture of fly artefacts and bloodstains.

As a result of these studies, three additional criteria are proposed for addition to the current objective criteria:

1.) External factors impacting on overall pattern shape

2.) Morphology (shape) of individual bloodstains

3.) Colour of individual bloodstains

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1. Introduction

1.1 Biological functions and physical properties of human blood

Blood is a complex and unique substance that is fundamental to all human life and such is the importance of blood that its components have been isolated and synthesised, yet nothing currently available maintains life as efficiently (Wonder 2001).

Biological Functions of Human Blood

Human blood, a liquid connective tissue, serves the two primary functions of transport, and defence. The most well-known function of blood is its role in the transport of oxygen to eukaryotic cells and the transport of carbon dioxide to the lungs for elimination from the body through respiration (Waugh and Grant 2010). However, equally vital roles performed by blood are the transport of glucose, amino acids and lipids to the cells of the body from the alimentary canal, and to act as a carrying medium for hormones that have been secreted directly into the blood by endocrine glands. Electrolytes that serve to maintain the acid-base balance such as calcium (Ca^{++}), sodium (Na^{+}) and bicarbonate (HCO_3-) are also transported in the blood, thus sustaining life and neuroconduction (James *et al* 2005).

Despite the fact that blood serves as a supply function, it also carries out an essential role in waste removal. During metabolism, waste products such as carbon dioxide, urea, uric acid, lactic acid and creatinine are produced at cellular level and accumulate in tissue fluid. If not removed from the body, levels can become toxic and death can occur (James *et al* 2005).

In addition to the transport of essential substances to the organs of the body, and subsequent waste removal, blood also plays an important role in the regulation of body temperature. Highly active areas of the body produce more heat than others and the body requires this to be equalised in order to function correctly. This is achieved

by way of the blood distributing any excess heat as it moves throughout the body, thus allowing it to dissipate (James *et al* 2005).

An additional essential function of blood is the part it plays in the defence of the body. Leukocytes or white blood cells (WBC) are produced and stored in the bone marrow and lymphatic tissue until they are needed as replacement for other aging white blood cells, or in response to infection. In the latter case, they are circulated throughout the body in the bloodstream (Jackson and Jackson 2008).

One of the most complex functions of blood is its ability to clot. In response to injury, and to prevent excessive, potentially fatal blood loss, the clot formation process begins the instant an injury occurs, whilst still leaving the circulatory blood in a liquid state (Waugh and Grant 2010). The full mechanism of blood clotting is beyond the scope of this paper, however, clotting factors and the pathways of clot formation can be seen in Figures 1 and 2 respectively.

I	Fibrinogen
II	Prothrombin
III	Tissue factor (thromboplastin)
IV	Calcium (Ca^{2+})
V	Labile factor, proaccelerin, Ac-globulin
VII	Stable factor, proconvertin
VIII	Antihaemophilic globulin (AHG), antihaemophilic factor A
IX	Christmas factor, plasma thromboplastin component (PTA), antihaemophilic factor B
X	Stuart Prower factor
XI	Plasma thromboplastin antecedent (PTA), antihaemophilic factor C
XII	Hageman factor
XIII	Fibrin stabilising factor

(There is no factor VI)
Vitamin K is essential for synthesis of factors II, VII, IX and X.

Figure 1. Clotting factors found within human blood (Waugh and Grant 2010)

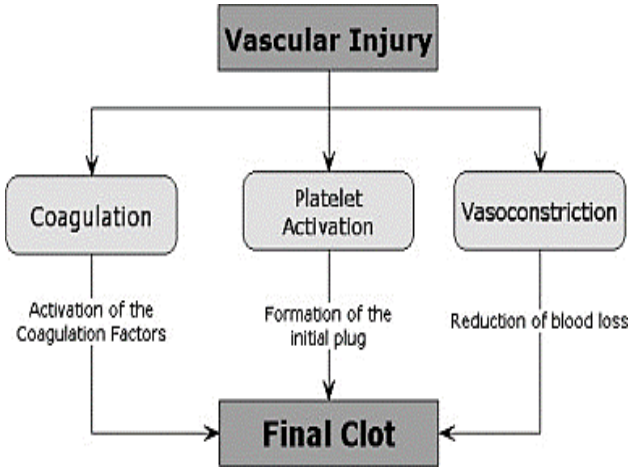


Figure 2. Pathways of blood clot formation (Acosta *et al* 2010)

It is clear that blood serves an abundance of vitally important functions, all of which are made possible by the almost unlimited access it is afforded to all parts of the human body through a complex network of arteries, capillaries and veins (James *et al* 2005; Waugh and Grant 2010).

Physical Properties of Human Blood

Whole blood is described as a viscoelastic, non-Newtonian, fluid connective tissue (Wonder 2001) and is comprised of the clear, pale yellow, watery liquid medium, plasma, within which is suspended several different types of blood cell (Houck & Siegel 2010). Red blood cells (RBC) or erythrocytes comprise approximately 44%-45% of whole blood volume (Jackson and Jackson 2008), with approximately 55% being plasma. Less than one percent of whole blood is comprised of white blood cells (WBC) and platelets (Jackson and Jackson 2008; James *et al* 2014; Waugh and Grant 2010).

RBC are heavier than plasma, as can be observed in post-mortem lividity (PML) when they settle at the lowest portion of a body following death (Wonder 2001). Similarly, under centrifugation, the denser RBC separate from the plasma, platelets and WBC and migrates to the bottom of the test tube (Figure 3).

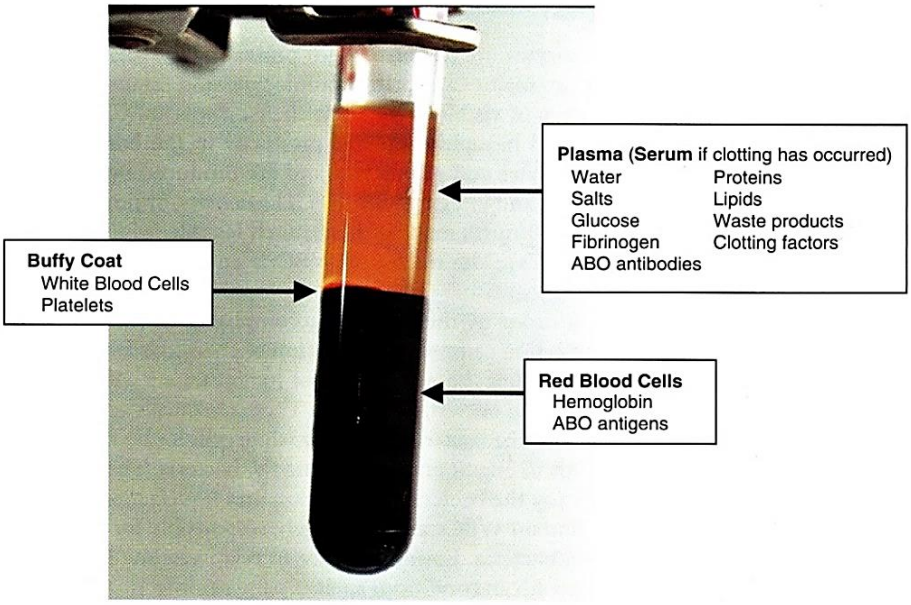


Figure 3. Components of whole human blood (James *et al* 2005)

Whole blood has a pH of 7.4 and contributes between 7 to 9% of the total body mass (James *et al* 2005; Waugh and Grant 2010). This is considered to equate to approximately 63g of blood per kg of body weight, or 60–66mL of blood per kg of body weight (James *et al* 2014).

The average adult has between 4.5 – 5.0L of blood; however, these volumes differ between males, females, children and infants (Waugh and Grant 2010), with males averaging 5.7L, females 4.3L, an 80lb child having 2.4-2.5L and an 8lb infant expected to have approximately 0.25L of blood (James *et al* 2005).

Whole human blood is a liquid that contains suspended biological cells and particles, dissolved substances, and sedimenting biological cells and particles, in addition to filterable and non-filterable material. Wonder (2001 p.7) claims that because of this unique chemical composition, blood can be described as a colloid, a true solution, not a true solution, a compound biochemical and many more such terms.

Outside of the, body blood droplets are held together by external surface tension created by strong cohesive molecular forces. This surface tension pulls surface molecules inwards and reduces the droplet surface area, and causes the liquid to resist penetration. Owing to this fact, no matter how far a drop of blood falls through the air, it will not break into smaller droplets or spatter unless the surface tension is disrupted by an object or a surface (James *et al* 2014). Furthermore, the extent to which a blood droplet separates upon impact is entirely dependent on the type of surface it strikes (Houck & Siegel 2010).

In the application of forensic science, two components of blood are the most important: the liquid (plasma) and the major particulate fraction (RBC), and are described as a percentage of the packed [red] cell volume (PVC). This is commonly described as the hematocrit. The human hematocrit is variable between individuals, their organs and their different blood vessels (Wonder 2001).

1.2 History of Bloodstain Pattern Analysis

Of the many types of physical evidence associated with the forensic investigation of death and violent crime, blood is one of the most frequently encountered and significant (James *et al* 2005). Bloodstain pattern analysis (BPA), is described by Bevel & Gardner (2002) as analysis of the shape, distribution, categorisation and relationship of bloodstain patterns at a crime scene, enabling reconstruction of events. Throughout history, blood shed as the result of violent acts have been widely depicted in iconographic form, providing some insight into the level of understanding of blood dynamics possesed by past populations.

Figure 4. The original publication, in German, of the works of Dr. Eduard Piotrowski (1895)

The first known systematic study of blood shapes and distribution that has been documented and preserved was carried out in 1895 by an assistant at the Institute for Forensic Medicine in Krakow, Poland, Dr Eduard Piotrowski.

Piotrowski's work (Figure 4), entitled *Uber Entstehung, Form, Richtung und Ausbreitung der Blutspuren nach Hiebwunden des Kopfes* (Concerning Origin, Shape, Direction and Distribution of the Bloodstains following Head Wounds Caused by Blows), was first published in 1895 in Vienna. The work was based on experiments conducted in which Piotrowski prepared sections of a room with sheets of white paper and observed and documented the blood patterns that resulted from beating anesthetised rabbits to death with a variety of different weapons (Figure 5). During these experiments he came to realise that bloodstains often appear with the second blow, after the blood source has been exposed.

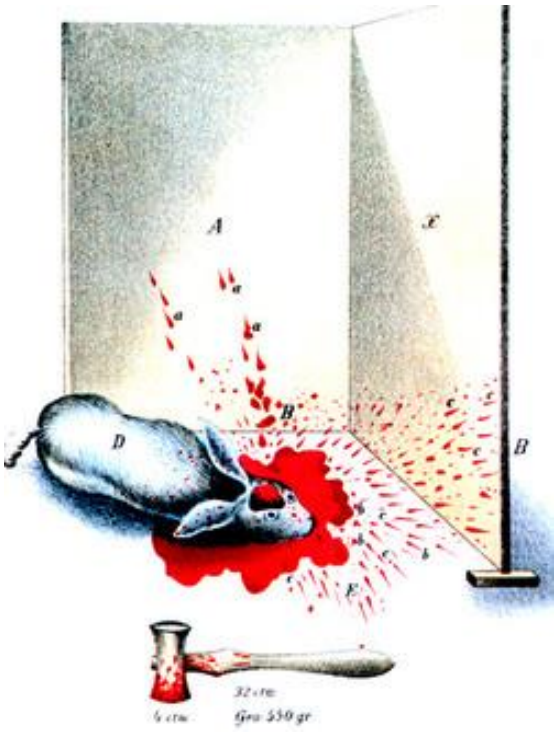


Figure 5. Colour diagram of experiment of Dr Piotrowski in which anesthetised rabbits were beaten with a variety of weapons (Piotrowski 1895)

Piotrowski recognised, and emphasised the importance of bloodstains in the forensic context, claiming that they throw light on a murder, and help to explain the most

essential moments of a murder. His work was fully translated from German to English and reprinted in colour through the efforts of the historian for the International Association of Bloodstain Pattern Analysts (IABPA), Professor Herbert Leon MacDonell from Corning, New York.

Professor MacDonell was quoted as saying of Dr Piotrowski, *"No one preceded Piotrowski In designing meaningful scientific experiments to show blood dynamics with such imagination, methodology and thoroughness"* (James *et al* 2005 p.4). Numerous significant works pertaining to BPA followed Dr Piotrowski's. These included those of Dr Paul Jeserich and the French scientist Dr Victor Balthazard. In 1900 Jeserich began the examination of many murder scenes, spanning the first decade of the 21st century, and later, Balthazard and associates undertook research, and conducted original experiments pertaining to blood trajectories and patterns. They subsequently published data (Jeserich 1930; Balthazard *et al* 1939) pertaining to the reconstruction of the angle of impact from the measurement of the length and width of small bloodstains found in blood patterns.

Dr Paul Kirk, considered by many, including Wonder (2001), to be the founding father of blood dynamics, achieved a milestone of great forensic significance in 1955 when bloodstain evidence was first recognised by the legal system in Ohio. Based on his findings, Dr Kirk prepared an affidavit for the court of common pleas in the case of *State of Ohio vs Samuel Shepard*, and was able to establish the relative positions of the victim and that of the attacker at the time of the assault (James *et al* 2014).

In 1971 with the aid of a Law Enforcement Assistance Administration (LEAA) grant, Herbert Leon MacDonell carried out research and experiments in which he was able to recreate and duplicate many of the commonly encountered blood patterns at crime scenes. This research resulted in MacDonell publishing '*Flight Characteristics and Stain Patterns of Human Blood',* which was one of the first modern books on blood pattern analysis (James *et al* 2005).

In the year 1973, Herbert MacDonell establish a basic bloodstain pattern interpretation program in Mississippi, including conducting his first Bloodstain

Institute, and also released his second publication, entitled *Laboratory Manual on the Geometric Interpretation of Human Bloodstain Evidence*. Since then MacDonell has, along with colleagues, spent his time conducting many basic and advanced bloodstain analysis courses, including the training of hundreds of crime scene investigators, police officers, crime laboratory technicians and forensic scientists (James *et al* 2005; James *et al* 2014).

Further activity of note by Herbert MacDonell includes the 1982 expansion of his original work in a paper entitled *Bloodstain Pattern Interpretation*, and in 1983 he conducted the first advanced Blood Pattern Analysis class, the members of which would later form the International Association of Bloodstain Pattern Analysts (James *et al* 2014).

In 2002, at a meeting held by the FBI Laboratory at the FBI Academy in Quantico, Virginia, the Scientific Working Group on Bloodstain Pattern Analysis (SWGSTAIN) was formed to standardise, and continue the development of BPA (James *et al* 2014).

Since then, numerous significant publications have provided further valuable contributions to the analysis and understanding of blood pattern evidence.

1.3 Bloodstain Pattern Analysis

Theory

The theory of blood pattern analysis is a relatively simple one: *Blood is a complex fluid, but it will respond to external and internal variations in a predictable manner* (Gardner 2012). As a consequence, the bloodstain analyst will be able to evaluate the numerous mechanisms and collapse mechanics of bloodstains, and compare that knowledge to known patterns that have been generated under controlled conditions (Peschel *et al* 2011; Gardner 2012).

Principles

In their approach to BPA, the analyst will adhere to the scientific method and the principles of mathematics, physics and pathology and possess a working knowledge of all three (Durdle *et al* 2013; James *et al* 2005).

Within the discipline of BPA, these principles are generally divided into three groups:

- The pattern diversity principle
- The physically altered bloodstain (PAB) principle
- The principle of stain shape and vector correlation (Gardner 2012).

The pattern diversity principle

Pattern diversity is the primary concept at the heart of BPA, and can be expressed as: *a variation in the combination of volumes of blood, and the forces acting on those volumes will ultimately result in identifiable classes of patterns* (Wonder 2001).

When a volume of blood is subjected to an external force, a recurring pattern is created. Different stain patterns are produced by different mechanisms, and these will display different class characteristics, however, these basic mechanisms are not exclusively crime oriented. For example, an assault, when someone is beaten, does not produce a beating-specific pattern. The same class of stain will be produced when

any static volume of blood is subjected to an external force (Gardner 2012; James and Eckert 2002; Peschel *et al* 2011).

The formulation of the basic bloodstain pattern types is governed by many principles centred in physics, and as a result the manner in which fluid masses are affected will relate these principles.

Gardner (2012 p.332) describes these event mechanisms in six primary categories:

1. Static blood that dispersed from a point source
2. Blood dispersed in a jet
3. Blood dispersed as a function of accelerated motion
4. Blood that accumulates or flows on a surface
5. Blood dispersed as a function of secondary contact with a surface
6. Blood dispersed as a function of gravity

The dynamics of these mechanisms are described more fully in section 1.4.

Through the identification of these basic pattern types, the blood pattern analyst will then be able to identify the causative mechanism of the pattern.

For example, having identified a particular stain as a contact pattern, then all other basic pattern types (drip, impact, cast-off, streaming ejection and accumulations) may be eliminated as the cause of the pattern.

A limitation of this principle however, is that not all blood patterns fit neatly into one single category. Complex patterns may exhibit traits of several different pattern types, thus confounding blood evidence for the analyst (Gardner 2012; James *et al* 2005; Peschel *et al* 2011).

The physiologically altered bloodstain (PABS) principle

The PABS principle is a simple acknowledgement that blood, when exposed to external forces, will react to environmental conditions in a predictable way (Wonder 2001).

PABS are more fully described in section 1.4.

The principle of stain shape, and vector correlation

The complete dynamics of blood, the scope of which is beyond this paper, are highly complex and can only be fully understood by a trained blood pattern analyst through many years of training and research. However, the basic principle of stain shape and vector correlation is an important consideration for the analysis of a variety of patterns encountered at crime scenes, and involves two primary sub-principles:

1. Directionality
2. Impact angle (Gardner 2012)

The Direction of Impact

The principle of the direction of impact is expressed by Gardner (2012 p.334) as: *the collapse of a droplet in free flight onto a surface will result in a stain with an elliptical or circular shape.*

When a blood drop in flight has separated from its source via gravitational or mechanical forces it will retake a spherical form, and upon contacting a surface it will leave a visual record in the form of a blood spatter (Wonder 2001). These blood spatter stains often display spines, satellite stains or scallops. If present, these characteristics can be useful in the determination of the direction of travel of the droplet at the exact moment of impact (James *et al* 2005). Even in the absence of these characteristics, the leading edge, or ‘tail’ is usually the narrowest, and can indicate direction of travel (Boonkhong *et al* 2010). An example of this is shown in Figure 6.

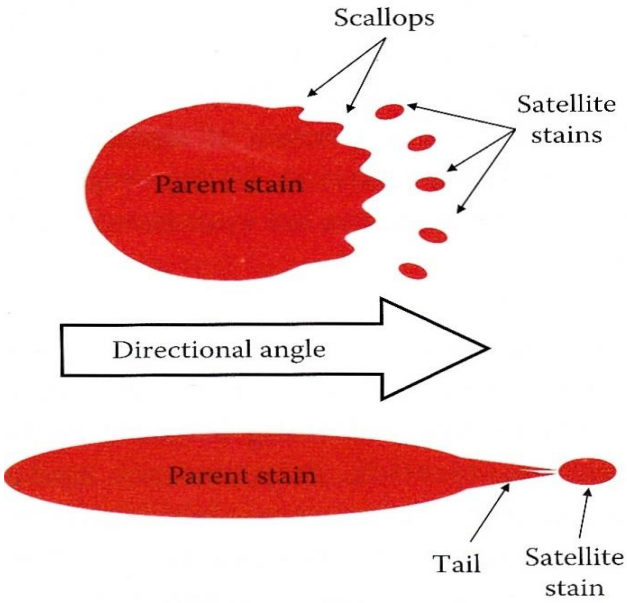


Figure 6. The directionality of stains showing scallops, satellite stains and tails (Gardner 2012)

Through the consideration of the direction of multiple stains at a crime scene, and if the stains are related, the blood analyst can determine the general area from which the stains originated by lining through their respective reverse vectors (Figure 7). This general area is known as the point of convergence (James *et al* 2005).

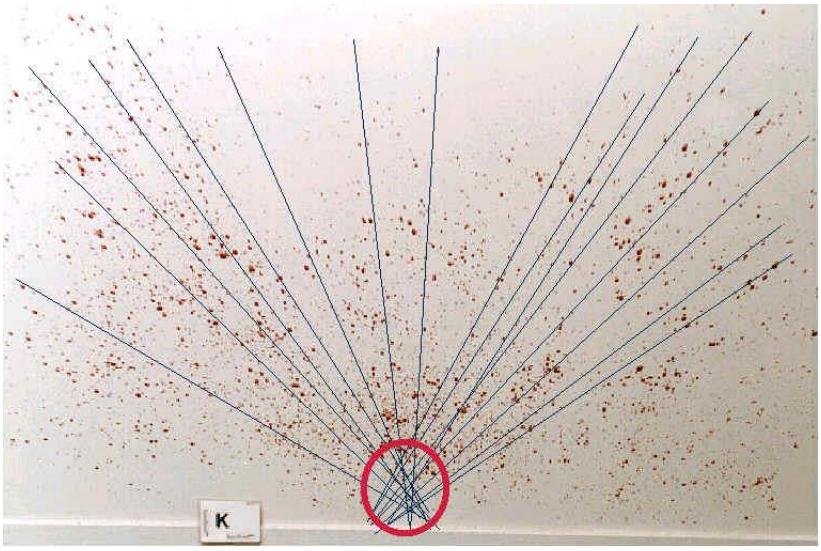


Figure 7. Blood spatter stains with lines showing area of convergence (Slemko 2015)

The one exception to this rule however, is claimed by Jackson and Jackson (2008) to be when smaller droplets of blood are thrown from a main droplet. The tail portion of

these smaller satellite stains will point toward the parent drop. Therefore, it is vitally important for the blood analyst to distinguish between satellite and parent stains when interpreting direction of travel.

The Angle of Impact

The impact angle principle is articulated by Gardner (2012) and James *et al* (2005) as: *the collapse of a droplet in free flight onto a surface will result in a stain with either an elliptical, or a circular shape*. *The compared ratios of the length of the long and the short axes of the resultant stain will have an empirical relationship to the angle at which the droplet contacts a surface.*

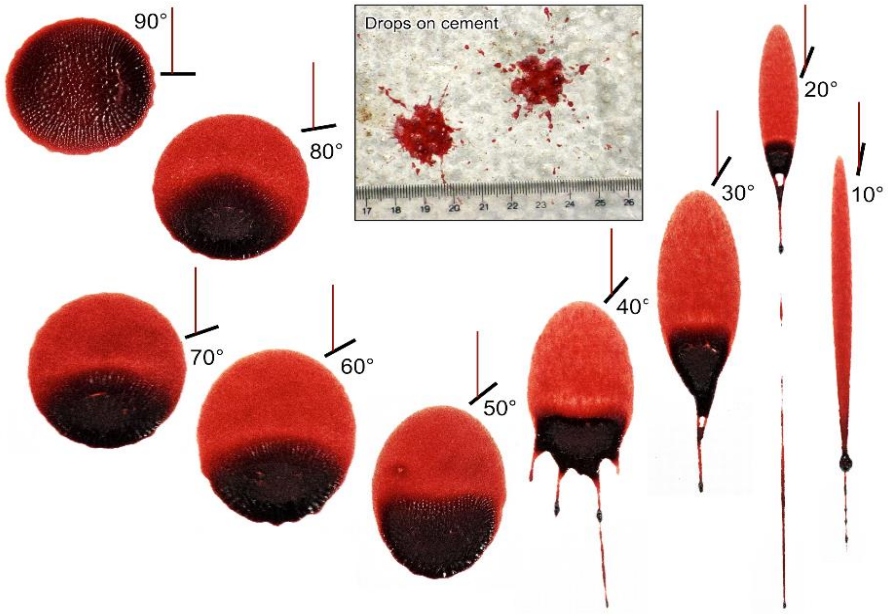


Figure 8. Droplets of blood dropped from angles of 10 - 90 degrees (Dr Joanne Millington 2017 – with permission)

When a viscous blood droplet makes contact with a surface creating a bloodstain, the resulting stain will exhibit both a minor and major axis, the length and width of which depend on the angle of impact (Figure 8). Using a trigonometric calculation (Figure 9), the approximate angle of impact is able to be calculated by the blood analyst using the measurements of these two axes of the bloodstain (Wonder 2001).

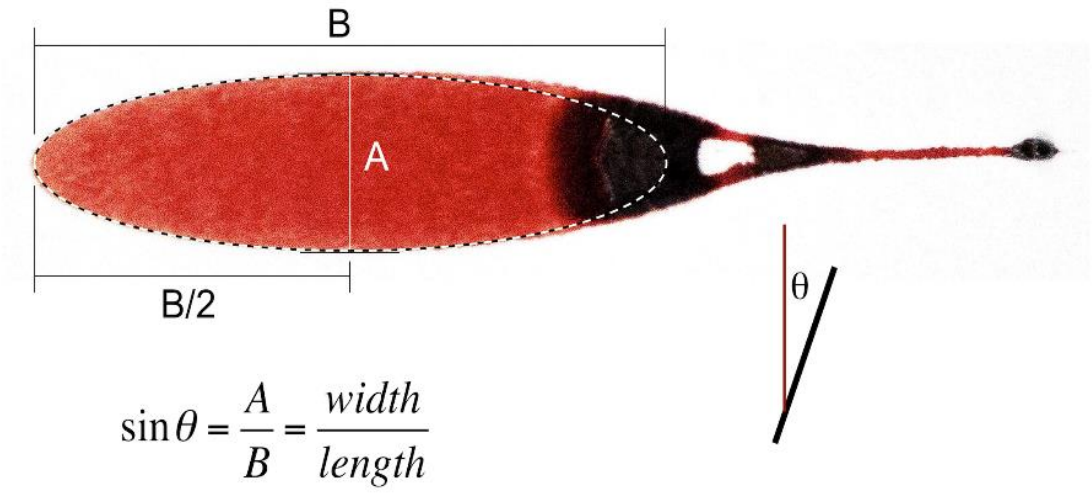


Figure 9. Measurement of the angle of impact of a bloodstain (Dr Joanne Millington 2017 – with permission)

First, the bloodstain width is divided by length excluding any portions of the tail, scallops or satellite spatter, and will always provide a value of <1. This is followed by determining the inverse sine of this number, thus providing angle of impact (Gardner 2012; James and Eckert 2002; Wonder 2001).

