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Forensic and ecological perspectives on insect succession on vertebrate remains

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Abstract

Entomological evidence is commonly used to estimate a post-mortem interval (PMI) in medicolegal investigations of deceased individuals. A PMI from insect-derived data can be estimated by either examining the thermal development rates of larvae or analysing the carrion insect succession process. The larval development method is well-established and reliable, while the succession method is less reliable as it depends on the predictable sequences of species arriving at a cadaver, which is a highly variable process. The effect of abiotic factors such as temperature and season on succession have been well documented, however the role of biotic factors has received far less attention. For the succession method to be reliably applied to forensic casework, a complete knowledge of all factors driving successional changes in insect communities needs to be known to identify and understand sources of variation.

Parallel to developments in forensic science, carrion ecologists have begun to quantify the biological sources of variation in carrion insect succession and identify the role carrion plays in ecosystem function. Importantly, the carrion resource and associated necrobiome have been identified as important ecological drivers of variation in carrion insect succession. Yet these ecological approaches and techniques have yet to be transferred to forensic entomology. For example, pigs are often used as substitutes for human cadavers in forensic entomology despite the relatively unknown effect of cadaver type on carrion insect succession. There is great potential, therefore, to examine how developments in ecology might be used to advance forensic entomology.

This thesis aims to learn from carrion ecology to advance forensic entomology by exploring ecological perspectives on how biotic factors such as cadaver type, carrion resource and species interactions drive variation in carrion insect succession. To do this, I conducted an innovative, multi-season experiment using pig and human cadavers at the Australian Facility for Taphonomic Experimental Research (AFTER).

First, I conducted an experiment comparing broad decomposition and entomological differences between pigs and humans (Chapter 2). I found that pigs generally decomposed faster, with insects arriving earlier and in higher abundance. This suggested that pigs may not be ideal proxies for humans in forensic entomology, and a correction factor may need to be developed to reliably apply pig-derived data to human cadavers in casework. This experiment also identified several important forensic and ecological questions that I answered throughout the rest of the thesis.

I further explored decomposition differences between pigs and humans by comparing mass loss, a decomposition metric seldom used in forensic entomology (Chapter 3). Mass loss was more rapid for pigs during early decomposition, and by the end of the experiments pigs had lost more mass than humans. I also compared mass loss to total body score (TBS) and recognized mass loss was able to provide additional information during advanced decay which was not evident when relying solely on TBS. This highlights the usefulness of combining decomposition metrics in forensic research for modelling decomposition progress. I extended my forensic approach by examining Diptera succession on human cadavers in more detail by comparing previous casework data to my field succession experiments (Chapter 4). I found carrion breeding Diptera did not oviposit on human cadavers at AFTER until day two or three, which should be taken into consideration when determining a PMI from insect-derived data.

I next took an ecological approach to determine what factors are driving carrion insect succession (Chapter 5). I used TBS as a measure of carrion resource change and examined how TBS and a combination of other factors drive changes in insect composition on carrion. I identified carrion resource change to be a key driver of species turnover, and highlight the usefulness of TBS as a semi-continuous measure enabling succession to be examined as a continuum. To complement this finding, I also examined the role of carrion resource quality, represented by TBS, in driving individual species abundance patterns (Chapter 6). I found that abundance patterns differed, as some species were abundant throughout decomposition, while others varied with abundance spikes and narrow windows of resource exploitation. Abundance data, unlike species occurrence data, can reveal greater nuance about species exploitation of carrion. Finally, I investigated how priority effects and larval density promote co-existence between two competing blowfly species, the facultative predator *Chrysomya rufifacies* and its competitor *Calliphora stygia* (Chapter 7). I found the species were either constrained by larval density requirements (*Ch. rufifacies*) or priority effects (*C. stygia*), thereby enabling the two species to coexist and share broadly similar niches.

A major conclusion from my research is the important role the carrion resource and associated necrobiome has in driving variation in carrion insect succession. I have linked insect activity with key aspects of the necrobiome and displayed the general applicability of the associated framework in understanding the complexities of decomposition and carrion insect succession. Integrating other aspects of the necrobiome, such as a range of other cadaver types and microbial activity, into future forensic entomology research will continue to increase our understanding of carrion insect succession.

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I am extremely grateful to my PhD supervisors Professor James Wallman, Dr Philip Barton and Dr Bethany Hoye. James, for the last five years you have been a pillar of support, providing continuous guidance and facilitating my transition from an undergraduate student to a knowledgeable scientist. Thank you for introducing me to the fascinating world of insects and for finding the time to consistently provide edits and feedback. I am truly thankful for all you have taught me. Philip, you have been a wonderful fountain of information and I have been constantly impressed with your breadth of knowledge about ecological patterns in nature. I hope you have enjoyed our many discussions about science and ecology as much as I have. Thank you for always guiding me in the right direction. Bethany, I am grateful for your ongoing encouragement, advice and support. I look forward to our future collaborations.

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Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university of other tertiary institutions by Blake M. Dawson and, to the best of my knowledge and belief, contains no material previously published or written by any other person, expect where due reference has been made in the text. I give consent to this copy of my thesis when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968. The author acknowledges that copyright of published works contained within this thesis (as listed below) resides with the copyright holder (s) of those works. I also give permission for the digital version of my thesis to be made available via the University's digital research repository, the Library catalogue, the Australasian Digital Theses Program (ADTP) and also through web search engines, unless permission has been granted by the University to restrict access. This thesis contains six data chapters, each written as a manuscript for publication, and therefore intended to stand alone. Some information, particularly the materials and methods may be repetitive between chapters and formatting may differ, especially referencing and spelling as they adopt the requirements of their respective journals. While the candidate made substantial contributions to the manuscripts and is fully responsible for the work presented in this thesis, where the first person is used in the manuscripts it is used in the plural ('we') to reflect contributions from co- authors. Professor James F. Wallman and Dr Philip Barton helped conceive each study, were involved in project design and contributed to the writing of manuscripts in this thesis. Dr Maldwyn Evans also assisted with data analysis and Dr Nathan Butterworth assisted with laboratory experimental design. Unless stated otherwise, all data presented in this thesis were collected during the period of PhD candidature.

Certifications

I, Blake Dawson, declare that this thesis submitted in fulfilment of the requirements for the conferral of the degree Doctor of Philosophy, from the University of Wollongong, is wholly my own work unless otherwise referenced or acknowledged. This document has not been submitted for qualifications at any other academic institution.

_____

Blake M. Dawson
Date: 03/12/21

List of publications included as part of this thesis

Paper	Chapter	Status	Journal
Dawson, BM, Barton, PS, Wallman, JF (2020) Contrasting insect activity and decomposition of pigs and humans in an Australian environment: A preliminary study. *Forensic Science International* 316: 110515.	2	Published	*Forensic Science International*
Dawson, BM, Wallman, JF, Barton, PS How does mass loss compare with total body score when assessing decomposition of human and pig cadavers?	3	In review	*Forensic Science International*
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Dawson, BM, Wallman, JF, Evans, MJ, Barton, PS. Abundance patterns reveal how insects perceive carrion resource quality on vertebrate remains.	6	Revisions received	*Oecologia*
Dawson, BM, Wallman, JF, Evans, MJ, Butterworth, NJ, Barton, PS. Priority effects and density promote coexistence between the facultative predator *Chrysomya rufifacies* and its competitor *Calliphora stygia*.	7	In review	*Oecologia*

Statement of candidate contribution

As the primary supervisor, I, Professor James Wallman, declare that the greater part of the work in each article is attributed to the candidate, Blake M. Dawson. In each of the papers that constitute this thesis, Blake led conceptual development, study design and was primarily responsible for the data collection, data analysis, and data interpretation. The first draft of each manuscript was written by the candidate, who was then responsible for responding to the editing suggestions of his co-authors. The co-authors were responsible for assisting with the study design, data analysis, interpreting data, and editing of manuscripts where necessary.

_____

Professor James Wallman

Principal Supervisor

Date: 03/12/2021

Additional publications and conferences

In addition to the manuscripts listed above, during the course of my PhD I have co-authored two journal articles and presented data from my thesis at two international and three domestic conferences.

Additional publications

Paper	Journal
Barton, PS, Reboldi, A, **Dawson, BM,** Ueland, M, Strong, C, Wallman, JF (2020) Soil chemical markers distinguishing human and pig decomposition islands: a preliminary study. *Forensic Science, Medicine and Pathology* 16: 605-612.	*Forensic Science, Medicine and Pathology*
Barton, PS, **Dawson, BM,** Barton, AF, Joshua, S, Wallman, JF (2021) Temperature dynamics in different body regions of decomposing vertebrate remains. *Forensic Science International* 325: 110900.	*Forensic Science International*

Conference presentations

Presentation	Conference
Dawson, BM, Barton, PS, Wallman, JF (2020) Contrasting insect activity and decomposition of pigs and humans in an Australian environment.	Digital World Congress of Forensic Entomology
Dawson, BM, Barton, PS, Evan, MJ, Wallman, JF (2020) Is carrion resource quality a useful predictor of insect succession on pigs and humans?	UOW postgraduate conference. Online
Dawson, BM, Barton, PS, Wallman, JF (2019) Contrasting insect activity and decomposition of pigs and humans in an Australian environment. Awarded Best Abstract	UOW postgraduate conference. Kioloa, Australia
Dawson, BM, Barton, PS, Wallman, JF (2018) Are flies fussy? Comparing fly assemblages attracted to dead pigs and humans.	9th International Congress of Dipterology. Windhoek, Namibia.
Dawson, BM, Barton, PS, Wallman, JF (2018) Are insects fussy? Comparing insect assemblages attracted to dead pigs and humans. Awarded Best Initial Seminar	UOW postgraduate conference. Kioloa, Australia

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Chapter 1. General introduction

1.1 Preamble

For carrion ecologists and forensic entomologists, the process of succession in carrion is fundamental to understanding the insect communities associated with this limited resource. The temporal patterns exhibited by insects during succession can enable researchers to determine the critical role carrion plays in ecosystem function and potentially assist forensic casework. Despite analysing the same insect community patterns, the approaches taken in carrion ecology and forensic entomology differ widely. Combining research in these fields can bring their methodologies closer together and thereby benefit forensic investigations.

1.2 Ecological succession

Succession is a natural process in which the community of organisms colonising a resource shifts over time (Sousa, 1979). The process is widespread and varies both temporally, from hours to decades, and spatially, from a single piece of dung to entire forests (McCook, 1994). Succession can be either primary, occurring on a resource that has not previously been colonised, or secondary, occurring after a disturbance event removes the pre-existing community from a resource, enabling new species to colonise (Prach & Walker, 2019). The successional process follows the general pattern of a pioneer species arriving first at a resource, followed by the arrival of mid- and eventually, late-stage colonisers. In many systems, succession will lead to a climax community, where species changes no longer occur at the community level, unless another disturbance event transpires (Clements, 1916). Ecological succession was first described in terrestrial plant communities on sand dunes (Cowles, 1899), and since then, plant communities have been fundamental to the development of successional research (Chang & Turner, 2019). Three mechanistic models of succession have been proposed and established from observations based on plant communities: facilitation, tolerance and inhibition (Connell & Slatyer, 1977).

- Facilitation suggests that pioneer species will colonise a new resource that is less hospitable to other species. Once established, these species alter the local environment making it more suitable for mid and late stage successional species. After the arrival of later species, competition will generally result in the loss of the original pioneer species.
- In the tolerance model, all species are able to colonise a resource at the same time, resulting in a 'free for all', where environmental conditions are favourable to all species from the onset of colonisation. From the beginning, all species thrive when space and resources are not limiting. However, over time as density increases, high levels of interspecific competition and other biotic factors result in only those competitively superior species surviving and dominating the community.
- Inhibition, like tolerance, involves all species having the ability to colonise a resource at the same time. However, by contrast particular pioneer species can alter the surrounding local environment to make it less hospitable, preventing other species from establishing. These pioneer species eventually die off, providing an opportunity for other species to colonise the resource.

These models, although relatively simple, provide a mechanistic approach to analysing the successional process in natural systems. Succession is highly variable and may not always follow these distinct models, particularly when succession occurs in non-plant based systems (Walker & Del Moral, 2003). This is particularly relevant for succession on ephemeral resources – those that are limited, patchy and unpredictable, such as woody debris (Weslien *et al.*, 2011), dung (Sladecek *et al.*, 2013) and small water bodies (Vindstad *et al.*, 2020). Due to the finite nature of ephemeral resources, succession can never reach a climax community, and therefore may not follow the traditional models of succession developed by plant ecologists. Of all such ephemeral resources, carrion – the decomposing remains of dead animals – has been a favoured model system for descriptive studies of the successional process (Michaud *et al.*, 2015). Yet few studies have applied the mechanistic models of succession to carrion or attempted to explain the mechanisms driving succession on ephemeral resources (Michaud & Moreau, 2017).

1.3 Carrion insect succession

Despite its limited nature, carrion is able to host a diverse assemblage of species from a wide range of taxa, including vertebrates, invertebrates and microbial organisms, all of which make

up the necrobiome (Benbow *et al.*, 2019). Of these species, it has been well documented that insects (Anderson & VanLaerhoven, 1996), and to a lesser extent, microbes (Pechal *et al.*, 2014b) follow a predictable successional pattern. Research first describing temporal patterns of carrion insects originally focused on distinct decay stages: i.e., fresh, bloat, active decay, advanced decay and skeletonisation (sensu Payne, 1965). However, this 'broad-brush' approach does not accurately reflect the continuous changes occurring on carrion, with research now focused on analysing species turnover as a continuum (Schoenly & Reid, 1987; Michaud *et al.*, 2015). Carrion insect succession is a complex process and difficult to quantify as it involves several different orders of species using carrion for a range of functional purposes (Schoenly *et al.*, 1996).

1.3.1 Trophic structure

Moments after death, carrion begins to break down via microbial activity and then releases volatile organic compounds (VOCs) into the local environment (Pascual *et al.*, 2017). Carrion insects are able to detect VOCs using highly specialised chemoreceptors, and in some instances, may arrive at carrion within minutes after death (Dekeirsschieter *et al.*, 2009). This is common for necrophagous insect species, those which feed directly off the decomposing remains (Frederickx *et al.*, 2012). The most dominant necrophages are blow flies (Calliphoridae) (Fig. 1) and flesh flies (Sarcophagidae), which will arrive at fresh carrion to oviposit eggs or larvae (Smith, 1986). The larvae of these species will quickly colonise and consume the soft tissue of carrion, often forming extremely large maggot masses, which facilitates faster growth rates via thermal dynamics and collective exodigestive behaviour (Scanvion *et al.*, 2018; Charabidze *et al.*, 2021).

Figure 1 Necrophagous calliphorid flies on carrion.

Not all necrophages will arrive moments after death, as other Diptera species like cheese skippers (Piophilidae) and scuttle flies (Phoridae) do not oviposit until late in the decomposition process. These species feed off older carrion once it is in a more favourable nutritional state; for example, piophilid larvae cannot develop on carrion until fatty acids are present (Martín-Vega, 2011). Necrophages not only consist of Diptera, but also a number of Coleoptera species, such as hide beetles (Trogidae) and burying beetles (Silphidae) that arrive at varying times depending on their nutritional requirements (Horenstein & Linhares, 2011; Martín-Vega & Baz, 2012).

The large influx of necrophagous insects to carrion acts as an additional food resource, attracting a number of predators and parasites. Many of these species prey upon or parasitise Diptera larvae as they are the most abundant insects on carrion with little defence against predation (Braack, 1987). Therefore, predators and parasites do not arrive at carrion until early arriving necrophages have colonised and established themselves (Smith, 1986). Predators and parasites comprise a number of insect orders, but most commonly Coleoptera, such as rove beetles (Staphylinidae) and clown beetles (Histeridae), as well as Hymenoptera, such as parasitic wasps (e,g. Pteromalidae) (Voss *et al.*, 2009; Daria *et al.*, 2011; Mądra-Bielewicz *et al.*, 2017). Some omnivorous species feed off both the carrion directly and prey upon other

insects (Villet, 2011). These species often display a generalist feeding strategy and, due to their broad diet, do not necessarily demonstrate a predictable temporal association with carrion (Barton & Evans, 2017). Despite this, omnivores can have profound effects on the succession process. For example, ants (Formicidae) either feed on the epidermal tissue of carrion or on dipteran eggs, and if they arrive at carrion quickly enough in high abundance, can prevent necrophages from colonising, thereby disrupting or delaying the successional process (Barton *et al.*, 2017; Eubanks *et al.*, 2019). Numerous other species also take advantage of carrion, either as a food source or as habitat structure. These species are termed incidentals and are not dependent on carrion for their survival, but rather use carrion as an extension of their own habitat and therefore display no predictable temporal patterns (Smith, 1986).

1.4 Forensic application

Due to their close association with death, carrion insects, and in particular, necrophages, have become helpful tools in criminal investigations. Insects can provide information about corpse relocation, food contamination and mistreatment of both humans and other animals (Catts & Goff, 1992). Most importantly, insects can provide crucial information about the time of death, known commonly as the postmortem interval (PMI), which is the minimum (minPMI) and maximum (maxPMI) amount of time for which an individual has been deceased (Sharma *et al.*, 2015). Generally, only a minPMI can be inferred from insect data, though in some cases a maxPMI can also be determined if enough biological information about a species is known, such as the pre-appearance interval (PAI) or nocturnal oviposition preference (Wells, 2019).

A minPMI can be estimated from insect data either by using larval development rates or succession data. For larval development rates, insect larvae feeding on a cadaver are collected and their age determined. Diptera larvae are primarily used for minPMI estimation from development rates, but Coleoptera larvae can also be employed (Ridgeway *et al.*, 2013). Larval age is often determined using the thermal summation model, which incorporates growth rates under different constant temperatures, although other methods also exist (Villet *et al.*, 2009). Once the temperature at a crime scene has been calculated, it can be compared to the thermal development models to estimate growth rates, with the age of the oldest specimens collected from a cadaver representing the minPMI (VanLaerhoven, 2008). This method has been improved and refined over the years, with detailed developmental datasets available for multiple species (Nabity *et al.*, 2006; Richards *et al.*, 2008; Day *et al.*, 2021; Wang *et al.*, 2021). The larval development method is generally only applicable to the first few weeks of

decomposition while the first generation of primary colonising Diptera larvae and pupae are still developing (Amendt *et al.*, 2004).

