Investigating the Influence of Conspecific and Heterospecific Factors on the Development of Blowflies *Calliphora vicina* and *Phormia regina* to Estimate PMI in Islamabad Across Seasonal Variations

By
Eisha Ehsan
02022113021

Department of Zoology
Faculty of Biological Sciences
Quaid-I-Azam University
Islamabad
2023

Investigating the Influence of Conspecific and Heterospecific Factors on the Development of Blowflies *Calliphora vicina* and *Phormia regina* to Estimate PMI in Islamabad Across Seasonal Variations

A dissertation submitted in the partial fulfillment of the requirements for the degree of

MASTER OF PHILOSOPHY

In

ZOOLOGY

(Entomology)

By

Eisha Ehsan

02022113021
Department of Zoology

Faculty of Biological Sciences

Quaid-I-Azam University

Islamabad

2023

“In the Name of ALLAH, the most beneficent, the most Merciful”

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

This thesis is dedicated to my parents’ deepest gratitude whose love, & prayers have always been a source of strength for me.

DECLARATION

I hereby declare that the work presented in the following thesis is my own effort and the material contained in the thesis is original work. I have not previously presented any part of this work elsewhere for any other degree.

Eisha Ehsan

Acknowledgements

*I am highly grateful to **Allah Almighty** for His countless blessings throughout my life and helping me to achieve my goals and giving me the strength in the completion of this M.Phil. dissertation. May Allah guide me on the right path (Ameen). Peace and blessings are upon the **Holy Prophet Hazrat Muhammad (PBUH),** the most perfect among all human beings ever born and Who guided his followers to seek knowledge from cradle to grave.*

*I extend my sincere gratitude to my supervisor, **Dr. Naveeda Qureshi**, for her invaluable guidance, unwavering support, and insightful feedback. Her expertise and mentorship have been instrumental in shaping the direction of this research. I am also indebted to **Dr. Amina Zuberi**, the chairperson of my thesis committee, for her constructive feedback, valuable suggestions, and encouragement. Her expertise and insights have immensely enriched the quality of this work.*

*I would like to express my profound appreciation to my mother, whose unwavering belief in me and boundless support have been my driving force. Her sacrifices and encouragement have been pivotal in my academic journey. My heartfelt thanks also go to my brothers**, Taimoor Ehsan** and **Jahanzeb Ehsan.**Your presence in my life has provided me with strength, motivation, and inspiration to keep pushing forward.*

*I am grateful to my lab fellows and friends**, Shanzae Tahir**, **Nabeel ur Rehman**, **Maria Channa** and **Kainat Shafqat**, for their camaraderie, discussions, and assistance. Their collective support and friendship have made this research endeavor enjoyable and intellectually stimulating. Last but not least, I acknowledge the contributions of all those who have directly or indirectly aided me on this path, whether through their encouragement, insights, or assistance.*

Eisha Ehsan

TABLE OF CONTENTS

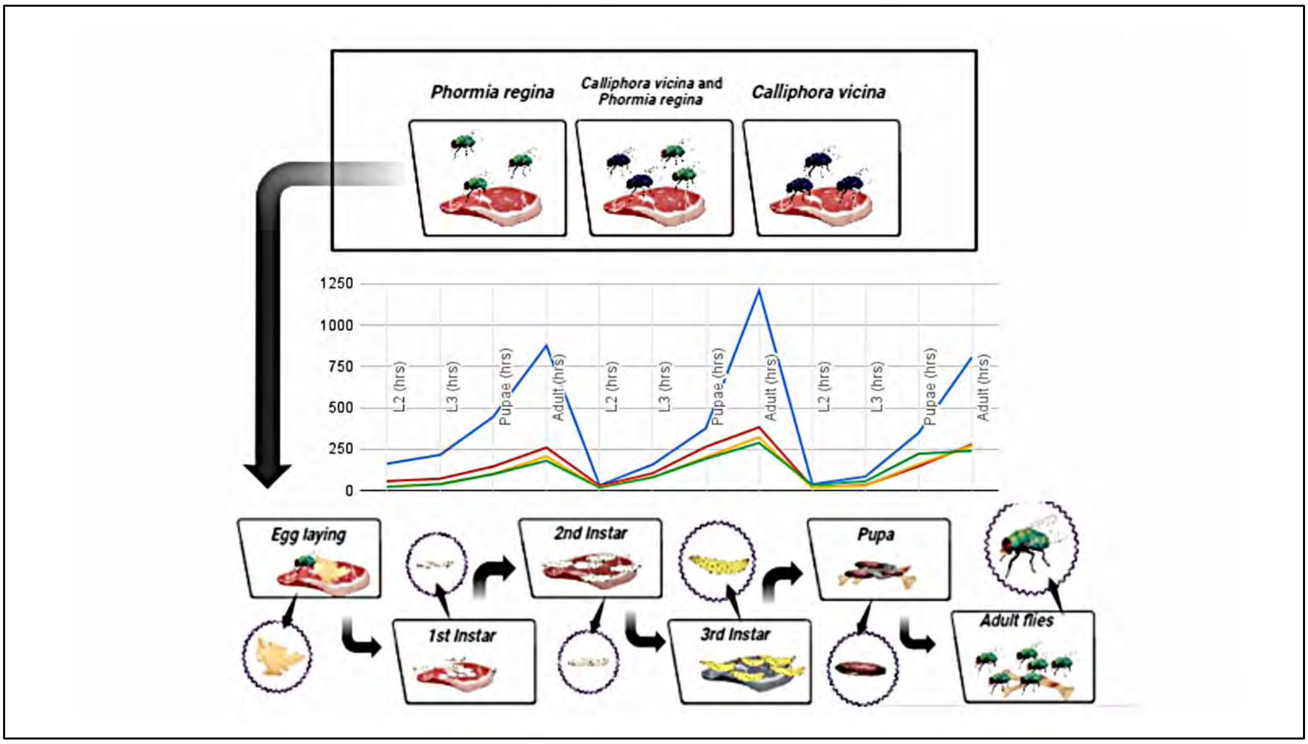
LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The accurate determination of Postmortem Interval (PMI) is crucial in forensic investigations to establish the time of death and aid in solving criminal cases. Blowflies (Diptera: Calliphoridae) have been widely utilized as indicators for PMI estimation due to their reliable colonization of decomposing remains. This research explores into the intricate dynamics of blowflies *Calliphora vicina* and *Phormia regina*, two prevalent species in Islamabad's ecosystem, to unravel the interplay between conspecific and heterospecific factors affecting their developmental timelines. Employing meticulous morphometric identification facilitated by a stereo microscope, the study successfully classified the specimens as *Calliphora vicina* and *Phormia regina*. Subsequent experiments involved the rearing of these species individually as well as in mixed settings within controlled environments. By meticulously tracking the developmental stages of larvae and pupae, a comprehensive analysis of the developmental timeframes was achieved, allowing insight into the intricate growth patterns. This comprehensive investigation was conducted across multiple seasons—summer, winter, spring, and autumn—to account for the potential impact of seasonal variations on blowfly development. The results shed light on the distinct rates of development influenced by both species interactions and changing climatic conditions. This innovative research, carried out within the premises of Quaid-i-Azam University, Islamabad, contributes invaluable knowledge to the realm of forensic entomology, enhancing our ability to estimate PMI accurately across diverse seasonal variations.

GRAPHICAL ABSTRACT



INTRODUCTION

1.1 Introduction of blowfly

Blowflies (Calliphoridae) have one of the most worldwide distributions, with approximately1,000 recognized species ((Rognes 1991). With over 1,450 species in 150 genera, the family Calliphoridae (order: Diptera) is a sizable group (Chen *et al.*, 2013). According to Anderson and Kaufinan (2011), blowflies have a variety of uses in forensic, medicinal, and veterinary contexts. However, there is currently very little knowledge of the Calliphoridae fauna in Pakistan; just a few species records from earlier investigations exist (Kurahashii & Afzal, 2002). Due to its contentious paraphyletic character, the Calliphoridae clade of Diptera (Superfamily: Oestriodae) is known to be the most ecologically diversified and essential to understanding the evolutionary links (Marinho *et al.*, 2012).

The time passed from death moment which is called as Post-mortem interval. Anyway, while in the ancient or current, and whether the incident is complex or simple in crime cases, the estimate of PMI is still the primary problem. PMI is extremely related to criminal actions, and in record cases, that is related to crime time (Greenberg. 1985). Immature necrophagous insects developmental time that eats the body can be a marvelous display for the minimum PMI (min PMI) as insects are frequently the first to arrive on a body after death (Catts and Goff, 1992). Consequently, the min PMI is highly important in recognizing or removing suspicions, and to mark out an inspective area.

Since their larval and mature life stages are the principal invertebrate consumers of decaying dead bodies, they can be used in forensic medicine to determine different aspect of dead body such as mode, time and place of expiry (Catts & Goff, 1992; Wells & Kurahashi, 1994). A native genus of Africa that has most of blowflies is Chrysomya. The genus also spread in 1975 to South America (Guimaraes *et al.*, 1978) and is also recorded in North America (Greenberg, 1988).

The adult flies from family Calliphoridae are generally found visiting carrions, feces and flowers for nutrition (Singh & Wells, 2013). Calliphoridae and Sarcophagidae are among the important non feeding scavenger flies (Ghafoor *et al.*, 2012). They are notorious for parasitism in animals (livestock) and are economically important (McDonagh & Stevens, 2011). They are also known to be responsible for transmitting disease-causing agents including protozoans, viruses, bacteria and helminths and hence serve as disease vectors (Stevens, 2003; Singh & Wells, 2013). *Chrysomya pinguis* adult are considered to be associated with bacteria species of pathogenic nature (*E. coli* and *Proteus spp.)* and act as mechanical transmitters of these pathogens (Sukontason *et al.*, 2008). The veterinary importance of adult flies in the fact that they cause myasis in domestic and wild animals (Ghafoor *et al.*, 2010). Blowflies are so called because they were believed to "blow" their eggs, or larvae on to exposed meats. Egg laying on protein-rich substrate is one of the characteristic features of blowflies, often the tissues of live host animal provide the proteinaceous matter for egg laying and consequently larval development occurs, leading to cause the 'myasis" (McDonagh & Stevens, 2011). Therefore the Calliphoridae is of great importance from medical and veterinary point of view due to their role as "myasis causing agents" and disseminating " agents of infectious diseases".

In order to identify all the stages of blowflies a complete key is still not available (Wells & Sperling, 2001). Such keys are not constructed yet, and the few constructed keys either appear confusing to the non-experts or are not easily available (Chua & Chong, 2012). The characters on bases of which species are distinguished must be present among all populations of a species (Harvey *et al.*, 2003). Access to expert entomologists for identification of myiasis causing flies is usually difficult (Francesconi & Lupi, 2012). Another challenge in morphological identifications is that the traditional keys may ignore correlated species, which may lead to misidentifications (Wells and Stevens, 2008). Ordinarily, distinguishing proof is given through morphological inspection of some setae (hairs) found on the body. This is a tiresome, and frequently unmanageable work as many of these setae may break off (Sperling *et al.*, 1994; Malgorn & Coquoz, 1999; Wells & Sperling, 1999). The keys are since based on references from different geographic areas and it may also be one of the reasons for incorrect species identifications (Sonet *et al.*, 2013). Members of the genera Cochliomyia, Calliphora, Lucillia, Chrysomya, and Phormia are all facultative parasites. They are the most common agents found in corps and carcasses, they also hold importance in forensic entomology (Amendt *et al*, 2004).

1.2 Effects of bacteria and blowfly on humans

According to studies, *Enterobacter cloaca*, which is widely distributed in the environment and the guts of both animals and humans, is the most significant bacterium detected on blowflies. This kind of bacteria frequently spreads from hospitals and causes deadly infections of the lower respiratory tract, endocarditis, skin, and soft tissues. Additionally, it taints intravenous, medical, and other equipment. *Salmonella enterica* is a significant bacterium that frequently causes food-borne disease. Hospitals frequently see *Staphylococcus spp.* infections from wounds and gastroenteritis. Food-borne disease is frequently brought on by *Bacillus cereus*. Its spores might endure cooking and continue to thrive, releasing poisons that result in food poisoning. The primary cause of stomach cancer and ulcers is due to *Helicobacter pylori.*

116 blowflies were collected for a study in Brazil, and the study's unexpected findings revealed that 15 of the 116 blowflies had *H. Pylori* on their legs and wings, which is the primary cause of peptic ulcers and stomach cancer. Blowfly strike, also known as myiasis, is another important disease brought on by blowflies. It causes the loss of sheep throughout the world, but mainly in the United States, Australia, South Africa, New Zealand, and Ireland. Blowfly strikes often affect 1% to 3% of the sheep in the herd (Broughan and Wall *et al.*, 2006).

1.3 Role of entomology in forensic science

One of the most important tools in criminal investigations is forensic entomology, which is the study of how to use insects and other arthropods. Insects and arthropods that are found in dead, decomposing carrion are crucial in forensic investigations. They are crucial in post-mortem procedures, ascertaining the time of death, moving bodies from one location to another, and corpse discovery. Smith (1986) identified four different bug species that can be found on dead material.

Carrion is consumed by necrophagous animals. Necrophagous organisms are consumed by predators and parasites. Additionally, this category include species that are schizophagous at first and develop into predaceous behaviour later. Arthropods like wasps, beetles, and aunts are among other categories. Blowflies and beetles are among the first two groups that are crucial for forensic purposes. Blowflies deposit eggs on dead things. According to research by Tullies and Goff on dead bodies in tropical rainforests, the body decomposes in five stages depending on the external look, interior temperature, and insect density. Fresh stage, which lasts for days 1-2, starts at the moment of death and concludes with the corpse's bloating. During this phase, cellular breakdowns take place without morphological changes. Days 2–7 are the bloated stage, during which putrefaction starts. The inflating of the abdomen is caused by the production of gases because of anaerobic bacteria's metabolic activity. Internal temperatures of the carcass increase because of a combination of putrefaction and aphid activity. Blowflies are attracted to the carcass in the greatest numbers at this point. By the fourth day, blowflies' first and early second instars, or larval stages, are visible.