The exact position of a victim at the time of impact can be determined by the blood analyst by combining the two above methods. The addition of the angle of impact to the point of convergence determination will add a third dimension to the analysis; this is known as the area of origin (Figure 10). The area of origin can provide important information pertaining to the relative position of the victim. For example, whether the victim was standing, kneeling, sitting or lying down at the time of bloodshed (James *et al* 2005).

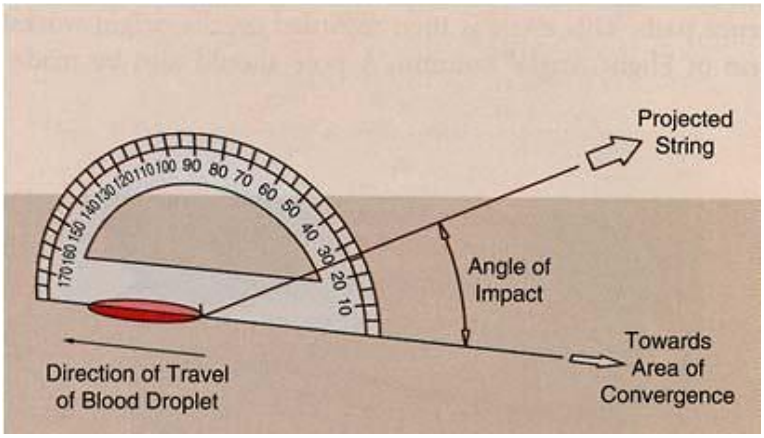


Figure 10. Protractor positioned at a stain showing determination of area of origin (James *et al* 2005)

Objectives of Blood Pattern Analysis

The blood pattern analyst may study evidence in two ways: by direct scene evaluation and/or careful examination of photographic evidence, the latter of which would ideally be in colour with a scale in the photo (James and Eckert 2002). These examinations may also be in conjunction with direct analysis of artefacts such as weapons and clothing, and information pertaining to hospital/medical records and photographs from the post-mortem and autopsy (Jackson and Jackson 2010).

In those cases where only photographic scene analysis is possible, the blood analyst must rely on carefully detailed sketches and diagrams, the reports of police/investigative officers, and laboratory reports (James *et al* 2005; Bartlett and Sutton 2009).

The aim of reconstructing a crime scene with the use of BPA is to provide answers to specific questions within a forensic investigation. Examples of the most common important questions to be answered are:

- What event(s) took place?
- Where did the event(s) take place?

- When and in what sequence did they transpire?
- Who was present at an event?
- Who was absent during an event?
- What did not take place?

These questions may be answered through the aid of the following information gathered during the BPA investigation:

- The point of convergence and origin of a bloodstain.
- The directionality, the type of impact, and causative mechanism that caused a bloodstain.
- Assistance with the determination of how blood stains were transferred onto items of evidence.
- The relative position of the victim, the suspect, objects or weapons at the scene during bloodshed.
- Possible movement of the victim, the suspect, objects or weapons at the scene after bloodshed.
- The support or the contradiction of statements given by the accused and/or any witnesses.
- Extra information on the post-mortem interval (James and Eckert 2002; James *et al* 2005; Bartlett and Sutton 2009).

1.4 Classification of Blood Pattern Groups

A key area of bloodstain pattern analysis lies in the evaluation of the physical characteristics of individual patterns, and the assignment of those patterns to a specific classification based on those characteristics.

According to Gardner (2012 p.340) some confusion arises from the fact that there are numerous classification systems being utilised today.

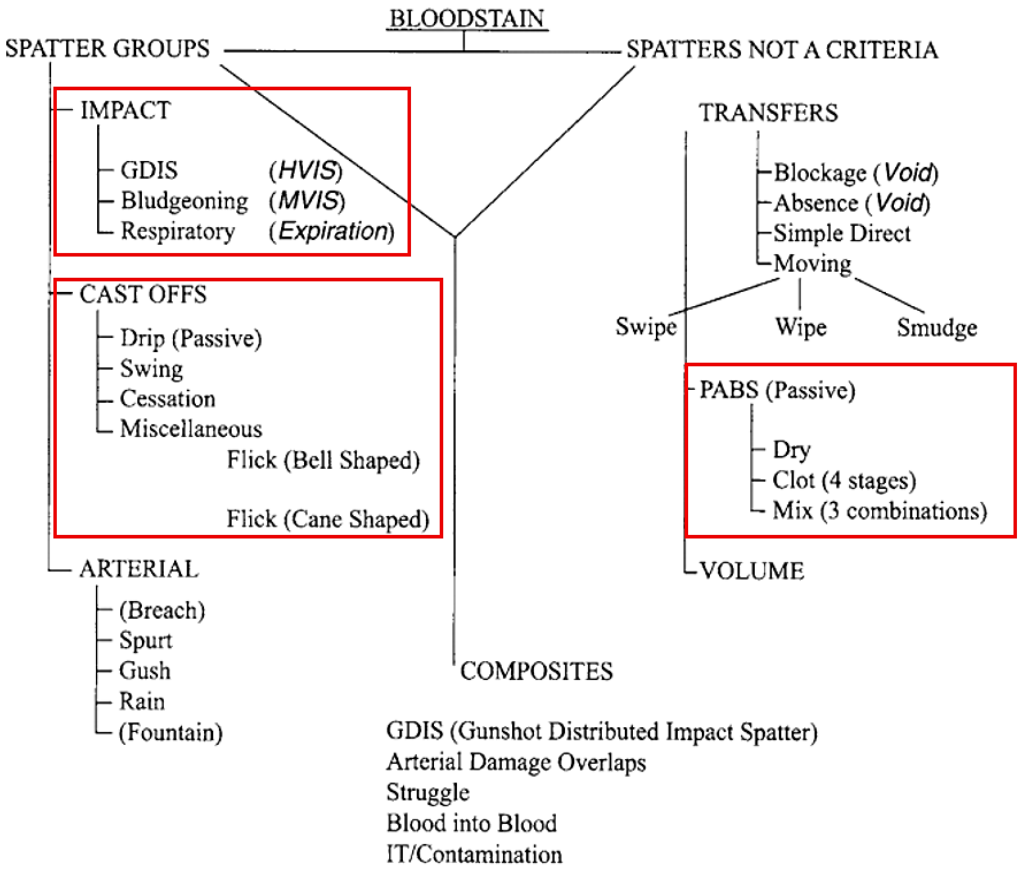


Figure 11. Flow diagram of blood stain pattern classes by Wonder (2001). Red boxed areas show the stain groups of interest to the current study

However, he claims that this is simply due to the different classification systems having different perspectives; for example, some are concerned with the size of the spatter, and others with the mechanism. Nevertheless, these eventually all lead back to the same basic pattern types. For the purpose of this paper, bloodstain pattern characterisation as shown in the flow chart of Wonder (2001) will be used as an example (Figure 11).

SWGSTAIN Terminology

Currently making its transition to the Organisation of Scientific Area Committees (OSAC), The Scientific Working Group on Bloodstain Pattern Analysis has developed

and defined a list of recommended terminology for use in BPA (SWGSTAIN 2013) which will be employed within this text.

The spatter groups

Spatter stains are defined as the result of blood being spread when a source of fluid blood has force applied to it (Peschel *et al* 2011), and include stains in which the primary stains are circular or elliptical (Gardner 2012). The three main categories of blood spatter are described as; Impact, cast-off and arterial/spurt (Wonder 2001).

Impact stains

Following the identification of a bloodstain as a 'spatter' based on its shape, size or distribution, the blood spatter analyst will then endeavour to further classify the pattern as an 'impact spatter'. Impact spatter is the result of an object directly striking a source of exposed blood (Gardner 2012), and can be divided into forward or backward spatter. Forward spatter is simply created by movement in the direction of the force, with backward spatter being created by force in the opposite direction (Gardner 2012), shown in Figure 12.

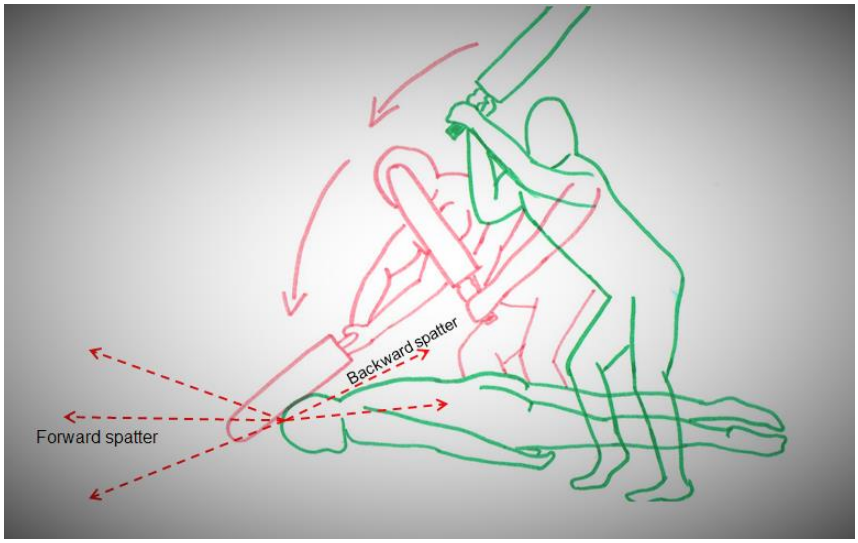


Figure 12. Impact spatter formation. Red dotted arrows indicate forward spatter and backward spatter

Impact spatter types were originally classified by Herbert MacDonell (1971) and accepted by the BPA community as being either low, medium or high velocity spatter which were solely dependent on the mechanism and velocity of the force applied to the blood source.

However, Laber (1985) found that this system of categorising impact spatter types was misleading due to large overlaps in stain sizes, such as those found in high and medium velocity spatter patterns.

Instead, he suggested a more flexible approach to categorising impact spatter based on the size of the stain:

- *Large [spatters* **>** *6mm]* - Blood dripping from an object (Cast-off) will usually produce spatter within this size range.
- *Medium [3mm – 6mm]* – Cast off bloodstains usually produce spatter within this size range.
- *Small [1mm – 3mm]* – Can contain overlaps of medium and fine spatters.
- *Fine [0.1mm – 1mm]* – Spatter resulting from a medium force impact, such as that from a beating, or spatter found in close proximity to a high force impact due to their low mass, and will usually only travel up to 4ft through the air. Typically, these types of spatter are associated with gunshot trauma (James *et al* 2005).
- *Mist [less than 0.1mm]* – Spatter found in close proximity to a high force impact, such that from a gunshot. Owing to the minute size of this spatter, it will travel only a short horizontal distance of approximately 6 –12 inches when in flight (Laber 1985).

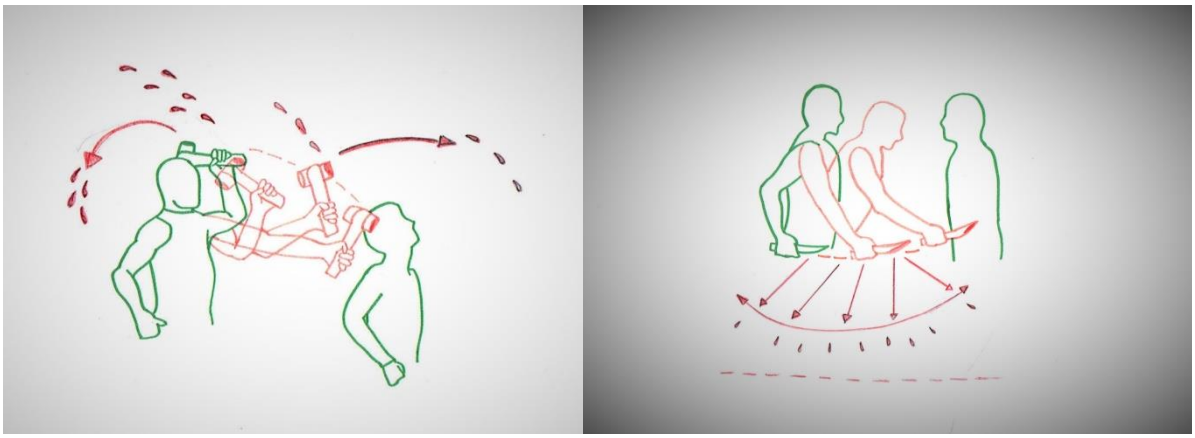
Because it is not practical to differentiate between the causative mechanisms of spatter stains based on size alone (James *et al* 2005), using Laber's more flexible classification system the sizes of the stains or mixture of stains is first defined and is then characterised by the type of impact (low, medium or high forces) that may have produced that type of spatter (Laber 1985).

Expirated impact spatter

Expirated (Respiratory) impact spatter can be observed when blood, and air from the lungs have been expelled upward, forward or downward from the mouth, nose or an open wound under force following impact to the body or respiratory illness (Wonder 2001; James *et al* 2005; Peschel *et al* 2011).This type of spatter can be created by numerous different mechanisms such as: the exhalation of blood, the coughing, sneezing, wheezing or spitting of blood through a bloody nose or mouth (Denison *et al* 2011) or even through paramedic intervention during resuscitation (James *et al* 2005). According to Peschel *et al* (2011), expired patterns often exhibit bubble rings as a result of a blood-air mixture.

Cast-off stains

Cast-off stains are created on occasions where blood is flung or propelled onto a surface by an object or other source in motion that has blood on it (Gardner 2012), such as a hand or weapons such as a knife, bat or a hammer (Figure 13). This is due to the whip-like action and the generation of centrifugal force being greater than the adhesive force that has caused the adherence of blood to the object, and finally being influenced by gravitational force (James *et al* 2005; Wonder 2001).



a **b**

Figure 13. Diagram showing the mechanism of cast-off bloodstain formation from (a) beating and (b) stabbing

According to Wonder (2001), for a cast off stain to be formed a source of blood must be exposed, and blood must have previously accumulated. This is made possible through three possible sources: an actively bleeding wound, a blood coated weapon or blood accumulation on materials.

Cast off stains are frequently encountered within impact spatter stains and may also overlap in size with impact spatter stains (James *et al* 2005).

Arterial (spurt) stains

Arteries are the major blood vessels in the human body that carry newly oxygenated blood from the lungs, via the heart, and away to the rest of the body. Arterial walls are much thicker and more muscular than veins because the transfer of blood away from the heart requires higher and continuous pressure (Waugh and Grant 2010). Examples of arteries that may be breached, their location and type of assault associated with each injury are shown in Table 1.

Table 1. Areas and actions associated with arterial damage (Wonder 2001)

Artery	Location	Probable action
Facial	Mouth/lips	Fist, beating, crush
Temporal	Head/temples	Gunshot, crush, (rare stab)
Carotid	Neck, front throat	Knife, gunshot, decapitation
Subclavian	Under collar bone	Gunshot, body crush
Aorta	Chest, upper tummy	Gunshot, stab
Brachial	Arm/elbow	Gunshot, bone break
Radial	Wrist	'Slit wrist', bone break, gunshot (defense wound)
Femoral	Groin	Gunshot, bone crush, stab
Tibial	Ankle	Bone break, crush, firearms
Deltoid	Upper arm muscle	Gunshot, stab/cut

Arterial bloodstain patterns produce a spray-like pattern, similar to that of a garden hose, and will contain distinctive physical characteristics, including the presence of bright red oxygenated arterial blood (Peschel *et al* 2011).

James *et al* (2005 p.149) states that arterial spatter stains range in size from less than 1mm to larger than 1cm in diameter, may exhibit satellite spatters around the

centre of the stain and have elongated spines. Whilst Gardner (2012 p.342) noted that the general characteristics found in arterial spatter cases are:

- A series of related spatter stains.
- Large volumes evident in the individual stains, demonstrated by flows from individual stains or a large volume in the overall pattern.

And any of the following:

- Lines of stains or overlapping stains deposited in V's, arcs, or a snake-like pattern.
- Large elliptical stains.

With the aid of these spatter characteristics the blood spatter analyst can gain vital information pertaining to the movement of a victim following an arterial injury (Peschel *et al* 2011; Gardner 2012).

Non-spatter groups

Apart from being bloodstain patterns, the non-spatter groups have little in common with the spatter groups, and the division of the groups relates more to the individual group composition of the stain rather than the dynamic behind it (Wonder 2001p.83).

Three categories separate the non-spatter group: (James *et al* 2005).

- Transfers
- Physiologically altered bloodstains (PABS)
- Volume

Transfer stains

According to Wonder (2001) the transfer bloodstain patterns can be divided into two groups:

- Blockage: Indirect pattern transfer
- Contact: Evidence in contact with a surface

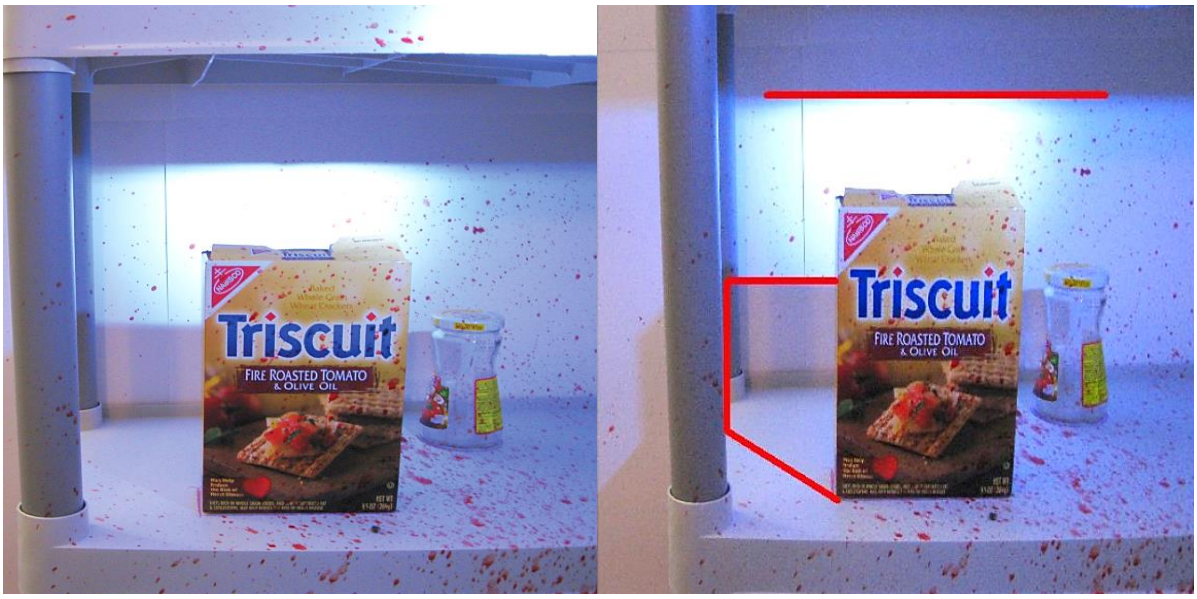
Figure 14. Void area on a floor after the removal of a victim (James *et al* 2005)

Blockage Stains

Blockage (void) stains are somewhat different to other types of trace evidence in that they do not require any contact between an obstruction, and a recording target surface (Wonder 2001p.84). According to Peschel *et al* (2011 p.264) and James *et al* (2005 p.210) if an object lies between a source of blood and the projection area it is likely that it will receive a percentage of the stain which, as a consequence, creates an absence of stain within an otherwise continuous bloodstain pattern.

Blockage stains are encountered most commonly in spatter events, when hundreds or thousands of small drops are forced onto the scene and surrounding surfaces (Gardner 2012). In some cases, if the secondary (blockage) object has been removed, it may still be possible to recognise the object (James *et al* 2005), or for an analyst to reconstruct its shape using the outskirt of the stain (Peschel *et al* 2011) as

shown in Figure 14. Blockage stains are also useful for determining direction of impact (Wonder 2001) as shown in Figure 15.



a **b**

Figure 15. Void patterns (a) multiple patterns caused by both the upper shelf and the food box, are shown to correspond with shadows created within the target area by use of a small LED flashlight (b) Red outlines illustrate the void patterns and corresponding shadows (Saviano *et al* 2010)

Contact Transfer patterns

Contact transfer patterns are created when a wet, bloody surface comes into direct contact with a secondary surface (Figure1), and the original surface may be able to be identified in the pattern as a whole, or as a recognisable image (Peschel *et al* 2011).

As with other stain groups, contact transfer patterns can be subdivided into further categories of:

- Simple direct transfer
- Moving transfer – wipe/swipe/smudge (Wonder 2001)

Simple direct transfers are merely the direct transfer of blood during contact with a surface. These surfaces may also be prone to uneven transfer or receipt of blood, depending on the type, and adherence of the surface or material (Wonder 2001). According to James *et al* (2005 p.92), the most common types of transfer stains are palm, finger, feet or footwear impressions, fabric impressions and weapon impressions.

Figure 16. Simple direct transfer in blood of left trainer showing pattern detail (Connolly 2012) - footwear impression used as evidence in the conviction of Brittany Norwood in 2012

Figure 16 shows a good example of simple direct transfer, however, variation in substrate material can cause stains to smudge or become distorted compared to the original surface. This is the case with surfaces such as laminate wallpaper, textured walls, knit fabrics, unfinished wood and carpets (Wonder 2001).

Moving Transfer Patterns

Moving transfer pattern (smear) categories are generally considered to be wipe, swipe or smudge.

Wipe patterns are simply where a hand or object has been smeared through a pre-existing bloodstain or pattern. Wonder (2001) describes this action as blood that is deposited along the path of movement, and increasing in stain depth as it progresses.

Wipe patterns can usually allow the analysis to determine the sequence of events, and the direction of the motion of the smear (Gardner 2012).

Swipe patterns are similar to simple direct transfer patterns in that they both involve the transfer of blood from an already bloodied surface onto a clean secondary surface. However, in contrast to simple direct, swipe patterns are created when a bloodied hand, foot or object comes into contact with another surface with associated lateral motion (Gardner 2012; James *et al* 2005). In the course of this action, blood is deposited along the path of motion and decreases in red blood cells as it proceeds. When movement stops, at lift off, a faint 'feathered edge' may be formed, thus providing an analyst with direction of travel for the action (Wonder 2001).

Smudge patterns are generally encountered with obvious contact stains, but which cannot be identified as simple direct or moving contact stains. Common causes may be that both surfaces are too bloody, movement of blood in more than one direction, which may occur during an attempted clean-up of a scene, or where a material is very absorbent, causes the stain to become distorted (Gardner 2012; Wonder 2001).

Volume Blood Stains

According to Wonder (2001), of all the blood stain pattern groups volume blood stains are perhaps the easiest to recognise. Determination of these stains usually requires no specific training and they can therefore be identified even by those unfamiliar with BPA. Bartlett and Sutton (2009 p.174) however, argue that the definition of a volume stain is rather vague, and state that methods of estimation or quantification should be treated with caution.

High volume blood stains are frequently encountered before the drying process has completed, which may allow an estimation of time since death, and may indicate that the individual died in the position the body was found in. Alternatively, the absence of large volumes of blood from the body, but not present at the crime scene, may provide an indication that the body was moved to the scene from another location following death (Wonder 2001).

Blood may also be more easily distinguishable in large volumes than other fluids such as oils or other aqueous fluids (James *et al* 2005), this is because during its drying process, blood exhibits changes from being shiny and highly reflective immediately after being shed, to a dull, non-reflective patina. In contrast, aqueous fluids dry, leaving no shine or patina, and oils do not fully dry, and will maintain their shine for a much longer time than it takes blood to dry (Peschel *et al* 2011; Wonder 2001).

Physiologically altered bloodstains (PABS)

The fundamental principle of PABS is described by Gardner (2012 p.339) as: Once blood has been exposed, it will react to environmental conditions such as airflow, temperature, humidity, and variations of surface in a predictable manner.

Blood will clot or coagulate once it has left the body, and can form recognisable bloodstain patterns from blood which has become mixed with another bodily fluid, such as saliva found in exhaled blood, cerebrospinal fluid, mucus or stomach contents (Fraser and Williams 2009). Non-physiological admixtures often encountered at crime scenes included the beverages tea, coffee, beer and wine, as well as cleaning products used in an attempt to clean a crime scene such as disinfectant or bleach (Fraser and Williams 2009).

Flies and other insects will also alter the composition and/or colour of blood at a crime scene through the process of blood feeding (Byrd & Castner 2010).

Fly Artefacts

The activity of insects, such as flies or beetles, at a crime scene can confound a crime scene (Benecke & Barksdale 2003).

Artefacts produced by flies, known as fly spots, during the process of blood feeding can often mimic other relevant bloodstain patterns (Houck & Siegel 2010). Several types of fly artefact have the potential to be confused with bloodstains encountered at the scene of a crime. Most commonly, the stain types confused with fly spots are small, fine and mist sized specs created by expirated blood, and medium to high force impact trauma (Benecke & Barksdale 2003; Byrd & Castner 2010), as both regurgitated blood droplets and faecal matter can mimic blood spatter. Furthermore, these types of stains are *usually* not able to be interpreted through the reconstruction of angle of impact (Gennard 2012), and are often inconsistent with blood spatter associated with injuries sustained by a victim (Benecke & Barksdale 2003).