The second method of determining a PMI from insect data, and the main focus of this thesis, is using the predictable temporal pattern of carrion insects colonising a cadaver (Goff & Flynn, 1991). Originally, simple descriptive succession tables were constructed to display species composition changes across decay stages; however, more complex models of succession have been developed to improve accuracy (Michaud & Moreau, 2009), although their reliability has been questioned and their applicability requires further research (LaMotte & Wells, 2017; Moreau & Michaud, 2017). Unlike the age development method, the succession method can be used to estimate a PMI several weeks to months after death, although the accuracy in older cadavers decreases as species community changes occur less frequently (Schoenly *et al.*, 1996). For accurate PMI estimation, an extensive record of carrion-associated taxa in the local environment in which a cadaver has been located is required (Gelderman *et al.*, 2021). Carrion insect succession is also a highly variable process influenced by several abiotic and biotic factors. The effects of a number of these factors have already been well documented, particularly environmental factors such as temperature (Archer, 2003), habitat (Matuszewski *et al.*, 2011), season (Benbow *et al.*, 2013), clothing (Matuszewski *et al.*, 2016), shading (Sharanowski *et al.*, 2018) and burial (Pastula & Merritt, 2013). The influence of biotic factors on the succession process has received less attention, most likely due to the complex nature of these factors and the difficulty in quantifying them. These biotic factors can include various species interactions within the necrobiome, such as competition, or factors relating to the cadaver itself, such as the cadaver type and associated microbiome (Benbow *et al.*, 2019). Identifying how these biological factors influence the succession process is key to incorporating them into PMI estimates to reduce error and uncertainty in future casework (Tomberlin *et al.*, 2011).

1.5 Biological sources of error in PMI estimates based on succession data

Cadaver type is an important biological factor that may be influencing carrion insect succession patterns. In a forensic context, this is of particular importance as most succession research has been conducted on pigs due to the ethical and logistical constraints of using human cadavers (Tomberlin *et al.*, 2012). Initially, it was shown that decomposing pigs display similar insect succession patterns to humans due to similar physiological and anatomical structure (Schoenly *et al.*, 2007). However, the authors did not assess PMI prediction performance or compare

between seasons. More recent research has identified differences in the decomposition pattern between pigs and humans (Connor *et al.*, 2017; Dautartas *et al.*, 2018), as well as differences in VOCs emitted during decomposition (Knobel *et al.*, 2019). Due to the insect succession being highly dependent on the decomposition progress, and important role VOCs play in the attraction of insects to decomposing remains, it can be hypothesised that the succession process may indeed differ between the two cadaver types (Benbow *et al.*, 2013; Pechal *et al.*, 2013). Despite these apparent differences, pigs are still useful in forensic research for testing new methods and protocols (Matuszewski *et al.*, 2020). However, before applying any data from pigs to casework, results must first be validated on human cadavers due to the current uncertainty of pigs as a suitable model for humans (Miles *et al.*, 2020).

With progression of decomposition not just varying between pigs and humans but also between other vertebrate species, it can be assumed that the carrion itself plays a crucial role in the decomposition process and subsequent insect succession (Watson & Carlton, 2005; Parmenter & MacMahon, 2009). Decomposition is a complex process driven in part by vertebrate and invertebrate scavengers, as well as microbiota consuming the remains (Barton & Bump, 2019). However, as carrion decomposes, the remains shift in both quantity (mass loss) and quality (nutritional value and digestibility) over time, thereby driving community changes in the species consuming the remains (Benbow *et al.*, 2019). For insects, some species prefer fresh remains, and once the remains shift in quality and are no longer in an optimal state, they depart and are replaced by species that prefer older carrion. Therefore, decomposition is both driving and driven by entomological activity, making it a complex process to disentangle. This interaction between the carrion and necrobiome has begun to be explored in carrion ecology, with some researchers identifying the need to examine the physical and molecular changes occurring on carrion in finer detail (Schoenly & Reid, 1987; Benbow *et al.*, 2019). More research is still needed on the intricacies of decomposition and carrion insect succession, particularly in a forensic context (Tomberlin *et al.*, 2011).

Species interactions between insects on carrion and the mechanisms allowing co-existence to occur is another important factor influencing the succession process that is often overlooked in forensic research. Interspecific competition is a common interaction on carrion due to the high species diversity and limited resources available (Charabidze *et al.*, 2021). Competition can often lead to temporal partitioning as species alter their behaviour to survive against competitively dominant conspecifics, thereby allowing co-existence between species sharing broadly similar niches (Hanski, 1987; Ives, 1991). For example, some blowfly species are unable to survive in the presence of the competitively dominant blowfly *Chrysomya*

rufifacies, which exhibits facultative predatory behaviour once it has reached the third instar (Baumgartner, 1993). Therefore, other blowfly species have adapted to arriving early at carrion before *Ch. rufifacies* has had time to colonise and develop (Brundage *et al.*, 2014). Research has also shown interspecific competition to have the potential to alter species development rates (Swiger *et al.*, 2014; Carmo *et al.*, 2018). However, species interactions can also be favourable, since increased growth rates may be facilitated among larvae that aggregate together, despite the increased interspecific and intraspecific competition (Charabidze *et al.*, 2021).

Biological sources of variation in carrion insect succession have begun to be investigated by carrion ecologists interested in quantifying the successional process, to identify the role carrion plays in maintaining biodiversity and ecosystem function (Baton *et al.*, 2013; Pechal *et al.*, 2014a; Barton & Evan, 2017; Benbow *et al.*, 2019). Currently the two fields of carrion research (carrion ecologists and forensic entomologists) often employ different approaches, with carrion ecologists focusing on quantifying the ecological drivers of insect succession, and forensic entomologists focusing on identifying general patterns in insect succession (Tomberlin *et al.*, 2011; Michaud *et al.*, 2015). Better integration of the two fields would be beneficial for forensic entomologists, as understanding the mechanisms driving carrion insect succession can only improve the reliability and accuracy of PMI estimates derived from insect data.

1.6 Thesis objectives and structure

In this thesis, I aim to bridge forensic and ecological disciplines to explore how cadaver type, the carrion resource and species interactions are driving variation in carrion insect succession. By understanding the ecological underpinnings of succession, forensic investigations can be enhanced by reducing sources of error and variation in estimating the PMI.

To meet this aim, I primarily conducted a multi-season field experiment using pig and human cadavers at the Australian Facility for Taphonomic Experimental Research (AFTER) (Appendix Table S1). The facility is the first of its kind in the southern hemisphere, allowing for the decomposition progress of human cadavers to be observed in a natural environment (Fig. 2). Specifically, I determined the effect of cadaver type and the carrion resource on the community composition and abundance of carrion insects. In a forensic context, I investigated different methods of modelling decomposition and provided forensically relevant information about carrion-associated Diptera. I also complemented my fieldwork with laboratory

experiments to examine interspecific competition and its role in shaping succession and coexistence on carrion.

Figure 2 Outside (A) and inside (B) the Australian Facility for Taphonomic Experimental Research (AFTER), Sydney, NSW.

Chapter 2 details a preliminary study on the effect of cadaver type on broad decomposition patterns and entomological activity. Here I challenged the long-held assumption that pigs are accurate proxies for human cadavers in forensic entomological and taphonomic

research. This chapter identifies important questions that I answer throughout the thesis with two parallel investigation topics: forensic and ecological aspects (Fig. 3).

Forensic aspects:

Chapter 3 builds upon the previous chapter by analysing decomposition patterns on pigs and humans in further detail. Here I examined decomposition patterns between pigs and humans by using mass loss to model decomposition. Mass loss is commonly used as a direct measure of decomposition in carrion ecology but seldom used in a forensic context. I also compared mass loss with other common decomposition metrics, such as total body score (TBS) to determine the usefulness of mass loss for forensic casework.

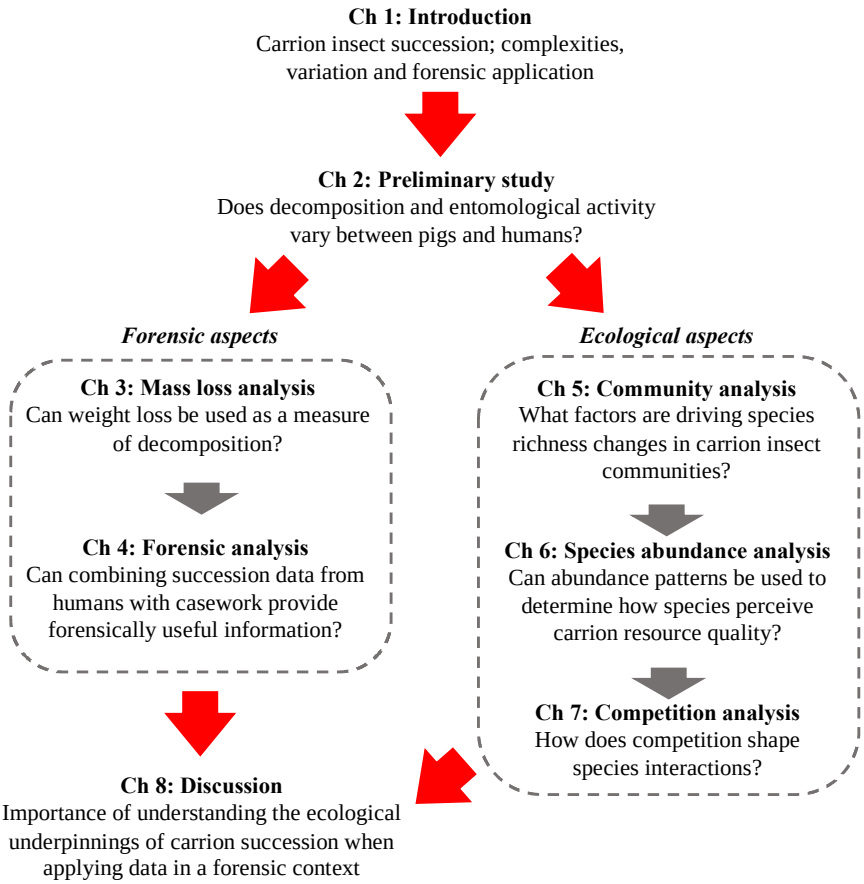

Figure 3 Thesis structure and relationship between chapters.

Chapter 4 examines Diptera succession on human cadavers in more detail, as Chapter 2 identified the need to focus more on human cadavers for forensic research. Here I also combined data from previous casework with my field succession experiments to determine

which species are forensically useful. This chapter highlights the benefit of linking casework and succession experiments, as I provide new biological information about several Diptera species, which, based on entomological evidence, might improve PMI estimates.

Ecological aspects:

Chapter 5 investigates the factors driving carrion insect succession, specifically species richness, on pigs and humans. I aimed to determine the effect of TBS, season, cadaver type, ambient temperature and initial starting mass on the community composition of carrion-associated Diptera, Coleoptera and Hymenoptera. I used TBS as a continuous measure of resource change to reflect the qualitative and quantitative changes occurring on carrion.

Chapter 6 follows on from Chapter 5 by examining the role TBS plays in driving individual species abundance patterns. I focused on the most common carrion insects and modelled their abundance over the course of decomposition. I used TBS as a metric for resource quality, and interpreted the results as phases of increasing abundance likely indicating perceived high resource quality, and length of abundance maxima indicating optimal oviposition and feeding time. I demonstrated how different species perceive resource quality and how this perception varies between pigs and humans.

Chapter 7 explores the factors that facilitate coexistence between the facultative predator *Chrysomya rufifacies* and its prey *Calliphora stygia*. Specifically, I investigated how interspecific competition is influenced by priority effects and larval density. To do this, I undertook a series of laboratory experiments to determine how adult oviposition, larval survival, developmental duration, and adult fitness were affected by the presence of differently aged heterospecific larval masses, and how these measures varied under three larval densities. This chapter explores the mechanisms allowing species to coexist on a competitively intense and limiting resource such as carrion.

As this is a ‘thesis by publication’, I remind the reader that Chapters 2-7 may contain repetition and variation in formatting, especially referencing and spelling, since they follow the requirements of their respective journals. Each paper needs to be self-contained, and necessary details have been provided in each chapter, so they are able to stand alone (see declaration on page v).

In Chapter 8, I summarise my main findings from each chapter and highlight their importance to the fields of both carrion ecology and forensic entomology. I also discuss the importance of understanding the ecological factors driving carrion insect succession and how

incorporating this knowledge into forensic research can improve PMI methods derived from insect data. I argue that ecological knowledge about decomposition and carrion insect succession processes is fundamental for forensic entomologists, and researchers must move from descriptive studies to incorporate the quantitative and experimental approaches developed in the ecological disciplines to further our understanding of successional processes.

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Chapter 2. Contrasting insect activity and decomposition of pigs and humans in an Australian environment: A preliminary study

2.1 Abstract

Non-human vertebrate animals, primarily domestic pigs, have been widely used in forensic science research as analogues for humans due to ethical and logistical constraints. Yet the suitability of pigs to mimic human decomposition and entomological patterns remains largely untested, and explicit comparative research in this area is lacking. We compared the decomposition rates and insect communities found at pig and human remains during summer and winter at the Australian Facility for Taphonomic Experimental Research (AFTER). Pigs decomposed faster than humans, with pigs entering active decay earlier in both summer and winter, and humans undergoing desiccation rather than skeletonisation. There was also a delay in the colonisation of humans by both flies and beetles. Species richness of these necrophagous taxa was between two and five times higher during the first two weeks of decomposition on pigs compared to humans during both summer and winter. Insect species composition was also significantly different between pigs and humans in each season. We interpret our findings to mean that the difference between humans and pigs, such as their mass, diet, medical history, or their microbiomes, might be causing different decomposition processes and altered timing or production of chemical cues for insect colonisation. Although preliminary, our results suggest that pigs might not be accurate substitutes for humans in particular fields of taphonomy and forensic entomology. Our findings also have broader implications for the reliability of forensic studies using pigs as models for humans, and highlight the need to recognise intrinsic differences between animal models and humans.

2.2 Introduction

The use of non-human vertebrate animals as proxies for humans is common in a number of research fields, including the forensic sciences, due to the ethical and logistical constraints associated with using human cadavers [1,2]. Research on such animal models in human taphonomy and forensic entomology has provided much of the fundamental knowledge relating to decomposition rates and entomological patterns on vertebrate carrion [3–5]. Numerous types of animal models have been employed, such as rabbits [6], guinea pigs [7], dogs [8], monkeys [9], impalas [10], rats [11] and pigs [12]. However, there has so far been very little research attempting to verify if other animals are reliable proxies for humans in forensic science research [13–16].

The most common animal model used in forensic studies is the domestic pig (Sus scrofa L.), which is assumed to have similar anatomical and physiological characteristics to humans, such as body mass, skin and hair coverage/composition [17]. The domestic pig is also easily acquired, relatively cheap, has simpler ethical considerations, and can be obtained in large numbers, which allows for experiments with appropriate replication to be conducted [18,19]. This contrasts with humans, which have obvious ethical constraints and are in limited supply, as well as there being few licensed facilities for their study. For these valid reasons, pigs are often the best candidate to act as proxies for humans in decomposition and forensic entomological experiments [20].

To date, only two studies have examined differences in insect communities found on decomposing pigs and humans: one in the USA [14] and one in China [15]. Schoenly et al. [14] conducted a 35-day summer entomological comparison of two decomposing pigs and one human at the Anthropology Research Facility (ARF) in Knoxville, Tennessee. The results revealed minimal differences between arthropod species attending the two types of remains. The authors confirmed the assumption that pigs are appropriate substitutions for humans. The authors did not however examine decomposition differences and presented only a checklist of insect species present. Wang et al. [15] conducted a summer decomposition and entomological comparison between one human and four pigs. They found that decomposition occurred at a faster rate in the human than the pigs, with skeletonisation occurring first in the human. The insect assemblages were similar in their composition between the human and the two larger pigs, while the smaller pigs hosted fewer similar assemblages. Unlike Schoenly et al. [14], Wang et al. [15] examined the change in insect species composition over an extended decomposition process, but both of these entomological studies were conducted during

summer. More work is needed to explore the diversity and composition of insects at pig and human remains over the extended decomposition process, across contrasting seasons, and in the southern hemisphere.

In addition to differences in insect assemblages, Wang et al. [15] showed differences in the way pigs and humans decompose. Other recent decomposition studies have revealed significant differences in the rate, pattern and variability of decomposition between pigs and humans, as well as differences in the volatile organic compounds (VOCs) produced during the decomposition process [21–23]. However, some of these studies have been criticised for their experimental design and findings, and more research is needed to verify their results [20]. The arrival, departure and residency times of necrophagous insects are dependent on the rate and progress of decomposition [24–26]. This is because insects use olfactory cues from VOCs to detect the decomposing remains, orient themselves, and disperse towards the remains for ovipositing and/or feeding [27]. The combination and concentration of VOCs that are emitted change as decomposition proceeds [23], and this is thought to attract different suites of insects at different stages of decomposition [28–29]. A contributing underlying mechanism for this is the microbiome present in the decomposing remains, which is critical to initiating many of the biochemical decomposition processes and the emission of the VOCs [27,30]. The interdependencies between necrophagous insects, VOCs, and the microbiome are complex [31], and are only now being examined in detail [32]. Our hypothesis, therefore, was that if the rate of decomposition varies between pigs and humans, it is likely that the timing of arriving insects, particularly primary colonising flies, will also be different. The little research in this area, and lack of an explicit test of this hypothesis, are concerning considering the importance and extensive use of pig models, and other animals, in forensic research [33].