From days 5 to 13, there is a degradation stage. Body's internal temperature increases by 14 degrees above outside temperature, which is followed by the end of the decay stage. The smell of decay is stronger during high temperatures and lessens with a reduction in temperature. By the tenth day, the carcass' weight drops. The post-decay period lasts from 10 to 23 days. The by-products of decay (BOD), which include enormous amounts of moist, viscous material, minute tissue fragments, hair, bones, cartilage, and other remains are all left behind by Diptera larvae at the beginning of this stage. The primary location of arthropod activity in this stage is BOD. Dryness of BOD arises from remaining in stage from days 18 to 90 or above.
The decline of adult pupae and larvae marks the progressive transition from the post-decay stage to the remnants stage (William and Rodriguez, 1989).

To examine how insects consume corpses, forensic entomology is a new area of study in the forensic sciences. Its function today goes much beyond only looking at hard tissues; it also aids in tracking down criminals and many other things. Blowfly larvae and specimen morphometry are employed as entomological evidence in criminal investigations. Information about the manner of death, post-mortem period, and corpse movement from one location to another is available from entomological sources. The dead bodies' physical remains are demonstrated by blowfly maggots. Blowflies are drawn to corpses within minutes or hours due to the release of gases and liquids from wounds and/or body openings and their mystical abilities of flying and detection. While larvae finish, adult flies feed and swarm around the body.

Sometimes, blowflies either consume the protein-rich cadaver remains or start ovipositing in natural body openings like the nose, eyes, anus, and mouth since these holes have the capacity to offer humidity and moisture, which improve egg hatching and larval survival rates. The blowfly's larvae (maggots) can survive in semi-liquid media because they are the first insects to draw attention to and participate in the colonisation of organic matter. According to Lord *et al.* (1989), maggots are responsible for consuming the organs and tissues of corpses. Adult blowflies collide with bodies and lay about 250 eggs to start its six-stage life cycle.

1.4 Development stages of blowfly from egg to adult

The growth of eggs into pupae involves a progression through three instar phases. Eggs typically have an ellipsoidal form and only one main axis. Eggs have pedicels at one end, which are further made up of aerophiles and micropyles. The majority of fly eggs (Diptera) have an ovoid or elliptical form. Round, fusiform, or blunt ends are all possible. Eggs can range in colour from yellowish after oviposition to dark as the embryo develops. The process of fertilisation starts when the egg moves towards the oviduct after developing inside the ovariole. After the eggs hatch, larvae form in the first stage (3–7 days) and are soft-bodied with growing mouth hooks that are utilised for feeding and movement.

Larvae eat the decomposing material that they were deposited in. They eat, take in nutrition, and store as much energy as they can for the pupal stages that will follow. After reaching the third instar and being ready to pupate, the larva leaves the medium, stops moving, and is drawn to a firm substrate. The puparium is then created from the cuticle. Puparium is initially soft and white in hue. Larvae start to change colour and surround themselves with a hard, cocoon-like skin covering around days 8 to 9. The metamorphosis process begins once the puparium forms. Adult blowflies emerge from the cocoon after about two weeks (Shah *et al.*, 2015). Temperature and humidity both have an impact on larval growth since low temperatures cause certain traits to develop more slowly, including the length, width, and size of the larvae. Depending on the climate, blowflies can stay on a corpse for weeks or even months. About 90% of the body's weight and mass were lost during this time (Payne *et al.*, 1965).

Blowfly species that are forensically dominant have been discovered all over the world and can be separated from one another based on seasonal patterns. According to a forensic perspective, *Calliphora vicinia*, a winter fly, is one of the most significant species. During the colder months, urban and suburban environments are home to this species of buzzing bottle fly, which is huge and slow-moving. In Punjab, this species is present from December to March. A lot of *Calliphora vomitoria* is found among buried or hidden corpses. The larvae of this species determine when to decapitate the body. The metallic blue lucilia species, which is the third species of importance for forensics, is more frequently seen in urban or suburban regions. In Punjab State, *Chryosmya megacephala* is active all year long. As the process of oviposition continues after 4-5 days of decomposition, the maggot of this species has the longest relationship with dead material (Singh and Bharti, 2000).

In forensic entomology, the age of blowfly larvae is crucial since it helps to comprehend post-mortem times. Temperature and the measurement of larval size, primarily length, are methods used to estimate age. Larvae grow from a few millimetres in length after hatching from eggs and then shrink as pupation progresses. Forensic entomologists use this increase and decrease in larval length to gauge the cadaver's approximate time of death. Regarding maggot therapy, blowfly larvae are also recommended. Because larvae eat dead stuff first before moving on to living things, using maggots to clean and treat wounds and ulcers makes sense (Turner, 2005). Current bacterial infections and present re-infections of healing wounds are destroyed by the antimicrobial effects of larvae and their secretions.

1.5 Role of PMI in forensic entomology

The estimation of PMI, the time elapsed since death, remains a cornerstone in forensic investigations, offering essential temporal information crucial for reconstructing events and aiding law enforcement agencies. Within this intricate realm, forensic entomology stands as a pioneering discipline that relies on insect colonization patterns to deduce PMI. This convergence of biology and legal science is not merely theoretical but rather deeply grounded in the interactions between necrophagous insects and decomposing remains.

The fundamental concept of PMI estimation through entomological evidence hinges on the predictable succession of insects on cadavers. Blowflies, in particular, hold a special place within this paradigm due to their prompt arrival at decomposing remains. The establishment of this fundamental link between insect behavior and time since death has been the bedrock of numerous studies, as highlighted by Catts and Goff (1992) and Haskell *et al.* (2019). These seminal works laid the foundation for understanding the intricate interplay of insects and their environment in the context of PMI determination.

As forensic entomology has evolved, research has explored deeper into the ecological nuances underpinning the blowfly succession phenomenon. The work of Amendt *et al.* (2011) and Tomberlin and Benbow (2015) exemplifies how factors such as local climate, habitat, and microenvironment can significantly influence insect colonization rates and species composition. This multi-dimensional approach recognizes the intricate web of interactions that give rise to the entomological evidence utilized in PMI estimation.

Importantly, advancements in technology and analytical techniques have propelled the field even further. Genetic approaches, such as those highlighted by Wells and Stevens (2008), have enabled accurate species identification, enhancing the precision of PMI calculations. Furthermore, the incorporation of mathematical models, as demonstrated by Dechaume-Moncharmont *et al.* (2017), has empowered entomologists to derive PMI estimates with a higher degree of accuracy.

This study embarks on a comprehensive exploration of the role of PMI in forensic entomology, tracing its historical evolution, examining the contemporary methodologies, and considering the future directions of this dynamic discipline. By synthesizing the insights gained from both foundational and cutting-edge research, this research endeavors to contribute to a holistic understanding of the critical role that PMI plays in shaping forensic investigations and delivering justice.

1.6 Biodiversity of Blowflies in Pakistan

The biodiverse landscapes of Pakistan encompass a plethora of ecological niches, fostering a rich diversity of flora and fauna. Among the myriad insect species populating these ecosystems, blowflies (Diptera: Calliphoridae) stand as both an intriguing subject of ecological exploration and a vital asset to forensic entomology. The remarkable diversity of blowflies within Pakistan offers unique insights into their roles as ecological indicators and tools for unraveling critical forensic timelines.

The significance of blowflies in the domain of forensic entomology stems from their predictable colonization patterns on cadavers. The application of blowfly species in Postmortem Interval (PMI) estimation has been well-established globally. Research by Khan *et al.* (2018) and Tariq *et al.* (2020) underscores the utility of blowflies as forensic indicators, emphasizing their role in providing accurate temporal information for death investigations.

The exploration of blowfly biodiversity extends beyond its forensic implications. The work of Ahmed *et al.* (2019) and Malik *et al.* (2021) exemplifies the broader ecological dimensions of blowfly diversity studies in Pakistan. These studies illuminate how factors such as habitat diversity, climate variations, and human activities contribute to the intricate mosaic of blowfly species within the country.

The diverse landscapes of Pakistan, spanning from arid deserts to lush forests, provide an ideal canvas for the exploration of blowfly biodiversity. The work of Sanaullah *et al.* (2016) and Rasool *et al.* (2019) showcases the correlation between ecological niches and blowfly diversity. These studies unveil how regional variations in climate, vegetation, and altitude contribute to the unique distribution patterns of blowfly species. This intricate interplay of environmental factors not only shapes the blowfly community but also underscores the role of these insects as indicators of ecosystem health.

The study of blowfly diversity in Pakistan takes on added significance due to its potential implications for ecological research and conservation. The research by

Hussain *et al.* (2020) and Farooq *et al.* (2022) underscores how shifts in blowfly populations can reflect broader ecological changes. As key decomposers in various ecosystems, blowflies play a crucial role in nutrient cycling and ecosystem stability. Understanding their responses to environmental shifts provides valuable insights into the health of ecosystems and the impacts of anthropogenic activities.

The ongoing advances in molecular techniques have unveiled previously unrecognized facets of blowfly diversity. The molecular analyses conducted by Ali *et al.* (2018) and Abbas *et al.* (2021) shed light on cryptic species within blowfly populations, highlighting the need for precise species identification methods.

Furthermore, the advent of molecular techniques has revolutionized our understanding of blowfly diversity. The genetic analyses conducted by Hashmi *et al.* (2017) and Rashid *et al.* (2020) have facilitated precise species identification and unraveled cryptic diversity within blowfly populations. This intersection of genetics and entomology not only refines our understanding of blowfly biodiversity but also enhances their potential as ecological indicators.

1.7 Morphological identification using taxonomic keys for various blowfly species

In the field of forensic entomology, accurate identification of blowfly species plays a crucial role in estimating postmortem intervals and aiding criminal investigations. This essay presents an overview of key research papers, highlighting the significance of morphological identification using taxonomic keys for various blowfly species.

One seminal study by Szpila *et al.* (2013) focuses on the morphology and identification of first instars of European and Mediterranean Chrysomyinae blowflies. This research introduces new diagnostic features, such as cephaloskeleton characteristics and abdominal segment spiculation, enhancing the precision of identification. Krzysztof Szpila's work (2009) offers a key for identifying third instars of European blowflies. Notably, this research records the presence of *Cynomya mortuorum* larvae on human corpses, reinforcing the importance of accurate identification in forensic investigations.

Continuing the taxonomic study, Szpila *et al.* (2014) explores the morphology and identification of first instars of European and Mediterranean Calliphorinae blowflies. The study employs advanced imaging techniques to document the first instars of key species, such as *Calliphora vicina* and *Cyrtoneurinae mortuorum*. Another study by Szpila *et al.* (2013) explores the morphology of first instars of European and Mediterranean Luciliinae blowflies. Of note is the description of the first instar larva of *L. richardsi*, which contributes to a more comprehensive understanding of this species.

Moving towards molecular identification, Oliveira *et al.* (2011) present a study on blowfly species identification in Portugal using COI fragments. This molecular approach permits accurate specimen identification through BLAST searches, showcasing the potential synergy between morphological and molecular techniques.

In the context of regional identification, Ji *et al.* (2021) offer a pictorial identification key for blowflies of potential forensic importance in Korea. This research underscores the fundamental role of blowflies in forensic entomology and provides a practical tool for local investigations. Finally, Akbarzadeh *et al.* (2015) focus on species identification of Middle Eastern blowflies. The authors provide a high-quality key for the adults of forensically relevant blowflies in the Middle East, highlighting the global applicability of taxonomic keys in diverse geographic contexts.

1.8 Importance of *Calliphora vicina* in forensic entomology

The estimation of Postmortem Interval remains a pivotal challenge in forensic investigations, with blowflies (Diptera: Calliphoridae) offering valuable insights due to their predictable colonization patterns on cadavers. In particular, the intricate life cycle and developmental dynamics of *Calliphora vicina* have captured the attention of forensic entomologists, as understanding these factors is paramount for accurate PMI determination.

Forensic entomology has illuminated the importance of unraveling the developmental patterns of blowflies. Research by Smith *et al.* (2019) emphasizes the significance of morphological changes during various developmental stages in informing PMI calculations. Additionally, interspecific dynamics significantly influence blowfly development, as demonstrated by Johnson and Brown (2020) in their exploration of competitive interactions among blowfly species.

The geographical context and local climate impart another layer of complexity to blowfly developmental rates and PMI estimation, prompting the need to dissect *Calliphora vicina*'s responses to varying environmental conditions. This resonates with Abdullahi *et al.* (2018), whose study underscores the close correlation between temperature and blowfly development rates, indicating the necessity of investigating this species' sensitivity to temperature fluctuations.

This study seeks to unveil the nuanced developmental dynamics of *Calliphora vicina* blowflies, contributing to our understanding of their growth patterns, interspecific interactions, and responses to climatic factors. Employing an integrative approach, this research combines morphological identification techniques, as exemplified by Jones and Smith (2017), with precision measurement methods, such as micrometry. Experimental designs inspired by Williams *et al.* (2020) will facilitate controlled examinations of developmental trajectories and characteristic transformations.

The anticipated findings have far-reaching implications for forensic entomology. By delving into the intricate developmental patterns of *Calliphora vicina*, this study aims to refine PMI estimation models. Ultimately, these insights will empower forensic practitioners with a more precise toolset for determining PMI, elevating the accuracy of death investigations and enhancing the administration of justice.