The first known published observations pertaining to fly artefacts in a forensic context were by Lassainge (1856) when the marks created by insects on steel were noted whilst examining bloodstains. Since then, only a limited number of texts have more fully described fly artefacts (Durdle *et al* 2013).

1.5 Post-mortem Interval

The post-mortem interval (PMI) is a key factor for investigators in determining when an individual died. The decomposition of a body is considered to occur in five stages; fresh, bloat, active, advanced, and the dry remains stage (Cockle and Bell 2015), and of these, it is the fresh stage that is of most interest with regard to this text.

Commencing immediately following death, the fresh stage of decomposition occurs until the first signs of bloating. During this period, the body's internal organs are broken down and digested by enzymes and bacteria via cell necrosis and autolysis onset (Cockle and Bell 2015), causing an odour that attracts the first insects (Kreitlow 2010).

Typically, one of the first colonising insects in search of carrion to arrive at the body are the Calliphoridae (Diptera), known as blowflies (Cockle and Bell 2015), and much research on the PMI focuses on these flies as forensic indicators due to their ability to seek out a cadaver, and can often do so within minutes of death occurring (Langer and Illes 2015). Two such species of these forensically important flies (shown in Figure 17.a) are the 'bluebottle' flies, *Calliphora vomitoria* (Linnaeus, 1758) and the 'common greenbottle' fly, *Lucilia sericata* (Meigen, 1826)(Figure 17.b), hereafter referred to as *Calliphora* and *Lucilia.*

a **b**

Figure 17. *Calliphora vomitoria* (a) and *Lucilia sericata* (b)

Another fly family, the Muscidae, are considered slightly less forensically important than the blowflies because they generally follow at a slightly later stage of decomposition, and indicate that the earlier blow fly phase has ended (Kabkaew 2010). However, like other Diptera, they still have the *potential* to be found at a crime scene at any stage of decomposition (Byrd and Castner 2010).
One such member of the Muscidae family is *Hydrotaea capensis* (Weidemann, 1818), referred to hereafter as *Hydrotaea,* and is shown below in Figure 18.

Figure 18. *Hydrotaea capensis*

1.6 Basic mechanisms of fly artefact deposition

Flies are attracted to sweet food, and protein rich food such as meat (Byrd & Castner 2010; Gennard 2012), but also have a high affinity for some bodily fluids such as blood, semen and saliva when other food sources are absent (Durdle *et al* 2013). For this reason, they are frequently be found at crime scenes if they have access through open doors or windows, or in sealed areas if they were already present (Benecke & Barksdale 2003).

Because some foods are difficult for a fly to breakdown in their pure forms, it first uses the proboscis to draw it up (Figure 19), which it then mixes with digestive enzymes to break it down. This food is then expelled, again via the proboscis, and returned to at a later time when it is more easily consumed (Benecke and Barksdale 2003; Byrd &

Castner 2010). Often, this type of feeding behaviour will leave ‘spotting’ stains on a surface as the fly dabs a surface with the proboscis following ‘bubbling’, which involves the fly repeatedly expelling and reabsorbing a bubble of regurgitated liquid from its proboscis (Durdle *et al* 2013). A good example of ‘bubbling’ is shown in Figure 20 where *Calliphora vomitoria* can be seen feeding on a blood meal.

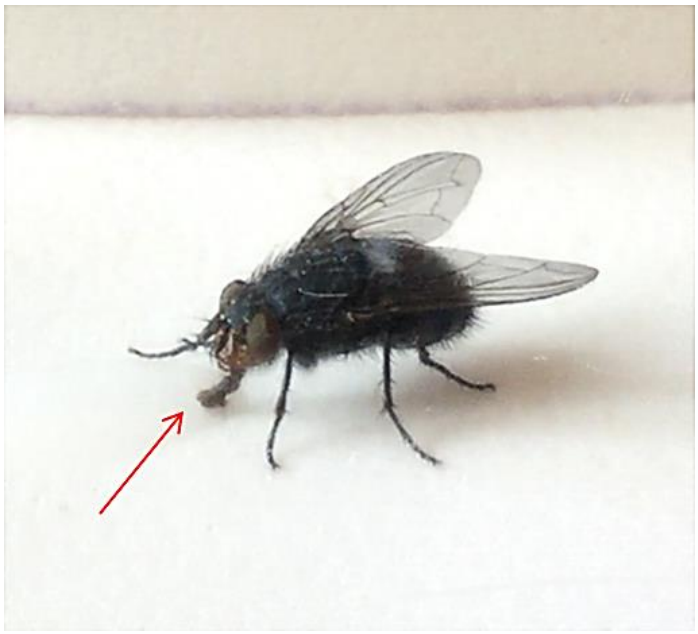


Figure 19*. Calliphora vomitoria* using the proboscis, highlighted by the red arrow, to feed on a sugar water solution

Figure 20. *Calliphora vomitoria* ‘bubbling’ a blood meal (Courtesy of Dr Joanne Millington 2015 - with permission)

Figure 21. Production of defecation artefact by *Lucilia sericata* (Durdle *et al* 2013)

A fly will defecate approximately 300 times in a single day, once every 4-5 minutes (Byrd & Castner 2010; Gennard 2012). These types of artefacts, often circular or teardrop shaped are formed through excretion (defication) when the fly is stationary, then deposited by way of the fly dragging its abdomen onto a surface as it moves away (Durdle *et al* 2013), seen in Figure 21. However, these artefacts can also be perfectly circular, and have the potential to be confused with similarly shaped impact-derived bloodstains at a crime scene.

2. Aim and Objectives

2.1 Aim

To investigate the 'confounding' effect that flies commonly associated with the post mortem interval can have on impact and expirated bloodstain spatter patterns following a blood meal. Assessment will be made with respect to overall pattern shape, artefact count, artefact density (per cm^2) and artefact size distribution, in relation to proximity from a blood source.

2.2 Objectives

1. In three separate experiments (500 flies per experiment) - expose approximately 500 each of the genera *Calliphora*, *Lucilia* and *Hydrotaea* to 500ml of horse blood, placed on a shallow tray on the floor, in a sealed 3m x 3m gazebo for a period of 72 hours, or until all flies have expired (whichever is sooner), and analyse deposited blood artefacts.
2. Recreate impact and expirated bloodstain patterns for comparison to fly artefacts.
3. Use the bloodstain size categorisation of Laber (1985) as a model to separate and categorise the deposited blood artefacts from all 3 fly genera, and the recreated impact and expirated blood spatter.
4. Employ the following objective criteria used by Wonder (2001) to analyse recreated impact and expirated, and compare to fly artefact patterns:
    - **S**hape of the overall pattern
    - **A**lignment of stains within the pattern – Informal observations only
    - **A**lignment of stains with respect to others – Informal observations only
    - **D**ensity of stains throughout the pattern
    - **D**istribution of stain sizes within the pattern
5. Make observations regarding the suitability of the current objective criteria above, and assess the need to recognise additional criteria to accommodate fly artefact patterns.

3. Methodology

3.1 Materials

- 1500 fly pupae from 3 different fly genera - 500 x *Calliphora*, 500 x *Lucilia* and 500 x *Hydrotaea* (respective species identified by project supervisor, Dr. Andrew Whittington, as: *C.vomitoria, L. sericata* & *H. capensis*)
  From: www.bugzuk.com (per pint of L3, post-feed stage maggots)
- White gazebo - 3m x 3m ($9m^2$) From: Amazon.co.uk
- White bug dorm/holding cage (large) – 60cm x 60cm x 60cm. From: watdon.co.uk
- Strong Duck® tape x 5 rolls - From local hardware store
- Plain white vinyl flooring – Approx. $40m^2$:
  $27m^2$ for fly experiments (3 x 3m x 3m)
  $4.5m^2$ for impact & expired blood spatter recreation (2 x $2.25m^2$)
  $6.75m^2$ for trial blood spatter recreation (3 x 1.5m x 1.5m)
  From: 247floors.co.uk
- 3 x 50cm x 50cm square trays covered with strong baking foil. Home made from: ply-wood, doweling and strong baking foil.
- 1500ml of defibrinated, oxalated horse blood - (3 x 500ml). From: www.tcsbiosciences.co.uk. Product code: HBO35
- Human Saliva (20ml) – Self collected over 8 hours
- Large wooden 'Victor' rat trap (modified) From: Amazon.co.uk
- Modelling plastic. From: Hobbycraft.co.uk
- Aluminium baseball bat. From: Amazon.co.uk
- Radox® body wash bottle with perforated nozzle (250ml). From: Asda
- Brother MFC – J6520DW Professional Series Scanner/printer. From: Currys PC World
- Image analysis computer program – MATLAB® (Courtesy of Dr. Hammadi Nait-Charif. Principal Academic, National Centre for Computer Animation. Bournemouth University)

3.2 Method

Fly experiment - *to be repeated for each of the 3 fly genera

A large plastic gazebo (Figure 22) was erected inside a 26.77m^2 room at the Crime Scene Training Facility (Figure 23) at Bournemouth University (Landsdown) and sealed. The internal floor of the gazebo (3m x 3m) was entirely covered with plain white vinyl floor covering, gridded out into 144 separate 25cm x 25cm squares (25cm x 12 = 3m) (12 x 12 = 144 tiles) also shown in Figure 23.

Figure 22. 3m x 3m gazebo set-up for fly experiments

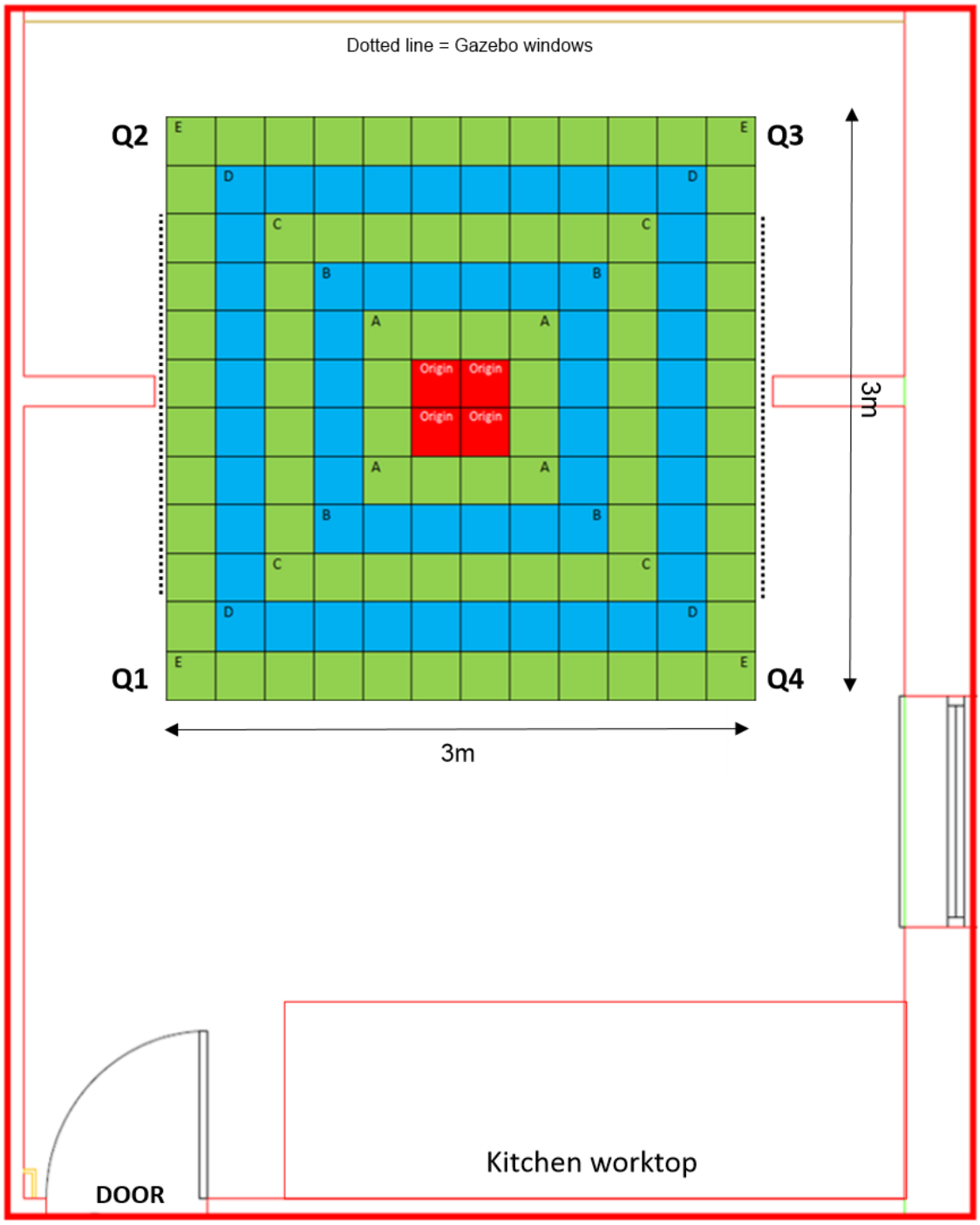


Figure 23. Floor plan of Bournemouth University Crime Scene Training Facility where the gazebo was situated for the fly experiments

Placed on the four centre squares was a 50cm x 50cm x 10ml tray, which was filled with 500ml of horse blood, creating the 'origin' around which five separate zones, A to E, were marked out on the grid. Zone A immediately borders the origin, with zone E being the farthest from it. The size of each surrounding zone will naturally increase

with greater distance from the origin; therefore will consist of more grid tiles and a greater area per cm^2 as follows:

- Zone A = 12 tiles (7,500 cm^2) - Single quadrant Zone A = 3 tiles (1,875cm^2)
- Zone B = 20 tiles (12,500 cm^2) - Single quadrant Zone B = 5 tiles (12,500cm^2)
- Zone C = 28 tiles (17,500 cm^2) - Single quadrant Zone C = 7 tiles (17,500cm^2)
- Zone D = 36 tiles (22,500 cm^2) - Single quadrant Zone D = 9 tiles (22,500cm^2)
- Zone E = 44 tiles (27,500 cm^2) - Single quadrant Zone E = 11 tiles(27,500cm^2)
- Per single quadrant = 35 tiles (21,875cm^2)

These larger areas were taken into account when calculating density per cm^2.

Five-hundred fly pupae were held in a bug dorm which was suspended in the corner of the gazebo (above quadrant 2). Once the majority of the flies were deemed to have emerged, the blood (500ml), which had remained in the refrigerator to keep it fresh prior to experimentation, was removed from refrigeration and poured onto the origin immediately before the flies were released from the bug dorm. The gazebo was then sealed for the duration of the experiment. No persons were inside the gazebo at any point during the experiment. The experiment was be deemed to be complete after a period of 72 hours, or when all flies had expired; whichever was sooner.

Impact Blood Spatter Recreation

Impact spatter recreation was carried out using one quadrant identical to that from the zoned gazebo floor plan (Figure 24), and a large ‘Victor’ wooden rat trap with the arm and base modified with modelling plastic to create a flat impact area for the blood to be set on. The trap was set on the ‘origin’ on a 23cm high wooden block, so as to replicate the length of the vertical axis of an average adult male head and set with 2ml of horse blood. This set up can be seen below in Figure 25. The trap was activated twice to simulate two strikes, with the 2ml quantity of blood being reloaded for the second strike.

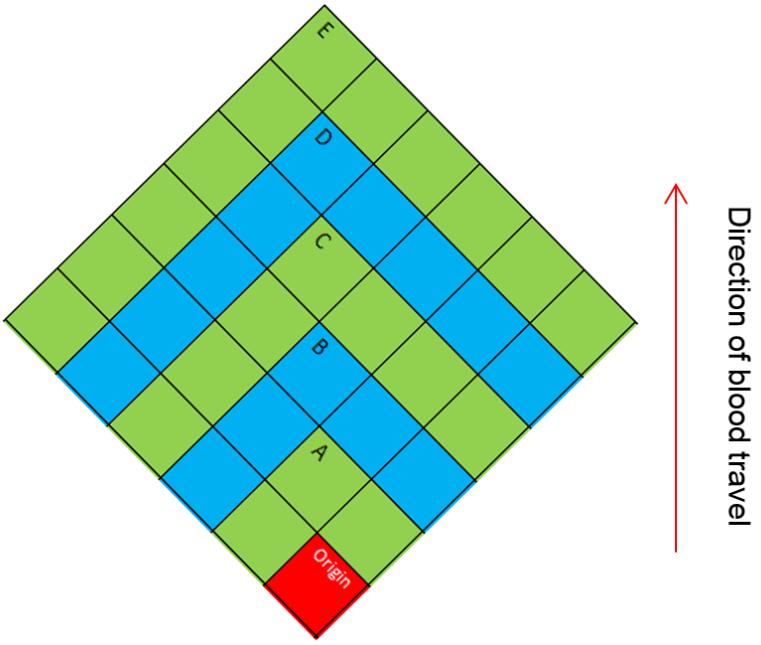


Figure 24. Zonal quadrant plan for impact and expired blood recreation

Figure 25. Rat trap device set-up for creation of 'impact spatter'

Expirated Blood Spatter Recreation

Expirated blood spatter recreation was also carried out using the one quadrant set-up identical to that from the zoned gazebo floor plan (Figure 24 & 25), with an empty body wash bottle replacing the rat trap device. The body wash bottle had a perforated nozzle to replicate the 'pursed lips' action often seen when spitting. A blood and self-collected human saliva mixture was added to the body wash bottle at a ratio of 5:2 (50ml of blood to 20ml of saliva), and as with impact spatter recreation, the bottle was set and secured to the wooden block at a height of 23cm on the 'origin'. The expired stains were generated by two hard strikes to the centre of the mixture filled bottle with an aluminium baseball bat.

Upon completion of the experiments, each individual tile was marked according to its zone and quadrant placement, as well as its orientation to the blood source. They were then removed and, once dry, boxed in cardboard boxes and separated with greaseproof paper. Each tile was then scanned using a large-bed scanner/printer under 1200 x 1200dpi resolution, to ensure uniform conditions for each blood tile image.

Analysis of results was carried out with a custom-built program for MatLab, courtesy of: Dr. Hammadi Nait-Charif, Principal Academic, National Centre for Computer Animation, Bournemouth University.

MatLab © Details

The MatLab program used in this study was primarily designed to count the number of artefacts on individual tiles, and to categorise those artefacts into one of the five size categories. It does not distinguish between different types of artefact.

Version: 8.3.0.532 (R2014a)

1. First the contrast of each image plane was adjusted using Ir = imadjust(Ir,[0.85 0.95],[]); The values in each image plane Ir, Ig, and Ib between 0.85 and 0.95 were mapped to new values in J such that values map to values between 0

and 1. Values below 0.85 and above 0.95 are clipped; that is, values below 0.85 mapped to 0, and those above 0.95 mapped to 1.

2. A histogram-based threshold was then applied to each image plane (red, green, and blue), then the image was converted in to a binary image.
3. As the blood stains are dark, the image was inverted using 'imcomplement' command such that black pixels become white and white become black. This is done in all three image planes.
4. All connected components (objects) that had fewer than 8 pixels were removed producing another binary image. This operation was intended to remove the noise.
5. Small objects from the binary image were removed using bw2 = bwareaopen (bw,8) which removes from the binary image all connected components (objects) that have fewer than 8 pixels, producing another binary image BW2. This operation is known as an area opening.
6. Morphological operations (closing & opening) are applied to remove holes in the foreground ("closing") and to remove stray foreground pixels in background ("opening")
7. For each object in the images the circularity was calculated: the command regionprops(I3,'MinorAxisLength','MajorAxisLength') was used to return the length of both the minor and major axis of each blood stain; the circularity of each stain is calculated using the following formula:

$$Cir = \frac{4\,\pi\,\mathrm{A}}{p^2}$$

Where A is the area, and p is the perimeter

8. The objects were then classified according to their minor axis:

- Large = minors >6mm
- Medium = minors 3 - 6mm
- Small = minors 1 - 3mm
- Fine = minors 0.1mm – 1mm
- Mist = minors < 0.1mm

9. All data from MatLab analysis is included in an excel spreadsheet on the compact disc accompanying this thesis, under the file named ‘Complete results.

Statistical Analysis

All statistical analysis was carried out in SPSS (version 23) and consisted of t-testing, ANOVA, and Chi-Square testing where artefact type, size, count, density and zonal distribution were all investigated in relation to one another.

In all instances where one-way ANOVA’S were carried out, and where Levine’s test for homogeneity of variances had been violated, Fisher’s Brown-Forsythe test for robustness was referred to before consulting the Games-Howell post-hoc test.

Raw data outputs from all statistical tests have been synthesised and can be located on the compact disc accompanying this thesis, with file names numbered accordingly.

Format/layout of Results Section

All results within the following section will be set out in the same format as the current objective criteria as Wonder (2001):

- **S**hape of the overall pattern (Shape of fly and recreated blood patterns)
- **A**lignment of stains within the pattern/with respect to others (Alignment of stains within recreated & fly blood patterns/with respect to others)
- **D**ensity of stains throughout the pattern (Density and count of stains throughout fly artefact & recreated blood patterns)
- **D**istribution of stain sizes within the pattern (Distribution of stain sizes within fly and recreated blood patterns

Reasoning for extended quadrant 4 analysis

Fly artefact numbers in Quadrant 4 were the most abundant. Furthermore, considering that the recreated spatter is contained within the equivalent area of a single quadrant from the fly experiment, the high amount of data, and that the “window-effect” appeared to relate best to crime scene investigation, only quadrant 4 was selected for comparison to the single quadrant of recreated spatter.

4. Results

4.1 Shape of fly and recreated blood patterns

Whilst the visual appearances of the overall shape of blood patterns produced by each fly genus were relatively similar, differences in stain dispersion can be seen immediately surrounding their origins, in zone A. Within this zone the greatest variation in artefact type was produced, and it consisted of visibly more dragging and tracking marks, giving a more irregular appearance to certain areas. Furthermore, artefacts from all 3 fly genera appeared to be much denser immediately surrounding the origin, and then dispersing as the stains radiate outward. In reality however, artefact numbers actually *increased* from zones B –E in all 3 fly genera (Figure 34 p.50) and only *appeared* lower in number due to the larger surface area as the zones moved away from the origin. The exception to this however, is the area immediately under the window, in zone E. On inspection, this area noticeably contained more artefacts, appearing denser, and gave an ‘unbalanced’ look to the overall pattern. Figure 26 shows zone A from the *Calliphora* experiment. As can be seen, the tile edges bordering the blood were more densely marked than the tile centres or the edges farthest away.

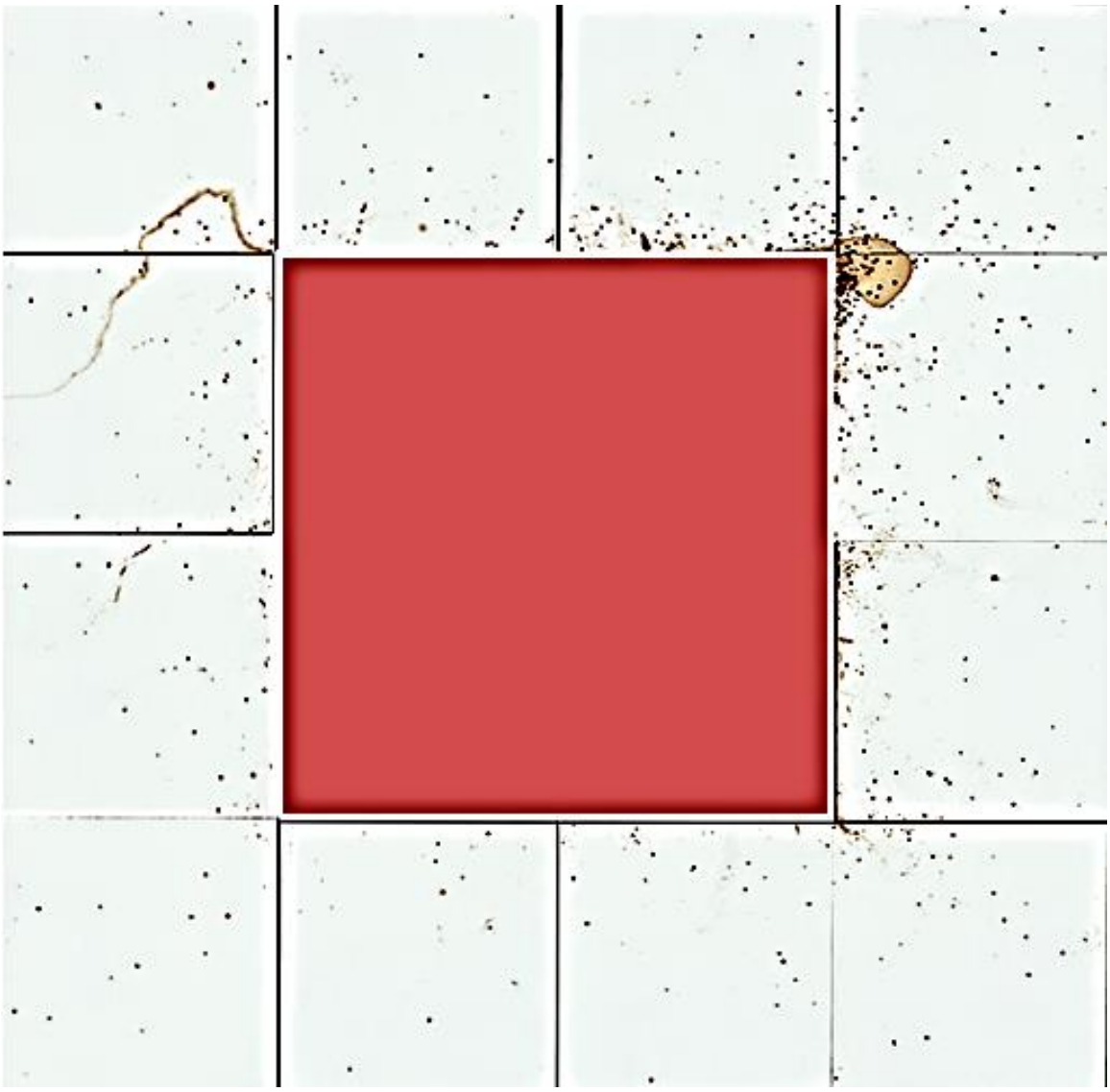


Figure 26. Fly artefact blood pattern in Zone A from the *Calliphora* experiment (Tiles = 25cm x 25cm)

In a visual sense, *Lucilia* appear to have deposited their artefacts in a much more uniform and spread out manner than that of *Calliphora*, and the tiles do not appear to be as densely marked on the origin border as can be seen in Figure 27. This appearance of lower density is confirmed in the ‘zonal density of fly genus artefacts’ graph (Figure 35) on page 52.