In this preliminary study, we compared the decomposition rate and insect communities between pigs and humans during summer and winter at the Australian Facility for Taphonomic Experimental Research (AFTER). We examined decomposition rates using measurements of total body score (TBS) [34], as well as temperature to determine the thermal contribution to decomposition. We assessed entomological activity by examining the species composition and richness of insect communities throughout the decomposition process. Our preliminary study provides the first assessment of decomposition and entomological differences between pigs and humans in the southern hemisphere.

2.3 Materials and methods

2.3.1 Study site

The study was conducted in two seasons, with a summer experiment lasting 56 days (12th January - 9th March 2018) and a winter experiment lasting 178 days (30th May - 23rd November 2018). The disparity in the length of the experiments was due to the slower rate of decomposition that occurred in the winter study. Both experiments took place at AFTER, operated by the University of Technology Sydney (UTS). The 4.86 hectare site is situated in the Hawkesbury region of western Sydney, consisting primarily of dry sclerophyll Eucalyptus forest with scattered rural housing in the nearby vicinity. Ambient temperature and humidity were recorded every fifteen minutes at the site using a HOBO MX2302 Ext temperature and relative humidity data logger (Onset) protected by a solar radiation shield.

2.3.2 Human and pig acquisition and set-up

One male human (age: 87 years, 85 kg) and one female pig (age: 4-6 months, 65 kg) were used in the summer experiment, while the winter experiment consisted of two female humans (human A – age: 53 years, 47 kg and human B – age: 86 years, 80 kg) and one female pig (age: 4-6 months, 67 kg). The humans were obtained through the UTS Body Donation Program, approved by the UTS Human Research Ethics Committee Program Approval (UTS HREC REF NO. ETH15-0029). The cause of death of the summer human donor was pneumonia and the cause of death of the winter donors was metastatic malignancy of the lung (human A) and chronic obstructive pulmonary disease (human B). Human A from the winter experiment had undergone chemotherapy treatment prior to death, while human B had been treated with numerous different antemortem pharmaceuticals. Data on antemortem treatment of the summer human donor were unavailable. After death, the human donors were kept refrigerated in mortuary-like conditions and delivered to AFTER within 48 hours. Once at AFTER, the human donors were placed on their backs directly on the ground in a designated 5 m x 5 m plot within the facility. Due to the number of other human donors placed within the facility, entomological independence in their placement was difficult to achieve [35]; therefore donors were placed closest to other donors that had already skeletonised or become desiccated, with little or no observable entomological activity.

The domestic pigs (Sus scrofa) were purchased from a licensed abattoir post-mortem, therefore requiring no ethics approval in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004). Pigs were killed by a captive head bolt and transported to AFTER within one hour of death. The pig carcasses were placed on their side and in direct contact with the ground outside the facility to comply with the facility licensing agreements. The pigs were located a minimum of 100 metres away from the human donors. All pigs and humans were placed on the ground in semi-shaded areas, with a metal cage placed over the top to prevent scavenging animals accessing the remains. On each pig and human, three HOBO MX2303 temperature data loggers (Onset Computer Corporation), each with two probes, were used to record temperatures every 15 minutes. The probes were positioned in the mouth, anus and beneath the shoulders and stomach of the pigs and humans (Fig. 4). Four pitfall traps were placed evenly around each pig and human and filled with a mix of propylene glycol and 70% ethanol to capture ground active insects [36].

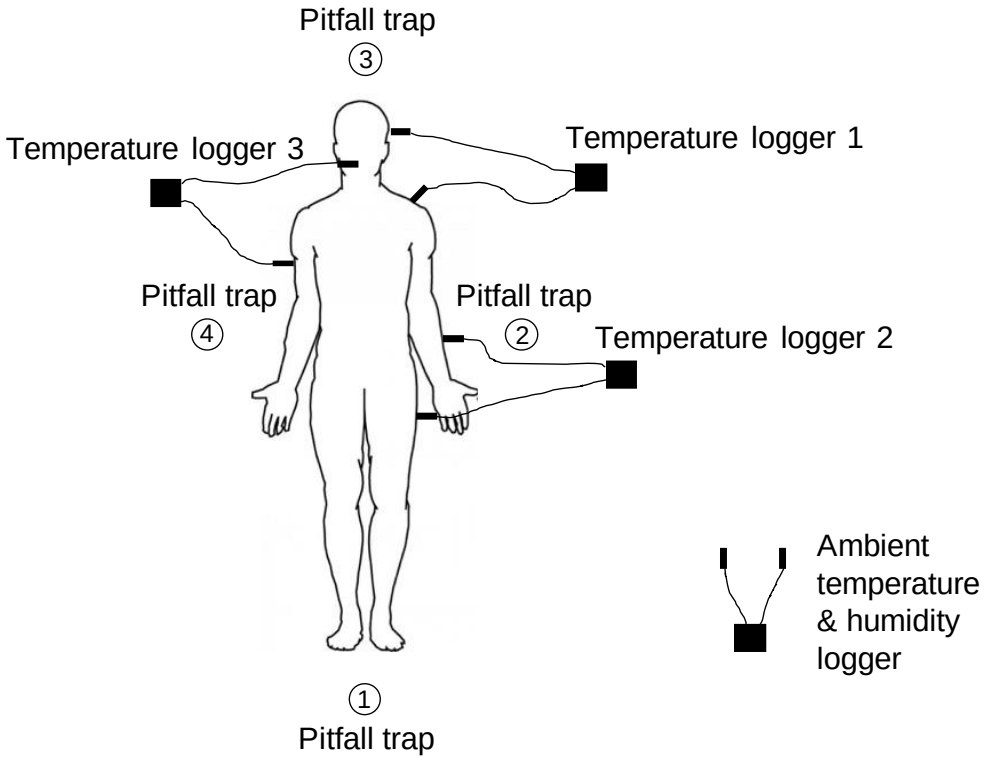

Figure 4 Example experimental set-up for each human, with locations of pitfall traps and temperature loggers. Traps and data loggers were arranged similarly at pig carcasses, but with each pig placed on its side.

2.3.3 Sampling procedure

Sampling frequency was dependent on the rate of decomposition and insect activity, with sampling occurring frequently (once a day in winter and every two days in summer) during the early stages of decay and gradually decreasing in frequency in the latter stages of decay. On every sampling day the decomposition progress and entomological activity were recorded between 11:00 and 13:00 h. Detailed descriptions of the decomposition process were made, with accompanying photos taken with a Canon EOS 70D camera with a 18–55 mm STM lens. The resident insect community at each cadaver was sampled with a combination of three different collection methods: manual sampling, sweep netting and pitfall traps. This combination of methods was to provide an unbiased and accurate recording of both ground-dwelling and airborne necrophagous insects present during the decomposition process [14]. Live maggots were also collected to determine the fly species breeding on the remains.

All collected insects were transported to the University of Wollongong (UOW) for identification. All insects were identified to species level, where possible, with the use of specialist dichotomous keys [37–42] and online LUCID keys [43–44]. All insects were preserved in 70% ethanol, with the exception of male sarcophagid flies, which were pinned, and their genitalia dissected out for identification purposes. Live maggots were taken to UOW's Ecological Research Centre (ERC) to be reared to adults for identification. Half of the collected maggots were killed in boiling water and preserved in 70% ethanol. The other half were placed in a plastic rearing container (130 x 190 x 70 mm) with a mesh top and provided with 100 g of kangaroo mince and wheaten chaff to act as a pupation substrate. Live maggots were kept at a constant temperature of 24 °C with a 12:12 h light/dark cycle. In some cases, when rearing was unsuccessful, molecular methods were used to confirm the identity of collected maggots. DNA was extracted, amplified and sequenced with the primers and methods outlined by [45]. The resulting COI sequences were submitted to the Basic Local Alignment Search Tool (BLAST) to compare against other sequences of identified flies.

2.3.4 Data analysis

To assess decomposition rates, a total body score (TBS) was calculated for both pigs and humans following the method of Megyesi et al. [34], by separating the body into three distinct segments (head, torso and limbs) and applying a numeric value to each. Scores were calculated

for the three segments on each visit and summed together to provide a numeric value to represent the decomposition rate. A value for accumulated degree days (ADD) was calculated for both the ambient temperatures and the temperatures recorded beneath the pigs and humans during decomposition. For the ambient ADD, the average ambient temperature for each day was summed. The ADD for the pigs and humans was calculated by first averaging across the six probes for each day, then summing the daily temperatures for each cadaver and season. To examine the overall progress of decomposition, the TBS was plotted against the ambient ADD, while the ADD of the remains was plotted against the total time elapsed. These comparisons were conducted for all pigs and humans in both the summer and winter experiments.

Since the replication of the humans and pigs was limited, as is typical for most studies of this kind [14,23], statistical models of our univariate data were not possible, and visual exploration was therefore most appropriate. We examined insect species richness by plotting the necrophagous flies and beetles present on the pigs and humans against total time elapsed. The time elapsed was grouped into weeks to more easily compare the insect activity between pigs and humans. To compare insect community composition, we used permutational multivariate analysis of variance (PERMANOVA) on Jaccard dissimilarity matrices of presence/absence data to test for differences, and non-metric multidimensional scaling (nMDS) ordinations to visualise the insect community data on each sampling day. Two PERMANOVAs and ordinations were conducted to compare insect community composition (i) between pigs and humans in summer only and (ii) between pigs and humans in winter only. A pairwise comparison was also conducted on the winter experiment to further identify differences in the insect communities among the two human replicates and the pig. All PERMANOVAs, nMDS ordinations and pairwise comparisons were performed in RStudio, using the vegan, RVAideMemorie and ggplot2 packages.

2.4. Results

2.4.1 Decomposition

During the winter experiment, we observed four stages of decay (fresh, bloat, active and advanced) for all pigs and humans. In the summer experiment, we observed the same four stages of decay for the human donor, but observed an additional decay stage (skeletonisation) for the pig. With the exception of the summer pig that skeletonised, all other pigs and humans became desiccated, with the skin remaining intact during the advanced stage of decay [23].

Decomposition (total body score) occurred at a faster rate for pigs than for humans in both summer (Fig. 5a) and winter (Fig. 5b) when plotted against accumulated degree days calculated from the ambient temperature.

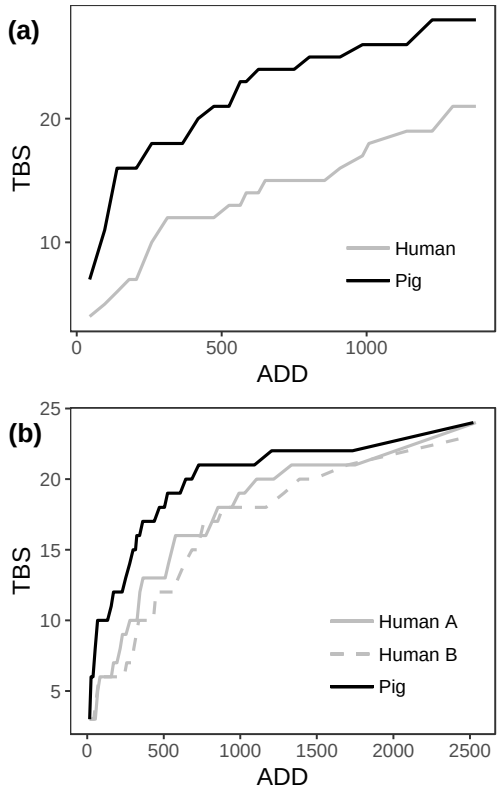

Figure 5 Total body score (TBS) plotted against ambient accumulated degree days (ADD) for pigs and humans in a) summer and b) winter experiments.

For the summer experiment, the average ADD beneath the pig and human were both above the ambient ADD throughout the entire experiment (Fig. 6a). The average ADD beneath the pig, however, was higher than that beneath the human. Similarly, the average ADD beneath the pig and humans in winter were above the ambient ADD for the whole experiment (Fig. 6b). The average ADDs in the winter experiment followed the same pattern as the summer experiment, however the ADD values were much closer together, particularly for the two humans, having almost the same average ADD throughout the experiment. The ADD paths for the humans and pigs in both seasons never become parallel with the ambient temperature, indicating that the cadavers continued to produce a heat 'island' effect, insulating the temperature probes from cooler ambient temperatures.

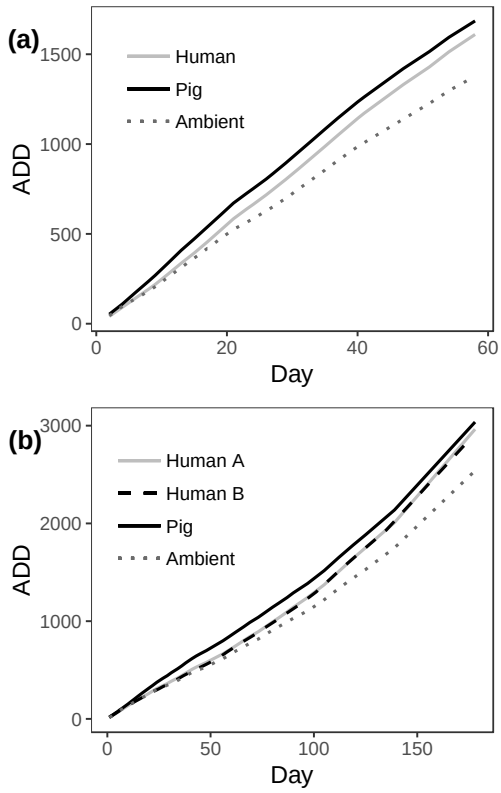

Figure 6 Accumulated degree days (ADD) calculated from temperature probes beneath the pig and humans plotted against time (days) for a) summer and b) winter experiments.

2.4.2 Entomology

In total, 40 species of flies and beetles were collected in this study, with the summer experiment totalling 35 species, the winter experiment totalling 30, and 25 species occurring during both experiments. The summer experiment had 10 unique species, while the winter experiment had only five. The occurrence patterns of all species of flies and beetles, as well as fly larvae, can be viewed in Appendix Tables S2 & S3. During the summer experiment, there were clear differences in the temporal trend in fly species richness between the pig and human, with the pig generally having more fly species during early decomposition than the human (Fig. 7a). The species richness in the pig decreased steadily as the decomposition process approached the latter stages, while the number of species remained relatively constant on the human after the delayed colonisation. We found that beetles followed a similar pattern to the flies during summer, but arrived slightly later than the early colonising flies (Fig. 7b). The summer pig had a higher richness of beetles, but this slowly declined over time as decomposition progressed.

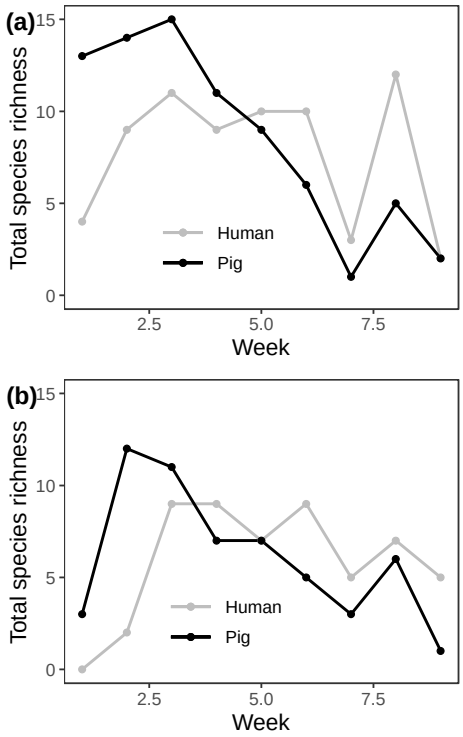

Figure 7 Total species richness of a) flies and b) beetles collected from the pig and human plotted against time (weeks) for the summer experiment.

We recorded more pronounced differences between the pig and humans in the winter experiment, especially in the early stages of decay because the slower decomposition process enabled a more detailed examination. Fly species richness was almost always higher on the pig compared to the humans (Fig. 8a). This was most obvious during the first two weeks of decay, where the fly species richness was over five times higher on the pig compared to both humans. Colonisation of the humans was limited to only one fly species for the first two weeks of decomposition, while the pig hosted many more species of flies within the first few days of decomposition. Even after more species arrived on the humans, the number of species during early decay stages never reached the richness of flies on the pig during early decay. Although similar in pattern, the number of fly species on human A was higher than human B, with human B only occasionally having more than two species present (Fig. 8a). Beetles displayed a delay in the colonisation of both humans relative to pigs, and did not colonise the human remains until week 5, compared to the pig, when they arrived during week 2 (Fig. 8b). The species richness of beetles on the human and pig fluctuated throughout decomposition, with neither human nor pig continuously dominating in their species richness.

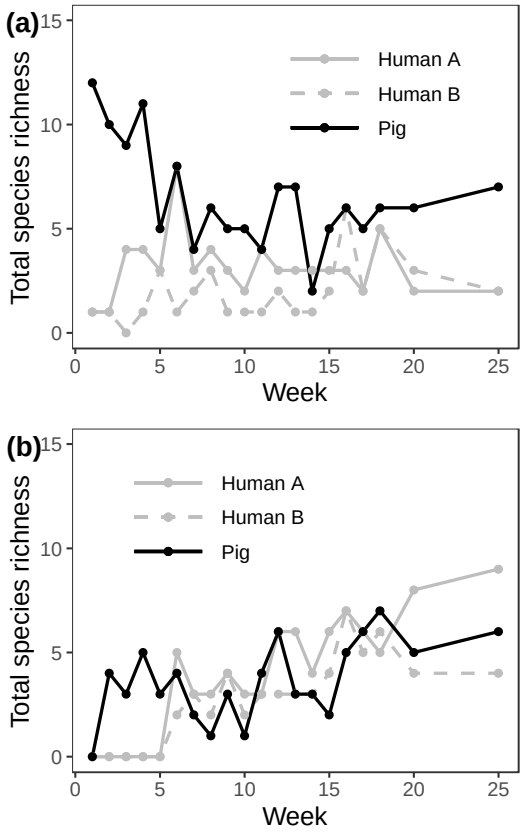

Figure 8 Total species richness of a) flies and b) beetles collected from the pig and humans plotted against time (weeks) for the winter experiment.