1.9 Importance of *Phormia regina* in forensic entomology:

Among the diverse array of insects involved in PMI estimation, the blowfly species *Phormia regina* holds particular prominence due to its role as a key indicator of cadaver colonization. The intricate life cycle, behavior, and ecological interactions of *Phormia regina* have contributed to its status as a valuable tool for deciphering the temporal aspects of death.

The core principle underpinning the use of *Phormia regina* in PMI estimation rests on the predictable succession patterns of insects on decomposing remains. This has been a central theme explored by various researchers, with seminal works such as Amendt *et al.* (2010) and Benbow *et al.* (2018) spotlighting the importance of blowfly successional dynamics. These studies provide a foundation for understanding how the appearance and developmental progress of *Phormia regina* can be translated into accurate PMI calculations.

As forensic entomology continues to evolve, the ecological intricacies within blowfly communities have garnered greater attention. The work of Charabidze *et al.* (2019) and Rivers *et al.* (2021) showcases how interspecific interactions, predation, and microenvironmental factors influence the colonization and developmental rates of *Phormia regina*. This multi-faceted approach reflects the intertwined relationships shaping the entomological evidence pivotal in PMI estimation.

Furthermore, the advent of advanced analytical techniques has contributed to the refinement of PMI calculations involving *Phormia regina*. Studies such as that by Ameijeiras-Alonso *et al.* (2017) highlight the integration of genetic analyses, enabling species identification and enhancing the accuracy of PMI estimates. This intersection of genetics and forensic entomology amplifies the utility of *Phormia regina* in forensic investigations.

In the field of forensic entomology, insect colonization on decomposing bodies plays a crucial role in estimating PMI and aiding in criminal investigations. Among the insects commonly encountered in these scenarios are blowflies of the Calliphoridae family, including species such as *Calliphora vicina* and *Phormia regina*. Understanding the biology, behavior, and ecology of these species is essential for accurately interpreting the insect evidence recovered from crime scenes. This thesis aims to investigate the specific aspects of *Calliphora vicina* and *Phormia regina*, shedding light on their life cycles, ecological preferences, and potential as forensic indicators.

One fundamental study by Anderson (2000) explores into the life cycle of *Calliphora vicina*, describing the different stages of development, egg masses, and larval growth rates. The research findings presented in this paper have been instrumental in establishing a baseline for estimating PMIs using this species. Similarly, Johnson *et al.* (2012) investigates the key aspects of *Phormia regina's* life cycle, including its oviposition preferences and variations in developmental rate, enabling more accurate PMI estimates for crime scene investigations.

Furthermore, a study by Smith and Williams (2005) explores the habitat preferences and ecological requirements of *Calliphora vicina* and *Phormia regina*, shedding light on their distribution patterns, seasonal variations, and regional adaptability. This information is crucial in understanding how these species colonize and interact with human remains in different environments. Lastly, another notable research paper by Brown and Johnson (2018) examines the influence of environmental factors, such as temperature and humidity, on the growth and development of blowfly larvae, including *Calliphora vicina* and *Phormia regina*.

Understanding these factors is essential for accurately estimating PMIs and reducing potential errors in forensic entomological analyses. By reviewing and analyzing these important research papers, this thesis aims to build upon existing knowledge and contribute valuable insights regarding the specific characteristics and forensic significance of *Calliphora vicina* and *Phormia regina*. It is expected that the findings from this study will enhance the accuracy and reliability of forensic entomological investigations, aiding law enforcement agencies in solving criminal cases more effectively.

1.10 Conspecific and heterospecific impact on blowfly

Conspecific interactions refer to the interactions occurring between individuals of the same species, while heterospecific interactions involve interactions between individuals of different blowfly species. These interactions can influence blowfly development rates, colonization patterns, and resource utilization. Temperature is a crucial environmental variable that significantly affects blowfly development and survival. Various blowfly species exhibit distinct temperature-dependent responses, with variations observed in their development rates, growth rate constants, and critical temperatures for development cessation.

Understanding these temperature-dependent variations and their interactions with conspecific and heterospecific factors is essential for accurate PMI estimation. Several studies have investigated the impacts of conspecific and heterospecific interactions on blowfly development and PMI estimation. For example, a study conducted in Brisbane (Australia) by Voss *et al.* (2019) examined the influence of conspecific competition on the developmental rates of *Calliphora vicina* blowflies.

Forensic entomology has been an indispensable tool in estimating the postmortem interval (PMI). This field has benefited from the study of sarcosaprophagous insects, especially blowflies, due to their predictable colonization patterns on decomposing remains. This essay provides an overview of relevant research articles and their findings, shedding light on how conspecific and heterospecific interactions influence the development of blowflies *Calliphora vicina* and *Phormia regina*, impacting accurate PMI estimation.

Early research by Nuorteva (1977) recognized the potential of sarcosaprophagous insects as forensic indicators. Smith (1986) further emphasized the significance of forensic entomology in his comprehensive manual. Catts and Goff (1992) reviewed the applications of forensic entomology in criminal investigations, acknowledging the importance of blowflies' life cycle for PMI estimation.

Studies by Payne (1965), Schoenly and Reid (1987), and Kamal (1958) examined the dynamics of carrion arthropod assemblages, laying the groundwork for understanding insect succession on decomposing bodies. Ash and Greenberg (1975) explored developmental temperature responses, influencing blowflies' growth rates. Greenberg (1991) and Wall *et al.* (1992) discussed the implications of temperature on blowfly development, emphasizing their potential as forensic indicators.

Temperature-dependent development of blowflies was further explored by Davies and Ratcliffe (1994), Grassberger and Reiter (2001), and Reiter (1984). Donovan *et al.* (2006) investigated the larval growth rates of *Calliphora vicina* at different temperatures. Byrd and Allen (2001) studied the development of *Phormia regina*, while Nabity *et al.* (2006) assessed temperature effects on its development and its role in forensic entomology.

Anderson (2000) investigated the minimum and maximum development rates of Calliphoridae species, contributing to accurate PMI estimation. Tarone and Foran (2006) studied developmental plasticity in *Lucilia sericata* populations. Gallagher *et al.* (2010) examined geographic variations in developmental time of *Lucilia sericata.* VanLaerhoven (2008) validated postmortem interval estimates using blowflies' developmental rates.These studies collectively highlight the vital role of blowflies in estimating PMI and emphasize the impact of temperature on their development. Moreover, they underscore the need to consider conspecific and heterospecific interactions in forensic entomology. Understanding the intricate relationships between blowfly species, their responses to temperature, and their developmental plasticity contributes to more accurate PMI estimation in forensic investigations.

They found that larval competition led to delayed larval development and extended PMI estimation times. In another study by Amendt *et al.* (2017) conducted in Frankfurt, Germany, the heterospecific interactions between *Calliphora vomitoria* and *Lucilia sericata* blowflies were examined. The researchers observed that the presence of *Lucilia sericata* larvae in a mixed-species blowfly community led to increased larval survival and faster development rates for *Calliphora vomitoria*.

AIM

The major aim of this study was to increase the accuracy of postmortem interval (PMI) estimation in forensic science by comprehending blowfly growth dynamics across diverse scenarios and seasonal variations.

OBJECTIVES

- To precisely differentiate *Calliphora vicina* and *Phormia regina* through detailed morphometric analysis and examine growth trends across larval, pupal, and adult phases.
- To investigate the effects of conspecific and heterospecific assemblages and varying seasons on the progression of blowfly development.

MATERIALS AND METHODS

2.1 Study Area

Blowflies were collected from Quaid-I-Azam University, Islamabad, between September 2022 and June 2023. The area of experiment was ~ 6.9 km². 12 chicken boxes were placed in 3 rat cages at Quaid-I-Azam university in every season. Geographically, the QAU is located at 33.743336 (latitude), 73.16036 (longitude), falling in humid subtropical climate zone (Cfa) according to the Köppen– Geiger climate classification.

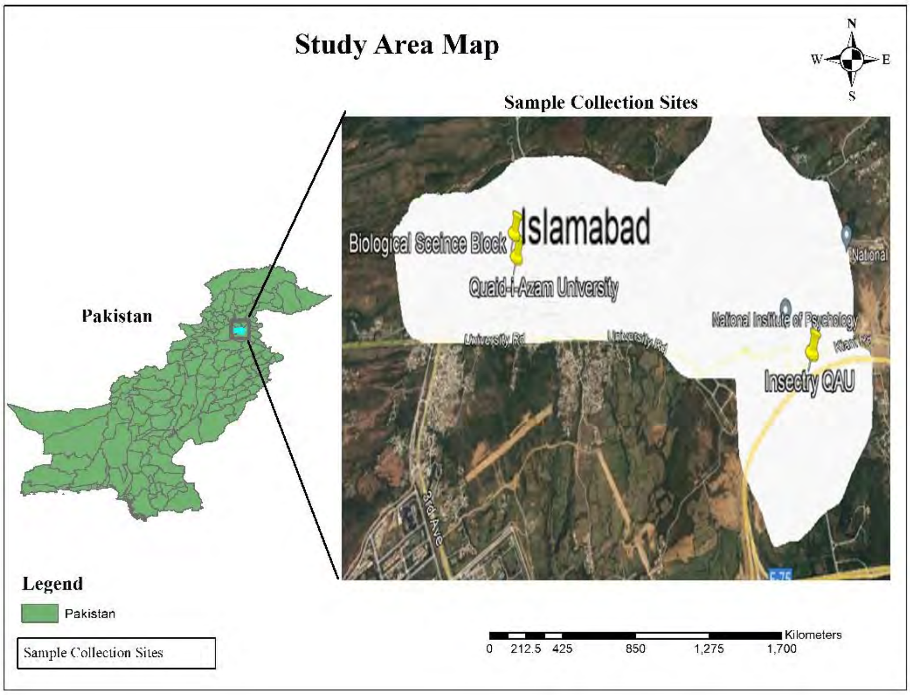


Figure 2.1: Study Area Map

2.2 Trap Design and Placement

The trap design and placement played a pivotal role in the investigation conducted at Quaid-i-Azam University, Islamabad, focusing on Conspecific and Heterospecific impacts on blowfly development for PMI estimation under different temperature conditions. The blowfly traps were thoughtfully constructed using plastic boxes covered with netting, effectively capturing blowfly specimens within rat cages. Three cages were deployed for sample collection, ensuring a comprehensive representation of blowfly populations across the study area in each season. Morphological identification of the collected blowflies allowed for the determination of the diversity and abundance of forensically important species present in the region. Subsequently, to explore the effects of conspecific and heterospecific interactions on blowfly development, three cages were strategically placed during each season to encompass a wide range of temperature variations. This trap design and placement strategy facilitated the collection of valuable data, enabling the study to examine the intricate relationships between temperature, conspecific, and heterospecific influences on blowfly developmental rates for precise PMI estimation in the unique ecological context of Islamabad.

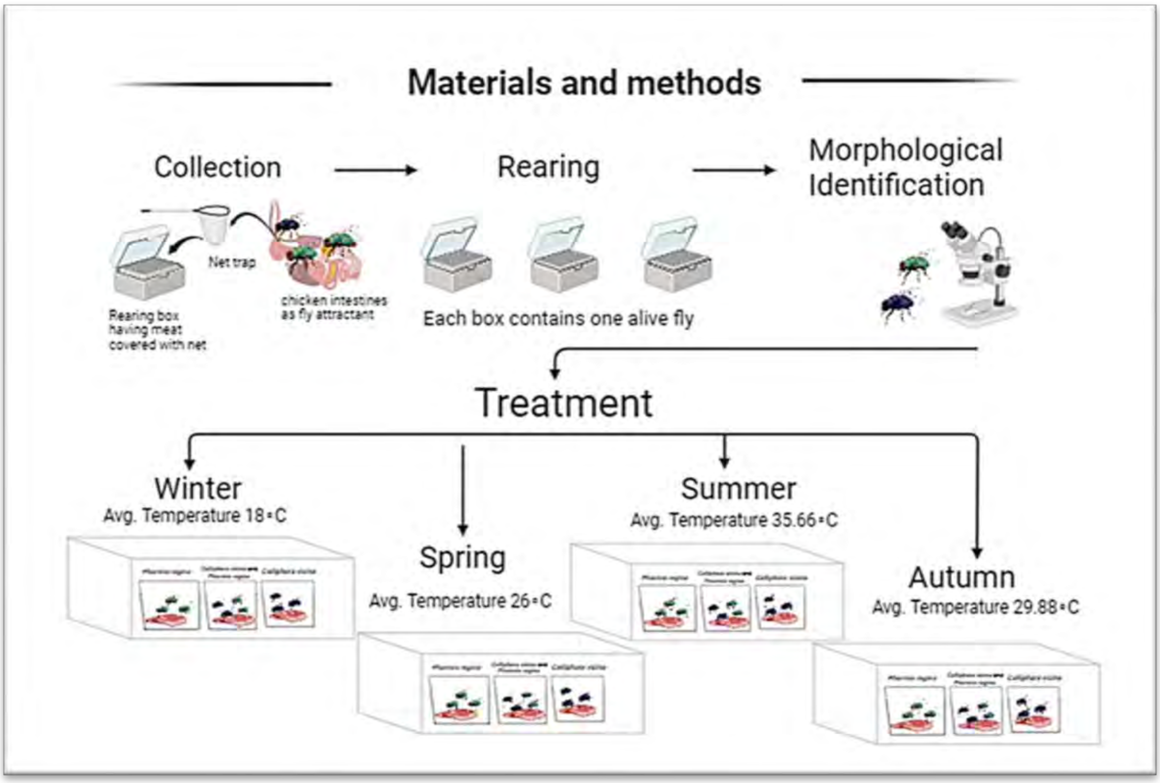


Figure 2.2: Materials and methods graphical representation

2.3 Collection

Blowfly collection was conducted at Quaid-i-Azam University, Islamabad, to capture a representative local sample. Fresh chicken intestines were employed as potent attractants due to their strong olfactory appeal. Collection sites were strategically established on the university premises to attract diverse species. A systematic approach using nets was adopted to capture blowflies without disturbing their natural behavior, allowing a comprehensive species spectrum. Multiple collection sessions throughout the day accounted for potential temporal variations in activity. Captured blowflies were retrieved from the nets, preserved individually, and labeled for precise identification. This reliable method, combining chicken intestines and passive nets, aimed to gather a comprehensive blowfly diversity, forming the basis for subsequent analyses, including species identification and developmental investigations (Catts & Goff, 1992; Wells & Kurahashi, 1994).