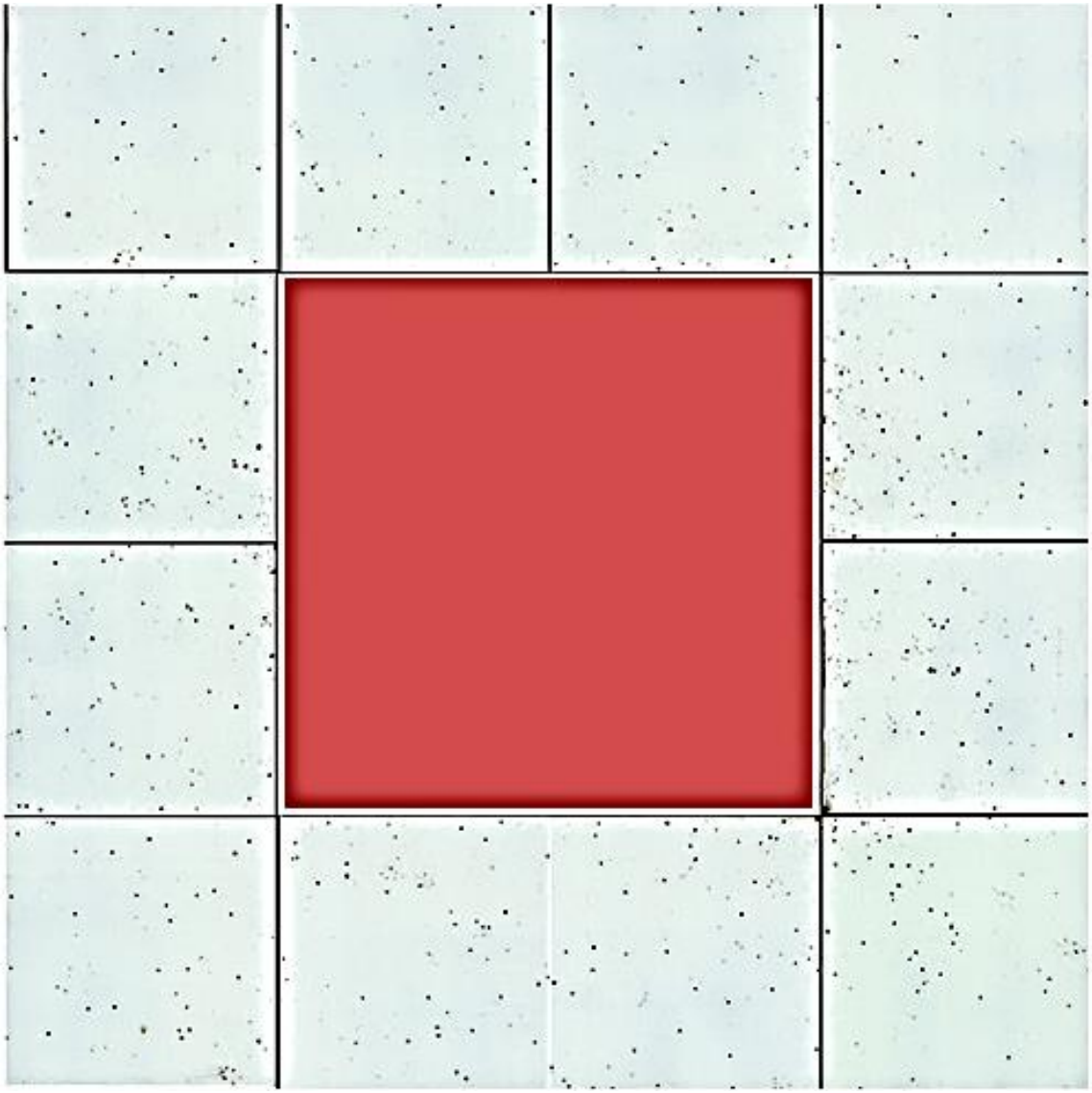


Figure 27. Fly artefact blood pattern in Zone A from the *Lucilia* experiment (Tiles = 25cm x 25cm)

Hydrotaea produced considerably fewer artefacts than *Calliphora* or *Lucilia*, and this is highlighted in Figure 28 where the tiles are much less densely marked and more open space can be seen. The exception to this is at the top of the image, where *Hydrotaea* have fed on a small amount of blood that has spilled or been dragged from the tray onto the tile, where higher amount of artefacts can be seen. This occurrence can also be seen above in the *Calliphora* image (Figure 26).

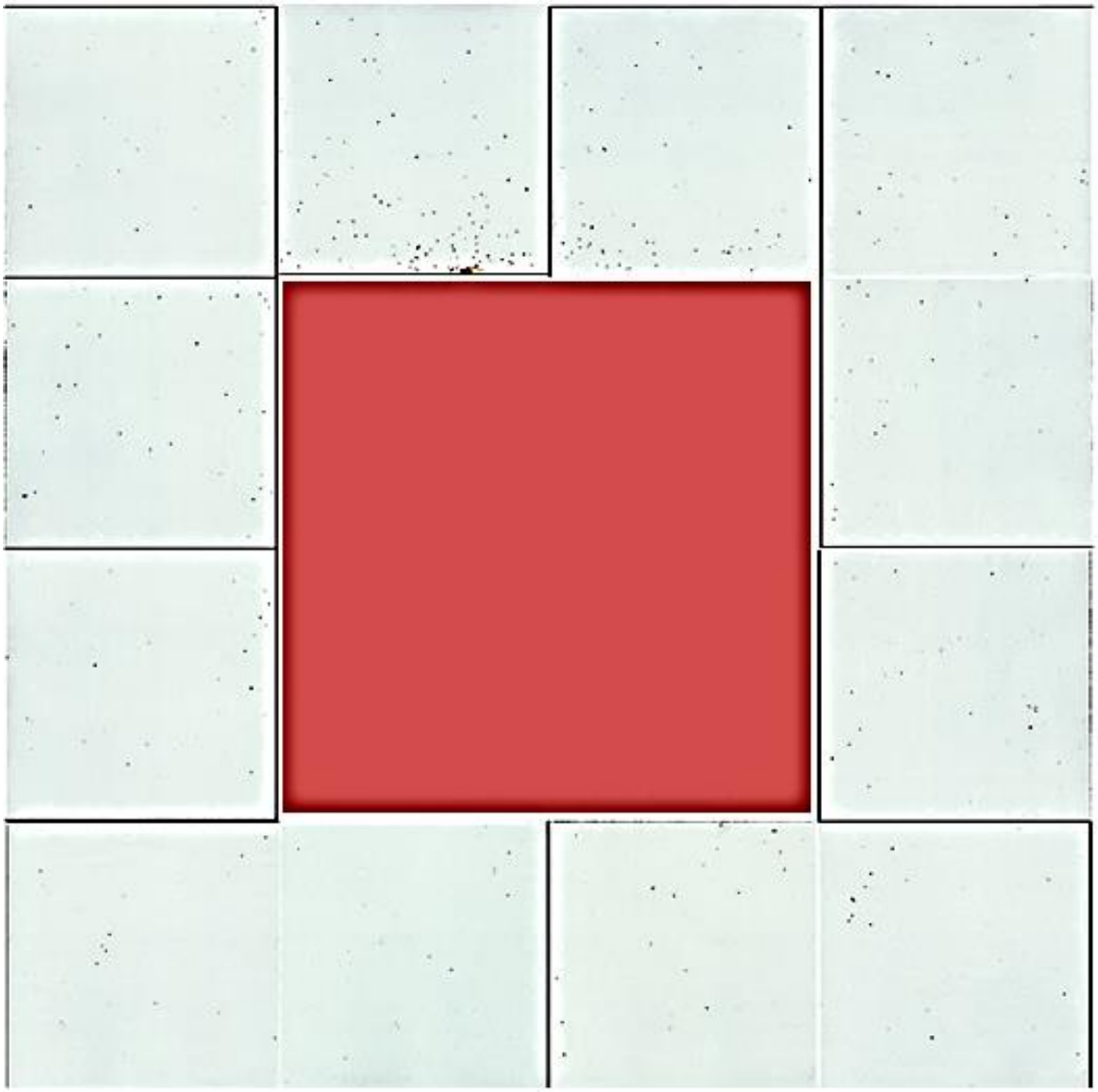


Figure 28. Fly artefact blood pattern in Zone A from the *Hydrotaea* experiment (Tiles = 25cm x 25cm)

Example tiles from the *Calliphora*, *Lucilia* and *Hydrotaea* experiments in more detail can be seen in appendices I, II and III respectively.

The recreated impact and expirated blood spatter pattern shapes exhibited very similar patterns. Both displayed the typical ‘fan’/’cone’ shape (discussed in section 5.0) in their dispersion from the point of origin, and were relatively wide spread as the

pattern reached zone E. However, expirated and impact stains differed from one another in that the expirated pattern (Figure 29) appeared to be primarily situated around a centralised ‘spine’ of much larger stains, and contained an enormous number of fine and mist sized stains within zones A-C, whereas impact stain (Figure 30) sizes were more evenly distributed, with the majority of the larger, more visible pattern occurring in zones C-E. However, impact spatter stain numbers were highest in zones A and B, which can primarily be attributed to the large number of fine sized stains in these zones.

Figure 29. Recreated expirated blood spatter stain in zones A – C, showing ‘spine’ of larger stains. Arrows show direction of blood travel. Tiles = 25cm x 25cm

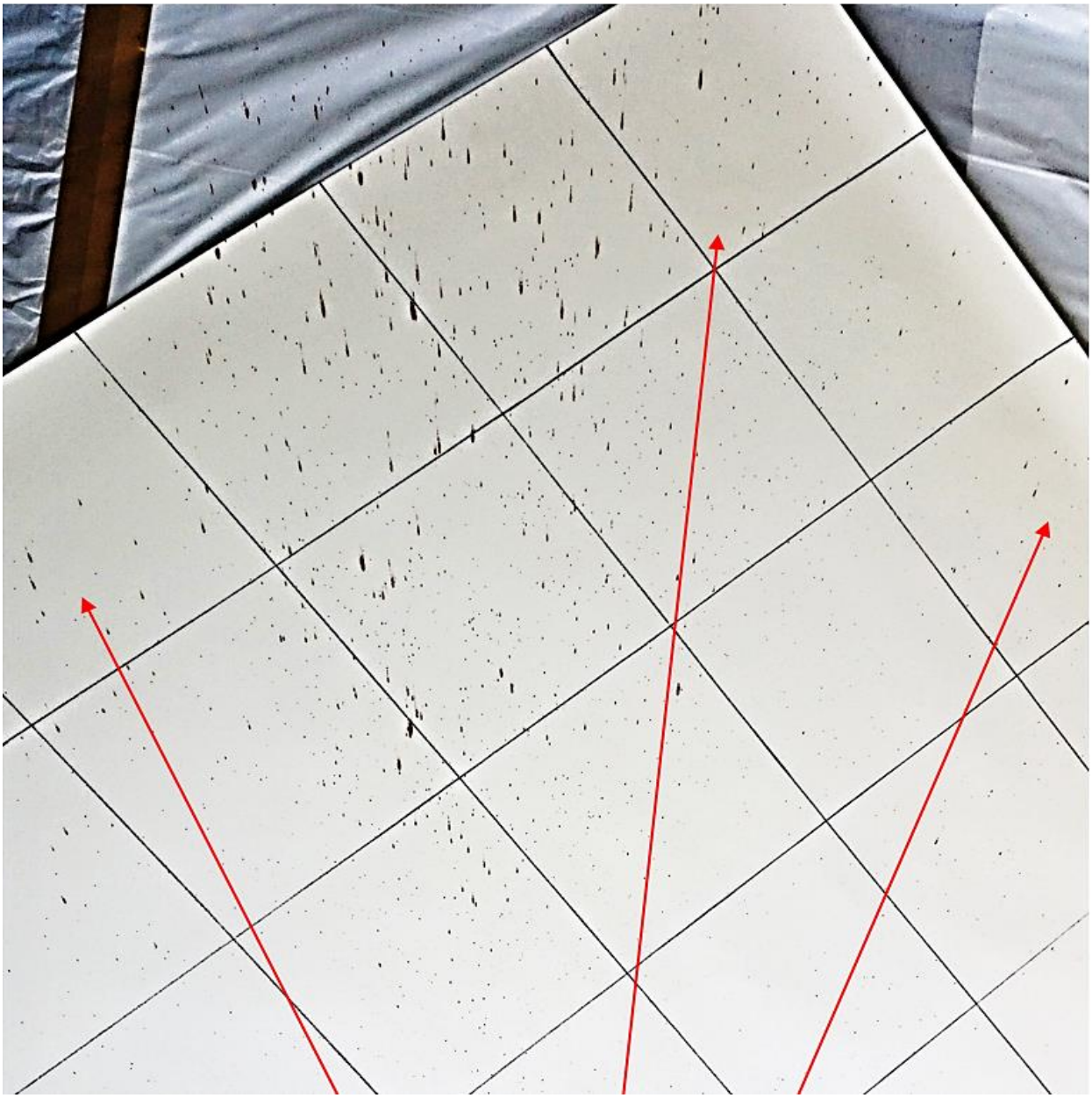


Figure 30. Recreated impact bloods spatter stain in zones C - E, showing the uniform distribution of stains. Arrows show direction of blood travel. Tiles = 25cm x 25cm

4.2 Alignment of stains within recreated & fly blood patterns & with respect to others

Objective interpretation of the images from the current experiments indicate that fly artefacts have high variation in their alignment to both the blood origin, and other artefacts within that pattern. It appeared that the majority of 'elliptical' or 'tadpole' shaped artefacts within the fly artefact patterns were angled in positions that were not consistent with the origin; however, several could be found that appear to align perfectly as seen in (Figure 31).

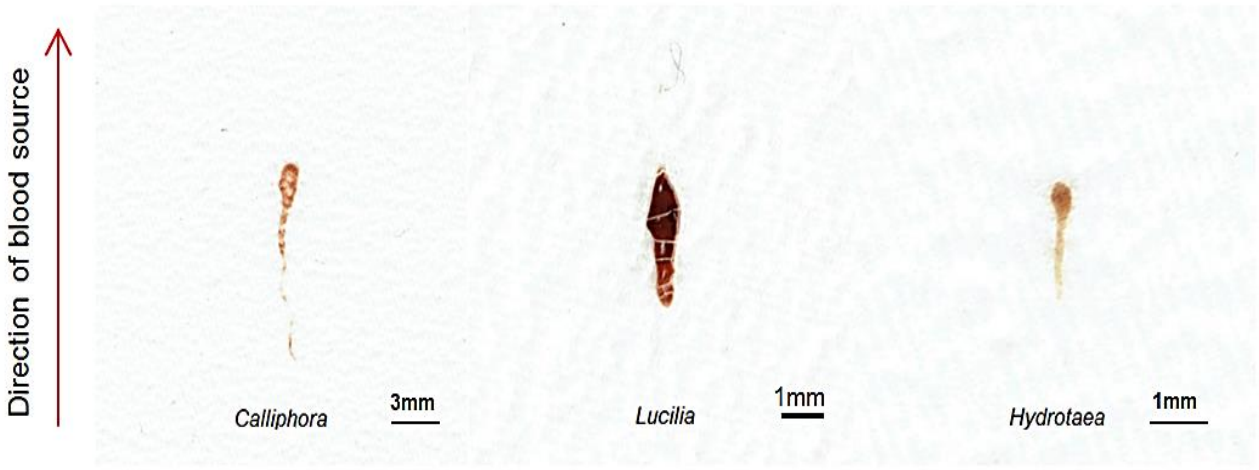
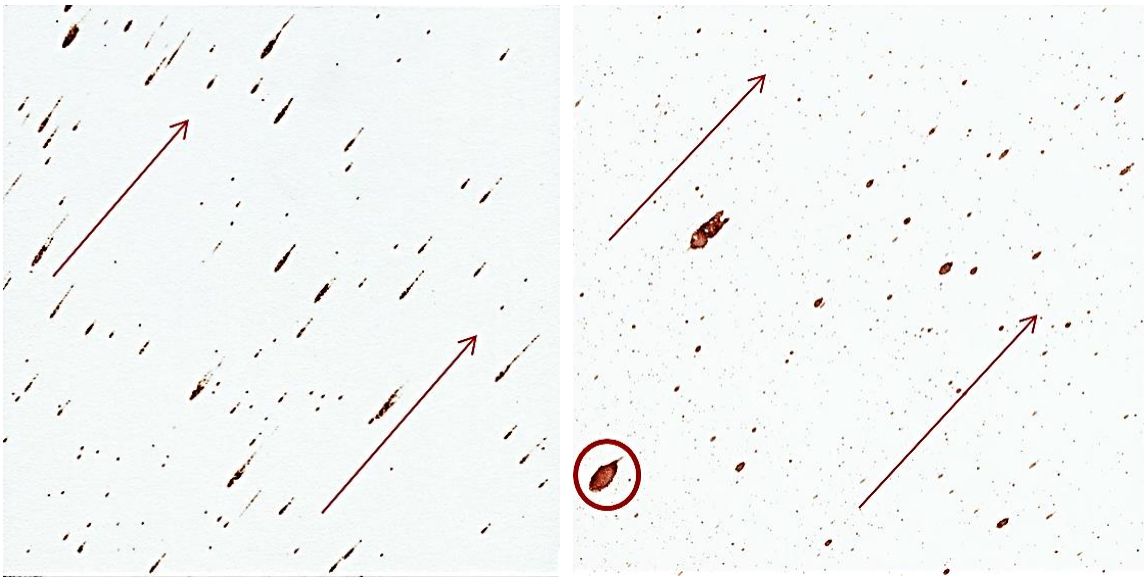


Figure 31. Blood artefacts from *Calliphora*, *Lucilia* and *Hydrotaea*. Arrow indicates direction of blood source

As expected, recreated bloodstain patterns showed directionality, and individual stains within these patterns align with other stains in the pattern. Impact spatter (Figure 32.a) is the truest example of this feature. The exception to this lays in certain expirated bloodstain patterns. Figure 32.b shows a circled stain with the tail sweeping slightly off to the right, and is one of many within the pattern that exhibit this feature. Figure 33 shows this stain enlarged, and that the tail angle is off of the stains major axis by 17°.



a **b**

Figure 32. Tiles from recreated (a) Impact spatter and (b) expirated blood showing circled stain with non-linear tail. Arrows show direction of blood travel. Tiles = 25cm x 25cm

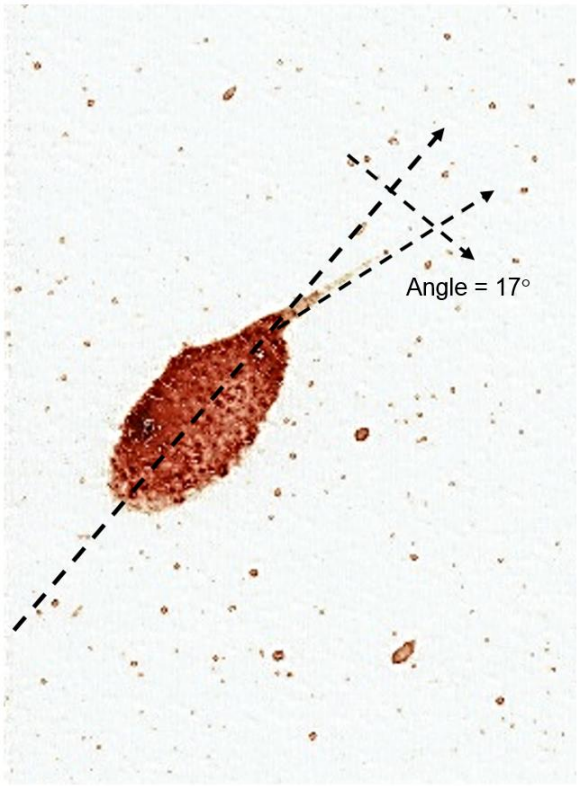


Figure 33. The angle, in degrees, of the tail of the expirated stain with non-linear tail from Figure 32.b above

4.3 Density and count of stains throughout recreated & fly blood patterns

Flies – Quadrant 1-4

Across quadrants 1 -4, the three fly genera produced an extremely high number (539,507) of post –blood meal artefacts over a 72 hour period. *Calliphora* (231,962) produced the highest total number of artefacts, followed by *Lucilia* (169,756) with *Hydrotaea* (137,789) producing the fewest ($F_{0.05,\ 2,\ 519924} = 19929$, $p < 0.01$). Furthermore, all 3 genera showed significant differences in distribution density across all quadrants in zones A –E ($F_{0.05,\ 2,\ 538278} = 77680$, $p < 0.01$).

As seen in the histogram below (Figure 34), fly artefact count generally increased as the zones moved away from the blood source ($F_{0.05,\ 4,\ 476025} = 137082$, $p < 0.01$) with zone A for *Calliphora* and *Lucilia* the exception of where feeding activity immediately adjacent to the blood tray elevated the count. By far, the greatest numbers of artefacts were deposited in zone E from all 3 fly genera.

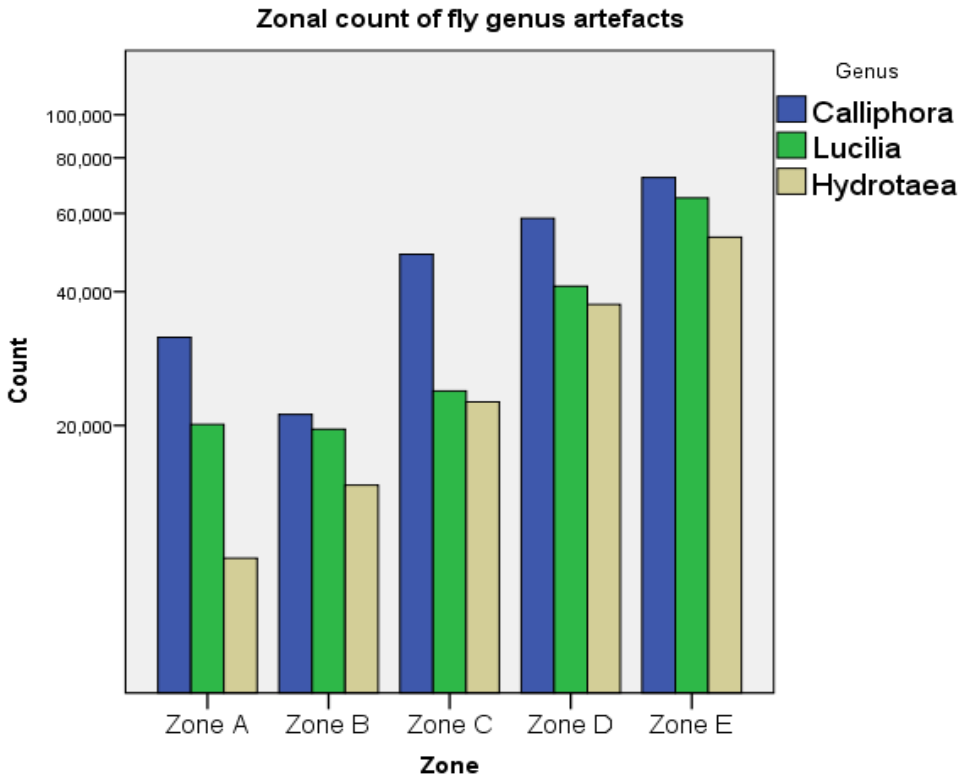


Figure 34. Histogram showing zonal artefact count of each fly genus (log.scale)

Table 2 displays the total percentage of all artefacts deposited from *Calliphora* ($\chi^2_{0.05, 4} = 36076$, $p < 0.01$), *Lucilia* ($\chi^2_{0.05, 4} = 44501$, $p < 0.01$) and *Hydrotaea* ($\chi^2_{0.05, 4} = 45122$, $p < 0.01$) per zone, as well as the residual percentages from the chi-square tests.

Of note in this table are the high fly artefact percentages, and residual values from zone E, all of which increase from zone C outward. These residual values are calculated on the assumption that all five zones have an equal artefact distribution of 20% per zone. For example, in Table 2, *Hydrotaea* produced 92.5% *more* artefacts than was expected in zone E, whereas in zone B *Calliphora* produced 54.4% *less* artefacts than the expected 20% if all zones were equal.