The summer insect community composition was significantly different between the pig and human (PERMANOVA: $F_{(1,51)} = 2.898$, $p < 0.005$). The nMDS showed clear differences in the insect community composition, however some overlap can be observed (Fig. 9a). We also found that the winter community composition was significantly different between the pig and humans (PERMANOVA: $F_{(1, 111)} = 5.945$, $p < 0.001$). A further pairwise comparison revealed the insect community of human A to be significantly different from human B ($P < 0.05$), while both human A and human B each showed significantly different insect communities compared with the pig remains ($P < 0.005$). Visually, however, the nMDS showed that the insect communities on the humans were much more similar to each other than either was to the community on the pig (Fig. 9b).

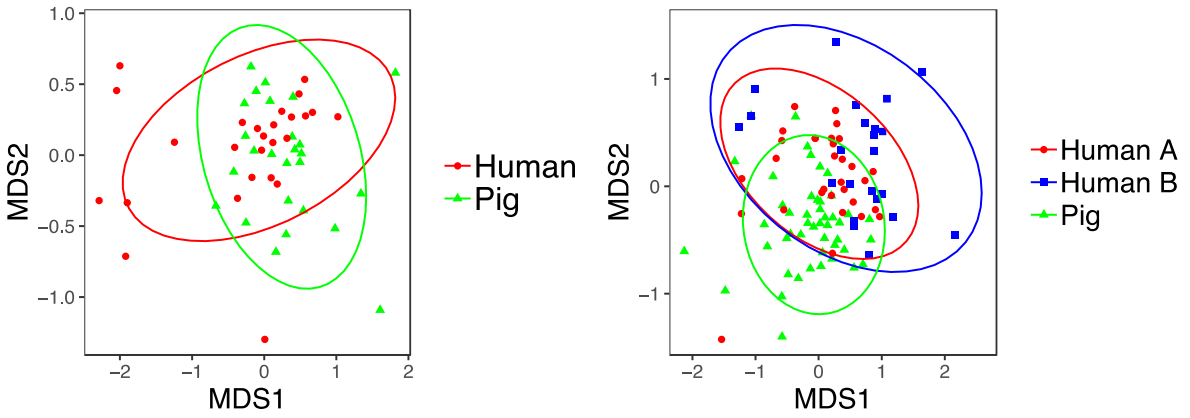

Figure 9 nMDS ordination comparing the insect community composition between the pig and human/s during the decomposition process for the a) summer and b) winter experiments.

2.5. Discussion

Our preliminary study revealed that very few species of primary colonising necrophagous flies arrived during early decomposition on the human remains compared to the pigs. This delay and lack of fly species in the initial stages of decomposition of humans occurred in both seasons, however the length of delay was more pronounced in winter, with fewer species colonising the humans in winter than summer. The pig remains in both seasons exhibited a higher number of insect species during decomposition, particularly during the early stages of decay when compared with the humans. The species community composition also differed between pigs and humans, especially in winter, where the two humans differed from one another and both differed from the pig. Our decomposition results, based on the TBS comparison of the Megyesi et al. [34] method, indicate that pigs decomposed faster than humans, with the humans desiccating, rather than becoming skeletonised. Although not used here, there is also a modified TBS scoring method available for the pig model that recognises the potential differences between pigs and humans that we observed in our study [46].

Our decomposition results are similar to those of other human and animal-model comparisons, in which humans also decomposed at a different rate [21–23]. However, Wang et al. [15] found that humans decomposed faster than pigs, which is contrary to our findings. This difference in the rate of decomposition may have been caused by numerous factors, the simplest of which could be stochastic variation due to low sample size. Although our study had a low sample size, this was similar to or greater than past studies involving human and pig entomological comparisons [14– 15], and is the first to provide evidence that pigs and humans differ in their associated insect communities over the course of the decomposition process. The reason for these differences remains unclear and requires further investigation. Possible

explanations relate to the systematic differences between the pigs and humans in our study, as well as the structure and function of their associated necrobiome affecting the decomposition process [31,47].

There were several systematic differences between the pigs and humans used in these experiments, such as age, sex, diet, mass and cause of death, which might have influenced our results. These factors are difficult to control for when conducting decomposition and entomological experiments on human donors, especially when comparing human donors to animal models, as discussed in detail by Matuszewski et al. [20]. In the context of our study, the body mass was different between the pigs and humans, with pigs ranging from 65–67 kg and humans ranging from 47–85 kg, with humans having a greater average body mass. Body mass is known to influence decomposition and insect activity, with larger pig carcasses generally decomposing slower and able to maintain more diverse insect assemblages [48–49]. The variability in body mass of the pigs and humans in our study could therefore have affected the species richness of insects and the decomposition rate of the pigs and humans. This is evident in the large disparity in body mass between the two humans in our winter experiment and the significantly different insect communities colonising them. However, the species richness of both flies and beetles of the two winter humans, as well as their decay rates, remained similar throughout the decay process despite their disparity in body mass.

Another systematic difference that could have influenced the decomposition and entomological differences observed in this study is the history of each of the humans and pigs, including cause of death, diet and drug ingestion [20]. We attempted to control for this by using human donors that were recently deceased and intact, but there is inevitably considerable physiological and toxicological variation between donors, since each has a unique medical background. The influence of the medical treatment experienced by donors during the peri-mortem period (just prior to death) on their subsequent decomposition, and the associated entomological activity, remains largely unknown. It has already been suggested that medical treatment affects human decomposition. For example, Hayman and Oxenham [50] noted differences in decomposition patterns and rates between two humans despite each being in the same environmental conditions. The differences in their case were attributed to the humans being variously treated with cytotoxic and antibiotic drugs. Pigs, by comparison, would typically have had no equivalent veterinary treatment or foreign chemicals in their systems, although some may be treated with antibiotics.

The colonisation pattern of maggots and the different physiological structure of pigs and humans might have also caused differences in decomposition rate. Primary colonising flies tend to favour the open orifices of dead animals, such as the mouth, nostrils and ears, for oviposition, leading to maggot masses first forming around the head region [51]. This pattern was observed on both the pigs and humans, with large maggot masses not forming elsewhere on the humans, although the pigs hosted such masses along the entire carcass. This could be attributed to the condensed body structure of the pig, with the pigs placed on their side on the ground. This placement creates a microhabitat for insects by allowing them to shelter beneath the stomach, protecting them from desiccation while providing access to soft tissue and larger internal organs to feed upon, resulting in large maggot masses forming. In contrast, the humans are placed on their backs on the ground, with their solid spine acting as a barrier between the internal organs and the soil, making it more difficult for insects to exploit the remains and causing smaller maggot masses to form. This limited maggot activity would allow the soft tissue to desiccate, possibly resulting in the desiccation of humans observed in this study and others (e.g. [23]).

The internal communities of microbes (microbiome) found within the pigs and humans could be another factor influencing the decomposition rate and colonisation of insects. The microbiome of pigs will be different from humans due to their different diet and environments [52,53]. Research has already confirmed that the microbiome of humans is different to that of guinea pigs [54], and even individual humans display unique microbiomes [55,56]. From the onset of death, microbial communities are the first organisms in the necrobiome involved in the decomposition of organic matter, and are the first to emit VOCs [57]. Since the internal microbial communities differ between pigs and humans, the initial VOCs emitted will be different in their concentration and composition [23]. Carrion-breeding insects, such as blowflies, are highly specialised and able to use pheromones and chemical cues (VOCs) to locate decomposing remains [27,58]. The internal microbiome of the humans in our experiments at the onset of death might have emitted VOCs unattractive to colonising necrophagous flies, resulting in the delayed colonisation we observed [59,60]. This delay might have been exacerbated in our experiments due to the human donors being refrigerated for a maximum of 48h after death, possibly altering the bacterial community [61].

These different variables and associated interactions make comparing humans with pigs difficult. Differences between individual pigs and humans encompass a whole suite of physiological, microbial, and antemortem treatments that likely combine to affect decomposition and insect colonisation.

2.5.1 Implications and conclusions

The results of our preliminary study show that decomposition rate and the species richness of insects is different between decaying pigs and humans. These results suggest that pigs might not be suitable analogues for humans in reliably replicating the decomposition process and associated entomological activity. This could have implications for various taphonomic or forensic studies, including research to improve estimates of the post-mortem interval (PMI). This hypothesis was not tested here, however, as we only examined broad entomological patterns, rather than larval development data, which are more commonly used in PMI estimations. Although our data show this in an Australian context, they might also apply in other situations or environments where humans are sourced from donor programs where individuals vary significantly in their medical history, diet or cause of death. Due to these reasons, humans sourced through body donation programs might also not be appropriate models to accurately represent a 'healthy' human in forensic casework. Further comparative experimentation is required to evaluate the influence of these systematic differences with a larger sample size, and to determine if data derived from the pig model are applicable to humans in forensic casework.

2.6. Acknowledgements

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2.7 References

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Chapter 3. How does mass loss compare with total body score when assessing decomposition of human and pig cadavers?

3.1 Abstract

Providing accurate and reliable measures of decomposition is paramount for forensic research where decomposition progress is used to estimate time of death. Mass loss is routinely used as a direct measure of biomass decomposition in ecological studies. Yet, few studies have analysed mass loss in a forensic context on human cadavers to determine its usefulness for modelling the decomposition process. We examined mass loss in decomposing human and pig cadavers, and compared our measures with other common decomposition metrics, such as total body score. We conducted one summer and one winter field decomposition experiment using human and pig cadavers, as pigs are often used as proxies for human cadavers in forensic research. The two measures of decomposition revealed two contrasting patterns of decomposition on pigs and humans, particularly in winter where TBS stabilised at similar values, but mass loss differed greatly. We found mass loss to be faster in pigs than humans during early decomposition. Pigs lost 75% of their mass in winter, while humans lost less than 50%, however in summer both lost around 80% of their mass. Total body score displayed similar patterns in both experiments, with TBS increasing more rapidly in pigs compared with humans but both eventually reaching similar TBS values in late decomposition. Measuring mass loss can provide additional information about decomposition progress that is missed if using TBS only. Our research also highlights key differences between cadaver types, suggesting caution when extrapolating data from pigs to humans for forensic research and decomposition modelling.

3.2 Introduction

Decomposition is a natural process whereby organic matter is broken down and consumed, releasing a pulse of nutrients back into the local environment (Carter *et al.* 2007). Knowledge of the decomposition process can aid forensic investigators in estimating a post-mortem interval (PMI), which is the minimum and maximum amount of time for which an individual has been deceased (Megyesi *et al.* 2005). Decomposition is a highly variable process and is influenced by numerous factors such as ambient temperature, habitat, carrion mass, scavenger activity and microbes (Komar & Beattie, 1998; Parmenter & MacMahon, 2009; Matuszewski *et al.* 2010; Lauber *et al.* 2014; Barton & Evans, 2017; Benbow *et al.* 2019). Due to the variable nature of decomposition, determining a universal and standardised metric for quantifying the decomposition process for forensic (and ecological) purposes has been challenging (Connor, 2019).

Traditionally, the decomposition process has been divided into distinct categorical decomposition stages based on visual morphological changes in the soft tissue of remains, such as the onset of bloat or bone exposure (Goff, 2009). The advent of decay 'stages' enabled other post-mortem processes, like insect activity, to be temporally linked to decomposition, thereby providing new sources of PMI estimation (Payne, 1965). Although these decay stages were fundamental in providing the groundwork for categorising decomposition, they have several associated issues. Firstly, numerous studies have used different numbers of decay stages, with no universally accepted number of decay stages (Goff, 2009), and secondly, decomposition is a continuous process and should therefore be treated as a continuum (Schoenly and Reid, 1987).

The total body score (TBS) metric was developed to improve upon the decay stage approach and provide a semi-quantitative measure of decomposition (Megyesi *et al.* 2005). This method separates a cadaver into three distinct regions (head, torso and limbs) and provides each region with a numeric value again based on the visual changes occurring during decomposition (Megyesi *et al.* 2005). The scores from each region are summed together to provide a numeric value representing decomposition progress. TBS can be compared with temporal measures of time or other measures incorporating time and temperature (accumulated degree days, ADD) to provide a model-based approach for PMI estimation (Franceschetti *et al.* 2021). The reliability of this method to produce accurate PMI estimates has been questioned (Suckling *et al.* 2016; Wescott *et al.* 2018), which has led to the development of more complex TBS models to improve accuracy and reliability (Moffatt *et al.* 2016; Connor *et al.* 2019).

Though TBS more accurately reflects decomposition progress than decay stages, it is still an indirect measure of decomposition, being based on morphological changes rather than any quantifiable ones, such as chemical, microbial, or physiological changes in the cadaver (Gill-King, 1996; Janaway *et al.* 2009).

A direct measure of decomposition needs to incorporate the bio-physical changes occurring in a cadaver. As a cadaver decomposes, biomass is lost via fluid leakage into the soil, desiccation and consumption by organisms, eventually leading to complete mass loss once the skeleton breaks down (Cater *et al.* 2007; Barton *et al.* 2019). Mass loss during decomposition is a continuous process and an ideal metric that directly reflects decomposition progress (Adlam & Simmons, 2007; Spicka *et al.* 2011). However, collecting mass loss data can be difficult as most studies rely on small-bodied animal cadavers, as periodically weighing large cadavers can be logistically challenging and may disturb the decomposition process and associated entomological activity (Parmenter & MacMahon, 2009; Maile *et al.* 2017). Despite this, mass loss has often been used in ecological research as a means of quantifying the decomposition process and to determine how nutrients and biomass is redistributed back into the local environment (Parmenter & Lamarra, 1991; Chaloner *et al.* 2002; Parmenter & MacMahon, 2009; Barton *et al.* 2019). However, in a forensic context, mass loss is not often used as a measure of decomposition, or incorporated into PMI calculations. The few forensic studies that do analyse mass loss are often conducted on animal models, of which the domestic pig is the most common (Cross & Simmons, 2010; Matuszewski *et al.* 2014; Lynch-Aird *et al.* 2015). Mass loss in human cadavers has seldom been studied, and to date, no research has been undertaken on comparing mass loss between pigs and humans.

In this study, we examined mass loss in decomposing humans and pigs over the course of one winter and one summer field decomposition experiment. We also compared mass loss with another measure of decomposition, TBS, to determine what similarities or differences occur among the different measures of decomposition. We also describe a novel field method for weighing cadavers and collecting mass loss data during the decomposition process for large-bodied vertebrate remains. Both human and pig remains were used in this study as pigs are often used as proxies for humans in forensic research (Tomberlin *et al.* 2012; Matuszewski *et al.* 2020). By assessing both types of cadaver we were able to investigate whether a direct measure of decomposition showed similar patterns between the two, which may be important for interpreting TBS or mass loss data when estimating the PMI or other aspects of decomposition (Matuszewski *et al.* 2020).

3.3 Material and methods

3.3.1 Study site

We conducted one winter experiment (8th May to 2nd October 2019) and one summer experiment (9th November to 16th December 2019) at the Australian Facility for Taphonomic Experimental Research (AFTER), a 4.86ha site located in the Hawkesbury region of Sydney, Australia. The facility is operated by University of Technology Sydney (UTS) and allows for human decomposition to be examined in a natural environment. The local vegetation in the facility is dominated by dry sclerophyll *Eucalyptus* forest with scattered urban housing in the nearby vicinity.

3.3.2 Human and pig cadavers

In the winter experiment, two male human donors (Human 1: age 57, 66 kg, placed 8/5/19 & Human 2: age 74, 51.7 kg, placed 7/6/19) and two female pigs (Pig 1: age 4-6 months, 70.6 kg, placed 8/5/19 & Pig 2: age 4-6 months, 57.7 kg, placed 11/6/19) were used, while the summer experiment consisted of two female humans donors (Human 3: age 82, 60.5 kg, placed 9/11/19 & Human 4: age 97, 46.8 kg, placed 14/11/19) and two female pigs (Pig 3: age 4-6 months, 102.9 kg, placed 11/11/19 & Pig 4: age 4-6 months, 63.5 kg, placed 18/11/19). Human donors were obtained through the UTS Body Donation Program, approved by the UTS Human Research Ethics Committee Program Approval (UTS HREC REF NO. ETH15-0029). Domestic pigs (*Sus scrofa*) were purchased post-mortem from a licensed abattoir, therefore requiring no ethics approval in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004). Pigs were euthanised by a captive head bolt and transported to AFTER within 1 h of death, while humans were delivered to AFTER within 48 hrs of death and kept refrigerated for the duration of transportation.

Once at AFTER, all human cadavers were placed on their backs in 5 x 5 m plots within the facility. Pig carcasses were placed on their sides along the outside of the facility to comply with licencing agreements. Pigs were placed a minimum of 100 m away from the human cadavers and a minimum of 20 m from one another. Within the facility, human cadavers were placed at least 30 m apart. Thin metal mesh was also placed beneath each pig and human to enable the lifting and weighing of remains throughout decomposition. A scavenger proof cage

was placed over each pig and human to prevent scavenging by larger animals. A HOBO MX2302 Ext logger with a solar radiation shield was placed on site to record ambient temperature and humidity every 15 minutes throughout both experiments.

3.3.3 Measuring decomposition

Direct mass loss: To measure mass loss, a single aluminium scaffold unit with a platform ladder (2.5 m length x 1.3 m width) was converted into a lightweight, mobile weighing mechanism (Fig. 10). First, the scaffold unit was altered to remove the outer platform, leaving only the ladder which runs along the top of the scaffold connecting the two ends. A pulley system was attached to the ladder by attaching a boat winch (Jarrett Trailer Winch) onto one end of the ladder. The winch wire was replaced with a polyester belt and fed into a metal pulley, which was welded to the centre of the ladder. This allowed the mass of the cadavers to be centred in the middle of the scaffold. At the end of the polyester belt, a small hook was attached, allowing for a digital hanging scale (Wedderburn, 150 kg) to be connected.