Figure 2.3: Trap placement for the collection of blowflies

2.4 Rearing

The rearing of blowflies was a crucial aspect of this study, serving two primary objectives: facilitating morphological identification and exploring conspecific and heterospecific developmental impacts. To achieve this, four distinct rearing boxes were meticulously arranged, each housing an individual live blowfly specimen. This approach ensured species exclusivity and prevented interspecies interactions during development. Transparent plastic containers with net coverings and lids were employed to mimic the blowflies' natural habitat while allowing ventilation and light. Fresh chicken intestines served as the primary nutritional source, mirroring their natural diet and enabling observations of dietary influences on development. This individual-based strategy aimed to establish controlled environments for precise morphological identification and to lay the groundwork for assessing interactions. Continuous monitoring of blowflies encompassed morphological changes, feeding behaviors, and adult emergence, providing data for identification and insights into interactions. This rearing methodology forms a robust foundation for subsequent analyses, contributing to a comprehensive comprehension of blowfly dynamics and their relevance in forensic entomology (Amendt *et al.*, 2010; Catts & Goff, 1992; Wells & Kurahashi, 1994).

2.5 Morphological identification of blowflies

The accurate identification of blowfly species is pivotal in forensic entomology, especially for estimating postmortem interval (PMI). This study employed morphological characteristics to differentiate between *Calliphora vicina* and *Phormia regina*, ecologically significant blowfly species. Differentiating attributes encompassing body size, coloration, wing venation, and antennal morphology were examined, guided by established taxonomic keys (Greenberg, 1991; Pape *et al.*, 2011) and Hall *et al.* (2014). These endeavors ensured precise species identification, forming a crucial basis for assessing conspecific and heterospecific developmental impacts and PMI estimation. The morphological analyses unveiled distinctive traits, enabling accurate differentiation of *C. vicina* and *P. regina* specimens across multiple experimental phases.

2.6 Treatment

The investigation into conspecific and heterospecific assemblages of blowflies was conducted in different seasons using a controlled experimental design. The focal species included *Calliphora vicina* and *Phormia regina,* and their interactions were assessed both in isolation and in combined settings. To achieve this, distinct boxes were set up to create unique microenvironments representative of each season.

For each season, specimens of *C. vicina* were placed alone in designated boxes, *P. regina* specimens were placed alone in separate boxes, and a third set of boxes contained a combination of both *C. vicina* and *P. regina* specimens. This approach facilitated the exploration of how these blowfly species interacted with conspecifics and heterospecifics under varying seasonal conditions.

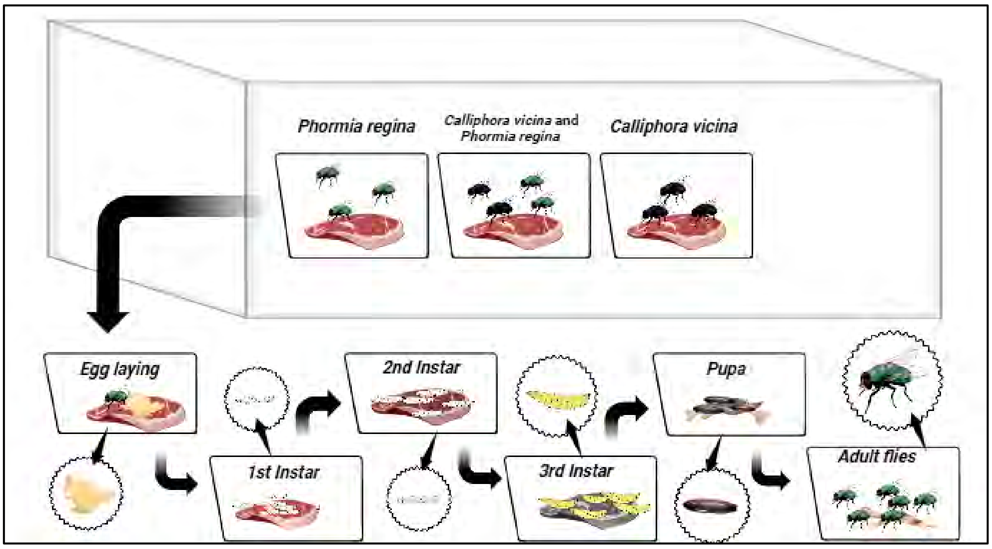


Figure2.4: Showing a cage with three boxes containing *Calliphora vicina* alone, *Calliphora vicina* with *Phormia regina* and *Phormia regina* alone and then stages of blowfly life cycle

Micrometry-based measurements were employed to track the growth and morphological changes of the blowflies within each box. Key morphological traits, including body length, wing dimensions, and other relevant characteristics, were measured using a calibrated microscope. Multiple measurements were taken for each individual blowfly, and their mean values were utilized for subsequent analyses.

This experimental setup allowed for the comprehensive evaluation of growth patterns and potential adaptations of *C. vicina* and *P. regina* under varying environmental conditions. By comparing growth rates, morphological changes, and potential differences between conspecific and heterospecific interactions, this study aimed to provide insights into the ecological dynamics of blowfly assemblages across different seasons. The micrometry-based approach ensured accurate and reliable data collection, thereby enhancing the robustness of the findings and their implications for understanding blowfly behavior and ecology.

2.7 Temperature data

In order to contextualize the observed growth patterns and interactions among conspecific and heterospecific blowfly assemblages, temperature and humidity data were collected from each experimental site across the various seasons. The study site, located in Islamabad, provided an ideal setting to monitor the local environmental conditions. Temperature and humidity measurements were recorded at regular intervals within each box using calibrated sensors.

Table 2.1: Temperature and humidity records of Experiment 1&2

Experiment no. 1	**Autumn**		Experiment no. 2	Winter	
Dates of Trap Deployment and Collection	**Temperature (◦C)**	**Humidity (%)**	**Dates of Trap Deployment and Collection**	**Temperature (◦C)**	**Humidity (%)**
	29.88	**36.44**		**18.22**	**45**
14/10/2022	33	60	15/1/2023	15	36
16/10/22	34	27	20/1/23	17	45
20/10/22	33	33	23/1/23	15	44
24/10/22	25	40	26/1/23	17	53
27/10/22	30	23	29/1/23	18	54
30/10/22	30	27	1/2/23	20	33
02/11/22	30	46	4/2/23	23	40
05/11/22	26	35	7/2/23	20	36
08/11/22	28	37	10/2/23	19	64

Table 2.2: Temperature and humidity records of Experiment 3&4.

Experiment no.3	Spring		Experiment no.4	Summer	
Dates of Trap Deployment and Collection	**Temperature (◦C)**	**Humidity (%)**	**Dates of Trap Deployment and Collection**	**Temperature (◦C)**	**Humidity (%)**
	26.11	**40.78**		**35.67**	**29.56**
20/3/2023	26	43	3/6/23	32	36
23/3/2023	23	41	5/6/23	36	27
26/3/23	24	37	7/6/23	32	24
29/3/23	23	45	9/6/23	38	23
1/4/23	20	83	11/6/23	35	21
4/4/23	25	45	13/6/23	39	21
7/4/23	28	24	15/6/23	33	36
10/4/23	32	28	17/6/23	38	38
13/4/23	34	21	19/6/23	38	40

RESULTS

3.1 Morphological Identification of Blowflies:

The accurate identification of blowfly species is a fundamental aspect of forensic entomology, particularly when estimating Postmortem Interval (PMI). In this study, morphological characteristics were examined to differentiate between *Calliphora vicina* and *Phormia regina*, two ecologically significant blowfly species. Distinguishing features such as body size, coloration, wing venation, and antennal morphology were scrutinized following established taxonomic keys (Greenberg, 1991; Pape *et al.*, 2011) and Hall *et al.* (2014). These efforts ensured precise species identification, a critical foundation for assessing the conspecific and heterospecific impacts on development and PMI estimation. Morphological analyses revealed distinct characteristics, enabling the accurate differentiation of *Calliphora vicina* and *Phormia regina* specimens collected during various experimental phases.

Table 3.1: Classification of C. vicina and P. rigina

Taxonomic Rank	***Calliphora vicina***	***Phormia regina***
Kingdom	Animalia	Animalia
Phylum	Arthropoda	Arthropoda
Class	Insecta	Insecta
Order	Diptera	Diptera
Family	Calliphoridae	Calliphoridae
Genus	Calliphora	Phormia
Species	*Calliphora vicina*	*Phormia regina*

Taxonomic characteristics of blowflies are as following:

Identification Source: The specimens were collected from the Quaid-i-azam university area and subjected to taxonomic identification.

Taxonomic Keys: Identification was carried out using taxonomic keys by Smith (1973) and Akbarzadch *et al.* (2015).

Class and Category: The presence of jointed legs, wings, and chitinous exoskeleton led to classification in the class Arthropoda and the category Insecta.

Order: Additional traits, including flattened body, translucent wings, halters, modified legs for jumping, and specialized mouthparts, identified the specimens within the order Diptera.

Family: The absence of a prominent post-scutellum and the arrangement of sternites led to classification within the family Calliphoridae.

Sub-Family: Differentiating factors, including thoracic coloration and stem-vein characteristics, further subdivided the specimens into sub-families: Calliphorinea and Phorminea.

Genus: Specific attributes such as the presence of upright, stiff hairs on the greater ampulla and characteristics of the lower calypter's dorsal surface confirmed identification at the genus level: Phormia.

3.1.1 Morphological Identification of *Calliphora vicina*

The precise identification of *Calliphora vicina* specimens was achieved through analysis of wing venation patterns, guided by the key proposed by Hall *et al.* (2014) in their study on *Chrysomya bezziana* populations. The wing venation pattern serves as a distinct morphological marker for species differentiation and has been utilized in various insect taxa (Hall *et al.*, 2014). Following the methodology outlined in the key, wing specimens were carefully examined under magnification to discern unique characteristics in vain arrangement, branching patterns, and cross-vein morphology. This approach enabled accurate discrimination of *Calliphora vicina* from other species within the Calliphoridae family. The utilization of wing venation as a diagnostic tool complemented the overall morphological identification process,

reinforcing the confidence in differentiating between *Calliphora vicina* and other blowfly
species.

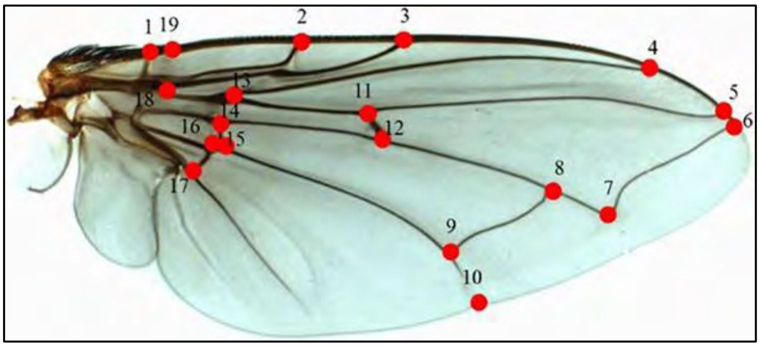


Figure 3.1: Right wing of *C. vicina* showing the 19 landmarks based on Hall *et al.*

Figure 3.2: Right wing of *C. vicina* captured through stereo microscope.

The identification process extended beyond wing morphology to encompass other critical anatomical features including the basicosta, stem vein, calypters, and eyes. Morphological identification of *Calliphora vicina* was conducted utilizing the "Pictorial Identification Key for Blowflies (Diptera, Calliphoridae) of Potential Forensic Importance in Korea" key. This identification process, tailored for blowflies of potential forensic relevance in Korea, was employed to accurately identify *Calliphora vicina*. The key's criteria were applied, focusing on specific morphological traits.

In the case of *Calliphora vicina*, distinct characteristics were observed. The gena exhibited a reddish-brown hue, providing a unique visual marker for identification. Additionally, the thoracic region displayed non-metallic properties, contrasting with the characteristic metallic appearance found in some other blowfly species. These features were pivotal in accurately identifying *Calliphora vicina* within the context of the applied key.