Table 2. Shows percentage of total fly artefacts per zone for per genus, and residuals showing percentages of more (+) than expected and less (-) than expected if distribution were assumed equal across zones A- E

	Calliphora		*Lucilia*		*Hydrotaea*	
Zone	% of total artefacts	Residual	% of total artefacts	Residual	% of total artefacts	Residual
A	13.6%	- 32%	11.85%	- 40.8%	7.3%	- 63.5%
B	9.13%	- 54.4%	11.55%	- 42.7%	10.7%	- 46.7%
C	20.9%	+ 4.62%	14.09%	- 29.5%	16.4%	- 17.9%
D	25.2%	+ 26%	24.24%	+ 21.2%	27.2%	+ 35.8%
E	31.14%	+ 55.7%	38.26%	+ 91.3%	38.5%	+ 92.5%

In contrast to the generally uniform *increase* in artefact count as zones outward from the origin, fly artefact densities from the three genera all exhibited significantly different trends ($F_{0.05, 4, 381058} = 12925$, $p < 0.01$). This can be seen below in Figure 35 where *Calliphora* generally decreased, *Lucilia* curved downward from zone A to C,

and then upward from zone C to E, and *Hydrotaea* density generally increased from zone A to E.

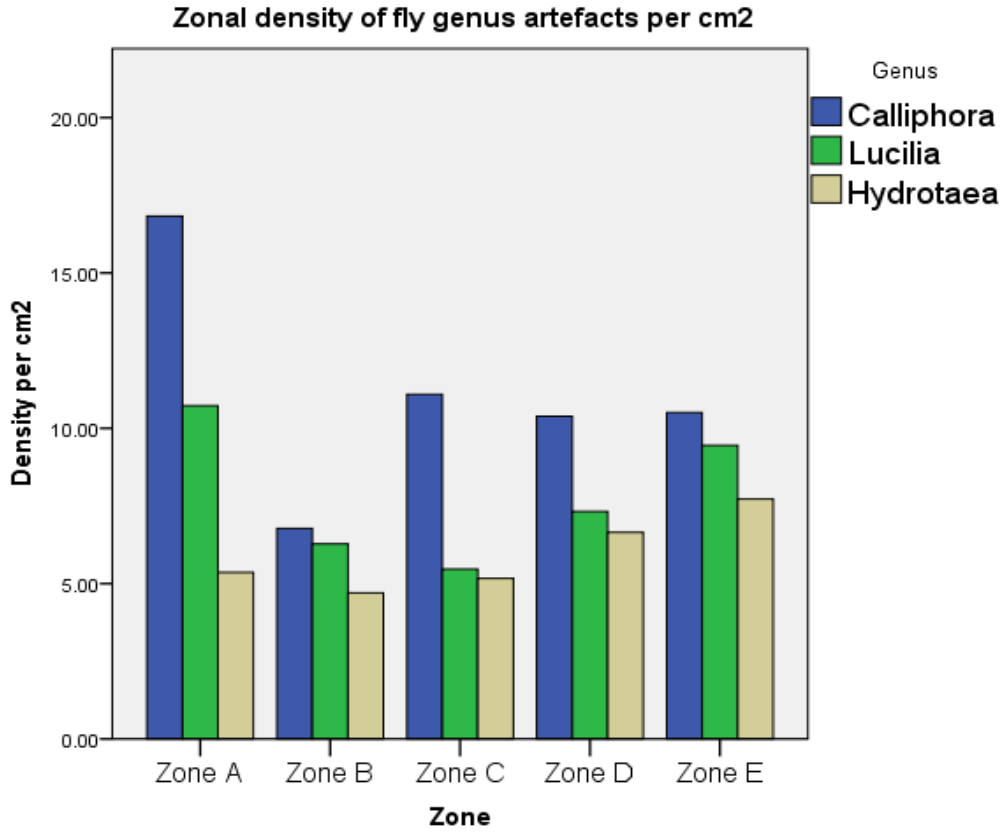


Figure 35. Histogram showing zonal artefact density per cm^2 of each fly genus

Figure 36 below displays the total number of artefacts produced by all flies in quadrants 1-4, as well as the percentage of their total artefacts deposited in each respective quadrant. Although total artefact count and all *zonal* tile placement data was accurately recorded, an error in recording the tile placement *quadrant* data for *Calliphora* meant that the values for quadrants 1-3 were not able to be identified. The four quadrant plans in the Figure show that all flies deposited the highest percentage of their artefacts within quadrant 4, with *Calliphora* by far the highest total artefact percentage (33.9%).

Lucilia and *Hydrotaea*, for which all quadrant data was available, both follow the same trend in quadrant percentage decrease from Quadrant 4, Quadrant 1, Quadrant 2 then Quadrant 3.

Of note, is that this identical high-low percentage pattern follows the path of the light ingress from the window, which is discussed in section 5.

	Q1	Q2	Q3	Q4
Calliphora	-	-	-	78,638
Lucilia	45,302	41,342	33,751	49,361
Hydrotaea	40,098	27,974	26,524	42,894

Figure 36. A table showing the total number of artefacts in quadrants 1-4 from the respective fly genera, and individual quadrant plans displaying percentage of total artefacts deposited within those quadrants from each genera: Blue = *Calliphora*, Green = *Lucilia*, Red = *Hydrotaea*. Arrows indicate position of the window

Figure 37 shows the zonal densities of *Lucilia* and *Hydrotaea* in quadrants 1-4. Of note are the higher densities in quadrants 1 and 4, which were situated where the main area of light entered the room from the window and across the kitchen area.

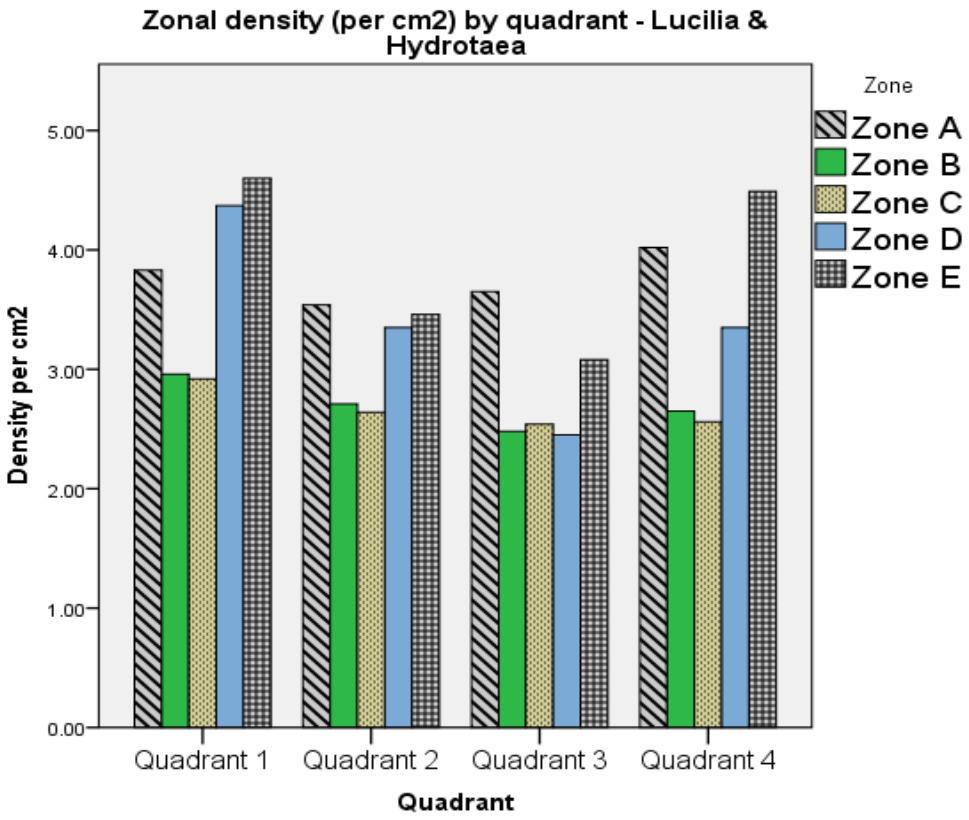


Figure 37. Histogram showing zonal artefact density per cm^2 in quadrants 1 – 4 from *Lucilia* and *Hydrotaea*

Recreated Spatter – Single Quadrant

All recreated bloodstain patterns generated a high number of stains with expirated producing by far the highest number (244,401), followed by impact spatter (60,098). Both of these recreated patterns exhibited significant differences in their respective zonal distribution count; impact ($\chi^2_{0.05,\,4} = 8980$, $p < 0.01$) and expirated ($\chi^2_{0.05,\,4} = 215107$, $p < 0.01$).

These differences are highlighted below in Table 3, which displays the total percentage of impact and expirated blood stains per zone, as well as the residual percentages from the chi-square tests.

As seen below in Figure 38, impact spatter count gradually decreased with distance from the blood source, only increasing slightly in zone E. Conversely, expirated

spatter count was lower in zone A before rising to enormous numbers in zones B and C, then decreasing gradually down to zone E.

Table 3. Shows percentage of total impact and expirated stains per zone, and residuals showing percentages of more (+) than expected and less (-) than expected if distribution were assumed equal across zones A- E

	Impact		Expirated	
Zone	% of total stains	Residuals	% of total stains	Residuals
A	30.1%	+ 50%	9.7%	- 51%
B	28.2%	+ 41%	53.6%	+ 168%
C	12.7%	- 36.5%	27.3%	+ 36.6%
D	11.8%	- 40.8%	5.2%	- 74%
E	17%	- 14.6%	4.1%	- 79.2%

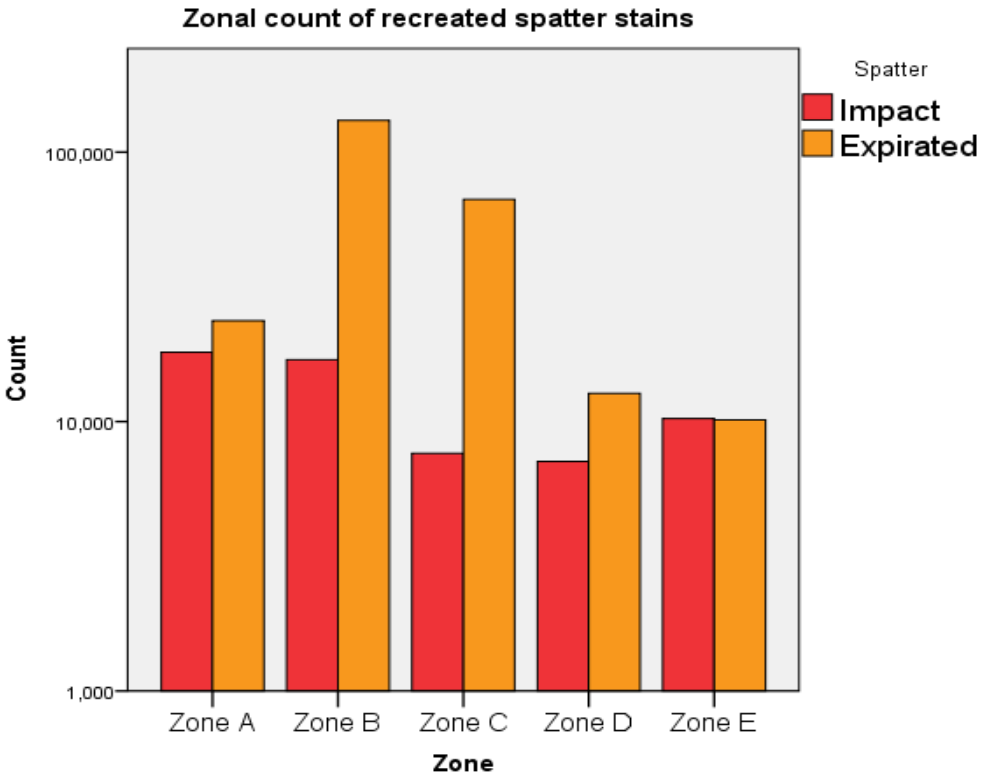


Figure 38. Histogram showing zonal recreated spatter stain count per zone (log.scale)

Spatter stain density per cm ^{2} of impact and expirated blood follows a very similar trend as spatter stain count (Figure 38) across the five zones, as can be seen in Figure 39.

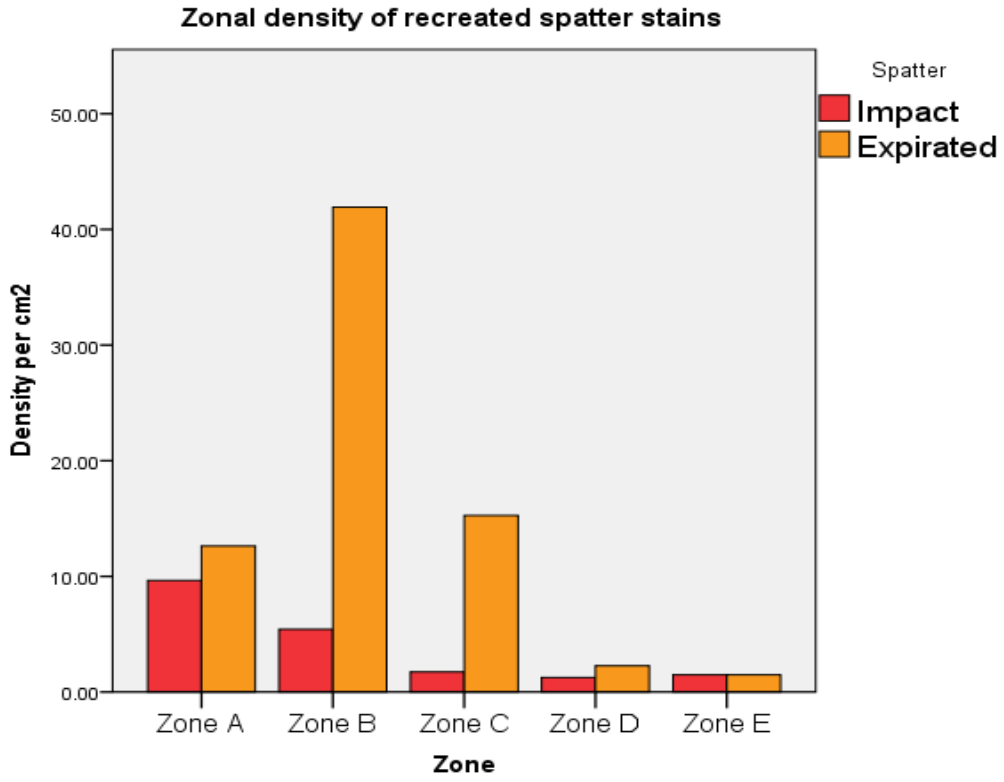


Figure 39. Histogram showing zonal recreated spatter stain density per cm²

Flies – Quadrant 4

Within quadrant 4, and out of a total 170,893 artefacts, *Calliphora* produced the most (78,638) followed by *Lucilia* (49,361), with *Hydrotaea* producing the fewest (42,894) ($F_{0.05,\ 2,\ 161620}$ = 12091, $p < 0.01$). Naturally, the same trend was observed with regard to density in quadrant 4, where *Calliphora* had the highest (3.59 artefacts per cm^2), followed by *Lucilia* (2.26 artefacts per cm^2) with *Hydrotaea* (1.96 artefacts per cm^2) having the lowest ($F_{0.05,\ 2,\ 170095}$ = 37571, $p < 0.01$).

Moreover, significant differences were found between *Calliphora*, *Lucilia* and *Hydrotaea* ($\chi^2_{0.05,\ 2}$ = 13215, $p < 0.01$) in their artefact count totals, in addition to

significant differences in their respective zonal (A-E) counts ($\chi^2_{0.05,\,4} = 53015$, $p < 0.01$), shown in Figure 40. Disregarding the immediate activity adjacent to blood source (Zone A), the abundance of artefacts increased from zone B to Zone E. This is likely to be a result of light ingress from the window.

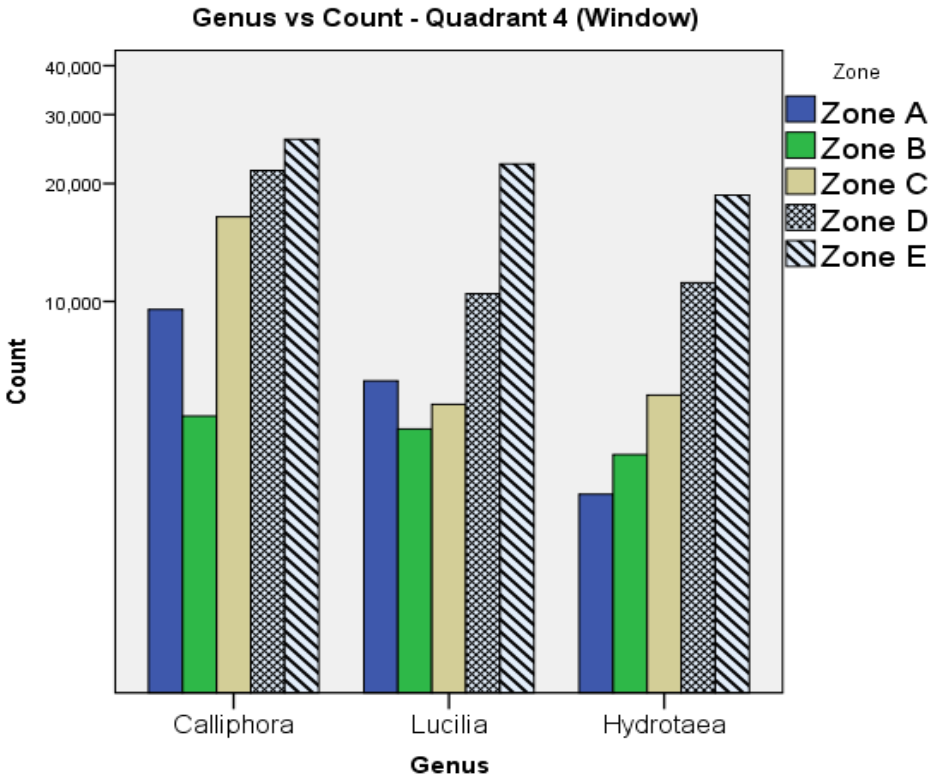


Figure 40. Histogram showing the total count of artefacts from individual genera per zone (log.scale)

Fly artefact density within quadrant 4 (Figure 41) was largely dominated by *Calliphora* in zones A, C, D and E, with only zone B displaying values closer to the other two genera.

Again, different trends can be observed between the three genera, where *Calliphora* artefact density decreased over zones A-E overall, *Lucilia* displays a 'U' shaped curve from A-E and *Hydrotaea* artefact density increased.

Of note, is that the trends from the respective genera within quadrant 4 are similar to the trends they displayed across all quadrants (1-4), as shown previously on page 52 (Figure 35).

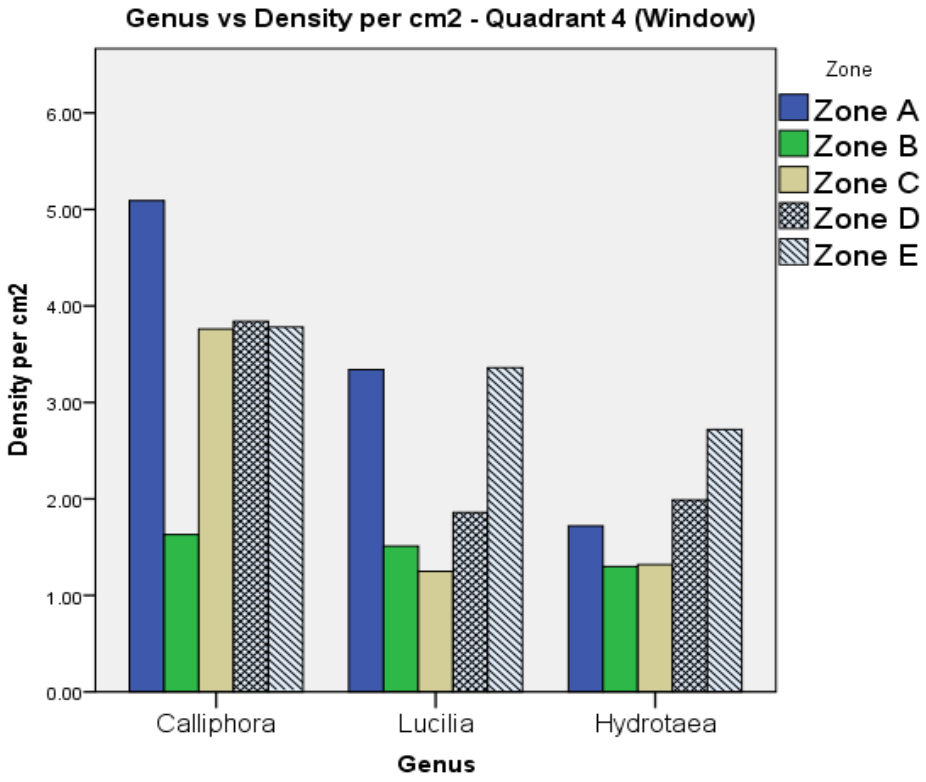


Figure 41. Histogram showing the total density per cm^2 of artefacts from individual genera per zone

Fly (Quadrant 4) vs Recreated (Single Quadrant)

Within quadrant 4, all five artefact categories (*Calliphora*, *Lucilia*, *Hydrotaea*, impact spatter and expirated spatter) displayed significantly different count totals in quadrant 4 ($F_{0.05,\ 4,\ 310417}$ = 364774, p <0.01) as well as significantly different respective densities ($F_{0.05,\ 4,\ 284227\ =}$ 360217, p <0.01). Additionally, Impact and expirated spatter and the fly artefacts from the three genera exhibited significant differences in their respective zonal distribution (A-E) in both artefact count ($F_{0.05,\ 4,\ 120369}$ = 38787, p < 0.01) and artefact density ($F_{0.05,\ 4,\ 107509\ =}$ 4597, p < 0.01).

The density of the recreated impact spatter pattern (Figure 42) reduced as the stain radiated away from the origin outward from a high density of 9.65 stains per cm^2 in zone A, 5.43 stains per cm^2 in zone B, to a much lower density of 1.74 stains per cm^2

in zone C, after which it generally became more evenly distributed (D=1.26 per cm^2. E=1.49 per cm^2). Fly artefact density followed a very similar profile to impact spatter in zones A (9.11 per cm^2) and B (4.71 per cm^2), before sharply increasing in zones C (6.32 per cm^2), D (7.01 per cm^2) and E (8.27 per cm^2).

Expirated spatter (Figure 43) density was significantly higher than fly artefact density primarily in zone B, but also in zone C. This is largely due to the enormous number of fine (93,105 out of 131,045 = 71%) and mist (37,766 out of 131,045 = 28.8%) sized stains generated in zone B, and fine (38,523 out of 66,785 = 57.7%) and mist (28,084 out of 66,785 = 42.1%) sized stains generated in zone C. Furthermore, the density of fly artefacts was much higher in zones D (7.01 per cm^2) and E 8.27 per cm^2) compared to that of expirated spatter (D = 2.26 per cm^2 & E= 1.48 per cm^2).