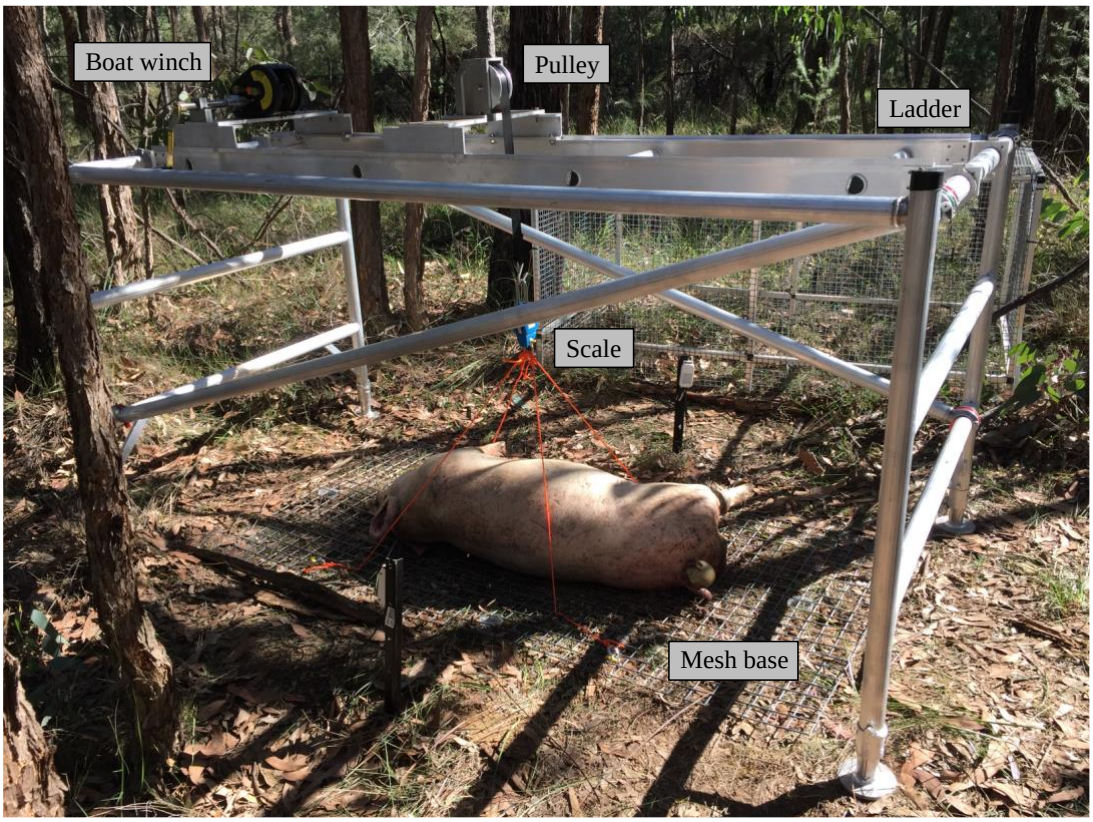

Figure 10 Modified scaffolding unit set up over a decomposing pig to measure mass loss.

Sampling occurred every 2-3 days during the first 1-2 weeks while decomposition progressed rapidly. Once decomposition slowed, sampling occurred every 5-7 days until the experiment was concluded. For the winter experiment, all individual pigs and humans were sampled a total of 11 times each. For the summer experiment, sampling intensity varied between replicates with a range of 3-6 measurements taken per replicate. This discrepancy was due to the quick rate of decomposition observed in summer with skeletonization occurring in one pig carcass within 8 days.

On each sampling day, the scaffold unit was placed directly over a cadaver and four hooks connected to rope were attached to the corners of the wire mesh, while the other end of the rope was attached to the hanging scale. Using the lever, the mesh was lifted slightly above the ground (approximately 2 cm) for no more than 10 seconds to record mass loss. The mesh had also been weighed prior to cadaver placement so the cadaver mass could be determined by subtracting the mesh mass from the sampling day mass.

Total body score: We also visually assessed the decomposition stage of every sampling day using the TBS method of Megyesi *et al.* (2005). The cadavers were divided into three distinct body regions (head, torso and limbs) and provided with a numeric score representing decomposition progress. These scores were summed together to provide a numeric value (TBS) for the total decomposition progress.

3.3.4 Data analysis

We used linear regression models to determine relationships between mass remaining (hereafter referred to as 'mass loss') and TBS with the post-mortem interval (PMI) (Moffatt *et al.* 2015). For PMI, we used accumulated degree days (ADD), which were calculated by determining the average ambient temperature each day and cumulatively summing them together. We used three different decomposition metrics to compare against ADD: mass loss, TBS and adjusted TBS. For adjusted TBS, TBS was transformed using the formula: TBS_{adj} = TBS – 3 and compared against logADD to create a more linear relationship between TBS and ADD (adapted from Moffatt *et al.* 2015). Values equal to 0 ADD were removed from the adjusted TBS analysis as log transforming these data was not possible and no decomposition had yet occurred. Mass loss was converted into a proportion of initial mass to standardise among the different starting masses of the cadavers. An additional linear regression model was also constructed comparing mass loss to TBS. Four separate linear models were constructed

for each cadaver type, resulting in a total of eight models. We then visually compared regression models and the R-square value of each model to assess how much variation in the model is explained by the independent variable (Marhoff *et al.* 2016). We conducted all regression models using the R base package (R Core Team, 2019) and lme4 package (Bates et al. 2016), while plots were created using the ggplot2 package (Wickham, 2016).

3.4. Results

3.4.1 Winter

We found mass loss to be significantly negatively correlated with ADD for both humans and pigs (Fig. 11a). Overall, pigs lost more mass than humans, with pigs reaching around 75% mass loss while humans lost no more than 50% of their mass. We also found TBS to be significantly positively correlated with ADD for humans and pigs (Fig. 11b). Unlike the humans, TBS values rose rapidly on pigs, reaching above 20 TBS before plateauing. Despite the more rapid rate of TBS increase on pigs, humans also eventually plateaued at the maximum recorded TBS of 24. When comparing mass loss to TBS, we found humans and pigs to be significantly negatively correlated (Fig. 11c). Mass loss occurred at a faster rate in pigs with around 25% of the mass loss occurring at 24 TBS. The adjusted TBS models were also positively significantly correlated with ADD with high R square values (Fig. 11d). In general, pigs displayed more unexplained variation in the data, with lower R-square values and more measurements lying outside the SE range compared with humans.

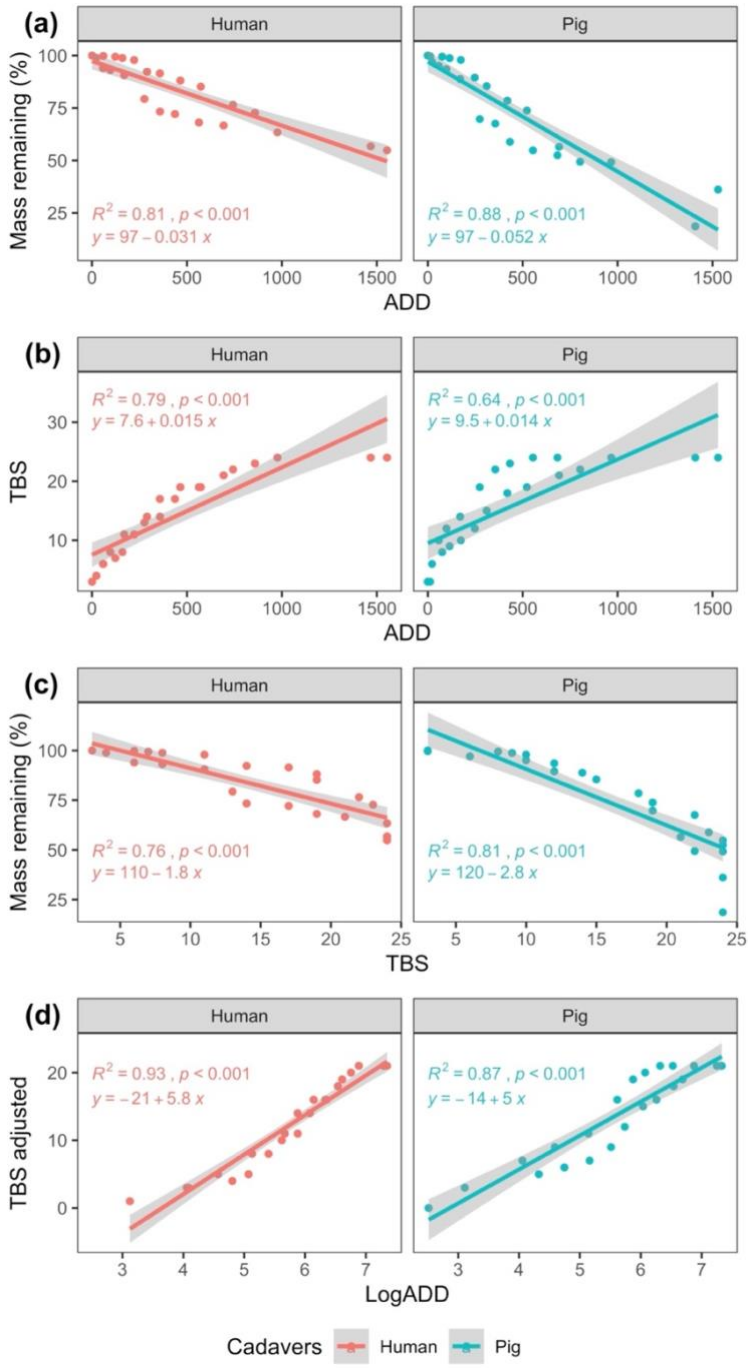

Figure 11 Decomposition progress measured as (a) mass remaining (%) compared against accumulated degree days (ADD), (b) total body score (TBS) compared against ADD, (c) mass remaining (%) compared against TBS and (d) adjusted TBS (TBSadj = TBS – 3) against ADD for human (red) and pig (blue) cadavers in the winter experiment. Grey bands represent SE.

3.4.2 Summer

In summer, we found mass loss to be significantly negatively correlated with ADD for humans and pigs (Fig. 12a). For the first 100 ADD, pigs lost roughly 50% of their mass, while humans

did not reach 50% mass loss until around 200 ADD. Despite this initial mass loss difference, both humans and pigs reached around 80-90% mass loss by the end of their decomposition. We found TBS to be significantly positively correlated with ADD for both humans and pigs (Fig. 12b). Total body score exhibited a similar pattern to the mass loss model with TBS initially increasing rapidly on pigs to 20 TBS at 100 ADD, while humans did not reach the same TBS until 200 ADD, but both reached similar TBS values by the end of their decomposition. Mass loss compared with TBS was significantly negatively correlated with a surprisingly strong linear relationship for humans and pigs, as most of the variation in the model was explained with a R square of 0.94 and 0.97, respectively (Fig. 12c). We found adjusted TBS to also be significantly positively correlated with ADD humans and pigs. More variation in the human model was explained relative to the pig model with a R square of 0.92 for humans and 0.72 for pigs (Fig. 12d).

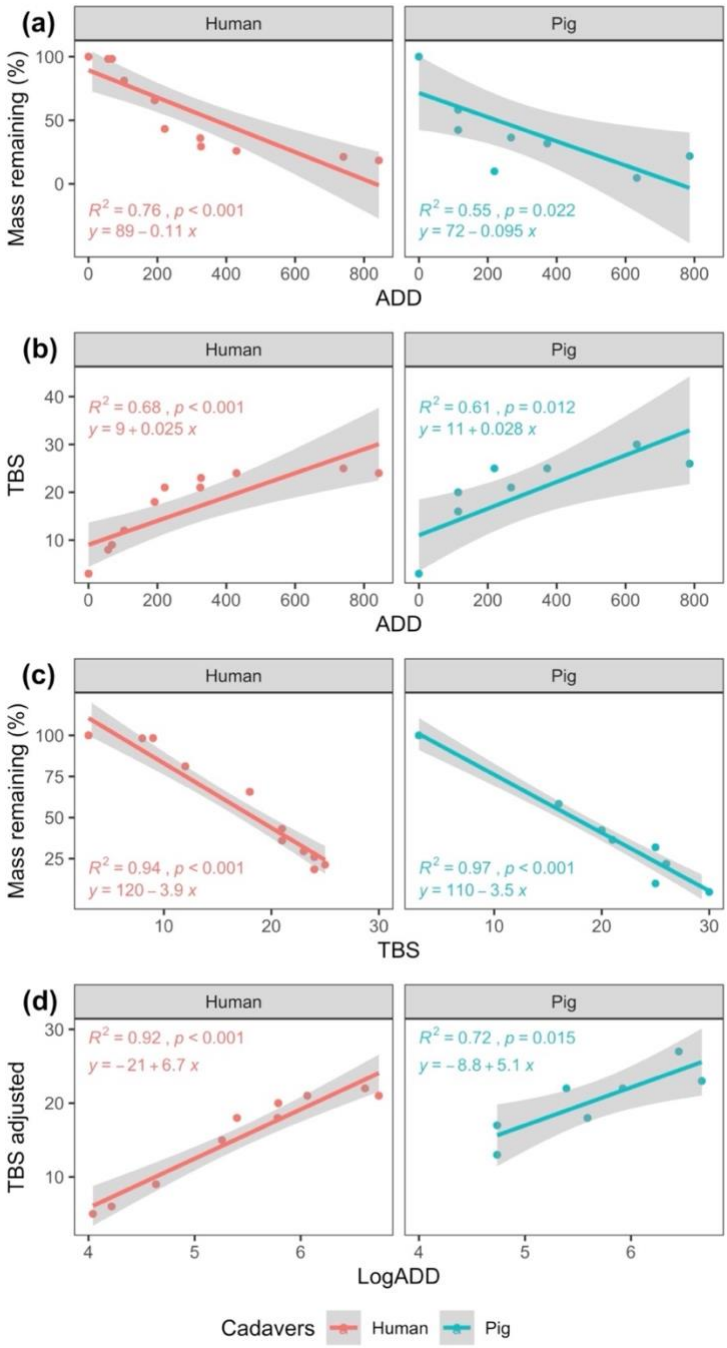

Figure 12 Decomposition progress measured as (a) mass remaining (%) compared against accumulated degree days (ADD), (b) total body score (TBS) compared against ADD, (c) mass remaining (%) compared against TBS and (d) adjusted TBS (TBSadj = TBS – 3) compared against ADD for human (red) and pig (blue) cadavers in the summer experiment. Grey bands represent SE.

3.5. Discussion

We set out to measure mass loss in decomposing humans and pigs, as well as compare mass loss with a commonly used but indirect measure of decomposition, TBS. Our findings indicated variation in mass loss patterns between seasons and cadaver types. At the beginning of decomposition, pigs lost mass more rapidly than humans in both experiments. In winter, pigs had lost more mass than humans by the end of decomposition, but in summer, mass loss was similar between humans and pigs by the end of their decomposition. Total body score was also found to increase more rapidly in pigs compared with humans at the start of decomposition, but both eventually plateaued and reached similar TBS by the end of decomposition during both seasons. Notably, mass loss had a strong linear relationship with TBS in summer when decomposition was more rapid, but in winter mass loss progressed more slowly with a large portion of mass loss occurring at high TBS values, particularly for pigs. Our study provides new insight into mass loss and TBS patterns in pigs and humans, highlighting how they represent different aspects of decomposition. Our results also provide the first mass loss benchmark for TBS observations for both human and pig cadavers in warm and cool weather conditions.

3.5.1 Mass loss between cadaver types

Pigs lost mass quicker than humans in both experiments, and by the end of the winter experiment, pigs had lost 75% of their mass while humans had lost 50%, but by the end of the winter experiment, both pigs and humans had lost 80-90% of their mass. This suggests that the decomposition progress and rate was different between the two cadaver types, which is similar to previous research comparing cadaver types using TBS (Connor *et al.* 2018; Dautartas *et al.* 2018; Knobel *et al.* 2019; Dawson *et al.* 2020). Mass loss is driven by environmental factors (desiccation via evaporation) and organisms consuming and dispersing the remains (Barton *et al.* 2019). Humans and pigs were placed around the same time and experienced similar ambient temperatures, therefore environmental desiccation rates would likely also have been similar between cadaver types. Insect activity, however, was different between the cadaver types, as previous research using the same cadavers as this experiment showed differences in species richness of insects, particularly in Diptera between pigs and humans (Dawson *et al.* 2021). Insects have the ability to remove a significant amount of biomass from decomposing remains

and therefore, are likely one of the key reasons why mass loss was different between pigs and humans (Payne, 1965).

The anatomy and physiology of humans and pigs has often been stated to be similar in terms of skin composition, fat to muscle ratio and proportional organ size. Despite this, there are some differences between pigs and humans that may have contributed to total mass loss differences observed in our study, particularly water content (humans often have a higher percentage than pigs) and the condensed body structure of pigs (Sheng & Huggins, 1979; Pearce *et al.* 2007; Dawson *et al.* 2020). However, total mass loss was only different in the winter experiment; therefore if body composition was influencing mass loss, we would expect similar results in summer. It is likely mass loss rates were driven by other factors, such as insect activity or potentially microbial activity and the peri-mortem treatment of the cadavers (Carter *et al.* 2007; Knobel *et al.* 2019; Dawson *et al.* 2020).