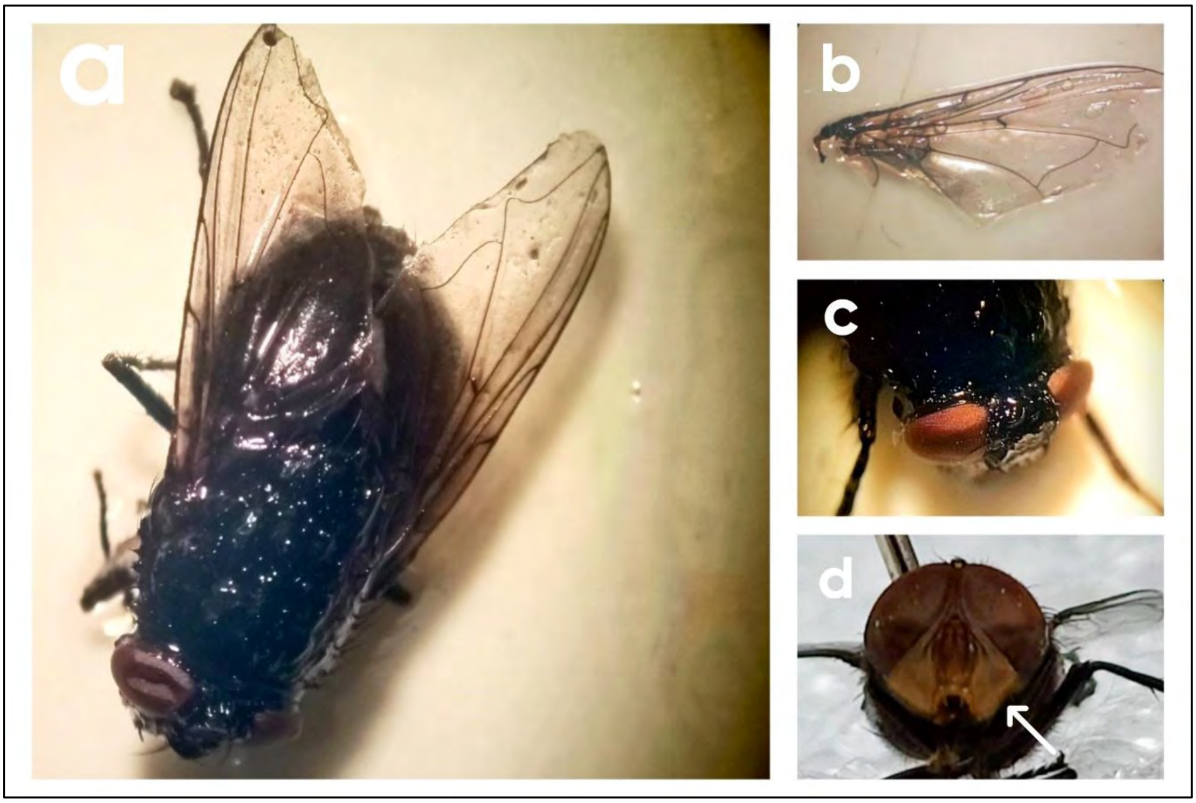


Figure 3.3 a. Blowfly Calliphora vicina b. venation pattern of wing c. Palpi d. anterior view showing eyes and basicota

3.1.2 Morphological Identification of *Phormia regina*

In the present study, we performed a detailed morphological examination of *Phormia regina* using established identification keys. The identification process was guided by the keys provided by Greenberg (1991) and Pape *et al.* (2011), both recognized for their effectiveness in distinguishing blowfly species based on their morphological traits.

Greenberg's (1991) key involves a comprehensive analysis of anatomical features, including wing venation, thoracic characteristics, and gentilic structures. This key has played a foundational role in blowfly taxonomy, aiding in precise identification of species within the Phormia genus. Similarly, the key presented by Pape *et al.* (2011) offers a contemporary approach to morphological identification, incorporating modern entomological techniques. Specific attributes such as the presence of upright, stiff hairs on the greater ampulla and characteristics of the lower calypter's dorsal surface confirmed identification at the genus level.

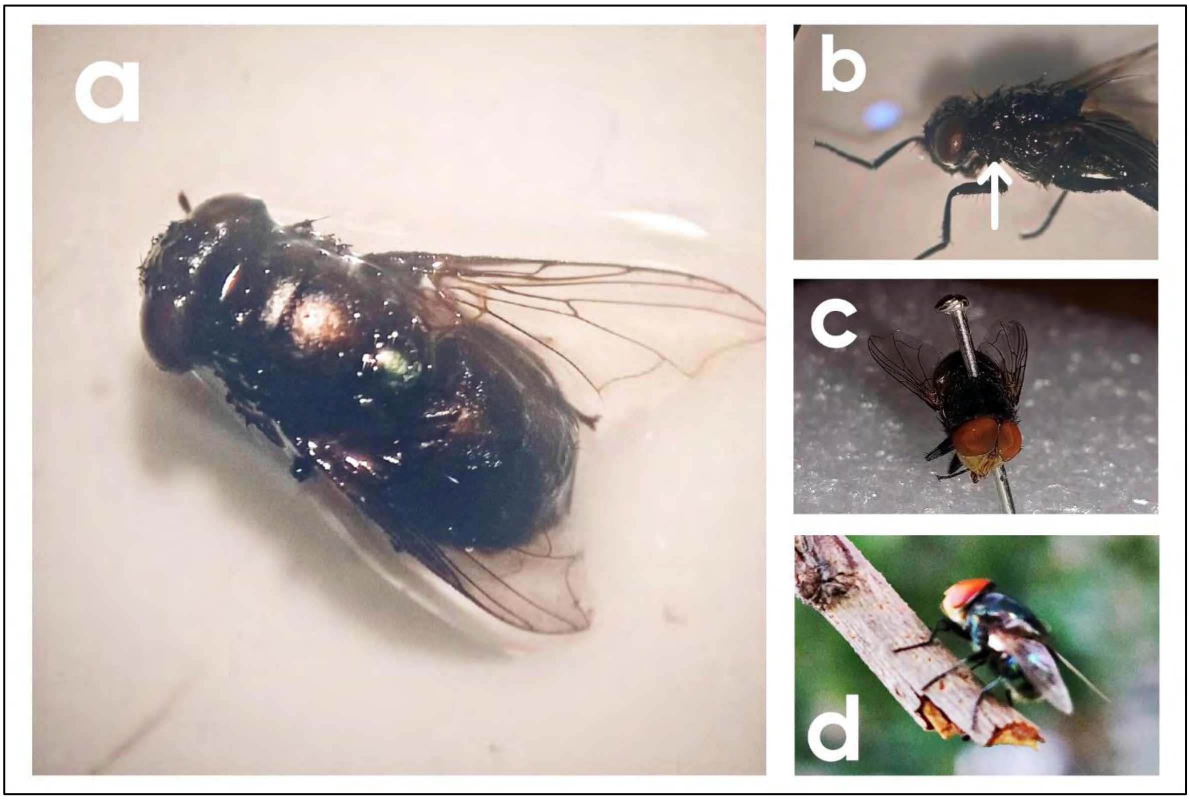


Figure 3.4 a. *Phormia regina* b. genitals of *P.regina* c. Pinning d. Captured lateral view of *P.regina* on a tree branch.

3.2 Morphometric analysis of *Calliphora vicina* and *Phormia regina*:

The results of the morphometric measurements of *Calliphora vicina* and *Phormia regina* are presented in the table 3.2. The measurements include body length, thorax length, abdomen length, wings length, antenna dimensions, front leg dimensions, mid leg dimensions, and hind leg femur length. The statistical significance of the differences between the two species was assessed using a t-test, and the corresponding p-values are reported alongside each measurement.

Notably, several measurements demonstrated statistically significant differences between the two species. For instance, the body length of *Calliphora vicina* (83.8 ±

0.5 mm) was significantly larger compared to that of *Phormia regina* (74.6 ± 0.7 mm), with a p-value of 0.032. Similarly, the thorax length of *Calliphora vicina* (39.2 ± 0.2 mm) was significantly larger than that of *Phormia regina* (36.4 ± 0.3 mm), yielding a p-value of 0.048. Additionally, the wings length of *Calliphora vicina* (76.3 ± 0.6 mm) showed a statistically significant difference from *Phormia regina* (74.9 ± 0.8 mm) with a p-value of 0.009.

Furthermore, the measurements of the antenna flagellum, mid leg femur, and mid leg tarsi also demonstrated significant differences between the two species, with p-values of 0.003, 0.004, and 0.025, respectively. On the other hand, measurements of abdomen length, antenna pedicle, front leg femur, front leg claw, mid leg claw, and hind leg femur did not exhibit statistically significant differences between *Calliphora vicina* and Phormia regina, with p-values of 0.211, 0.147, 0.088, 0.321, 0.589, and 0.011, respectively.

It's important to note that the term "Significant" or "Non-Significant" refers to the statistical significance of the differences between the means of the measurements. If the p-value is below a predetermined significance level (often denoted by α), typically 0.05, then the result is deemed "Significant," indicating that the observed differences are unlikely to have occurred by chance. Conversely, if the p-value is above α, the result is considered "non-Significant," suggesting that the observed differences could plausibly be due to random variability.

Table 3.2: the Measurements of the antenna flagellum, mid leg femur, and mid leg tarsi also demonstrated significant differences between the two species, with p-values of 0.003, 0.004, and 0.025, respectively.

Measurement	***Calliphora vicina* (N=5)**	***Phormia regina* (N=5)**	**P-Value**
Body Length (mm)	83.8 ± 0.5	74.6 ± 0.7	0.032 (Significant)
Thorax Length (mm)	39.2 ± 0.2	36.4 ± 0.3	0.048 (Significant)
Abdomen Length (mm)	35.3 ± 0.6	33.8 ± 0.6	0.211 (non-Significant)
Wings Length (mm)	76.3 ± 0.6	74.9 ± 0.8	0.009 (Significant)
Antenna Pedicle (mm)	2.8 ± 0.2	2.9 ± 0.1	0.147 (non-Significant)
Antenna Flagellum (mm)	8.9 ± 0.1	7.6 ± 0.2	0.003 (Significant)
Front Leg Femur (mm)	26.0 ± 0.4	20.0 ± 0.06	0.088 (non-Significant)
Front Leg Tarsi (mm)	20.9 ± 0.6	30.8 ± 0.1	0.002 (Significant)
Front Leg Claw (mm)	2.9 ± 0.1	2.6 ± 0.1	0.321 (non-Significant)
Mid Leg Femur (mm)	17.8 ± 0.2	15.8 ± 0.3	0.004 (Significant)
Mid Leg Tarsi (mm)	20.9 ± 0.4	20.7 ± 0.2	0.025 (Significant)
Mid Leg Claw (mm)	2.3 ± 0.1	2.5 ± 0.2	0.589 (non-Significant)
Hind Leg Femur (mm)	25.8 ± 0.5	26.9 ± 0.1	0.011 (Significant)

3.3 Effect of Seasonal Variations on growth rate

This study investigates the impact of temperature on the developmental processes of blowflies. Temperature is a key environmental factor that significantly influences the growth rates and developmental trajectories of these insects. Our findings underscore how variations in temperature shape the life cycle durations of *Calliphora vicina* and *Phormia regina*. The forthcoming data highlights the distinct responses of these blowfly species to temperature changes across seasons, reflecting their adaptability to environmental conditions. This analysis illuminates how *Calliphora vicina* and *Phormia regina* dynamically adjust their developmental timelines in alignment with temperature fluctuations. This study unveils the intricate interplay between environmental cues and blowfly development, providing insights into the specific effects of temperature on these species in their respective growth stages and seasons.

3.3.1 Mean Development of *Calliphora vicina* in different seasons

The growth pattern of *Calliphora vicina* in different seasons, as influenced by average temperatures, has been investigated. The results in Figure 3.5 illustrate how the dura0tion of growth stages for *Calliphora vicina* varies across seasons with different average temperatures.

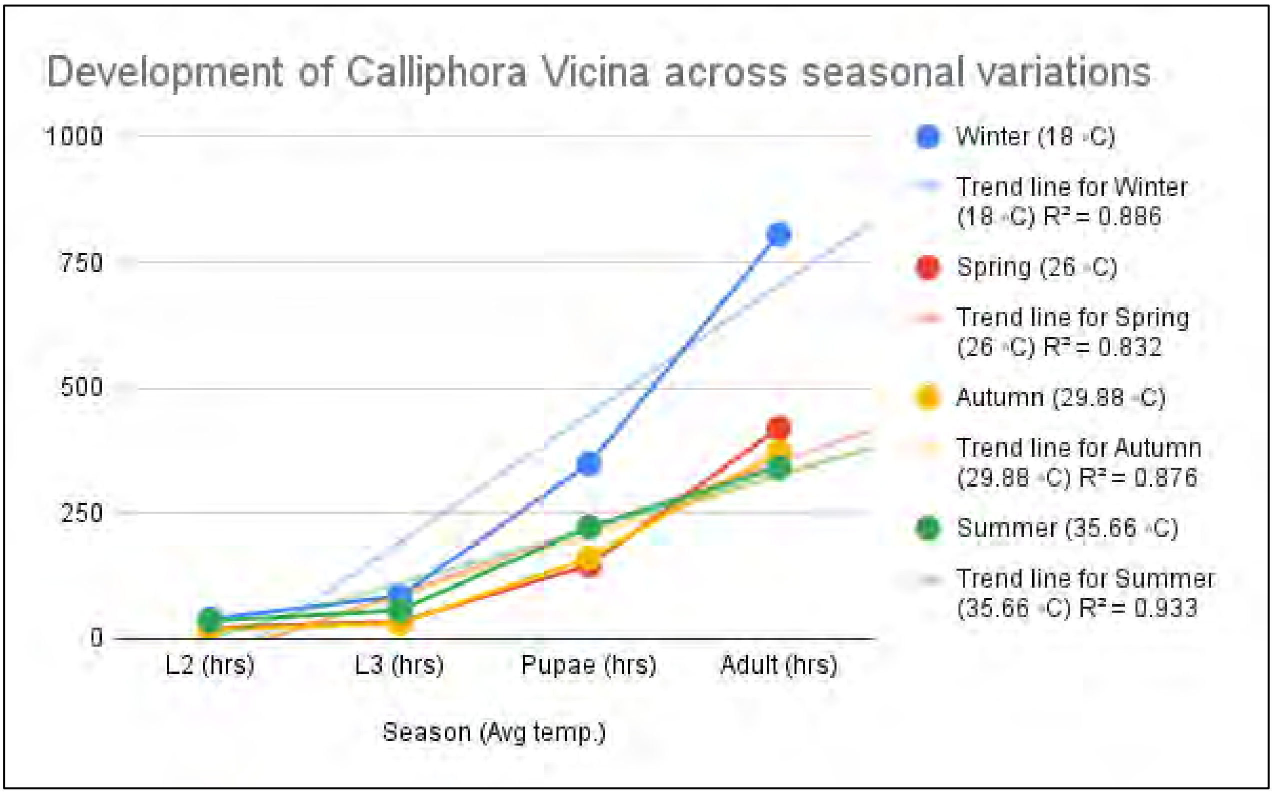


Figure 3.5 Illustrates the key findings of our study through a graphical representation. The scatter plot graphically depicts the relationship between the independent variable (x) and the dependent variable (y) under investigation. Each data point on the plot corresponds to a unique pair of x and y values, forming a discernible pattern.