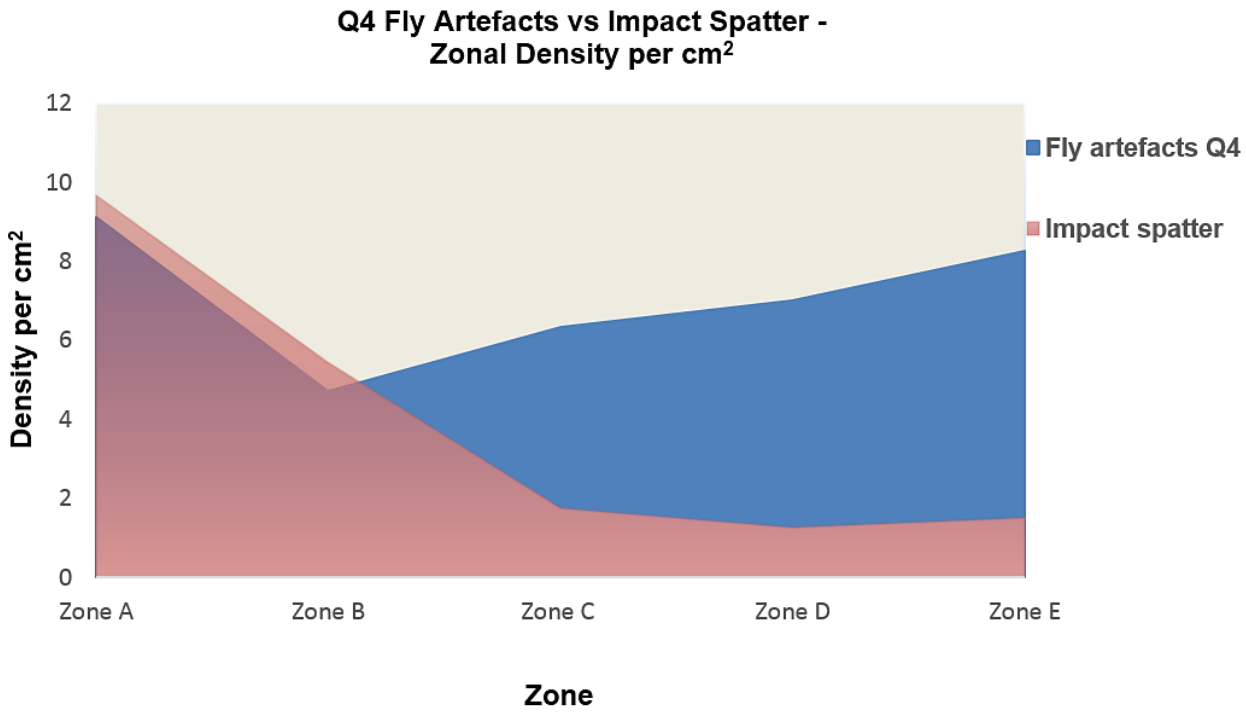


Figure 42. Graph showing an overlay of the respective densities per cm^2 of quadrant 4 fly artefacts and impact spatter in zones A-E

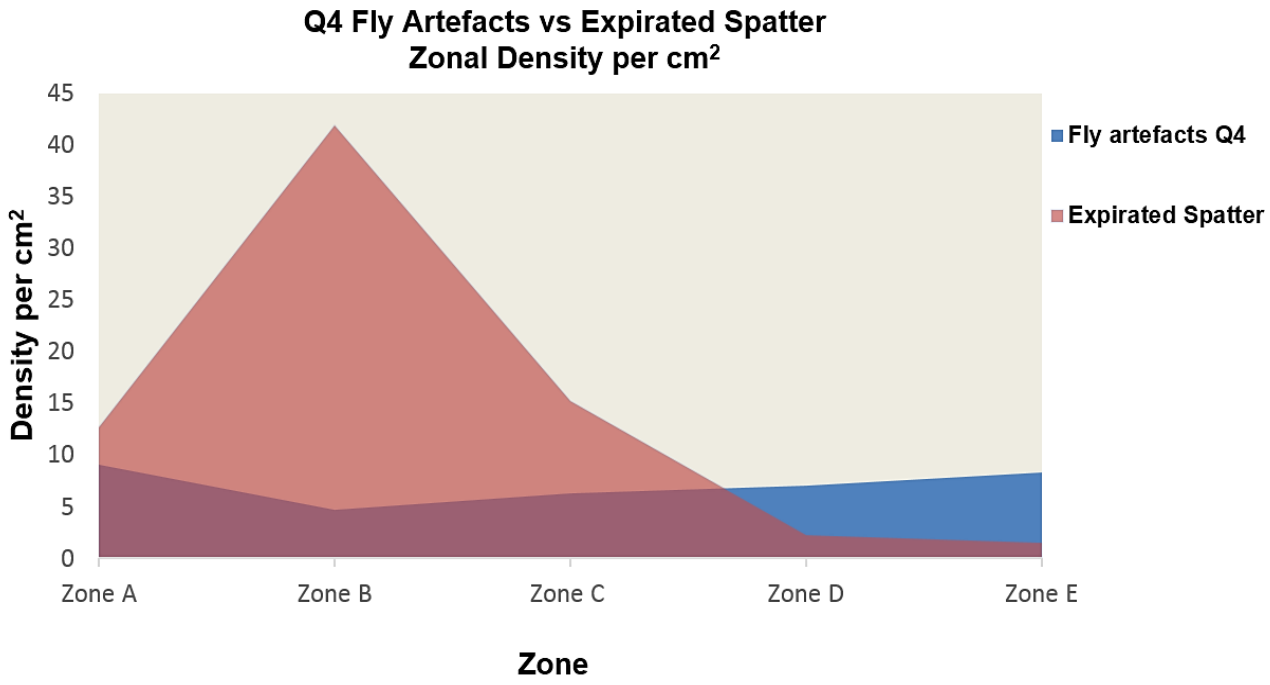


Figure 43. Graph showing an overlay of the respective densities per cm^2 of quadrant 4 fly artefacts and expirated spatter in zones A-E

4.4 Distribution of stain sizes within fly and recreated blood patterns

Throughout all fly and recreated bloodstain patterns, the five artefact size categories (large, medium, small, fine and mist) all exhibited significant differences to one another in both count ($F_{0.05,\ 4,\ 537934} = 678320$, $p < 0.01$) and density per cm^2 ($F_{0.05,\ 4,\ 253001} = 547784$, $p < 0.01$).

For example, large fly artefact sizes, seen in Figure 44, were primarily distributed in zones A and E, with only 2 other artefacts occuring in zones C and D respectively, with the majority being produced by *Calliphora*.

A similar pattern was observed with medium fly artefacts (Figure 45) which again, had distribution heavily weighted in zones A and E, and were primarily produced by *Calliphora*

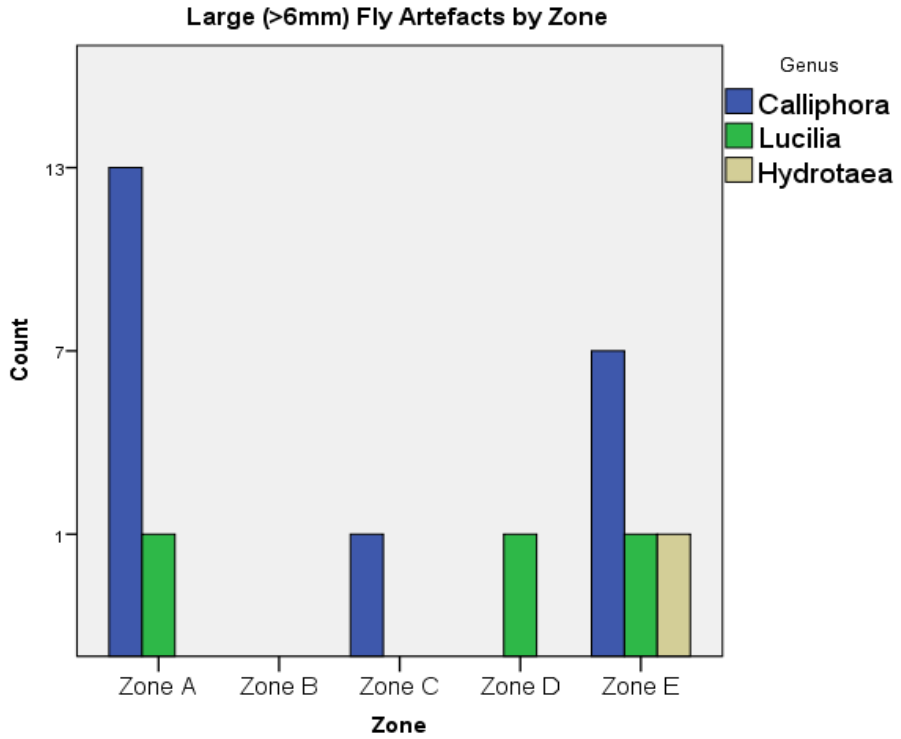


Figure 44. Histogram showing large (>6mm) fly artefact distribution by zone

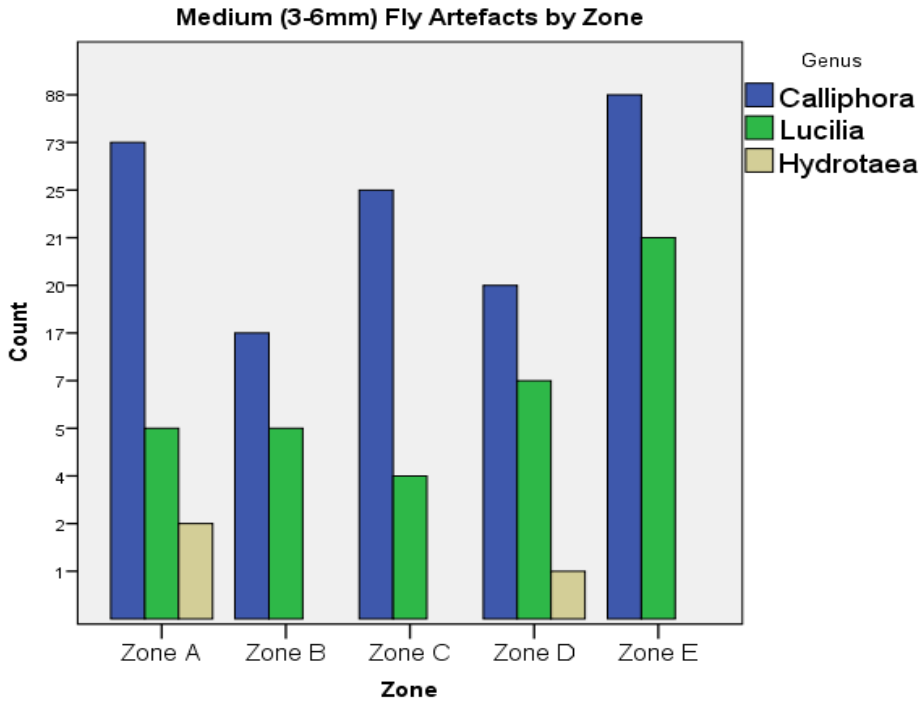


Figure 45. Histogram showing medium-sized (3-6mm) fly artefact distribution by zone (log.scale)

Small stains (Figure 46) occurred in much higher numbers than large or medium, and although they were slightly weighted in zones A and E, were produced much more frequently and evenly in zones B, C and D.

Fine sized fly artefact (Figure 47) numbers were enormously higher than large, medium and small stain numbers. Aside from *Calliphora* and *Lucilia* artefacts in zone A, the fine stains increased almost uniformly from zone B onward to zone E, which had the highest number.

Mist sized stains (Figure 48) showed an almost identical distribution and growth pattern to that of fine sized stains across all zones.

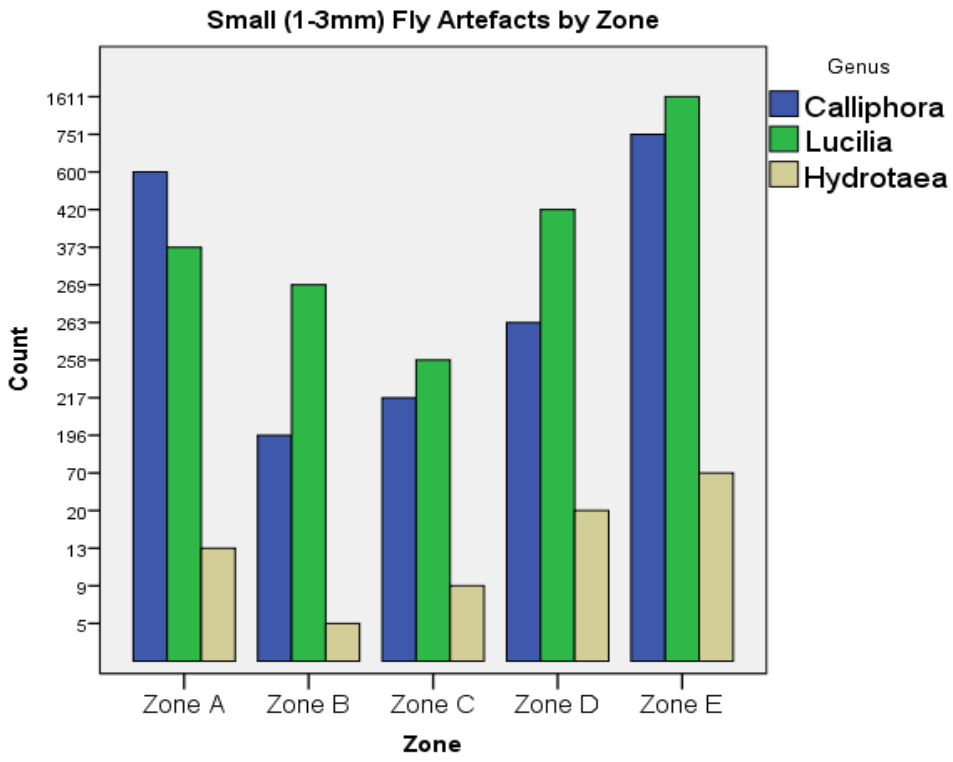


Figure 46. Histogram showing small-sized (1-3mm) fly artefact distribution by zone (log.scale)

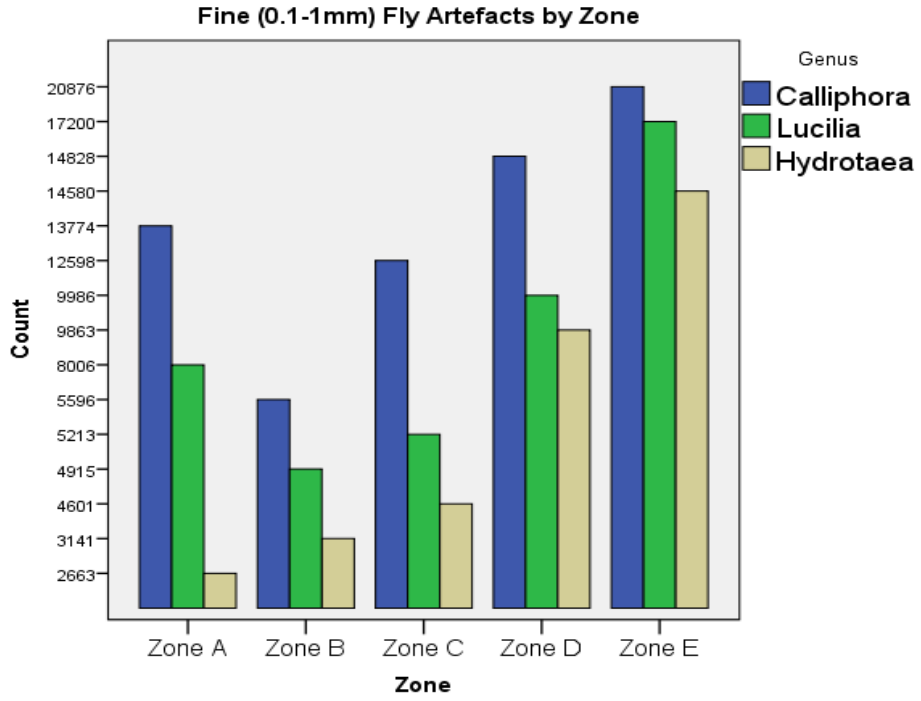


Figure 47. Histogram showing fine-sized (0.1-1mm) fly artefact bloodstain distribution by zone (log.scale)

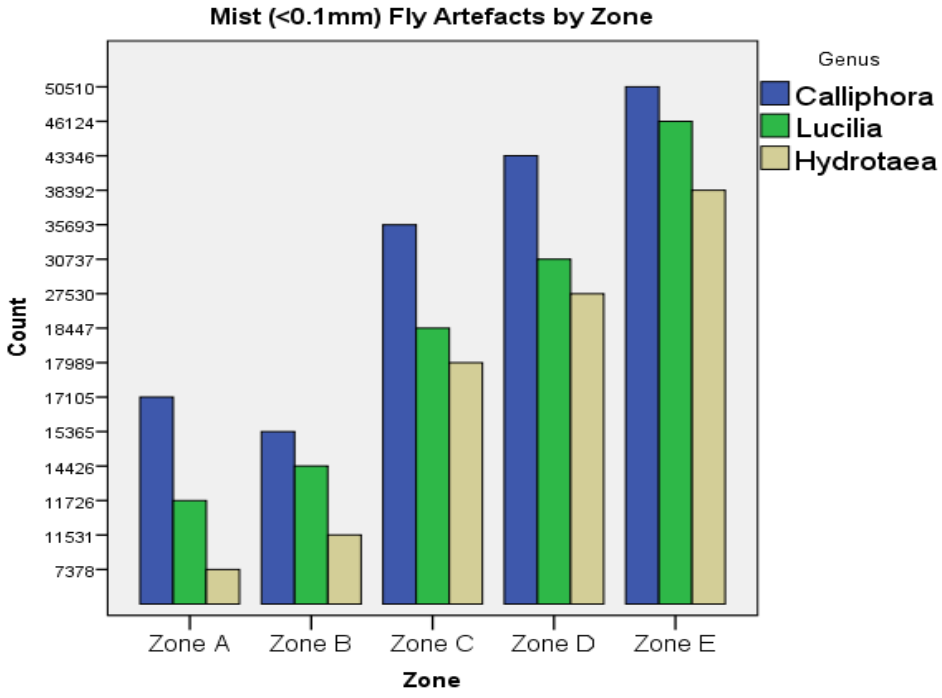


Figure 48. Histogram showing mist-sized (<0.1mm) fly artefact bloodstain distribution by zone (log.scale)

Quadrant 4 - flies

Within quadrant 4 a significant difference in artefact number was found between *Calliphora*, *Lucilia* and *Hydrotaea* ($\chi^2_{0.05,\,2} = 13215$, $p < 0.01$), in addition to a significant difference in the size of the artefact (Large –mist) they deposited ($\chi^2_{0.05,\,4} = 314093$, $p < 0.01$). Furthermore, the 5 artefact size categories (large-mist) all showed significant differences in both count ($F_{0.05,\,4,\,170241} = 133247$, $p < 0.01$) and density ($F_{0.05,\,4,\,119116} = 143910$, $p < 0.01$) within this quadrant.

The respective zonal counts of each artefact size category for *Calliphora*, *Lucilia* and *Hydrotaea* within quadrant 4 can be seen below in histograms (Figure 49, Figure 50 and Figure 51).

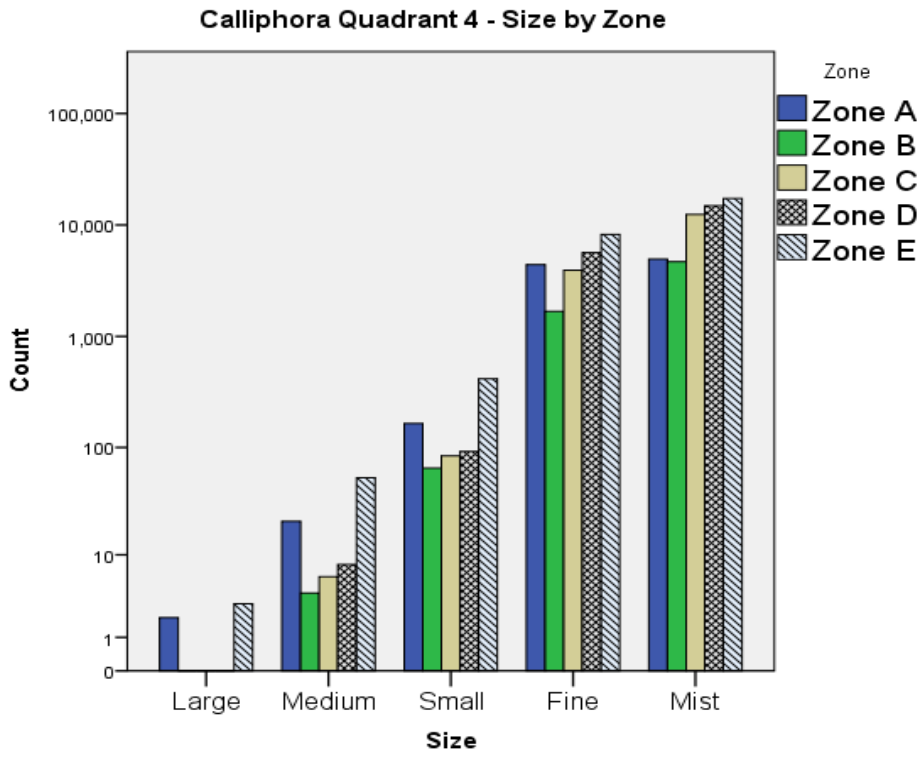


Figure 49. Histogram showing the size count totals of *Calliphora* artefacts by zone in quadrant 4 (log.scale)

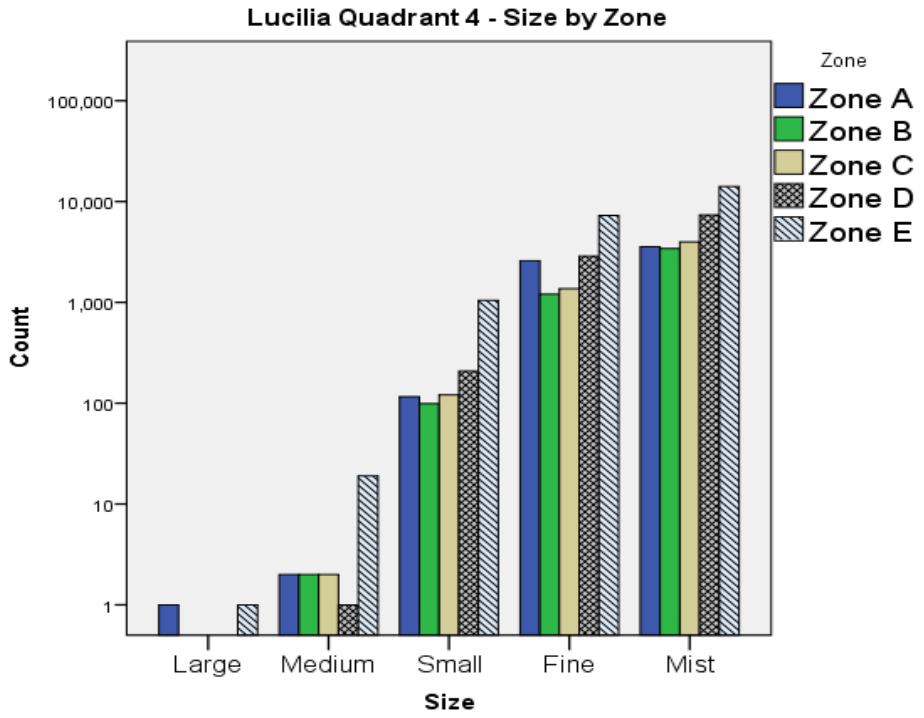


Figure 50. Histogram showing the size count totals of *Lucilia* artefacts by zone in quadrant 4 (log)

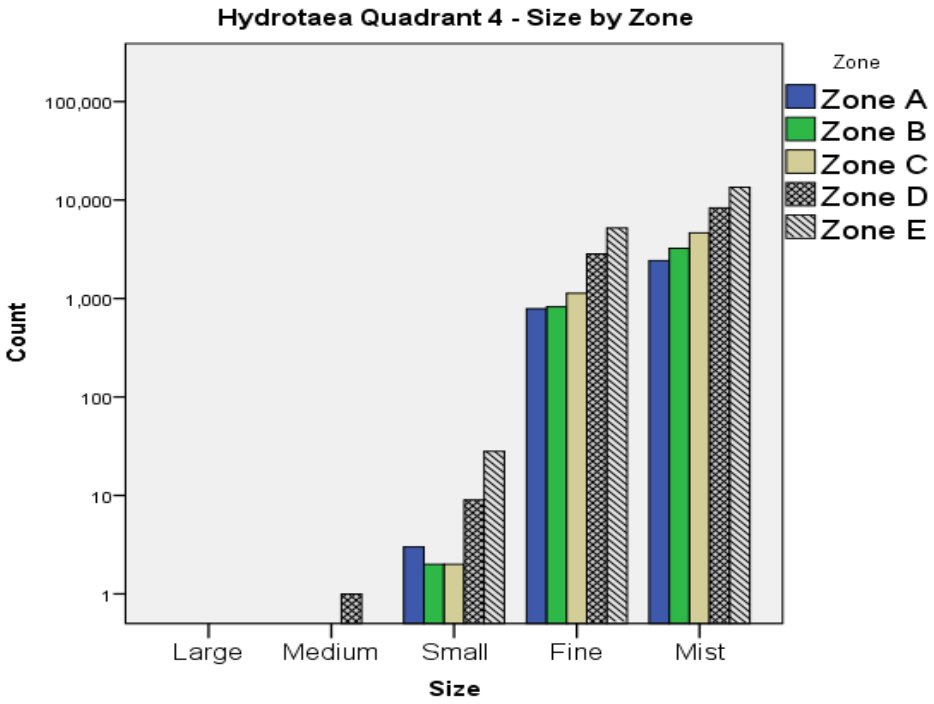


Figure 51. Histogram showing the size count totals of *Hydrotaea* artefacts by zone in quadrant 4 (log.scale)

Recreated Spatter – Single Quadrant

Within the recreated spatter patterns, significant differences (p <0.01) were found between the sizes ($\chi^2_{0.05, 4} = 92470$, $p < 0.01$) of individual impact stains and between the zones ($\chi^2_{0.05, 4} = 8984$, $p < 0.01$) in which they occurred, in addition to the sizes ($\chi^2_{0.05, 4} = 403155$, $p < 0.01$) of individual expirated stains and between the zones ($\chi^2_{0.05, 4} = 215128$, $p < 0.01$) in which they occurred.

Within the impact spatter pattern, large stains were scarce, with only a single stain produced in zone D. In contrast, a total of 13 large expirated stains were found within zones A, B and C, and are likely made up of the central 'spine' of large stains previously reported.

Medium-sized stains were distributed irregularly amongst the zones where both categories produced identical numbers (2) in zone A. However, zones B and C had a high contrast between them with 13 expirated stains and no impact stains in B, and in zone C only a single impact stain and 7 expirated. The remaining zones shared higher numbers of stains between them.

Small-sized recreated stains steadily increased in number across all zones except for a slight drop in impact numbers in zone B, and a drop in expirated numbers in zone D.

The distribution of fine-sized stains was very similar between the impact and expirated spatter patterns; both categories had the highest stain numbers in zone B, where the stain numbers of both categories decreased steadily outward to zone E, where impact spatter had a slight rise in number.

Mist-sized stains exhibited an almost 'mirror image' of one another in the trend of their spatter size distribution. Impact spatter numbers decreased from zone A outward to zone C, and then increased to zone E, whereas expirated spatter stains saw an increase out to zone B, before decreasing steadily outward to zone E.