3.5.2 Mass loss compared with TBS

Total body score exhibited similar patterns to mass loss when compared with ADD, displaying a more rapid increase in TBS on pigs at the start of decomposition. Both measures of decay accurately reflect the more rapid decomposition progress in pigs compared with humans at the start of decay. However, in both experiments, TBS plateaued at 24 TBS on humans and pigs for roughly 500 ADD at the end of decomposition. Over those 500 ADD in winter, pigs lost roughly 25% of their mass, but this decomposition progress was not reflected in the TBS measure, which stayed at 24 as no morphological changes occurred on the remains. Mass loss was therefore able to detect continual differences in decomposition progress between cadaver types that was unable to be detected using the TBS method. Total body score relies on visual morphological changes occurring on a cadaver during decomposition, which occur less frequently in advanced decay (Megyesi *et al.* 2005). Mass loss on the other hand is a continuous process occurring throughout decomposition, even when limited visual changes in the cadaver are occurring (Barton *et al.* 2019). Total body score may therefore be unreliable during advanced decay for detecting decomposition changes on cadavers, while direct measures like mass loss are potentially more reliable.

Table 1 Estimated mass remaining for different total body scores derived from linear model equations.

	HUMAN	**HUMAN**	**PIG**	**PIG**
	Winter	Summer	Winter	Summer
TBS	Mass Remaining	Mass Remaining	Mass Remaining	Mass Remaining
5	100	100	100	93
10	92	81	92	75
15	83	62	78	58
20	74	42	64	40
25	65	23	50	23
30	56	3	36	5

Although differences were observed between mass loss and TBS in winter, the data for summer showed a much more linear relationship between mass loss and TBS. As decomposition progressed more rapidly in summer, humans and pigs were at similar mass loss and TBS values by the end of decomposition, unlike in winter, where mass loss differed at the end of decomposition. Due to this close association, TBS can be used as a benchmark to quantify possible mass loss progress in cadavers. For example, based on the model formulas derived from our experiments, we have determined predicted mass loss at different TBS intervals (Table 1). Knowing how TBS correlates with mass loss could open new avenues of mass loss estimation for other researchers unable to directly measure this variable in their experiments. The relationship between mass loss and TBS differs between season and cadaver type as observed here, but also likely differs between habitats and climates; therefore validation of these relationships is required in other localities (Carter *et al.* 2007).

3.5.3 Implications and conclusion

Decomposition is a complex process influenced by a diverse combination of biotic and abiotic factors. Having a reliable measure of decomposition is key to the development of accurate PMI models for forensic investigators. Our study has shown that mass loss can be used as an accurate measurement of decomposition progress. Mass loss can provide more quantitative information about the decomposition progress during advanced decay that is not evident in indirect measures such as TBS. Although mass loss is difficult to use in casework due to the often unknown or unreliable starting mass of a cadaver, it is still a valuable tool to incorporate

in forensic research and casework. A combination of direct and indirect decomposition measures is recommended to ensure reliable assessment of the decomposition progress. We have provided some estimated values of percentage mass remaining for a range of different TBS values. These may be useful for estimating mass loss from TBS assessments, and we welcome further research that tests the robustness of TBS-mass loss relationships in a range of other environments. Our study also highlights the differences in decomposition progress between pigs and humans, suggesting caution when relying solely on pig models for extrapolating to human forensic research, with some differences in mass loss occurring in different seasons.

3.6 Acknowledgments

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Chapter 4. Field succession studies and casework can help to identify forensically useful Diptera

4.1 Abstract

Fly development rates, and to a lesser extent succession data, can be used to provide an estimate of a minimum postmortem interval (mPMI). Yet, these data are most useful when a full account of species' ecology, seasonality, and distribution is known. We conducted succession experiments on human cadavers over different seasons near Sydney, Australia, to document forensically useful information, including the pre-appearance interval for carrion flies. We also compiled a detailed record of flies identified in casework collected in 156 cases distributed across New South Wales, Australia. We then compared the occurrence of fly species from both field and casework datasets to identify any consistencies or gaps to determine how useful species might be for forensic investigations. In the field experiments, we found differences in species diversity and abundance between seasons, as well as yearly variation between two winter seasons. Most fly species we recorded ovipositing showed a 2- or 3-day delay between adult arrival and oviposition in summer, with a longer delay in winter. Species that were previously encountered in casework, such as *Calliphora augur* (Fabricius, 1775) and *Calliphora ochracea* Schiner, 1868, were confirmed as forensically useful, with their colonization behavior and seasonal preferences documented here. Although not encountered in casework, we confirmed *Hemipyrellia fergusoni* Patton, 1925 as a primary colonizer of human cadavers. Our study emphasizes the need to link field and casework data for a complete understanding of all aspects of a carrion fly's ecology to assist forensic investigators in mPMI estimations.

4.2 Introduction

Particular species of flies (Diptera) can provide information about the time of death of deceased individuals due to their specialized adaptations that enable them to rapidly locate decaying animal matter (carrion) and oviposit on it [1–3]. By examining growth and development rates of fly larvae in human remains, a minimum postmortem interval (mPMI) can be estimated, which is the minimum amount of time for which a person may have been deceased, and when combined with a maximum PMI, can provide an interval for time of death [4]. Development times of fly larvae are commonly used to estimate a mPMI in forensic entomology casework worldwide [5]. Extensive research has been conducted to establish development times for numerous fly species under different rearing temperatures and conditions [6–7].

The other main insect-based method to estimate the mPMI (and in some cases the maximum postmortem interval) is to examine the insect community succession process occurring on carrion [8]. Carrion insects colonize decomposing remains in a broadly predictable sequence that depends on the rate of decomposition [9], and this sequence can be used to estimate the PMI [10]. The succession of insects, however, can be influenced by a wide range of biotic and abiotic factors such as temperature, microclimate, season, clothing, cadaver body mass, and shade [11]. This leads to substantial variation among studies of carrion insect succession and can make it difficult to use succession data for PMI estimations [12–15]. The validity of carrion succession research for forensic casework also remains in doubt due to the use of non-human animals in succession experiments, with emerging research suggesting important differences in insect activity between decomposing pigs and humans due to the many sources of variation among these cadaver types [16–18].

Despite these issues, succession experiments provide a source of fundamental information about carrion insect visitation patterns, colonization behavior, spatial distribution, seasonal preference, interspecific interactions, and carrion resource preferences [19–23]. These species characteristics, in addition to larval development data, can provide a fuller picture to forensic investigators that estimate a mPMI from insect evidence. This is of particular importance with the pre-appearance interval (PAI) of some primary colonizing flies, which can exhibit a delay in arrival at carrion of several days under different circumstances. If this delay is considered when calculating a PMI, then the time interval between the mPMI estimate and the actual time of death may be reduced [24].

In terms of the forensic applicability of a given fly species, species characteristics and larval development times provide only part of the picture of how useful a species might be in

a forensic investigation. Both types of data need to be examined to gain a comprehensive understanding of the sequence of events occurring in the context of the mPMI. A good knowledge of the ecological species characteristics defining a carrion-associated species as forensically useful, and filling knowledge gaps relating to such useful species, will improve the accuracy of investigations.

Forensic casework typically leads to the identification of fly species found on human remains and can therefore identify species that might benefit from additional ecological information. Undertaking field succession studies with such species in mind can help improve their usefulness where this ecological information is lacking, particularly for species that are localized [25]. With casework exclusively conducted on human remains, it is important to also determine a species' ecology and succession patterns on human remains, rather than on non-human animals that are often used as proxies [26]. This enables a more robust link to be established between useful species identified in casework and ecological species characteristics observed in field succession studies on human cadavers. In Australia, carrion succession work has mostly been conducted on animal models, with few human succession studies undertaken and little work attempting to assess ecological information of forensically useful species identified in casework [27–31].

The aim of this study was to provide ecological species information for carrion flies that were previously collected in forensic casework, thereby determining how useful they are for forensic investigations. Forensically useful species are common, in high abundance, and display little or no delay in colonisation. Information on fly species' ecology was collected during field succession experiments on human cadavers at the Australian Facility for Taphonomic Experimental Research (AFTER). Here, we provide new information on species in the Sydney region, their seasonal preferences and colonization behavior, as well as their abundance and pre-appearance interval.

4.3 Materials and Methods

We obtained data on flies in three ways: field experiments, casework, and a literature review.

4.3.1 Field data

Two winter and one summer human decomposition experiments were conducted over a 2-year period (May 2018–December 2019). The two winter experiments lasted 139 days ('Winter A':

30th May– 15th October 2018) and 148 days ('Winter B': 8th May–2nd October 2019), while the summer experiment lasted 38 days ('Summer': 9th November–16th December 2019). The disparity in the length of the experiments was due to the slower rate of decomposition in the cooler winter experiments. All experiments took place at the Australian Facility for Taphonomic Experimental Research (AFTER), operated by the University of Technology Sydney (UTS). The 4.86 hectare site is situated in the Hawkesbury region of western Sydney, consisting primarily of dry sclerophyll *Eucalyptus* forest with scattered rural housing in the nearby vicinity. Ambient temperature and humidity were recorded every 15 min at the site using a HOBO MX2302 Ext temperature and relative humidity data logger (Onset) protected by a solar radiation shield.

Experiment Winter A consisted of two female human donors (Human 1: age 53, 46.8 kg, placed 30 May 2018 & Human 2: age 86, 80 kg, placed 2 June 2018), Winter B consisted of two male human donors (Human 3: age 57, 66 kg, placed 8 May 2019 & Human 4: age 74, 51.7 kg, placed 7 June 2019) and Summer consisted of two female human donors (Human 5: age 82, 60.5 kg, placed 9 November 2019 & Human 6: age 97, 46.8 kg, placed 14 November 2019) (see Appendix Table S4 for additional details). All human cadavers were obtained through the UTS Body Donation Program, approved by the UTS Human Research Ethics Committee Program Approval (UTS HREC REF NO. ETH15– 0029). Cause of death of the cadavers included cancer, pneumonia, and heart failure. They were refrigerated (4°C) immediately after death and delivered to AFTER within 48 h. Cadavers warm to ambient temperature within 24–36 h of placement at AFTER [32].

Once at AFTER, the cadavers were placed on the ground in a semi-shaded 5 m × 5 m designated plot. The cadavers in Winter A were placed directly onto the ground, while those in the Winter B and Summer experiments were placed atop thin metal mesh and raised off the ground briefly at intervals throughout the experiment to collect weight loss data for another study. This brief lifting did not impede any fly colonization or insect activity on the cadavers but we acknowledge that the mesh may have introduced some variation in decomposition rates, but this was likely to be trivial compared with variation due to differences in cadaver weight, diet, or cause of death. Due to the number of cadavers placed within the facility, entomological independence in terms of their placement was difficult to achieve [33]. Prior to the experiments, almost all other cadavers in the Facility had become desiccated or skeletonized with minimal associated insect activity. Any new cadavers placed during our experiments were located at least 30 meters away from others, with the same inter-cadaver distance used for within-season replicates. Cadavers were also separated temporally, with at least 1 month between placement.

A cage was placed atop all humans to prevent access by scavenging animals such as birds. Four pitfall traps (250 ml each) were placed evenly around each human and filled with a mix of propylene glycol and 70% ethanol [34]. Although pitfall traps are generally used for ground dwelling invertebrates, we used them in our study as they are effective at trapping flies as they crawl around the cadavers looking for suitable oviposition sites. When combined with sweep netting (see below), pitfall traps were able to supplement our fly collections.

Insect sampling occurred between 11:00 and 13:00 h once every day for the first week, every second day for the following 3 weeks, every fourth day for the following 3 weeks and then every fifth day until no changes in the level of insect activity were recorded. As cadavers were placed at different times, occasionally extra sampling days occurred to synchronize sampling intervals between cadavers. Sampling occurred every day for the first week to ensure accurate documentation of the PAI of early colonizing flies. Insects (both adults and larvae) were collected on each sampling day using a combination of timed manual sampling (10 min search time, including beneath cadavers), sweep netting (10 min), and pitfall traps, with observations of egg and larval masses also documented every sampling day. Identification and rearing procedures of collected insects followed the methods described in Dawson et al. [18].

For this study, only flies were included in any data analysis to allow comparisons with forensic casework. For each cadaver, the total abundance of adult flies was recorded for each species by combining all flies collected across all sample days and collection methods. An average abundance for each species was calculated for each succession experiment by averaging the total abundance for each cadaver (two per season). The PAIs of adult flies and larvae (the minimum amount of time before first observed, regardless of replicate) were also recorded and tabulated, along with information on seasonal preference and colonization behavior for each seasonal experiment.

4.3.2 Casework data

Data on fly species collected in NSW police investigations were collated from the past casework of Levot [35] and one of the authors (Wallman). A total of 156 cases, including both indoor and outdoor cases between the years 1984 and 2018 were used in this study. Insect samples were collected either by police investigators in situ or later by the police and/or forensic pathologists and sent to the forensic entomologist for analysis. We compiled data on collected fly species in casework for all cases to assess the commonalty of species colonizing

human remains. For the Wallman cases only, data on seasonal preference and distribution were also examined and compared with data collected in our field succession experiments.

4.3.3 Literature review data

Lastly, a literature review was conducted on previously recorded colonization behavior for all 28 species captured in the field experiments and casework. For each species, Google Scholar was searched using the terms "genus and species" and if more than 20 papers appeared, the search was further narrowed by adding the term "succession." Papers were examined and information relating to a species' colonization behavior was obtained. Colonization behavior was categorized as primary (first species to arrive and colonize carrion from the onset of decay), secondary (species that colonize carrion after primary colonizers have already established themselves), and tertiary (species that colonize later in the decomposition process after the primary and secondary flies). This information was compared with the colonization behavior of species recorded in our field experiments. All graphs and maps were completed using the R base package [36], ggplot2 [37], sf [38], ggspatial [39], and cowplot [40] packages.

4.4 Results

A total of 21 species of flies associated with carrion were collected throughout all field experiments, with 11 of those species observed as larvae. The average ambient temperature was similar for the two winter experiments at 17.2 and 17.8°C for Winter A and Winter B, respectively. The summer experiment recorded warmer ambient temperatures with an average of 26.5°C (Appendix Fig. S1). A summary of each experiment is given below.

4.4.1 Winter A

We recorded 11 species as adults and four species as larvae (Table 2). Between the two humans the average abundance of adults was low, except for *Piophila casei* (Linnaeus, 1758) and *Australophyra rostrata* (Robineau-Desvoidy, 1830). Although these two species arrived relatively early and in high abundance, larvae of *P. casei* and *A. rostrata* were not recorded until late decay at days 97 and 87, respectively. The first species recorded colonizing humans were *Calliphora hilli hilli* Patton, 1925 and *Sarcophaga impatiens* Walker, 1849 on day 22. Despite larvae being collected, no adult *S. impatiens* were observed on the cadavers and larvae

of *C. hilli hilli* were first collected 5 days before adults were first documented. Adults of known early stage carrion-breeding species, such as *Calliphora ochracea* Schiner, 1868 and *Calliphora stygia* (Fabricius, 1782), were observed on the cadavers, but not recorded ovipositing. There was an overall delay in the PAI for all species, with *P. casei* being the first recorded adult on day 6.

Table 2 Information on fly species collected during the Winter A experiment, detailing the PAI for adults and larvae (days until first observed on any cadaver), and the average abundance of adults (n=2).

Winter A

Family	Genus	Species	Adult PAI (days)	Larval PAI (days)	Average adult abundance (and range)
Calliphoridae	*Chrysomya*	*incisuralis*	38	-	0.5 (0-1)
		varipes	17	-	3.5 (2-5)
	Calliphora	*augur*	32	-	0.5 (0-1)
		hilli hilli	27	22	2 (1-3)
		ochracea	15	-	3.5 (0-7)
		stygia	17	-	1.5 (1-2)
	Hemipyrellia	*fergusoni*	37	-	0.5 (0-1)
Sarcophagidae	*Sarcophaga*	*impatiens*	-	22	0
Muscidae	*Australophyra*	*rostrata*	36	87	25.5 (22-29)
	Hydrotaea	*chalcogaster*	38	-	0.5 (0-1)
		spinigera	73	-	2 (2-2)
Piophilidae	*Piophila*	*casei*	6	97	390 (179-601)
Total species richness/abundance			11	4	430

4.4.2 Winter B

We recorded a much higher average abundance of adult flies in this experiment, with 18 species collected, and seven of those recorded as larvae (Table 3). Only a slight delay in colonization was observed, with *Hemipyrellia fergusoni* Patton, 1925 larvae collected on day 3 and adults first observed on day 1. The arrival of the later stage colonizers also occurred sooner in Winter B with *A. rostrata* and *P. casei* larvae first recorded on days 12 and 60, respectively. A number of *Chrysomya* species had relatively high abundances, especially *Chrysomya varipes* (Macquart, 1851) but were not observed colonizing the humans. Similar to Winter A, the most abundant species recorded was *P. casei*, which arrived relatively early as an adult on day 6.

Table 3 Information on fly species collected during the Winter B experiment detailing the PAI for adults and larvae (days until first observed on any cadaver), and the average abundance of adults (n=2).