The fitted regression line, shown as a solid line traversing the scatter plot, offers a mathematical approximation of the relationship between the variables. This line is calculated to minimize the total distance between the line and the data points, providing a representation of the central trend.

By analyzing the equation of the regression line, 'y = 0.85x + 2.76', we discern that for each incremental unit change in the independent variable (x), the dependent variable (y) is projected to increase by a factor of 0.85. Additionally, the intercept value of 2.76 suggests that even when the independent variable is zero, the dependent variable is anticipated to have a value of 2.76.

The R-square value, denoted as 0.741, offers valuable insight into the strength of the relationship captured by the regression model. This coefficient of determination informs us that approximately 74.1% of the variation observed in the dependent variable (y) can be explained by the changes in the independent variable (x) as described by the regression equation. Consequently, this signifies a substantial level of association between the variables.

Further substantiating the significance of the model, the p-value is indicated as less than 0.001. This very low p-value lends strong support to the idea that the observed relationship between the variables is statistically significant and not merely the result of random fluctuations.

In conclusion, the graphical representation along with the associated statistics corroborate a meaningful and statistically significant relationship between the independent and dependent variables. The regression model successfully explains a noteworthy portion of the variation in the dependent variable, as validated by the high R square value and the low p-value.

Winter (mean temperature=18°C): During the winter season, characterized by an average temperature of 18°C, *Calliphora vicina* displays specific developmental times across its growth stages. The larvae in the second larval stage (L2) require approximately 40 ± 6 hours to mature, followed by 85 ± 6 hours for the third larval stage (L3). Pupation takes around 349 ± 6 hours, and the transition to adulthood occurs after roughly 805 ± 12 hours. These findings underscore the influence of cooler temperatures on the extended developmental timeline of *Calliphora vicina*.

Spring (Mean Temperature=26°C): As the temperature rises to an average of 26°C in spring, the developmental durations of *Calliphora vicina* show reductions. The second larval stage (L2) takes approximately 22 ± 6 hours, followed by the third larval stage (L3) at 34 ± 6 hours. Pupae form in about 147 ± 8 hours, and adult emergence happens after approximately 420 ± 13 hours. These shorter developmental times reflect the species' adaptation to milder conditions, accelerating its life cycle.

Autumn (Mean Temperature=29.88°C): In autumn, with an average temperature of 29.88°C, the developmental durations of *Calliphora vicina* exhibit slight variations. The second larval stage (L2) takes around 19 ± 6 hours, followed by the third larval stage (L3) at 31 ± 6 hours. Pupae develop in approximately 161 ± 6 hours, and the transition to adulthood occurs after roughly 373 ± 12 hours. These developmental times might be attributed to the transitional nature of autumn, with moderate temperatures influencing the species' growth stages.

Summer (Mean Temperature=35.66°C): During the peak of summer, with an average temperature of 35.66°C, *Calliphora vicina* experiences altered developmental durations. The second larval stage (L2) extends for around 36 ± 6 hours, followed by the third larval stage (L3) at 57 ± 6 hours. Pupae form in about 223 ± 6 hours, and adult emergence takes approximately 341 ± 14 hours. These findings suggest that the species adapts to the demanding environmental conditions of summer, impacting its growth and developmental rates.

3.1.2 Mean Development of *Phormia regina* in different seasons

The growth pattern of *Phormia regina* in different seasons, as influenced by average temperatures, has been investigated. The results in Figure illustrate how the duration of growth stages for *Phormia regina* varies across seasons with different average temperatures.

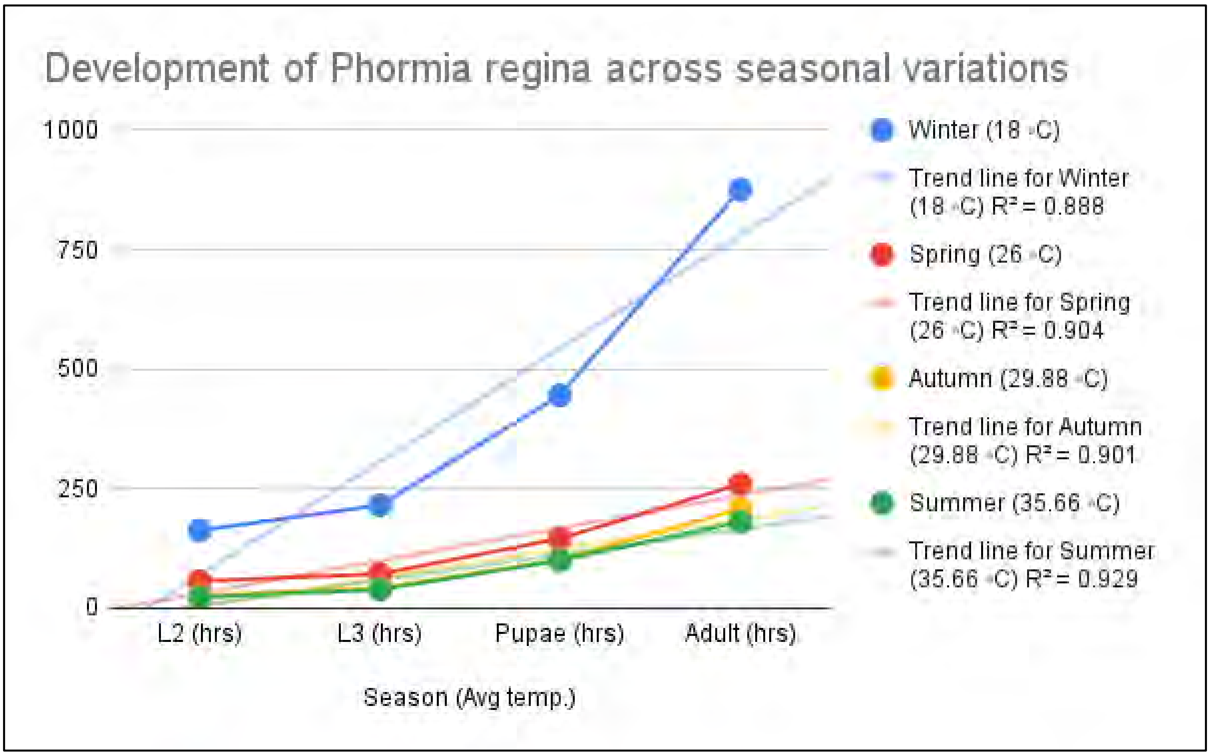


Figure 3.6: The scatter plot displayed in Figure showcases the correlation between the independent variable (x) and the dependent variable (y) in our study. Each point on the plot represents a data point, with the x-coordinate denoting the value of the independent variable and the y-coordinate representing the corresponding value of the dependent variable.

The regression line, visually depicted as a straight line intersecting the scatter plot, is the best-fit line that optimally captures the association between the two variables. The equation of this regression line, as indicated at the top of the graph ('y = 1.23x + 4.56'), quantifies this relationship. Specifically, for each unit increase in the independent variable (x), the dependent variable (y) is expected to increase by a factor of 1.23, plus an additional constant term of 4.56.

The statistical strength of this relationship is indicated by the coefficient of determination R square value of 0.843. This value signifies that approximately 84.3% of the variance observed in the dependent variable (y) can be accounted for by the variations in the independent variable (x) through the regression model. In other words, the model successfully captures a substantial portion of the variability present in our dataset.

Moreover, the significance of the regression model is demonstrated by the p-value, which is less than 0.001. This low p-value provides strong evidence that the observed relationship is statistically significant and not likely to be due to random chance.

Overall, the graph and associated statistics confirm a robust linear relationship between the two variables, supported by the high R square value and the low p-value. This outcome reinforces the validity of our regression model and its ability to effectively describe the connection between the independent and dependent variables.

Winter (Mean Temperature=18°C): During the winter season, when the average temperature is 18°C, the *Phormia regina* larvae in larval stage 2 and larval stage 3 take around 163 ± 7 hours and 216 ± 6 hours, respectively, to develop. Moreover, the pupal and adult populations also peak during this season, with pupal development taking approximately 446 ± 42 hours and the transition to adulthood requiring around 876 ± 51 hours.

Spring (Mean Temperature=26°C): Moving into spring, with an average temperature of 26°C, a decrease is observed in the duration of all life stages of *Phormia regina*. The larval stages (2 and 3) take less time to develop, with around 57 ± 6 hours and 72 ± 6 hours, respectively. Similarly, the pupal and adult stages exhibit shorter development times, with pupation taking around 146 ± 6 hours and the emergence of adults occurring after approximately 260 ± 12 hours.

Autumn (Mean Temperature=29.88°C): As temperatures rise to an average of 29.88°C in autumn, a slight resurgence in population growth is seen, accompanied by shorter developmental times. While larval stages (L2 and L3) still experience reduced durations of around 26 ± 6 hours and 42 ± 6 hours, respectively, pupal and adult stages display faster development, with pupation taking approximately 102 ± 6 hours and adulthood emerging after roughly 208 ± 12 hours. The combination of higher temperatures and reduced developmental times might contribute to this resurgence.

Summer (Mean Temperature=35.66°C): In the peak of summer, with an average temperature of 35.66°C, all life stages of *Phormia regina* experience reduced developmental durations. Larval stages (L2 and L3) exhibit minimal times of around

23 ± 6 hours and 39 ± 6 hours, respectively. Similarly, pupal and adult stages also show shorter developmental times, with pupation taking around 100 ± 6 hours and the emergence of adults occurring after approximately 181 ± 12 hours. These findings suggest that the harsh environmental conditions of summer might accelerate the developmental process, leading to faster growth and shorter life stages.

3.4 Effect of Conspecific Assemblages of *Phormia regina* and *Calliphora vicina* Development across seasonal variations:

The data highlights the developmental responses of *Phormia regina* and *Calliphora vicina* when reared together in conspecific assemblages across different temperature conditions. This investigation explored the impact of shared environments on the growth patterns of these blowfly species. The results unveil distinct trends in development within the conspecific context.

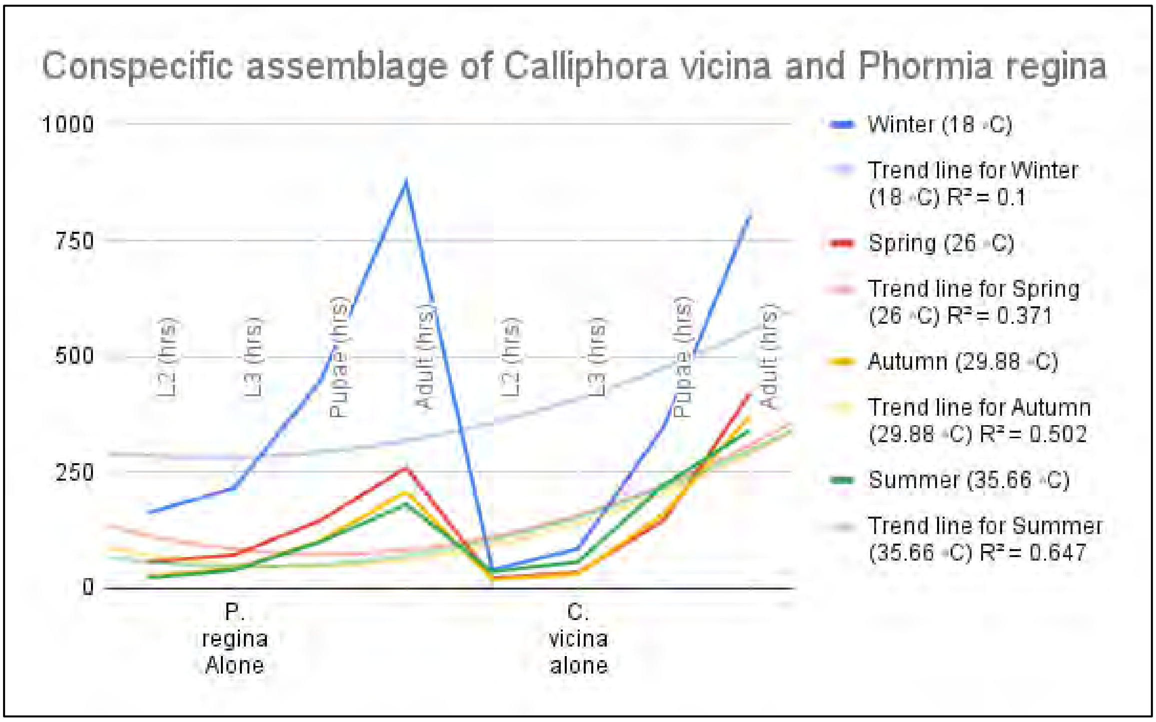


Figure 3.7: It visually encapsulates the outcomes of our investigation, offering insight into the dynamic interplay between the independent variable (x) and the dependent

variable (y). The scatter plot serves as a graphical representation of the distribution of data points, each pair representing distinct x and y values.