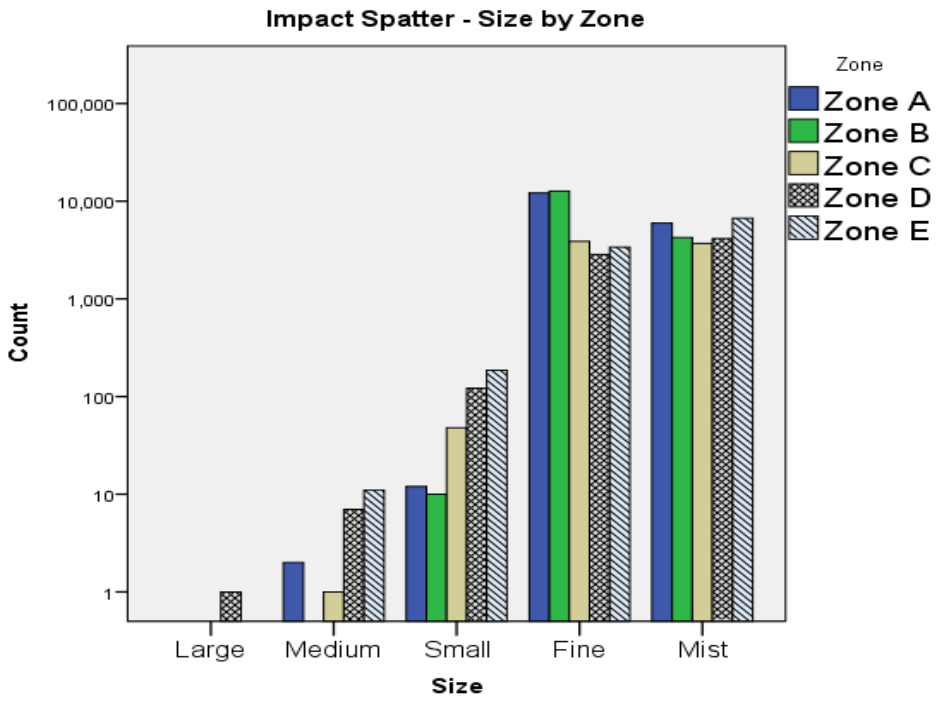


Figure 52. Histogram showing the size count of impact spatter by zone

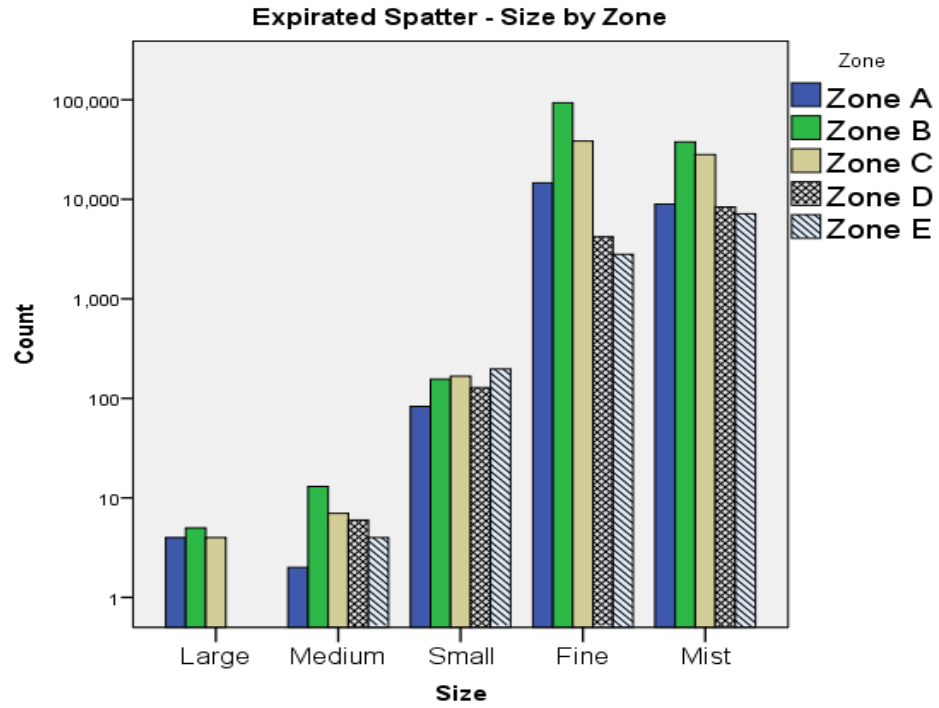


Figure 53. Histogram showing the size count of expirated spatter by zone

4.6 Artefact Morphology

Many similarities between the morphology of artefacts from the 3 fly genera and the recreated blood spatter stains were observed. *Calliphora*, *Lucilia* and *Hydrotaea* artefacts showed similarities to expirated and impact blood stains. C*alliphora* artefacts contained similar bubble rings to those seen in the expirated stains and *Lucilia* artefacts showed the same ‘doughnut-like’ appearance of other expirated stains. Similarities to expirated/impact mist-sized stain patterns were also observed in *Hydrotaea* artefact patterns. A selection of these types of artefacts can be seen below in Figures 54 & 55 respectively.

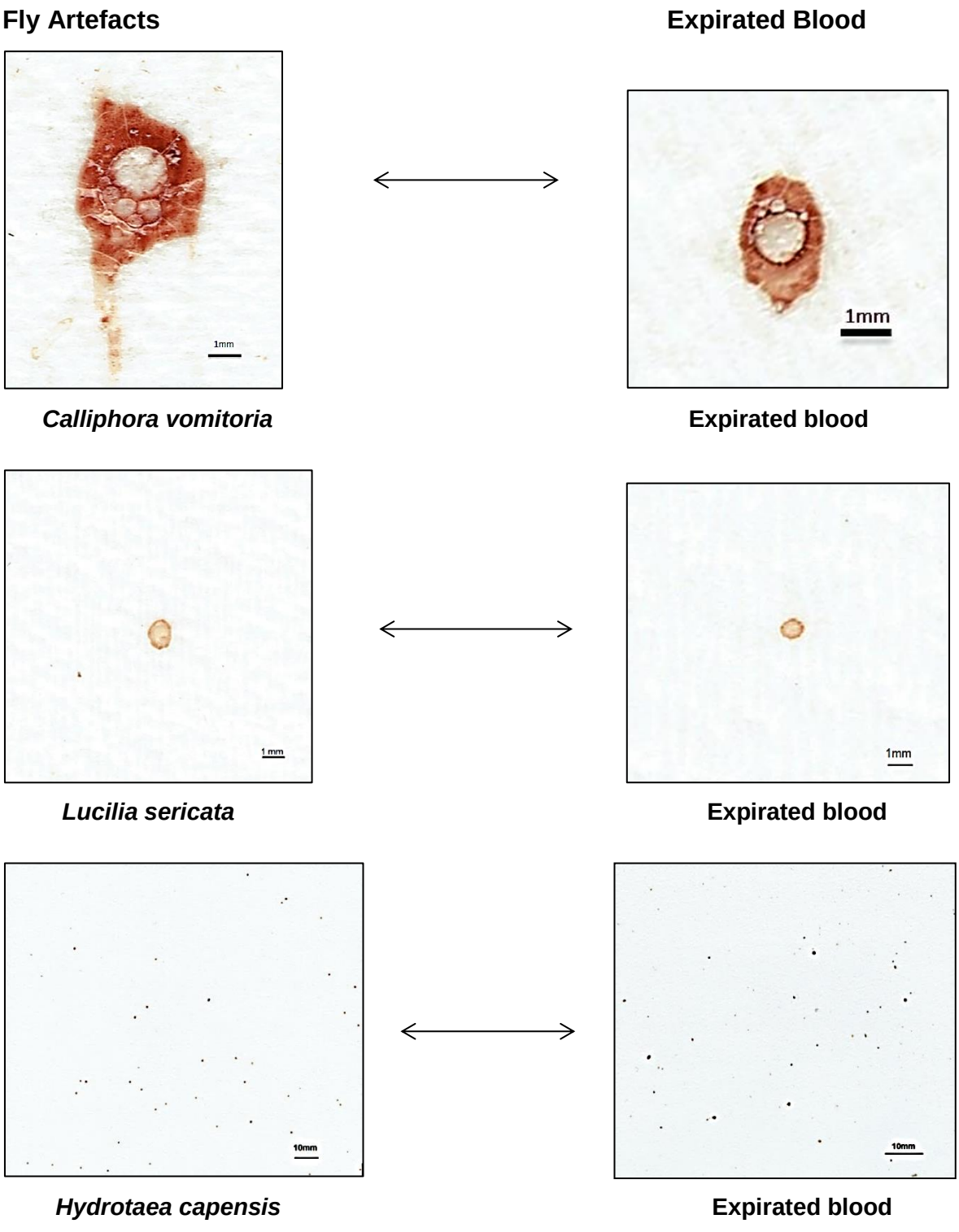
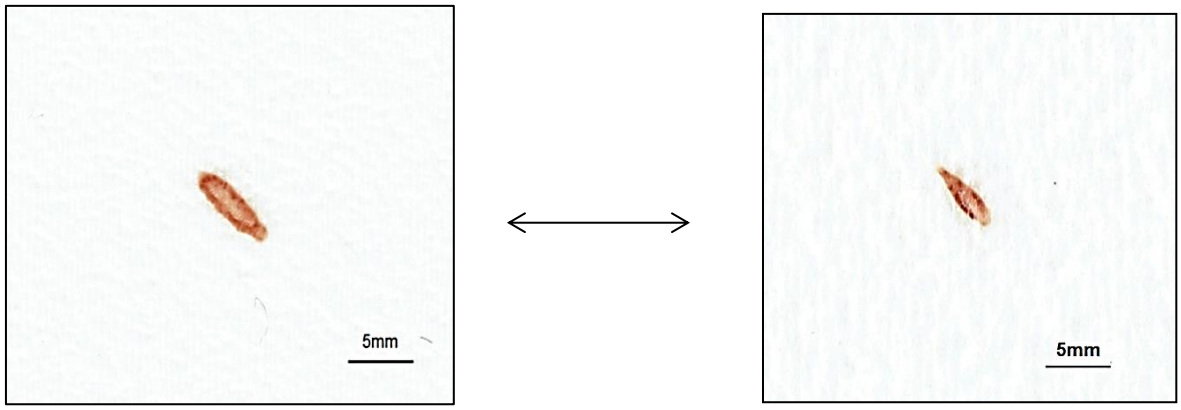
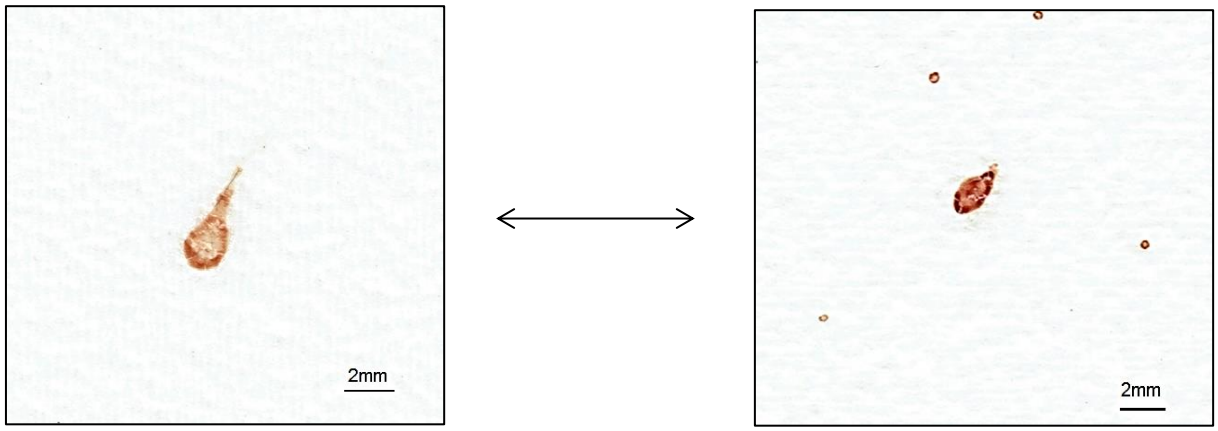


Figure 54. Fly artefacts vs Expirated blood stains

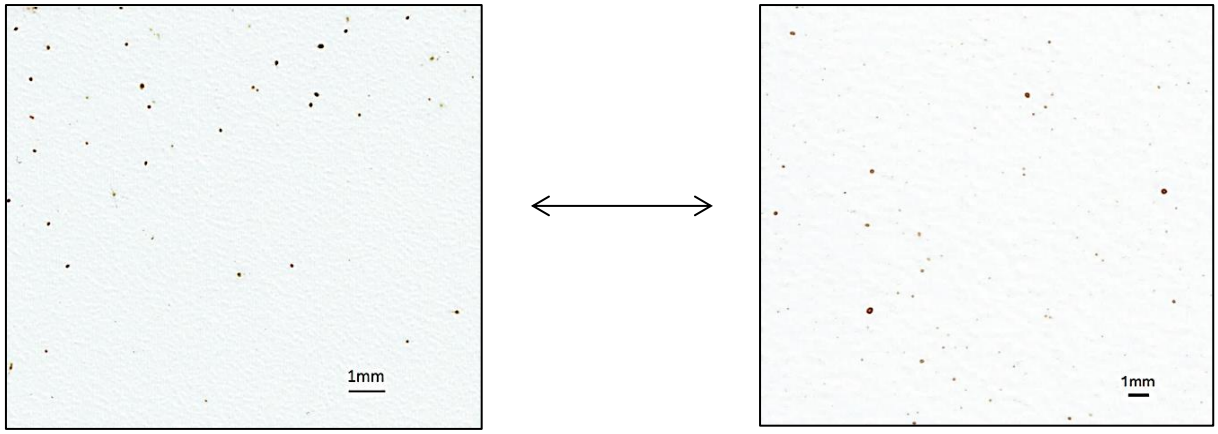
Fly artefacts **Impact spatter**



Calliphora vomitoria **Impact spatter**



Lucilia sericata **Impact spatter**



Hydrotaea capensis **Impact spatter**

Figure 55. Fly artefacts vs Impact spatter

5. Discussion

Background to the current study

This study aims to objectively investigate the 'confounding' effect that flies, that are most commonly associated with the post mortem interval, have on impact and expirated bloodstain spatter patterns following a blood meal, and to assess the impact of this on forensic bloodstain pattern analysis (BPA). The primary focus is on the deposited fly blood *artefacts* that could potentially occur at a crime scene, and it should not be confused with any type of analysis or comparison of fly *behaviour*. Because no two crime scenes are the same, and considering the fact that the data in this study were collected from three separate experiments consisting of three fly genera (albeit a high number of flies) it is reasonable to suggest that the scenario used for testing here is not only well within the realm of possibility, but is also operationally relevant in the field of BPA.

It is an extension of Dwen (2015), where an experiment was conducted to investigate whether the size and morphology of artefacts deposited by the common housefly *Musca domestica* compared to those within the literature. Additionally, the proximity at which artefacts were deposited in relation to a blood source and any similarities to assault derived spatter were also recorded. In that study a total of 13,355 blood artefacts were observed where artefact type, size range (in mm^2), and general colour of artefacts was recorded.

The results indicated that the type, size, and colour of artefacts deposited by the common housefly, *Musca domestica*, following a blood meal mimicked assault related blood spatter. Furthermore, the high frequency that artefacts were deposited within close proximity of a blood source could confound a crime scene owing to their similarity, in terms of stain features and distribution, with assault related spatter. Interestingly, the results from the current study support the findings of the previous study with regard to the proximity that artefacts were distributed in relation to a blood source. In the previous study, and contained within a 135mm wide 'microscene',

Musca domestica deposited its artefacts in much higher numbers within the first 45mm of the origin (blood source) border, and decreased in number with increasing distance from it. Whilst the current study employed zones that were 250mm wide, the results show that fly artefacts were much more numerous around the origin border in artefact generation from all 3 genera.

Objective bloodstain pattern classification

Although BPA is widely regarded as subjective there are a number of published approaches which support the objective classification and interpretation of bloodstains. Laber (1985) proposed a classification scheme based on measurement of bloodstain size and this was built upon by the Objective Criteria scheme that was formed by Wonder (2001). In the current study, a robust mechanism by which to assess, characterise and then compare the resultant stain patterns was required and the above schemes were used as a framework for this. The assessment of stains based on size provides a reproducible and auditable method of classification and the range of stain sizes devised by Laber were applied in this study. For this reason, artefact/stain categorisation by size was the key feature of the MatLab program.

Within the published BPA literature, the use of objective criteria has been widely employed, and is now a well-established and proven method of approaching the analysis of bloodstains in forensic casework. This objective approach is flexible enough to allow the employment of its criteria in the analysis of fly artefact patterns; however, it is important to recognise that there are substantial differences in the mechanisms by which bloodstain patterns are generated following an assault, for example, as compared to the way that a pattern consisting solely of blood artefacts is produced by flies. Firstly, an assault-derived bloodstain pattern may be generated by only a single, or several actions or strikes (James *et al* 2005). Conversely, a pattern consisting of only fly artefacts will potentially have been produced from thousands of different actions by numerous individual flies. Secondly, blood patterns are governed by blood dynamics, that is, the blood acts in a well-documented manner according to

the laws of physics (Wonder 2001; James 2005; Kabaliuk *et al* 2014), whereas fly artefacts are the result of the individual mechanism of the fly producing it. On this basis, whilst the objective criteria as published provide a clear framework under which complex pattern features can be assessed and recorded, including those generated by fly activity, the scheme has been further developed here in order to allow other features to be formally considered. This includes consideration of external factors, such as light ingress from a window, and stain appearance, both of which are significant observations in the assessment of fly artefacts, but which arguably are as relevant in the assessment of BPA more generally. Ultimately it is hoped that the revised scheme based on this study will be adopted by practitioners involved in the interpretation of all bloodstain patterns and not just restricted to the assessment of fly artefact patterns.

1. Shape of whole fly and recreated blood patterns

Currently, Wonder's (2001) recommended criterion for the analysis of whole impact *patterns* (which are different from individual stains/artefacts discussed later) involves recognising that patterns can appear in several descriptive shapes (Figure 56) which include: star-burst, pie wedge, cone, and trapezoid shaped. Additionally, that each blood pattern will reflect an origin and that each *individual* stain within that pattern will show directionality that is distributed away from that origin. Both the overall recreated impact and expirated blood pattern shapes observed in the current study closely resembled the cone shape (c) in Figure 56, which radiate outward from a narrow point of impact (POI).

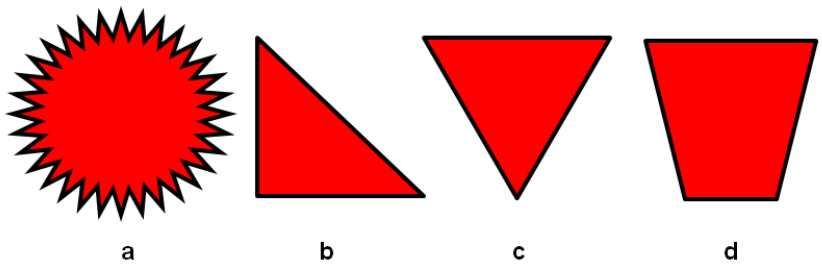


Figure 56. Overall blood pattern shapes as described by Wonder (2001) - (a) star-burst (b) pie wedge (c) cone (d) trapezoid (Wonder 2001)

All 3 genera of flies distributed their artefacts in a manner that was similar to the star-burst shape (Figure 56.a); radiating outward from the blood source in a somewhat uniform manner, albeit, differing slightly between genera. An example of this type of fly artefact pattern is shown in Figure 57, which is taken from the previous study by Dwen (2015). Often the artefact stains were distributed over a large area and exhibited a circular pattern. It was noted that this uniformity could be disrupted by external factors, most commonly the presence of a window which caused an asymmetrical appearance to the pattern.

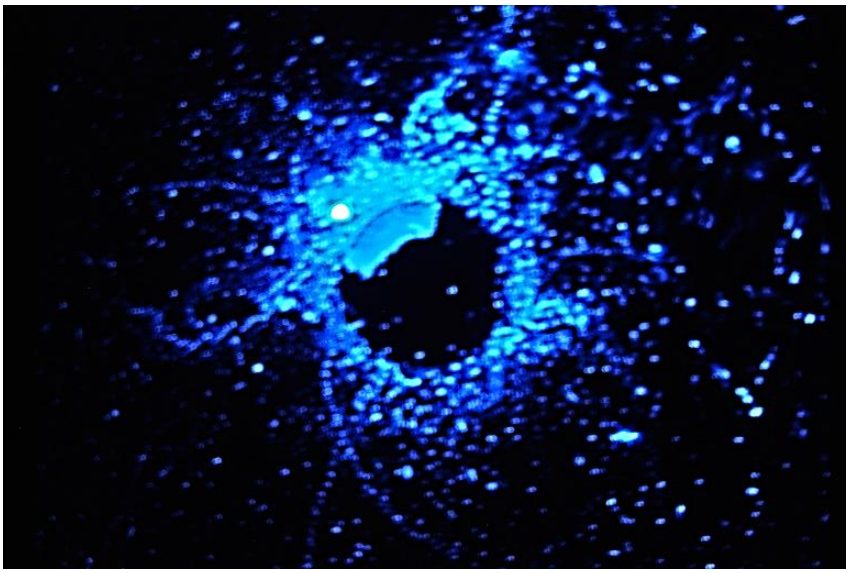


Figure 57. *Musca domestica* artefact pattern enhanced with Bluestar (Dwen 2015)

External factors causing alteration of pattern shape

This similarity between impact- and insect-derived patterns poses a significant problem in pattern interpretation. Because the star-burst shaped fly artefact patterns observed here can easily encompass any, or all of the pattern shapes identified within an impact-derived blood pattern, this has the potential to disguise important aspects of the analysis and the two patterns can be indistinguishable. The concept that fly

artefact patterns are often misclassified as impact has been recognised by works such as Benecke & Barksdale 2003 and Langer & Illes 2015, and these studies have shown that the rote application of objective criteria, as published, can lead to ambiguity. On this basis, it is important that objective approaches are expanded to include external factors which impact on stain pattern generation such as fly artefacts.

External factors, such as the light from windows or even objects in the path of blood in flight, have the potential to significantly alter the overall appearance of a blood pattern. A good example of this is the 'Blockage/void' pattern, described in section 1.4 (p.22), which demonstrates that only part of the pattern has been represented. Whenever possible it is important for the BPA analyst to understand the causative factor for these types of patterns. For example, at a crime scene, the reason for a void in an impact pattern may be obvious to the investigator, such as the physical presence of an object still *in situ*. However, these 'obvious' reasons can be disguised by the fact that fly artefact production is not governed by the same restrictions or laws of physics that influence a drop of blood in flight. A fly will deposit artefacts on any surface to which it has access, such as under a door, behind it, or on both vertical surfaces of that door. This can mean that a void that may have initially been present following the generation of the original, unaltered blood pattern may contain any number of fly artefacts by the time an examiner attends the crime scene. Consequently, the BPA analysts' interpretation must consider factors which are not explicitly outlined in the criteria and these could be non-physical or environmental in nature.

For example, in this study a high number of artefacts (539,507) were recorded from 3 fly genera. This high production rate suggests that if enough flies were present at a crime scene, a significant number of artefacts could be deposited behind an obstacle, such as a door. This could indicate to an investigator that the door may have been open when an assault took place, even if at the time of their examination it was closed. Studies by Benecke and Barksdale (2003) and Parker *et al* (2010), have demonstrated that fly behaviour is heavily influenced by external factors, and as previously mentioned, they are known to prefer to congregate and distribute their

artefacts, in areas of natural light such as around windows or near food sources. This is highlighted in the current results with artefact numbers being distributed in much higher numbers in quadrant 4 near the window area, with *Calliphora* depositing 33.9%, *Lucilia* 29.1% and *Hydrotaea* 31.1% of their total artefacts there. Singularly or collectively external factors can result in a heavily non-symmetrical appearance and yet they are not formally considered within the objective criteria that are currently available in a BPA context.

Morphology and colour of individual fly artefacts

Morphological differences and colour are beneficial for distinguishing between two types of fly artefact, defecated and regurgitated stains; but, it is also essential to acknowledge these two types of fly artefacts similarity to impact-derived bloodstains. By doing so, an investigator may be alerted to those artefacts that may require a closer examination. These results (p.69-70) illustrate several of the numerous similarities in artefact morphology observed between *Calliphora*, *Lucilia* and *Hydrotaea* and the recreated impact and expirated bloodstains. Similarities were observed between defecated *Calliphora* artefacts and some expirated stains. Typical features of expirated stains recorded were a stringing effect (interconnecting lines between individual bloodstains) and the presence of bubble rings within the individual stains (Figure 54 p.69).This has been well documented as a common feature of expirated blood (Millington 2016). Moreover, it has been reported that the behaviour of flies feeding on wet blood can cause a ‘cratering’ effect to occur in individual stains when dry (Striman *et al* 2011). This can mimic the crater effect which can result from bubble rings popping in expirated blood, potentially causing confusion for an investigator. Additionally, *Lucilia* and *Hydrotaea* both produced artefacts of a size that are comparable to expirated and impact-generated bloodstains; *Lucilia* produced the most *small* (1-3mm) artefacts, which when the enormous numbers of *fine* and *mist* stains (166,780) were eliminated from analysis, the morphologies of which are not distinguishable by eye, accounted for 98.5% of their total artefacts (Large = 1,

Medium = 42, Small = 2931). In comparison, *small* artefact percentages of *Calliphora* were 89.2% (Large = 21, Medium = 223, Small =2,027) and *Hydrotaea* 96.7% (Large = 1, Medium = 3, Small = 117). Many of these *small* artefacts were of 'doughnut-like' appearance with hollow centres. This effect may be the result of feeding activity by the individual fly, or simply a result of the natural drying process (centre desiccation) of the blood stain (Byrd and Castner 2010; Laan *et al* 2016). In general, all 3 fly genera produced very high percentages of *fine* and *mist* sized artefacts, with *Hydrotaea* (99.9%) producing the highest of these. *Calliphora,* a much larger fly, produced marginally less at 99% and *Lucilia* with the lowest percentage in these categories produced 98.2% of their total artefacts. This high percentage of *fine* and *mist* sized artefacts produced by *Hydrotaea* was likely a result of the small body size, approximately 4mm in length (Figure 58) and therefore is likely to be correlated to the size of the orifice/proboscis and its inability to produce larger artefacts.

Figure 58. *Hydrotaea capensis*

As a consequence of their size, the precise morphology of the *Hydrotaea* artefacts were indistinguishable by eye, and the characteristics of these could only be observed when images of the artefacts were enlarged digitally after scanning.