Winter B

Family	Genus	Species	Adult PAI (days)	Larval PAI (days)	Average adult abundance (and range)
Calliphoridae	*Chrysomya*	*incisuralis*	2	-	21 (3-39)
		latifrons	5	-	1 (0-2)
		megacephala	6	22	4.5 (4-5)
		nigripes	8	-	31.5 (1-62)
		rufifacies	8	-	16.5 (2-31)
		varipes	8	-	66.5 (9-124)
	Calliphora	*augur*	3	-	3.5 (0-7)
		hilli hilli	2	-	27.5 (14-41)
		ochracea	2	4	12.5 (5-20)
		stygia	1	-	15 (8-22)
	Hemipyrellia	*fergusoni*	1	3	6 (3-9)
Sarcophagidae	*Sarcophaga*	*impatiens*	8	15	0.5 (0-1)
Muscidae	*Australophyra*	*rostrata*	8	12	65 (65-65)
	Hydrotaea	*chalcogaster*	12	-	6 (5-7)
		spinigera	12	-	7.5 (5-10)
Piophilidae	*Piophila*	*australis*	8	-	0.5 (0-1)
		casei	6	60	406 (244-568)
Sepsidae	*Parapalaeosepsis*	*plebia*	10	-	253.5 (0-507)
Total species richness/abundance			18	7	944.5

4.4.3 Summer A

We recorded a high average abundance of adult flies, similar to that of Winter B, despite this experiment only lasting 38 days, with adults of 17 species and larvae of five species (Table 4). *Calliphora augur* (Fabricius, 1775) larvae were the first species recorded breeding on day 2, followed by *Chrysomya rufifacies* (Macquart, 1843) on day 3. Large maggot masses of *Ch. rufifacies* quickly formed on both cadavers, with decomposition progressing rapidly. The most abundant adult species was *P. casei*, with adults arriving on day 1 and larvae recorded on day 18. *Chrysomya varipes* was the second most abundant species, with adults recorded on day 2, but no larvae were recorded. The PAI for most species was short, with many arriving within

the first one to three days. It is notable that *Chrysomya* species did not arrive for up to 3 days in summer, and often later still in winter.

Table 4 Information on fly species collected during the Summer experiment detailing the PAI for adults and larvae (days until first observed on any cadaver), and the average abundance of adults (n=2).

Summer

Family	Genus	Species	Adult PAI (days)	Larvae PAI (days)	Average adult abundance (and range)
Calliphoridae	*Chrysomya*	*incisuralis*	3	-	2.5 (1-4)
		latifrons	2	-	1.5 (0-3)
		megacephala	3	4	1 (0-2)
		nigripes	3	-	12.5 (2-23)
		rufifacies	2	3	60.5 (44-77)
		varipes	2	-	82 (46-118)
	Calliphora	*augur*	1	2	5 (0-10)
		ochracea	12	-	0.5 (0-1)
	Lucilia	*cuprina*	2	-	1.5 (1-2)
Sarcophagidae	*Sarcophaga*	*africa*	2	-	2 (1-3)
		impatiens	27	7	1 (1-1)
		zeta	4	-	4 (3-5)
Muscidae	*Australophyra*	*rostrata*	2	-	81.5 (56-107)
	Hydrotaea	*chalcogaster*	3	-	6 (5-7)
		spinigera	15	-	0.5 (0-1)
Piophilidae	*Piophila*	*casei*	1	18	456.5 (281-632)
Sepsidae	*Parapalaeosepsis*	*plebia*	13	-	0.5 (0-1)
Total species richness/abundance			17	5	719

4.4.4 Casework

From the collective forensic casework of Levot and Wallman, a total of 22 Diptera species were recorded as larvae, with 10 species from the Sydney region. The casework was mostly distributed along the coast in urban locations, especially the Sydney region (Fig. 13), allowing for appropriate comparisons with data collected from AFTER. Almost all Sydney species occurred in our field succession experiments as adults (except *Sarcophaga crassipalis* Macquart, 1839 and *Synthesiomyia nudiseta* (Van Der Wulp, 1883)), and five of these species were observed as larvae (Table 4). The only species recorded as larvae in our experiments and

not documented in any past forensic case was *H. fergusoni*.

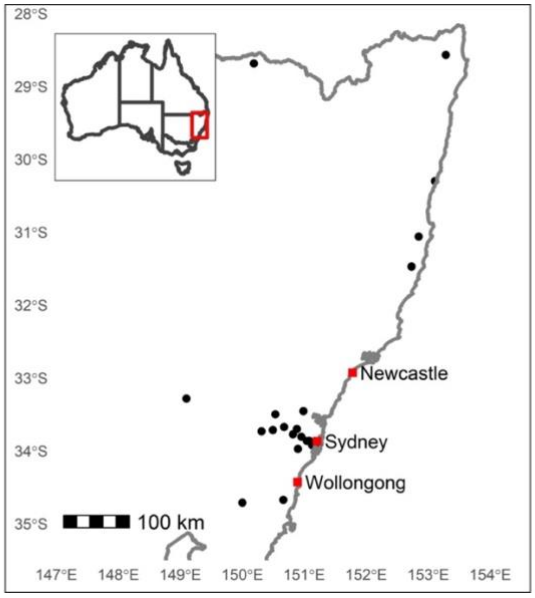

Figure 13 Distribution of forensic entomology cases in NSW, Australia, from the data of Wallman (n=24).

Seasonal data were available for 14 species from Wallman's casework, which included all species that he documented in the Sydney region (Table 5). Data from our field succession experiments showed all recorded *Chrysomya* species, as well as *A. rostrata* and *S. impatiens*, to be present in both summer and winter, while these taxa were only documented from casework. Our literature review identified 25 relevant papers that documented colonization behavior of fly species recorded in our field experiments or from casework; for a complete list of papers see Appendix Table S5. Information for known colonization behavior was available for most species documented in casework, except for some species of less common *Calliphora* and *Sarcophaga* (Table 5). Colonization behavior in our experiments mostly matched that documented in the literature, with a few exceptions. *Chrysomya megacephala* (Fabricius, 1794) was found to be both a primary and tertiary colonizer (though low in adult abundance), while *C. stygia* was found to be a secondary colonizer, despite previously being reported as primary. New records of colonization behavior for *H. fergusoni* and *C. ochracea* have been recorded in our experiments, with both occurring as primary colonizers.

Table 5 Comparison of species collected in forensic casework from Levot and Wallman with species observed in all succession experiments. Details from forensic cases include the number of times species were collected from all cases (Levot and Wallman) and whether they were collected in summer (S) or winter (W) (Wallman). Species names with * are those that were collected from casework in the Sydney region (Wallman). Details of species presence as adults (A) and immatures (I), seasonality and colonisation behaviour is also detailed for all succession experiments (exp) combined. Previously known colonisation behaviour collected from the literature is also listed (L).

Family	Genus	Species	Casework	Succession exp	Season (cases)	Season (exp)	Colonisation behaviour (Exp)	Colonisation behaviour (L)
Calliphoridae	*Chrysomya*	*incisuralis*	0	A		S/W		
		latifrons	0	A		S/W		
		*megacephala**	13	A/I	S/W	S/W	Primary Tertiary	Primary
		*nigripes**	10	A	S	S/W		Tertiary
		*rufifacies**	51	A/I	S	S/W	Primary	Primary Secondary
		saffranea	2	no	S			Primary Secondary
		*varipes**	21	A	S	S/W		Primary Secondary
	Calliphora	*augur**	52	A/I	S/W	S/W	Primary	Primary
		hilli hilli	2	A/I	W	W	Primary	Primary
		ochracea	8	A/I	W	S/W	Primary	
		nigrithorax	1	no				
		stygia	35	A/I		W	Secondary	Primary
		vicina	2	no				Primary
	Lucilia	*cuprina**	11	A	S	S		Primary
		sericata	8	no				Primary
	Hemipyrellia	*fergusoni*	0	A/I		W	Primary	
Sarcophagidae	*Sarcophaga*	*africa*	0	A		S		
		*crassipalpis**	1	no	S			Primary
		*impatiens**	3	A/I	S	S/W	Primary Secondary	Primary Secondary
		praedatrix	1	no	S			
		zeta	0	A		S		
Muscidae	*Australophyra*	*rostrata**	27	A/I	S	S/W	Secondary Tertiary	Secondary Tertiary
	Hydrotaea	*chalcogaster*	1	A		S/W		Tertiary
		spinigera	1	A		S/W		Tertiary
	Synthesiomyia	*nudiseta**	2	no	S/W			Secondary
Piophilidae	*Piophila*	*australis*	1	A		W		
		casei	7	A/I		S/W	Tertiary	Tertiary
Sepsidae	*Parapalaeosepsis*	*plebia*	0	A		S/W		

4.5 Discussion

Our field succession experiments revealed differences in the diversity and abundance of necrophagous fly species arriving on decomposing human cadavers in winter and summer, but also differences between the two winter experiments. Our results also showed that different species of primary colonizing flies initiated colonization on the cadavers in each experiment, thus highlighting significant variability in the succession process. All commonly documented species in previous forensic casework were observed in our field succession experiments, with these species generally having the highest adult abundance. The majority of species reported in casework in the Sydney region were also recorded in our experiments. This suggests that AFTER provides valuable entomological insights applicable to forensic casework in NSW. We also documented new colonization behavior and seasonal preferences for previously recorded flies in casework. New information is also provided for *H. fergusoni*, which has not previously been recorded in casework but occurred as a primary colonizer in our experiments, highlighting its potential as a forensically useful species in future casework.

Below we discuss our key findings relating to variability in field succession experiments, which species are potentially useful forensically, and how linking field and casework should improve mPMI investigations.

4.5.1 Variability among field succession experiments

The Winter A experiment had a remarkably lower adult abundance and a delay in both adult arrival and initial colonization compared with the other experiments, particularly Winter B. Our results confirm the findings of other studies that also identified a delay in Diptera colonization [31]. The delayed colonization and abundance differences could be attributed to the ambient temperatures to which the cadavers were exposed. Although the Winter A and B experiments had similar average temperatures, Winter B had higher temperatures in the first few weeks compared to Winter A. This initial higher temperature could have increased the insect activity and succession process on the cadavers in Winter B. Insect activity in general has been shown to be influenced by higher temperatures (within the thermal limits of a species) [41–42], with decomposition and insect succession also shown to progress more rapidly with higher temperatures [13,43–44]. The medical histories, physical characteristics, and volatile organic compounds (VOCs) released by the humans may also have influenced the initial

succession and species abundance on the humans in Winter A [18,45]. The effect of medical history on decomposition and insect communities remains relatively unknown, but it has been suggested that medical treatment during the peri-mortem period can influence decomposition [46]. However, the human donors in Winter B and Summer experiments exhibited no colonization delay or reduced abundance, despite having a range of body masses and medical histories. Species abundances are often neglected in forensic entomology, and usually do not form part of PMI estimates. More abundant species are however more likely than rarer species to be encountered in casework, and therefore more likely to be more forensically useful. Abundance data can provide a different perspective in the application of traditional succession models, in that the numbers of flies of a given species influences the numbers of eggs being laid and the likelihood of eggs and larvae being collected by forensic investigators.

4.5.2 Information on forensically useful species

Although a larger number of adult flies were arriving at the humans, only relatively few species were recorded as breeding. This was most obvious in the Summer experiment, with species that are known to be carrion breeders, such as *Ch. varipes* and *A. rostrata*, having high adult abundance but no immatures present. This is likely attributable to the dominance of *Ch. rufifacies*, which formed extremely large maggot masses beneath the humans. This species is known to be a facultative predator of other fly larvae, with other species avoiding oviposition entirely when *Ch. rufifacies* is present [47]. *Chrysomya rufifacies* was the second most recorded species in casework and has previously been documented as a secondary colonizer, though it can also act as a as a primary colonizer [48–49]. In the Summer experiment, this species was a primary colonizer and when it was not breeding, such as in Winter B, a more diverse range of other species was breeding. Breeding species, such as *S. impatiens, C. hilli hilli*, *Ch. megacephala*, and *H. fergusoni*, were often in very low adult abundances.

All five of the most commonly recorded species in casework were recorded in our experiments. The most common, *C. augur*, occurred in all experiments as an adult but was only documented breeding in summer. The seasonal preference, colonization behavior and PAI of *C. augur* seen in our study is similar to previously recorded observations of this species, with other studies documenting its predictable arrival sequence on carrion and larval development rates, making it ideal for mPMI estimations [50–51]. Another species with mPMI applicability is *C. ochracea*, which has previously been recorded in several police cases and is distributed

around the Sydney region [52]. In our experiments, *C. ochracea* displayed a high adult abundance, was a primary colonizer and had a short PAI, as it was generally one of the first adults to arrive. While these qualities give the species forensic potential, only very limited larval development data are available [53].

4.5.3 Linking field experiments and forensic casework

We found that *H. fergusoni* bred in our experiments, but it has not previously been recorded as larvae in casework. Adults of *H. fergusoni* were previously collected from an urban site in the Sydney region in high abundance [52] and from a human cadaver at AFTER [31]. This species was a primary colonizer in Winter B, making it valuable for mPMI estimates. Considering that *H. fergusoni* was observed initiating colonization on human cadavers in our experiments, it is surprising that the species has never previously been recorded in casework. A likely explanation for this is that it was previously misidentified due to its superficial morphological similarity to *C. augur*, which was the most commonly recorded species in casework. The amount of error in a mPMI estimate based on the misidentification of *H. fergusoni* as *C. augur* is however unknown due to the lack of developmental data for the former species. Such data are however available for the closely related species, *Hemipyrellia ligurriens* (Wiedemann, 1830), which has a quicker larval development cycle then *C. augur* [54–55]. If *H. fergusoni* has similar development times to *H. ligurriens*, the result would be an overestimated mPMI based on *C. augur* development data. This highlights the importance of correct identification of species using both morphological and molecular techniques when applying them to PMI estimations.

Australophyra rostrata has previously been documented as a summer species in casework, but was recorded breeding in winter during our experiments. This species has previously been documented as a tertiary colonizer, but can also be a secondary colonizer [56], as observed in our Winter B experiment. *Australophyra rostrata* arrives on carrion relatively early, with a short PAI as an adult in high abundance, but delays oviposition. This delay provides conditions on the cadaver that are optimal for the growth and survival of the larvae—dried remains and the absence of competition with larvae of primary colonizing species [50]. *Piophila casei* exhibited similar oviposition behavior, with the species having relatively short adult PAI and high adult abundance, but not ovipositing until late in the decomposition process. While these species can provide mPMI estimates from larval data, such estimates would be far

from the actual time of death due to the unpredictable timing of oviposition, which appears to be heavily dependent on the decomposition process.

4.5.4 Implications and conclusions

Our study has provided new information for forensically useful Diptera species in relation to their colonization behavior, seasonal preference, and adult/larval PAI on human cadavers in the Sydney region of Australia. Only one summer experiment was conducted for this study, with further replication required to confirm patterns and potential variation between seasons. PAI is a difficult metric to measure, particularly for adult flies whose presence may be missed while sampling. After commencing sampling every second day and despite the standardized sampling methods across all cadavers, it is possible that the PAI of later arriving species became slightly less accurate or some species might have been missed, thus affecting PAI estimates. This seems to have been the case in our study, as we sampled some species of flies as larvae prior to sampling them as adults. Despite this, our PAI measures still provide a valuable step toward determining the true PAI for species analyzed in our study and provides an indication of when these mid/late stage species are arriving. Real-world data from forensic casework are likely to be even less accurate than our data due to the *ad hoc* sampling of cadavers, with rare species even more likely to be missed. Combining data from succession models and casework can help reduce uncertainty. This information can assist forensic investigations in assessing what species may be of forensic value in mPMI investigations, particularly if succession data are used for estimations. Our research highlights the value of combining field succession work on cadavers with previous casework data in order to gain a better understanding of the potential forensic value of carrion species. Future work is needed on all aspects of a species' life history and ecology to determine what forensic information they can provide, as well as on succession patterns and larval development rates of less commonly encountered species.

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Chapter 5. Is resource change a useful predictor of carrion insect succession on pigs and humans?

5.1 Abstract

Carrion is a dynamic and nutrient-rich resource that attracts numerous insect species that undergo succession due to the rapid change in the carrion resource. Despite this process being well understood, few studies have examined resource change as a driver of carrion insect succession, and instead have focused on the effects of time per se, or on coarse, qualitative measures such as decay stage. Here we report on three field succession experiments using pig carcasses and human cadavers encompassing two winters and one summer. We quantified the effects of resource change (measured as total body score, TBS), carrion type, initial carrion mass, ambient temperature, and season on insect species richness and community composition. We found that all variables had an effect on different taxonomic or trophic components of the insect community composition, with the exception of initial carrion mass which had no effect. We found significant positive effects of TBS on beetle species richness and composition, while fly species richness was not significantly affected by TBS, but was by ambient temperature. TBS had a significant positive effect on all trophic groups, while ambient temperature also had a significant positive effect on the necrophages and predator/parasitoids. Our study indicates that resource change, as indicated by TBS, is an important driver of carrion insect species turnover and succession on carrion, and that TBS can provide information about insect ecological patterns that may not be evident when using other temporal measures.

5.2 Introduction

Carrion, the tissue of dead animals, is a unique, spatially sparse, and dynamic resource, playing a key role in nutrient recycling and ecosystem function (Beasley et al. 2012, Barton et al. 2013). Shortly after death, carrion is quickly colonized and consumed by a range of carrion-associated species, all of which interact forming a complex trophic web, referred to as the necrobiome (Benbow et al. 2019). The organisms at carrion (microbial, vertebrate, and invertebrate) digest and consume the different tissues, and this drives a rapid change in the resource itself. This resource change can be categorized as both quantitative change (i.e., through mass loss) and qualitative change (i.e., through changes in nutritional value or digestibility) (Benbow et al. 2019). Resource change also incorporates the microbial community colonizing carrion, as the microbes change in structure and function, and in many ways become the resource itself as tissue and microbial biofilms are ingested by insects (Pechal and Benbow 2015). Understanding the complex interactions of species on carrion, and the dynamics and change in carrion itself, is important and has a range of applications including forensic investigations (Byrd and Tomberlin 2019), mass mortality events (Tomberlin et al. 2017), biodiversity conservation (Barton et al. 2013), and ecosystem function (DeVault et al. 2003).