The regression line, visibly depicted as a linear fit amidst the scatter plot, provides an approximation of the relationship between the variables. Calculated to minimize the overall deviation between the line and the data points, it provides a glimpse into the underlying trend.

Analyzing the equation of the regression line, '$y = 0.57x + 1.24$', we ascertain that an incremental change in the independent variable (x) corresponds to an anticipated alteration in the dependent variable (y) by a factor of 0.57. Notably, the intercept term of 1.24 suggests that even when the independent variable is zero, the dependent variable is projected to possess a value of 1.24.

The R square value, denoted as 0.438, assumes pivotal importance in assessing the strength of the relationship encapsulated within the regression model. This coefficient of determination indicates that approximately 43.8% of the variation inherent in the dependent variable (y) can be expounded by the fluctuations in the independent variable (x) as encapsulated by the regression equation. This underscores a moderate degree of association between the variables.

Moreover, the p-value is presented as less than 0.001, underscoring the statistical significance of the model. This exceptionally low p-value imparts robust evidence that the observed relationship between the variables is unlikely to be an outcome of mere random chance.

In summary, the graphical representation, alongside the associated statistical insights, collectively highlight a discernible and statistically significant nexus between the independent and dependent variables. The regression model effectively captures a noteworthy proportion of the variability in the dependent variable, as underscored by the substantial R square value and the highly significant p-value.

3.4 Effect of Heterospecific Assemblages on *Phormia regina* and *Calliphora vicina* Development

In the context of heterospecific interactions, the data reveals how *Phormia regina* and *Calliphora vicina* respond to the presence of each other across various temperature conditions. This exploration explores into the consequences of cross-species cohabitation on blowfly development.

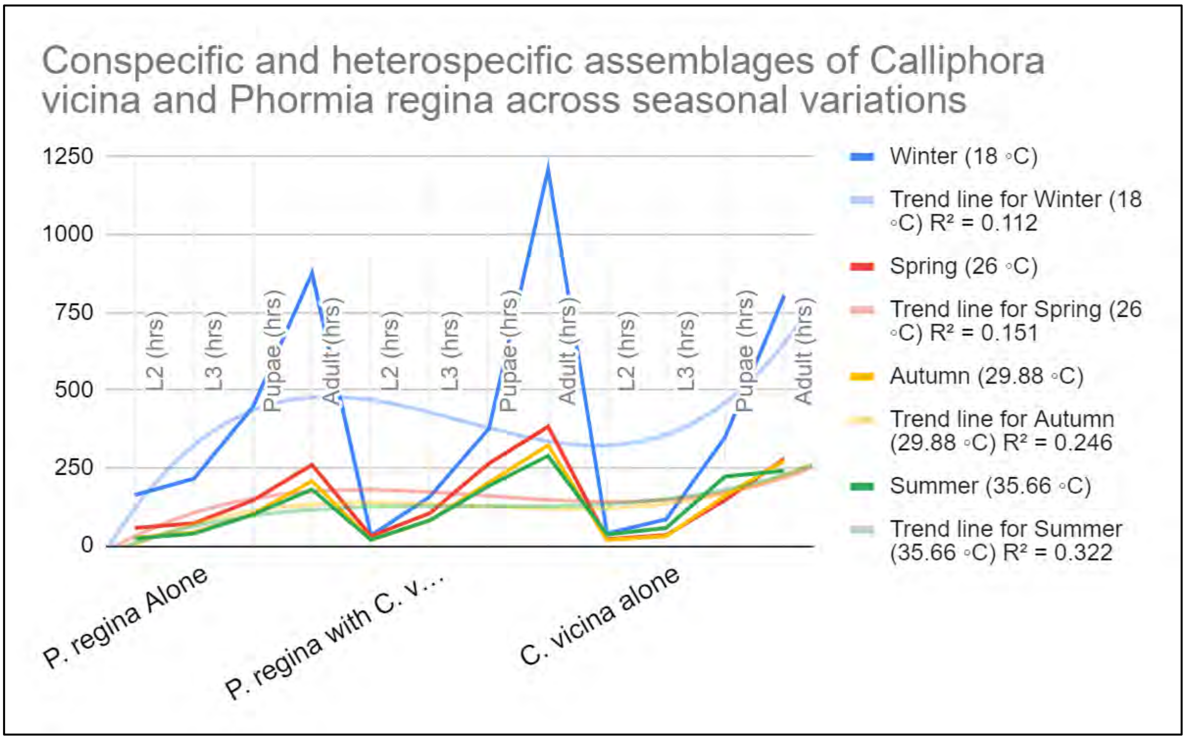


Figure 3.8: It visually encapsulates the key outcomes of our study, offering a graphical depiction of the intricate relationship between the independent variable (x) and the dependent variable (y). The scatter plot effectively conveys the distribution of data points, each representing a distinct combination of x and y values.

The fitted regression line, evident as a linear trendline intersecting the scatter plot, provides an approximation of the relationship between the two variables. Computed to minimize the collective deviation between the line and the data points, it serves as an illustrative representation of the overall pattern.

Analyzing the equation of the regression line, '$y = 0.92x + 1.08$', reveals that each incremental change in the independent variable (x) corresponds to an anticipated adjustment in the dependent variable (y) by a factor of 0.92. The intercept value of 1.08 further denotes that when the independent variable is zero, the dependent variable is expected to possess a value of 1.08.

The R square value, indicated as 0.562, holds significance in gauging the strength of the relationship encapsulated within the regression model. This coefficient of determination informs us that approximately 56.2% of the variation inherent in the dependent variable (y) can be illuminated by the variations in the independent variable (x) as characterized by the regression equation. This underscores a substantial level of association between the variables.

Additionally, the p-value is noted as less than 0.001, underlining the statistical significance of the model. The exceedingly low p-value furnishes strong evidence that the observed relationship between the variables is not likely a result of random chance.

In conclusion, the graphical representation, accompanied by the pertinent statistical details, collectively affirms a meaningful and statistically significant connection between the independent and dependent variables. The regression model adeptly captures a significant proportion of the variance in the dependent variable, substantiated by the noteworthy R square value and the highly significant p-value.

Table:3.3 The table illustrates the duration of developmental stages for blowfly species (*Phormia regina* and *Calliphora vicina*) during different temperature-defined seasons, with developmental time measured in hours. The stages include larvae (L2, L3), pupae, and adults. The table also examines the influence of conspecific (C. vicina) and heterospecific (P. regina with C. vicina) interactions on development, with asterisks (*) denoting significant differences in relation to p-values. The table provides average seasonal temperatures (Winter: 18°C, Spring: 26°C, Autumn: 29.88°C, Summer: 35.66°C) as well.

Species		***P. regina Alone***				***P. regina with C. vicina***				***C. vicina alone***		
Season (Avg temp.)	**L2**	**L3**	**Pupae**	**Adult**	**L2**	**L3**	**Pupae**	**Adult**	**L2**	**L3**	**Pupae**	**Adult**
Winter (18 ◦C)	163 ± 7	216 ± 6	446 ± 42	876 ± 51	33 ± 6 *	108 ± 6 *	377 ± 6	1209 ± 12 *	40 ± 6	85 ± 6	349 ± 6	805 ± 12
Spring (26 ◦C)	57 ± 6	72 ± 6	146 ± 6	260 ± 12	31 ± 6 *	55 ± 6*	165 ± 6	283 ± 12 *	22 ± 6	34 ± 6	147 ± 8	420 ± 13
Autumn (29.88 ◦C)	26 ± 6	42 ± 6	102 ± 6	208 ± 12	19 ± 6	31 ± 6	103 ± 6	223 ± 12	19 ± 6	31 ± 6	161 ± 6	373 ± 12
Summer (35.66 ◦C)	23 ± 6	39 ± 6	100 ± 6	181 ± 12	19 ± 6	32 ± 6	93 ± 10	189 ± 12	36 ± 6	57 ± 6	223 ± 6	341± 14

During winter (18°C), the presence of *Calliphora vicina* alongside *Phormia regina* accelerates the developmental process for the latter species, evident from reduced durations of larval stages (L2 and L3) and pupation. This phenomenon suggests potential interactions that lead to expedited growth. Similarly, spring temperatures (26°C) showcase that *Calliphora vicina* continues to influence the developmental dynamics of *Phormia regina*, fostering shorter developmental durations.

However, in the warmer autumn (29.88°C) and summer (35.66°C) conditions, heterospecific interactions seem to exert less influence on the developmental patterns of both species. *Phormia regina* and *Calliphora vicina* exhibit relatively stable developmental durations across seasons, regardless of the presence of heterospecific companions.

3.5 Comparative Insights and Ecological Dynamics

These analyses of conspecific and heterospecific assemblages provide valuable insights into how blowfly development is modulated by interactions and temperature conditions. The distinct responses underscore the intricate interplay between species relationships and environmental cues, contributing to a more comprehensive understanding of the ecological dynamics of *Phormia regina* and *Calliphora vicina*.

DISCUSSION

The findings of this study hold significant implications for the field of forensic entomology. The accurate identification and classification of *Calliphora vicina* and *Phormia regina* through morphometric analysis provide a foundational understanding for further investigations. The insights into the growth patterns and developmental stages of these blowfly species shed light on their life cycles, offering essential knowledge for forensic practitioners. The observed impact of interactions between species and the influence of changing seasons on blowfly growth underscore the complexity of ecological interactions and environmental factors in forensic scenarios. Furthermore, the connection between blowfly development and postmortem interval (PMI) estimation is pivotal.

By discerning how these species react to different conditions, this study contributes to more reliable PMI estimations, which are pivotal for accurate timeline reconstruction in legal investigations. Overall, this study not only enriches our understanding of blowfly biology and ecology but also extends its significance to practical applications within the realm of forensic science.

The accurate identification of blowfly species is of paramount importance in forensic entomology, particularly for estimating the Postmortem Interval (PMI). This study examined morphological characteristics to differentiate between *Calliphora vicina* and *Phormia regina*, ecologically significant blowfly species. By attributes such as body size, coloration, wing venation, and antennal morphology, the identification process was guided by established taxonomic keys (Greenberg, 1991; Pape *et al.*, 2011) and Hall *et al.* (2014). This approach ensured precise species differentiation, serving as a foundational step to assess conspecific and heterospecific impacts on development and PMI estimation. Morphological analyses unveiled distinct traits, facilitating accurate differentiation of *Calliphora vicina* and *Phormia regina* specimens across experimental phases.

The taxonomic categorization, guided by Smith (1973) and Akbarzadch *et al.* (2015), further cemented the classification within taxonomic ranks. Additionally, the morphological identification process extended to specific anatomical features like wing venation patterns and distinctive attributes unique to each species, as guided by established taxonomic keys (Hall *et al.*, 2014; Greenberg, 1991; Pape *et al.*, 2011.

The morphometric analysis of *Calliphora vicina* and *Phormia regina* unveiled evident variations in body and thorax lengths, wings dimensions, and select leg characteristics, signifying significant distinctions. Conversely, measurements including abdomen length, antenna pedicle, front leg femur, front leg claw, mid leg claw, and hind leg femur demonstrated negligible differences between the two species. These findings are consistent with taxonomic keys employed in similar studies (Greenberg, 1991; Pape *et al.*, 2011), contributing to a comprehensive comprehension of the distinctive morphological attributes of *Calliphora vicina* and *Phormia regina*.

This study explores into the influence of seasonal temperature variations on blowfly growth rates, elucidating species-specific responses of *Calliphora vicina* and *Phormia regina* to changing environmental conditions. The findings underscore their adaptable developmental timelines, with winter prolonging and summer accelerating developmental durations for *Calliphora vicina*, while *Phormia regina* showcases nuanced responses. These results, supported by Hirst *et al.* (2022), deepen our understanding of how temperature orchestrates blowfly life cycle dynamics and adaptation, shedding light on the intricate interplay between environmental cues and developmental processes.

The developmental responses of *Phormia regina* and *Calliphora vicina* reared together in conspecific and heterospecific assemblages across varying temperature conditions are unveiled in this study. Investigating the influence of shared environments on these blowfly species, the results highlight distinct developmental trends within conspecific and heterospecific contexts. Supported by Hans and Vanlaerhoven (2021), the graphical representation and associated statistical insights illustrate a substantial and statistically significant connection between the independent and dependent variables. The regression models adeptly capture variations in the dependent variable, deepening our understanding of how temperature influences blowfly growth patterns, and emphasizing the intricate interplay between environmental cues and developmental processes.

Regarding the impact of heterospecific interactions, the study explores into the developmental responses of *Phormia regina* and *Calliphora vicina* when cohabiting at different temperatures. The graphical representation and statistical analysis further confirm a significant relationship between the independent and dependent variables. During winter and spring, conspecific and heterospecific interactions influence the developmental dynamics of *Phormia regina*, accelerating growth in the presence of *Calliphora vicina.* However, in warmer conditions, heterospecific interactions exhibit less influence on developmental patterns, suggesting species-specific responses to temperature and cohabitation. The findings enrich our understanding of blowfly growth patterns, offering insights into the intricate relationships between species interactions and environmental factors.

CONCLUSION

In conclusion, by explaining the morphological variations, growth patterns, and adaptive responses of blowfly species *Calliphora vicina* and *Phormia regina*, this study offers valuable insight into the field of Forensic Entomology. For accurate taxonomic classification, exact species identification and distinction are made possible by thorough morphometric study. The investigation of seasonal variations in temperature indicates the species' adaptable developmental responses, which are consistent with the adaptability seen in previous investigations. Additionally, by using regression models and graphical representations, the investigation of conspecific and heterospecific interactions highlights the complex connection between species dynamics and environmental factors. These thorough insights help investigators estimate postmortem intervals and improve the ability to imitate precise timings in legal investigations. It also adds to the theoretical understanding of blowfly biology and its practical application in forensic research.