Additionally, the colour of these artefacts was indistinguishable from the colour of the fine/mist sized recreated spatter. For these reasons, an investigator at a scene may mistake *Calliphora*, *Lucilia* and *Hydrotaea* artefacts for the fine (0.1mm-1mm) or mist-sized (<0.1mm) bloodstains which can be deemed diagnostic of impact-generated blood patterns e.g. gunshot, or are consistent with blood that has been exhaled; which also had very high percentages (recreated expirated = 99.7% and recreated impact = 99.4%) of fine/mist sized stains within their patterns. As a consequence the investigator should be aware of the similarities in artefact/bloodstain morphology and take these into consideration when forming their conclusions.

Artefact colour was not formally recorded in the current study, but general visual observations were made following the experiments, and were largely the same as the subjective observations recorded by Dwen (2015). This study showed that faecal artefacts, which were typically teardrop (Figure 59) and elliptical in shape, were generally darker than suspected regurgitated artefacts; although some regurgitated stains exhibited a dark colour and/or were diluted in appearance. Without a reference set for colour, it is difficult to use this diagnostically. This is particularly significant when dealing with circular artefacts, irrespective of how they have been generated. The current study, and those by Striman *et al* (2011), Durdle *et al* (2013) and Fujikawa *et al* (2011) have demonstrated that circular fly artefacts produced by defaecation and regurgitation are virtually indistinguishable from circular blood spatter stains in morphology or colour. Examples of these circular artefacts from the current study are shown in Figure 60. Moreover they fall within the range of stains present within the recreated impact, and even more so, expirated patterns. These patterns also appear to contain stains which exhibit a colour which is visually similar to many of the fly artefacts recorded here. Therefore, whilst a quantitative scheme by which to differentiate stain colour could be probative, the qualitative assessment of stain colour should not be dismissed.

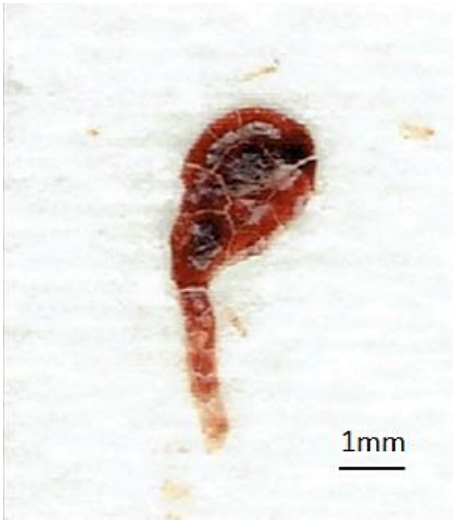


Figure 59. Dark coloured teardrop shaped faecal artefact from *Calliphora vomitoria*

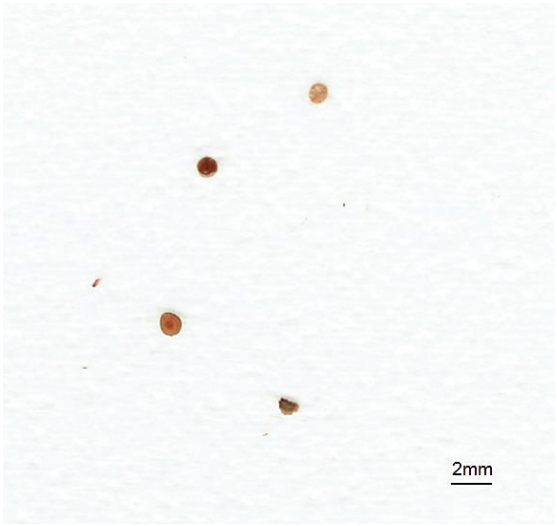


Figure 60: Spotting artefacts from Lucilia sericata showing variation in colour

2. Angle of fly artefacts and recreated bloodstains within the pattern and with respect to others.

A chief aspect of blood pattern interpretation is determining the angle of impact, point of convergence and the area of origin, and is achieved by recording the directionality of individual stains, calculating the angle of impact and tracing those stains back to a blood source or an approximate point of impact (Wonder 2007; Gardner 2012; Millington 2016). All recreated bloodstains observed in the current study showed directionality away from the known origin and were generally radiating in respect to each other. These features would support angle of impact calculations and origin interpretation. Although only observed visually, and a large majority of the fly artefacts recorded appeared to show random directionality, many artefacts showed directionality away from the blood source which was comparable with directionality shown in the recreated stains; which could thus, also potentially be traced back to the same origin using the calculations applicable in reconstruction. This has the potential to be problematic for an investigator dealing with a scenario where there are fewer impact-derived bloodstains and a large number of ‘directional’ fly artefacts. If the fly

artefacts are included in a reconstruction this has the potential to incorrectly indicate multiple origin sites that do not actually exist. This may be particularly the case if separate clusters of stains, each with their own 'origin' are present. In these circumstances the context of the overall scene, such as the presence of fly activity should be considered.

3. Density and count of stains throughout fly and recreated patterns

The relative distribution of fly artefacts, in terms of artefact count and density, with respect to the overall bloodstain pattern was analysed. One way ANOVA and Chi-square testing of *Calliphora*, *Lucilia* and *Hydrotaea* artefacts showed statistically significant relationships ($p < 0.01$) with regards to the zone in which flies deposited their artefacts. This indicated that the area in which the flies deposited their artefacts, and the frequency of distribution per genus was highly significant. For example, all three genera favoured areas around the blood source, and *highly* favoured the area's leading up to and around the window, all of which differed in artefact numbers. Interestingly, zone B, the second nearest zone to the blood source, contained the fewest artefacts in all genera except for *Hydrotaea* (14,677 of 137,789), indicating that *Calliphora* (21,174 of 231,962) and *Lucilia* (19, 615 of 169,756) prefer to move further away from the blood source to deposit after feeding, whereas *Hydrotaea* are not as inclined to do this. One possible hypothesis for this is that there is a relationship between the size of the fly, and the distance it travels after feeding; and as *Hydrotaea* is the smallest of the 3 genera, it naturally travels/covers the least distance. On average, in quadrants 1-4 fly artefacts became more numerous in each of the 3 fly genera with distance outward and away from the origin, whereas the density (number of stains) per cm^2 of these artefacts decreased. This decrease in density is due to the increase in surface area as the zones moved outward from A-E. Whilst, this density decrease can suggest visually that artefacts become less numerous with increasing distance from the blood source, in fact, the total artefact numbers are higher. However, when quadrant 4 was analysed alone, fly artefact

density actually *increased* outward from the blood source with a high density of artefacts immediately below the window in zone E, where fly artefacts were noticeably very dense. This could be potentially problematic in a BPA context as the densities of the recreated impact spatter, and recreated expirated spatter patterns peaked in zones A and B respectively, and fell rapidly in the following zones. In a broader sense, this suggests that in areas of light ingress flies can considerably alter the overall density of an impact/impact derived blood spatter pattern.

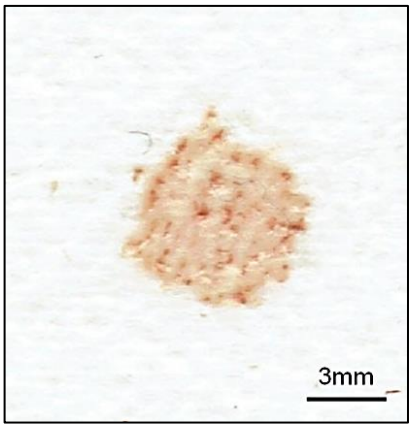
In their paper, Benecke and Barksdale (2003) described a case where fly artefacts were observed not only around a window area but also on a fan chain, two rooms away from where a body was found. This highlights the fact that flies will travel distances much further than normal impact-derived blood spatter, and therefore, further than the 3m x 3m gazebo set-up in the current study. This also suggests that in smaller rooms fly activity can be artificially confined and therefore fly artefact patterns that visually appear much denser than might develop in larger spaces could be observed. In light of these facts, the results from this study suggest that, in addition to the number of flies present, room size plays an important factor in the visual density of fly artefact patterns, and future studies should endeavour to investigate the blood feeding/deposition activity of flies in much larger areas.

4. Distribution of stain sizes throughout the pattern

Laber's proposed classification scheme based on bloodstain size (1985) has provided the BPA community with a more flexible and objective approach to the interpretation of bloodstain patterns than was possible under the previous velocity-based scheme. For example, under the previous non-flexible subjective approach many of the size categories generated from the recreated impact and expirated patterns in the current study would have been misclassified, given the high variation in stain sizes observed in each respectively.

The current results showed that statistically significant differences existed between the sizes of individual impact ($\chi^2_{0.05,\,4} = 8984$, $p < 0.01$) and expirated ($\chi^2_{0.05,\,4} = 403155$, $p < 0.01$) stains and also between the zones (impact = $\chi^2_{0.05,\,4} = 8984$, $p <$

0.01, expirated = $\chi^2_{0.05, 4} = 215128$, $p < 0.01$) in which they occurred. Equally, a significant differences ($p < 0.01$) were found between the 3 fly genera and the size of artefact distributed in each respective zone ($F_{0.05, 4, 170241} = 133247$, $p < 0.01$). This demonstrated that each spatter pattern type has its own unique form of stain size distribution and that individual fly genera will deposit artefacts of a certain size in higher number in specific areas around a blood food source. For example, *Calliphora* deposited 20 of its 21 (95.3%) *large* sized (remnant) artefacts in zones A (13) and E (7) respectively and only a single artefact in zone C.



61. Large >6mm Remnant stain by *Calliphora vomitoria*

These large 'remnant' artefacts (Figure 61) are the result of coprophagy (consumption of faeces) or hemetophagy (blood feeding) as described in the study by Durdle *et al* (2013). This suggests that *Calliphora* may feed on its own larger defecated or regurgitated artefacts in areas primarily near food, and secondly, in areas of warmth/light (window area). Interestingly, this occurrence was reversed for *Lucilia* in its deposition of *medium* sized artefacts; depositing a higher number in the window area (21) than in zone A (5), near the origin; and in its *small* sized artefacts, depositing 373 in zone A, and 1,611 in zone E.

Whilst only a single *large* sized stain from the impact pattern was observed, the expirated stain generated a higher number (13) in the zones where large fly artefacts were scarce; thus, widening the spread of large stains/artefacts in the overall pattern. This could potentially cause problems for an investigator owing to the size distribution

if overlaid patterns of fly artefacts and impact-derived spatter are present. The chance of pattern misinterpretation increases further with the fact that the *small*, *fine* and *mist* sized fly artefacts were much more numerous and spread out across the zones, as were those in the recreated blood patterns. As these results show, when overlaid on top of recreated impact/expirated bloodstain patterns, a fly artefact pattern containing high numbers of similarly sized artefacts could confound a crime scene owing to the large number of blood stains that occur in similar areas.

As a result of these studies, an expanded version of the Objective Criteria has been proposed which includes the requirement for formal observations/records to be made in relation to both individual stain characteristics and external factors. In the context of fly artefacts these are likely to focus on stain colour and environmental factors such as light ingress, but the new scheme is hoped to be sufficiently generic to encourage these factors to be recorded in all areas of bloodstain pattern classification. By way of example, it has been demonstrated here that colour change can assist in the recognition of fly artefact, but it is equally applicable in the identification of blood that has been altered due to other mechanisms, such as blood that has been diluted with saliva in exhaled blood. Although the effect of light can have an influence on fly artefact distribution, other external factors such as air flow or moisture should be considered and these may or may not be evident at the time of the examination. If external factors are formally considered as part of an objective approach, they will support bloodstain pattern analysts in their interpretation. In particular they may allow patterns, which would otherwise demonstrate overlapping features and may be wrongly classified or remain unclassified, to be robustly and consistently differentiated.

The author therefore proposes the addition of three additional criteria to the Objective Criteria scheme to account for this:

1. External factors impacting on overall pattern shape
2. Morphology (shape) of individual bloodstains
3. Colour of individual bloodstains

6. Conclusion

Based on the results from these studies and the enormous number of fly artefacts recorded therein, it is clear that the blood artefact patterns created by a high number of flies could considerably confound a crime scene. Owing to not only their colour and morphological similarities to impact-derived bloodstain patterns, but also the relative densities in certain areas of these patterns, errors with regard to the type of spatter pattern, and/or relative positions of the victim/offender could initially be made by an investigator. These combined factors could result in overall bloodstain pattern misinterpretation.

It has also been demonstrated here that external factors such as the presence of a window are determining factors in the formation of fly artefact patterns, and are not currently accounted for within the guidelines of the published Objective Criteria for the interpretation of blood patterns. It is hoped that the addition of the additional criteria, as proposed here, will add robustness to the objective approach for BPA that is already in place.

6.1 Recommendations for future research

It is evident from the results of this study, and those within the published literature, that the scope for further research in the behaviour of flies feeding on blood at crime scenes is considerable. Where the possibility of extensively observing or cataloguing the feeding behaviour of flies at real crime scenes is not possible, it is imperative that the manufactured scenes of those wishing to conduct similar studies are as close to a real scene as possible. This should include experiments in variety of scene sizes, such as a large warehouse for example, to observe changes in distribution behaviour and pattern density. Walls should also be examined, and preferably be covered with a suitable substrate to allow for easy visualisation of artefacts, such as the white lino flooring used in the current study. However, it must be noted that in real life settings, walls may be painted or papered, and artefacts may not be as easily visible as with a lino substrate.

In addition to this, a scheme by which to record, quantitatively, stain colour should be devised so that its suitability as a diagnostic tool can be assessed. Moreover, the current study should act as a catalyst for further, and perhaps more extensive testing, where the distribution of fly artefact stains should be correlated with those generated by common assault related mechanisms in order to present empirical data on which different pattern types may be distinguished.

7. References

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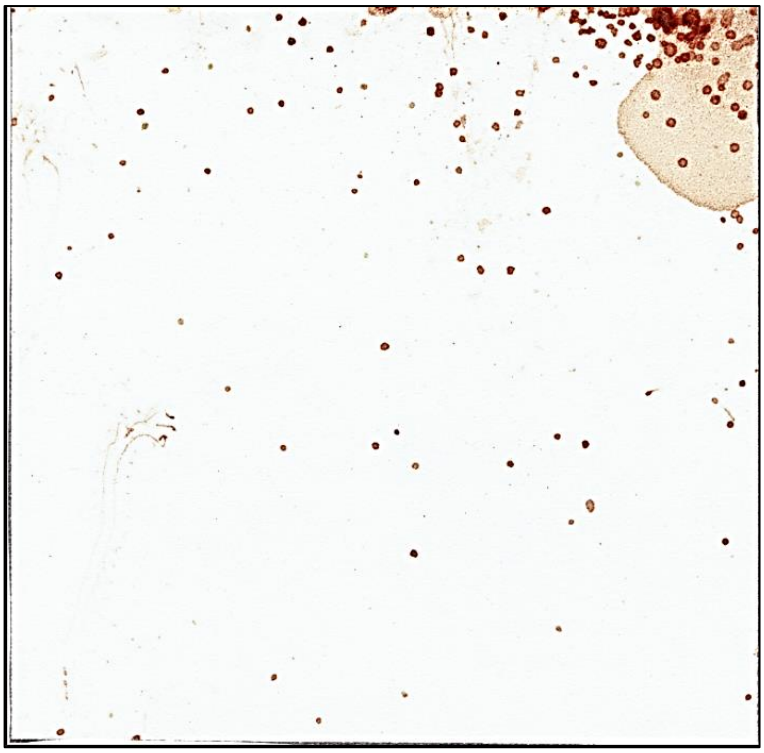
8. Appendices

Appendix I

Calliphora tiles – zone A (Tiles = 25cm x 25cm)



***Calliphora* tiles – zone A (Tiles = 25cm x 25cm)**

Calliphora tiles – zone E (within 25cm of window)

Calliphora tiles – zone E (within 25cm of window)

Appendix II

***Lucilia* tiles – zone A (Tiles = 25cm x 25cm)**

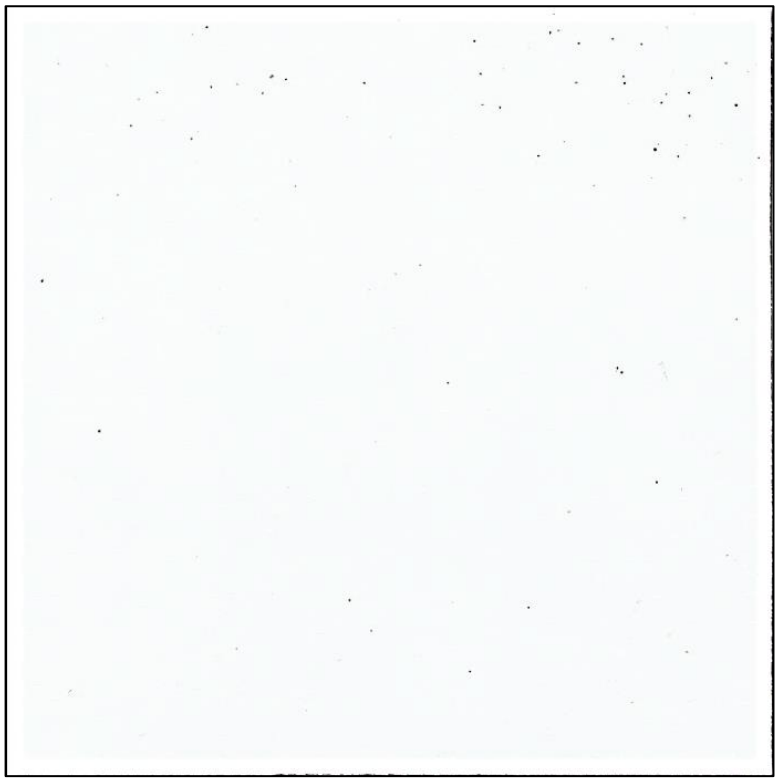
Lucilia tiles – zone A (Tiles = 25cm x 25cm)

Lucilia tiles – zone E (within 25cm of window)

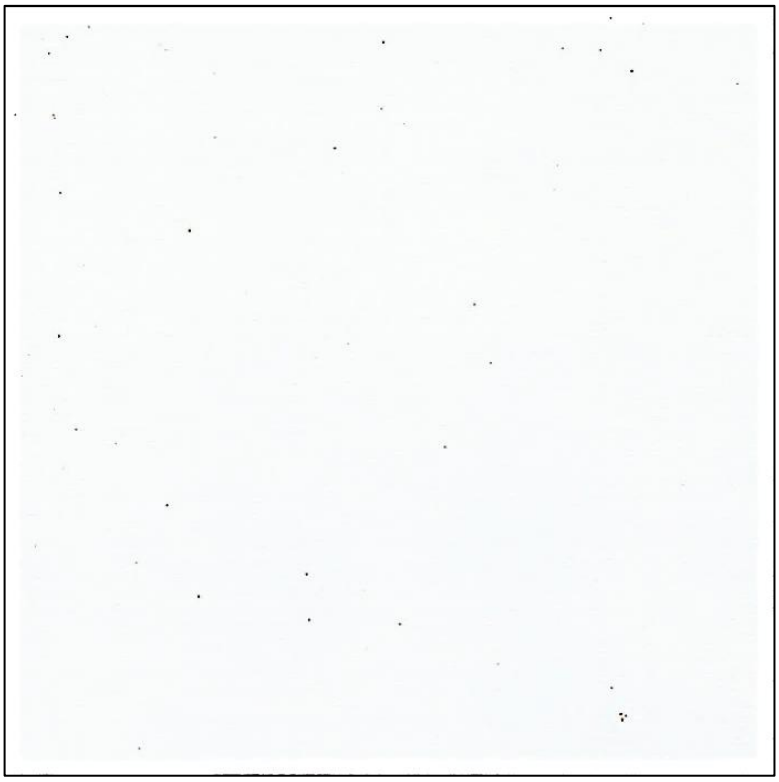
Lucilia tiles – zone E (within 25cm of window)

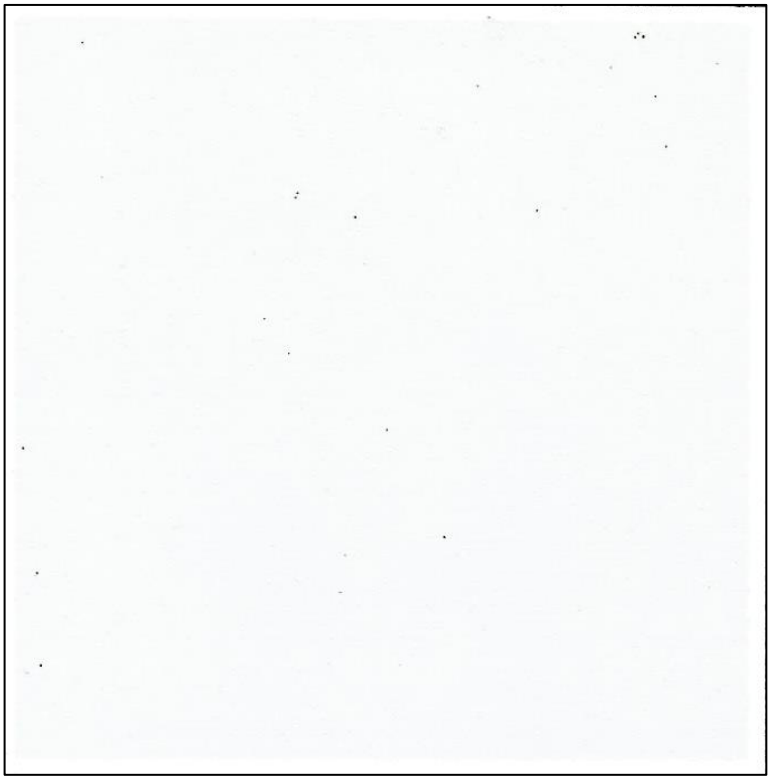
Appendix III

Hydrotaea tiles – zone A (Tiles = 25cm x 25cm)

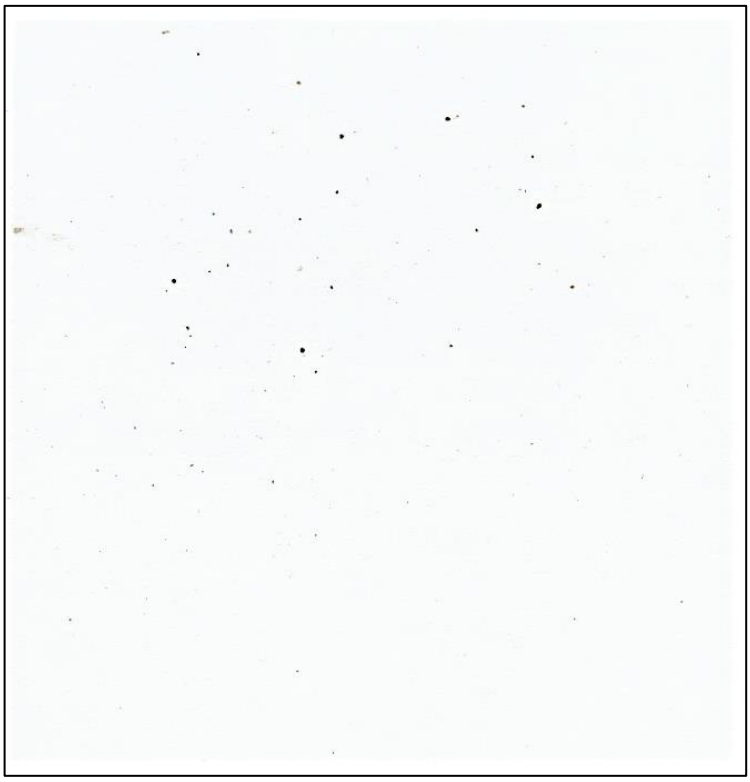


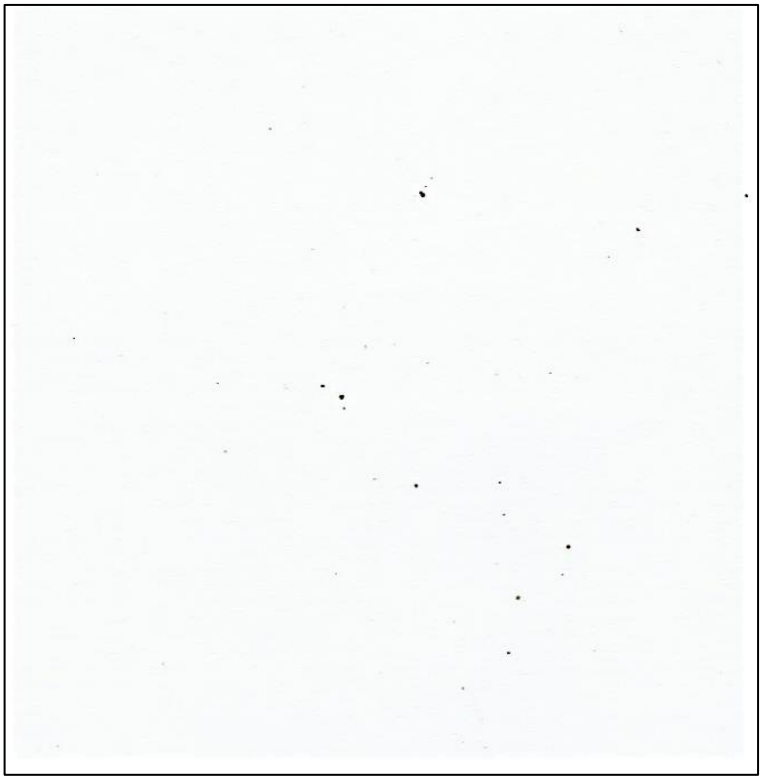
Hydrotaea tiles – zone A (Tiles = 25cm x 25cm)





***Hydrotaea* tiles – zone E (within 25cm of window)**





***Hydrotaea* tiles – zone E (within 25cm of window)**