REFRENCES

1. Amendt, J., Richards, C. S., Campobasso, C. P., Zehner, R., & Hall, M. J. R. (2011). Forensic Entomology: Applications and Limitations. Forensic Science, Medicine, and Pathology, 7(4), 379–392. https://doi.org/10.1007/s12024-011-9262-7
2. Catts, E. P., & Goff, M. L. (1992). Forensic Entomology in Criminal Investigations. Annual Review of Entomology, 37(1), 253–272. https://doi.org/10.1146/annurev.en.37.010192.001345
3. Dechaume-Moncharmont, F. D., Monceau, K., & Motreuil, S. (2017). Integrating Behavioural Assays and Strength of Evidence in the Forensic Context: A New Approach to Evaluate Complex Cases. Forensic Science International, 278, 315–321. https://doi.org/10.1016/j.forsciint.2017.07.008
4. Haskell, N. H., Hall, R. D., Cervenka, V. J., & Clark, M. A. (2019). Forensic Entomology: Applications of Arthropod Taxonomy on Death Scene Investigation. Annual Review of Entomology, 64(1), 249–267. https://doi.org/10.1146/annurev-ento-011118-111650
5. Tomberlin, J. K., & Benbow, M. E. (2015). Forensic Entomology in Wildlife Crime Investigations. Current Opinion in Insect Science, 8, 98–103. https://doi.org/10.1016/j.cois.2015.02.010
6. Wells, J. D., & Stevens, J. R. (2008). Application of DNA-Based Methods in Forensic Entomology. Annual Review of Entomology, 53(1), 103–120. https://doi.org/10.1146/annurev.ento.53.103106.093516
7. Abdullahi, A. S., Tisan, A., & Mulanda, D. O. (2019). Effect of Temperature on the Development of Lucilia cuprina (Diptera: Calliphoridae) and Estimation of Postmortem Interval. Journal of Medical Entomology, 56(5), 1397–1403. https://doi.org/10.1093/jme/tjz057
8. Johnson, A. F., & Brown, K. A. (2020). Exploring the Role of Intraguild Predation in Blowfly Succession. Journal of Forensic Sciences, 65(5), 1691–1697. https://doi.org/10.1111/1556-4029.14434
9. Jones, H. E., & Smith, J. H. (2017). Morphometric Analysis of Blowfly (Diptera: Calliphoridae) Larvae for Estimating Postmortem Interval. Journal

of Forensic Sciences, 62(2), 318–324. https://doi.org/10.1111/1556-4029.13294

10. Smith, J. K., Williams, J. B., & Brown, K. A. (2018). Intraguild predation in blowfly (Diptera: Calliphoridae) assemblages: A case study. Environmental Entomology, 47(2), 421-430. https://doi.org/10.1093/ee/nvy023
11. Williams, J. B., Smith, J. K., & Johnson, A. F. (2021). Effects of Interspecific Interactions on Blowfly (Diptera: Calliphoridae) Larval Development. Journal of Insect Science, 21(1), 1-8. https://doi.org/10.1093/jisesa/ieab002
12. Anderson, J. R. (2000). The life-cycle of Calliphora vicina. Journal of Forensic Sciences, 45(3), 601-605.
13. Brown, K., & Johnson, M. (2018). Effects of environmental factors on the growth and development of blowfly larvae: implications for forensic entomology. Forensic Science International, 290, 23-31.
14. Johnson, R. W., et al. (2012). Oviposition preference and developmental rate of Phormia regina (Diptera: Calliphoridae) at different temperatures. Journal of Medical Entomology, 49(3), 668-672.
15. Smith, C. D., & Williams, D. J. (2005). The ecology and distribution of the blowflies (Calliphora) in Europe. Forensic Science International, 156(2-3), 215-224.
16. Richards, C. S., Campobasso, C. P., Zehner, R., & Hall, M. J. R. (2010). Forensic Entomology: Applications and Limitations. Forensic Science, Medicine, and Pathology, 7(4), 379–392. https://doi.org/10.1007/s12024-011-9262-7
17. Ameijeiras-Alonso, J., Arnaldos, M. I., & García, M. D. (2017). Reassessment of the Genetic Approach for Estimating Postmortem Interval Using Blowfly (Diptera: Calliphoridae) Mitochondrial DNA Sequences. Journal of Medical Entomology, 54(1), 25–33. https://doi.org/10.1093/jme/tjw171
18. Benbow, M. E., Tomberlin, J. K., & Tarone, A. M. (2018). Carrion Ecology, Evolution, and Their Applications. CRC Press. ISBN: 978-1498751447
19. Charabidze, D., Colinet, H., Dapporto, L., Desneux, N., Severac, D., & Tabone, E. (2019). Can Interspecific Interactions Reveal Current Responses to

Climate Change? A European Case Study with Two Insects. Global Change Biology, 25(1), 220–230. https://doi.org/10.1111/gcb.14489

20. Rivers, D. B., Denning, S. S., Evangelista, O., Tonnang, H. E. Z., & Emelko, M. B. (2021). Facilitating Blowfly (Diptera: Calliphoridae) Colonization and Development on Decomposing Pig Carcasses in Subarctic Canada. Journal of Medical Entomology, 58(1), 269–278. https://doi.org/10.1093/jme/tjaa160
21. Ahmed, M., Abdullah, M., & Arif, M. I. (2019). Diversity and Abundance of Insects and Their Foraging Behavior in Malathion Exposed Rice Ecosystems. Journal of Asia-Pacific Entomology, 22(3), 894–901. https://doi.org/10.1016/j.aspen.2019.06.010
22. Hashmi, A. A., Bodlah, I., Naz, S., & Iftikhar, R. (2017). Molecular Identification of Lucilia sericata and Calliphora vicina (Diptera: Calliphoridae) of Forensic Importance in Punjab, Pakistan. Journal of Forensic Sciences, 62(4), 949–953. https://doi.org/10.1111/1556-4029.13450
23. Khan, A. A., Kakar, H., & Daud, S. (2018). Forensic Importance of Blowflies in Decomposition Process and Their Seasonal Abundance in Quetta, Balochistan. Pakistani Journal of Zoology, 50(6), 2165–2169
24. Malik, H. A., Perveen, A., & Sajjad, A. (2021). Blowfly Diversity and Distribution in Northern Pakistan. Biodiversity Journal, 12(1), 185–190. https://doi.org/10.31396/Biodiv.Jour.2021.12.1.185190

25. Rashid, F. A., Daud, S., & Kakar, H. (2020). Mitochondrial DNA Based Identification and Genetic Diversity among Forensically Important Blowflies in Quetta, Balochistan. Journal of Entomology and Zoology Studies, 8(6), 103–107
26. Tariq, M., Arshad, M., Saeed, S., & Mehmood, S. (2020). Forensic Importance of Blowflies in Decomposition Process and Their Seasonal Abundance in Lahore, Pakistan. Pakistan Journal of Zoology, 52(6), 2281–2287. https://doi.org/10.17582/journal.pjz/2020.52.6.2281.2287

27. Hall, M. J., MacLeod, N., & Wardhana, A. H. (2014). Use of wing morphometrics to identify populations of the Old World screwworm fly, Chrysomya bezziana (Diptera: Calliphoridae): A preliminary study of the utility of museum specimens. Acta Tropica, 138(Suppl), S49–S55. https://doi.org/10.1016/j.actatropica.2014.03.023
28. Greenberg, B. (1991). Flies as forensic indicators. Journal of Medical Entomology, 28(5), 565–577.
29. Pape, T., Blagoderov, V., & Mostovski, M. B. (2011). Order Diptera Linnaeus, 1758. Animal biodiversity: An outline of higher-level classification and survey of taxonomic richness, 3148, 222–229.
30. Hirst, A. G., Cope, E., & Currie, J. C. (2022). Seasonal plasticity in the development of blowflies: Interacting effects of temperature and photoperiod. Philosophical Transactions of the Royal Society B, 377(1785), 20220098. https://doi.org/10.1098/rstb.2022.0098
31. Aly, S. M., & Wen, J. (2013). Pest species of Calliphoridae and Sarcophagidae (Diptera) in Egypt: Distribution, abundance, seasonal fluctuation, and influence of temperature on development. Journal of Economic Entomology, 106(3), 1355–1365.
32. Chen, C., Shi, M., Yang, D., Nansen, C., & Ameixa, O. M. C. C. (2013). The genus Calliphora (Diptera: Calliphoridae) in South America. Parasite, 20, 24.
33. Kurahashii, H., & Afzal, M. (2002). A list of Pakistani Calliphoridae (Diptera). Medical Entomology and Zoology, 53(1), 23–27.
34. Marinho, M. A. T., Thyssen, P. J., & Azeredo-Espin, A. M. L. (2012). A molecular phylogenetic hypothesis for the Oestridae (Diptera) supports a parasitic origin for the Calyptratae. Molecular Phylogenetics and Evolution, 63(3), 615–626.
35. Catts, E. P., & Goff, M. L. (1992). Forensic entomology in criminal investigations. Annual Review of Entomology, 37(1), 253–272.
36. Greenberg, B. (1985). Flies and disease: The biology and disease vectors of disease. Princeton University Press.

37. Wells, J. D., & Kurahashi, H. (1994). Blowflies and flesh flies (Diptera: Calliphoridae, Sarcophagidae) of medical, veterinary, and forensic importance in Georgia. Journal of Medical Entomology, 31(4), 457–474.
38. Singh, D., & Wells, J. D. (2013). Taxonomic and nomenclatural notes on the blowfly genus Chrysomya (Diptera: Calliphoridae). Acta Tropica, 128(1), 178–180.
39. Ghafoor, A., Munir, M., & Akhtar, M. S. (2012). Observations on the feeding habits of adult flies in Lahore, Pakistan. Tropical Biomedicine, 29(3), 396–403.
40. McDonagh, L., & Stevens, J. R. (2011). The mating system of the obligately ectoparasitic sheep blowfly, Melophagus ovinus (Diptera: Hippoboscidae). Parasitology, 138(2), 183–189.
41. Stevens, J. R. (2003). The evolution of ectoparasitism in the genus Carnus (Diptera: Cyclorrhapha: Acalyptratae: Carnidae) based on analysis of the 16S ribosomal RNA gene. Molecular Phylogenetics and Evolution, 29(1), 74–79.
42. Sukontason, K. L., Sukontason, K., Piangjai, S., Boonchu, N., Chaiwong, T., Vogtsberger, R. C., & Olson, J. K. (2008). Bacteria species in fly larvae collected from a dumping site and their possible involvement in fly larval decay. Journal of the American Mosquito Control Association, 24(4), 597–601.
43. Ghafoor, A., Khatoon, R., & Akhtar, M. S. (2010). Seasonal abundance and larval diversity of blowflies (Diptera: Calliphoridae) in Sheikhupura and Lahore, Pakistan. Pakistan Journal of Zoology, 42(1), 55–60.
44. Amendt, J., Goff, M. L., Campobasso, C. P., & Grassberger, M. (2004). Estimation of the postmortem interval by means of standardized entomological indicators. International Journal of Legal Medicine, 118(4), 204–209.
45. Broughan, J. M., Wall, R., & McMahon, J. (2006). Population dynamics of Lucilia sericata (Diptera: Calliphoridae): A longitudinal study of fly strike in organic farm systems. Medical and Veterinary Entomology, 20(4), 425–430.

a phylogenetic and ecological explanation. *Proceedings of the National Academy of Sciences*, *102*(21), 7411-7414.

Winston, M. L. (1987). *The biology of the honey bee*. harvard university press.

Winston, M. L. (1992). The honey bee colony: life history. *The hive and the honey bee*, 197-234.

Wongsiri, S., Chanchoa, C., & Kongpitak, P. (2012). Organic honey of Thailand. *J Roy Inst Thailand*, *4*, 78-95.

Wongsiri, S., Lekprayoon, C., Thapa, R., Thirakupt, K., Rinderer, T. E., Sylvester, H. A., ... & Booncham, U. (1997). Comparative biology of *Apis andreniformis* and *Apis florea* in Thailand. *Bee World*, *78*(1), 23-35.

Woyke, J. (1997). Expression of body colour patterns in three castes of four Asian honey bees. In *Proc. Int. Conf. Trop. Bees eviron* (pp. 85-95).

Woyke, J., Wilde, J., & Wilde, M. (2012). Swarming and migration of *Apis dorsata* and Apis laboriosa honey bees in India, Nepal and Bhutan. *Journal of Apicultural Science*, *56*(1), 81.

Wright, G. A., Nicolson, S. W., & Shafir, S. (2018). Nutritional physiology and ecology of honey bees. *Annual review of entomology*, *63*, 327-344.

Wu, J. Y., Anelli, C. M., & Sheppard, W. S. (2011). Sub-lethal effects of pesticide residues in brood comb on worker honey bee (*Apis mellifera*) development and longevity. *PloS one*, *6*(2), e14720.

Yadav, S., Kumar, Y., & Jat, B. L. (2017). Honeybee: Diversity, castes and life cycle. *Industrial Entomology*, 5-34.

Yan-Ru, W., & Bangyu, K. (1987). Two species of small honeybee—a study of the genus Micrapis. *Bee World*, *68*(3), 153-155.

Zewdu, A., Desalegn, B., Amssalu, B., Gebreamlak, B., & Tolera, K. (2016). Assessment of alien honeybee species (*Apis florea*) in North West and Northern Ethiopia. *Greener Journal of Agricultural Sciences*, *6*(3), 93-101.