Among the different groups of species found at carrion, many have been observed to show a pattern of succession, and this is most notable for microbial (Pechal et al. 2014) and insect communities (Payne 1965). Community succession at carrion never reaches an equilibrium or stable community of species, as it does with plant communities, and therefore does not conform with traditional ecological succession theory (Schoenly and Reid 1987, Michaud and Moreau 2017). Due to the short-lived nature of carrion, interspecific and intraspecific competition for resources is high for both vertebrate and invertebrate carrion-associated species, particularly those that are dependent on carrion for reproduction and survival (Denno and Cothran 1976). Species need to be highly adapted to locating carrion or specialized in feeding on carrion in different conditions or environments (Denno and Cothran 1975, Tomberlin et al. 2011). As the carrion changes in physical and chemical composition, it offers distinct opportunities over time for species to colonize the remains, thereby driving succession. The quality of the resource is therefore dependent on the perspective of the insects colonizing the remains. For example, early colonizing species, such as sarcophagids (e.g., *Sarcophaga impatiens*) and some calliphorids (e.g., *Calliphora augur*), which display viviparous behavior (lay live larvae), have a narrow window of opportunity for optimal resource usage on carrion. This requires quick detection and colonization to gain an advantage

over other carrion-associated species, with recently deceased carrion being perceived as higher quality (Williams et al. 2017). Later colonizing species, on the other hand, make use of slightly different resources on carrion, but have a larger window of opportunity. For example, species like piophilids arrive only when fatty acids are present at the remains and perceive older carrion to be of higher quality (Martín-Vega 2011).

Insect species not only need to detect carrion from great distances away, but also determine the quality of the carrion to ensure that it is optimal for their survival. To locate carrion, insects, and in particular flies, use highly specialized chemoreceptors to detect volatile organic compounds (VOCs) emitted from the decomposing tissues (Frederickx et al. 2012). It is this chemical cue that attracts insects to carrion in a predictable sequence, with previous studies showing the change in composition and quantity of VOCs as decomposition of carrion progresses (Knobel et al. 2019). This predictable sequence of arrival and departure of insects at carrion can provide valuable information to forensic investigations (Anderson 2002). Most importantly, carrion insect succession can assist forensic investigators by providing an estimation of the postmortem interval (PMI), based on the relative abundance and presence/absence of certain species (Byrd and Tomberlin 2019). The succession of flies and beetles at carrion has been studied extensively, with numerous insect succession studies conducted in different biogeographic areas (Bourel et al. 1999, Carvalho et al. 2000, Tabor et al. 2004, Biavati et al. 2010). Further, the effects of a range of abiotic factors on the succession process, such as temperature, season, and climate, have been well documented (Archer and Elgar 2003, Martinez et al. 2007, Sharanowski et al. 2008). These studies all tend to have a forensic focus by examining species change in relation to direct temporal measures (e.g., days) or compound measures that incorporate time (e.g., accumulated degree days [ADD]) (Michaud and Moreau 2009). These approaches fail to explicitly incorporate resource change that occurs simultaneously on the carrion and often neglect the ecological aspects of carrion insect succession, such as how these abiotic factors influence the behavior and occurrence patterns of the insect trophic groups associated with carrion (Barton et al. 2013).

One of the key ecological drivers of carrion insect succession is the carrion itself, including all the factors relating to the resource quality and quantity of the remains (decomposition age, mass, and type of carrion). Previous studies have shown species richness and composition changes, as well as changes in oviposition preference as carrion decomposition progresses (Farwig et al. 2014, Kotzé and Tomberlin 2020). These studies however usually represent decomposition rate or carrion decomposition age as discrete decay stages (fresh, bloat, active, advanced, and skeleton), rather than as a continuum (Sharanowski

et al. 2008, Voss et al. 2009). Species turnover does change with these discrete stages of decay, but this 'broad- brush' approach lacks precision and does not reflect the continuous change in the carrion resource (Schoenly and Reid 1987, Michaud et al. 2015). It is important to recognize the continual change of carrion, especially when examining community assemblages on carrion and how species turnover occurs on a rapidly changing resource. Along with decomposition age, the biomass and type of carrion may also dictate species change and turnover (Watson and Carlton 2005). Biomass has been shown to influence the species richness of insects on carrion, with larger-bodied carrion able to host more diverse insect assemblages (Matuszewski et al. 2016). Other studies have shown species richness and community differences in carrion insects between carrion types, particularly pig and human remains (Dawson et al. 2020). However, few studies have examined these factors in combination and in relation to other key abiotic factors such as ambient temperature and season to determine if and how these factors are driving carrion insect succession.

The aim of this study was to determine how factors relating to resource change, such as total body score (TBS; measure of decomposition progress), initial carrion mass and carrion type (human compared to pig), and associated abiotic factors (ambient temperature and season), affected the dynamic change in species richness and community composition of carrion insects. We focused on how these factors drive changes in both taxonomic groups (flies, beetles, and ants) and trophic groups (necrophages, predator/parasitoids, and omnivores) of carrion-associated insects. TBS was used as the main proxy for resource change in this study as this measure of decomposition incorporates physical changes occurring on the carrion (i.e., desiccation of remains) and to some degree molecular changes (i.e., occurrence of bloating). We deemed TBS to be an appropriate measure of carrion resource change as it provides a semiquantitative and intuitive measure of decomposition progress. We tested three hypotheses: 1) that TBS (i.e., degree of resource change) would be a significant driver of insect community turnover for all trophic groups, and in particular for necrophages that specialize on decomposing remains as a food source, and are dependent on the decomposition stage of the carrion; 2) that insect species richness would be significantly affected by carrion mass as the size of the carrion would dictate the number of species it is able to support; and 3) that carrion type would have a significant effect on insect community composition and species richness as different VOCs are emitted by decomposing pigs and humans, and would therefore likely attract different suites of insects.

5.3 Materials and Methods

5.3.1 Study site

We conducted our experiments at the Australian Facility for Taphonomic and Experimental Research (AFTER), which is operated by the University of Technology Sydney (UTS). The 4.86-hectare facility is located in the Hawkesbury region of western Sydney with the surrounding vegetation categorized as dry sclerophyll *Eucalyptus* forest with scattered rural housing nearby. In total, three succession experiments with humans and pigs were conducted at AFTER: two in winter ('Winter A': 30th May to 15th October 2018 and 'Winter B': 8th May to 2nd October 2019) and one in summer ('Summer': 9th November to 16th December 2019). Only one summer experiment was conducted as no donors were available in summer 2018. The disparity in the length of the experiments was due to the slower rate of decomposition that occurred in the cooler winter experiments. Ambient temperature and humidity were recorded every 15 min at the site using a HOBO MX2302 Ext temperature and relative humidity data logger (Onset, Cape Cod, MA) protected by a solar radiation shield.

5.3.2 Human and pig setup and experimental design

We used a total of six human cadavers and five pig carcasses in the succession experiments (Appendix Table S6). Cadavers were obtained through the UTS Body Donation Program, approved by the UTS Human Research Ethics Committee Program Approval (UTS HREC REF NO. ETH15-0029). After death, the human cadavers were kept refrigerated and delivered to AFTER within 48 h. Domestic pigs (*Sus scrofa*) were purchased postmortem from a licensed abattoir, therefore requiring no ethics approval in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004). Pigs were killed by a captive head bolt and transported to AFTER within 1 h of death.

We placed all human cadavers on their backs within the facility in a designated 5 m × 5 m plot, while pig carcasses were placed on their sides along the outside of the facility to comply with licensing agreements. The pigs were placed a minimum of 20 m apart from one another, and were a minimum of 100 m away from the human cadavers. Due to the number of other human cadavers placed within the facility, entomological independence between humans was difficult to achieve; therefore, cadavers were placed closest to other cadavers that had already skeletonized or become desiccated, with little or no observable entomological activity.

Humans and pigs in the Winter A experiment were placed directly on the ground, while humans and pigs in Winter B and Summer experiments were placed on metal mesh and infrequently lifted slightly off the ground for a few seconds throughout the decomposition process to collect weight loss data for another study. All humans and pigs were placed in semishaded areas beneath metal cages to prevent scavenging animals accessing the remains. Four pitfall traps were placed evenly around each human and pig and filled with a mix of propylene glycol and 70% ethanol to capture ground-active insects. The layout of the traps around the humans and pigs followed the method described by Dawson et al. (2020).

On each sampling day, between 1100 and 1300 h, the decomposition progress was documented and photographed with a Canon EOS 70D camera with a 18- to 55-mm STM lens. We collected adult carrion insects on every sampling day using a combination of standardized methods including timed sweep netting and manual sampling, as well as pitfall traps. The identity of fly eggs and larvae was documented by collecting and rearing live samples. Sampling occurred once every day for the first week, every second day for the following 3 wk, and then gradually reduced depending on the progress of decomposition and level of insect activity. We identified all insects to species level where possible using morphological and molecular techniques, as described by Dawson et al. (2020).

5.3.3 Data analysis

We were interested in quantifying and comparing the effects of resource change and other abiotic variables on insect community change during the decomposition of pigs and humans. We used TBS as a measure of resource change and decomposition stage for the humans and pigs. TBS was calculated following the methods of Megyesi et al. (2005), by separating the body into three distinct regions (head, torso, and limbs) and applying a numeric value to each. Scores were calculated from the decomposition rate and summed together for each segment. Prior to any statistical analysis, all carrion insect species richness and community composition data were sorted by TBS by only using sampling days where a change in TBS was recorded. This meant that our data set reflected the insect communities and accompanying abiotic variables sampled at each step-change in TBS. Prior to the analysis of our insect data, we removed rare species from our data set that were recorded on two or fewer days (seven species removed, see Appendix Table S7). This was because they may have been random or transient species, but also can influence results by inflating richness or compositional difference among

samples.

First, we wanted to examine insect species richness. We used model averaging with Akaike's second-order information criterion (AICc) on generalized linear mixed models (GLMMs) to determine how resource quality and other abiotic variables predicted species richness of adult carrion insects (Barton and Evans 2017). The predictor variables incorporated into the models included TBS, ambient temperature, and initial carrion mass (kg) as fixed continuous effects, with carrion type (human compared to pig) and season (summer compared to winter) as fixed categorical effects. Accumulated degree days were not included as we wanted to determine the effect of just ambient temperature, without confounding it with time, while time is already inherently apart of TBS. Each replicate of human and pig (total replicates = 11), referred to here as 'replicate' number, was added as a random effect for all models. In total, seven different models were constructed, one for each of the following seven species richness response variables: total (all species), fly, beetle, ant, necrophage, predator/parasitoid, and omnivore. These response variables were chosen to represent how both taxonomic (flies, beetles, and ants) and trophic groups (necrophages, predator/parasitoids, and omnivores) are influenced by resource change. To allow direct comparisons between effects, we transformed all predictor variables to have a mean of 0 and a standard deviation of 1. A Poisson error distribution and a logarithmic link function were used for all species richness GLMMs, unless the data were overdispersed, in which case a negative binomial was used. We conducted model averaging based on Akaike's information criterion (AIC) (Burnham and Anderson 2002) using the 'MuMIn' package (Barton 2016). For each response variable, we fitted all subsets of a full model that contained all our predictor variables described above and performed model averaging on all models lower than $\Delta AIC = 6$ (Burnham and Anderson 2002, Anderson 2008). After averaging, we calculated the mean estimates and 95% confidence intervals for each predictor variable. We plotted our results of the modeling as effect sizes and interpreted estimates as significant if their 95% confidence intervals did not cross the zero-effect line (du Prel et al. 2009).

Second, we wanted to examine changes in insect community composition. To determine how resource change and other factors influenced the community composition, principal coordinate analysis (PCoA) was first used to derive new axes that represented important variation in insect composition, and to visualize the effect of the predictor variables; carrion type, TBS, season, and ambient temperature on the community composition of total (all species), flies, beetles, and ants. We conducted the PCoAs on presence/absence data using Jaccard dissimilarity matrices. We extracted the scores for axes 1 and 2 from the PCoAs and

used them as our 'community composition' response variables in model averaging on linear mixed models (LMMs). The same predictor variables described above were used for the species richness analysis, with 'replicate' fitted as a random effect. We constructed a total of eight models, with two models (axis 1 and axis 2) for each community composition in the ordination (total, fly, beetle, and ant). We followed the same model averaging procedure described above for the community composition analysis. Although common in community composition analyses, PERMANOVAs were not used in this study as they do not allow for fitting of multiple variables in one model, and the comparison of relative effect sizes. We conducted all mixed models and model averaging using the R base package (R Core Team 2019), glmmTMB (Brooks et al. 2017), and lme4 (Bates et al. 2016) packages, while ordinations and plots were created using ggplot2 (Wickham 2016), vegan (Oksanen et al. 2019), and ape (Paradis and Schliep 2019) packages.

5.4 Results

We collected a total of 52 carrion-associated species during our field succession experiments, and identified them to species level where possible, including 28 fly, 13 beetle, and eight ant species (Appendix Table S7). The relative species richness of each taxonomic group changed over time, particularly for beetles which displayed a large increase in species richness as TBS increased (Fig. 14a). The trophic groups displayed a less obvious change over time with TBS, with most maintaining a similar species richness throughout decomposition (Fig. 14b).

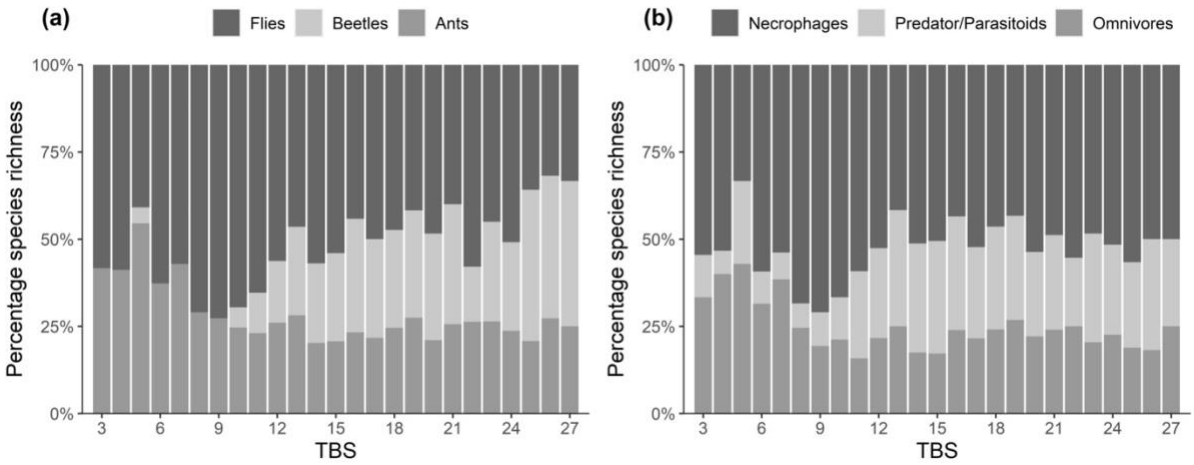

Figure 14 Relative species richness of the taxonomic groups (flies, beetles, and ants) (a) and the trophic groups (necrophages, predator/parasitoids, and omnivores) (b) collected from human and pig remains (n = 11). Data are combined together and sorted by TBS to include all seasonal field experiments and carrion types.

5.4.1 Species richness

We found that TBS had a significant positive effect on species richness of beetles, as well as all insect trophic groups (Fig. 15). Carrion type had a significant effect on species richness of flies, necrophages, and omnivores, with the richness of flies and necrophagous insects higher on pigs than humans, while omnivore richness was higher on humans than pigs. There was a notable absence of any effect of initial carrion mass on all insect taxonomic and trophic groups. We found a significant effect of season on beetles and ants, but not flies, with both having higher species richness in summer. We also found that ambient temperature had significant positive effects on fly, necrophage, and predator/parasitoid species richness.

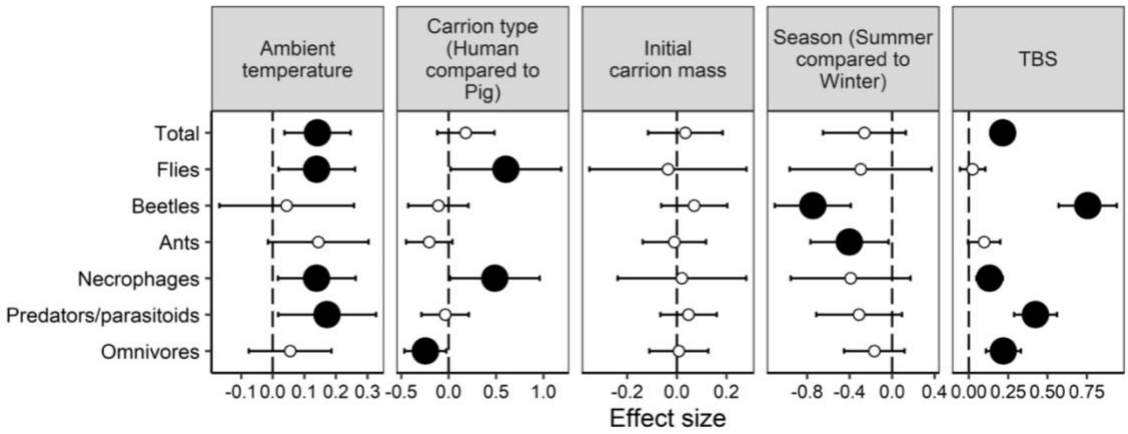

Figure 15 Effects of ambient temperature, carrion type (human compared to pig), initial weight, season (summer compared to winter), and TBS on carrion insect species richness. Significant effects are denoted by 95% CIs that do not cross zero (shown in bold). Effect sizes are derived from generalized linear mixed models and model selection of species richness data.

5.4.2 Community composition

We first examined the insect community visually using ordination plots, and found some structuring of the carrion insect community when viewed as a whole (all species) (Fig. 16a), as well as when examining flies only (Fig. 16b). For these communities, the composition changed with TBS (as indicated by increasing dot size) and ambient temperature (as indicated by degree of shading). There was also a visible difference in insect community composition between summer and winter (indicated by orange and blue, respectively), and humans and pigs (indicated by triangles and circles, respectively) for both the whole insect community and the fly-only community. In contrast, the structuring of the beetle (Fig. 16c) and ant (Fig. 16d) communities was less clear when viewed in the ordination plots, with a large amount of overlap in community compositions between sampling points, although some differences are evident between seasons for the beetles (Fig. 16c).

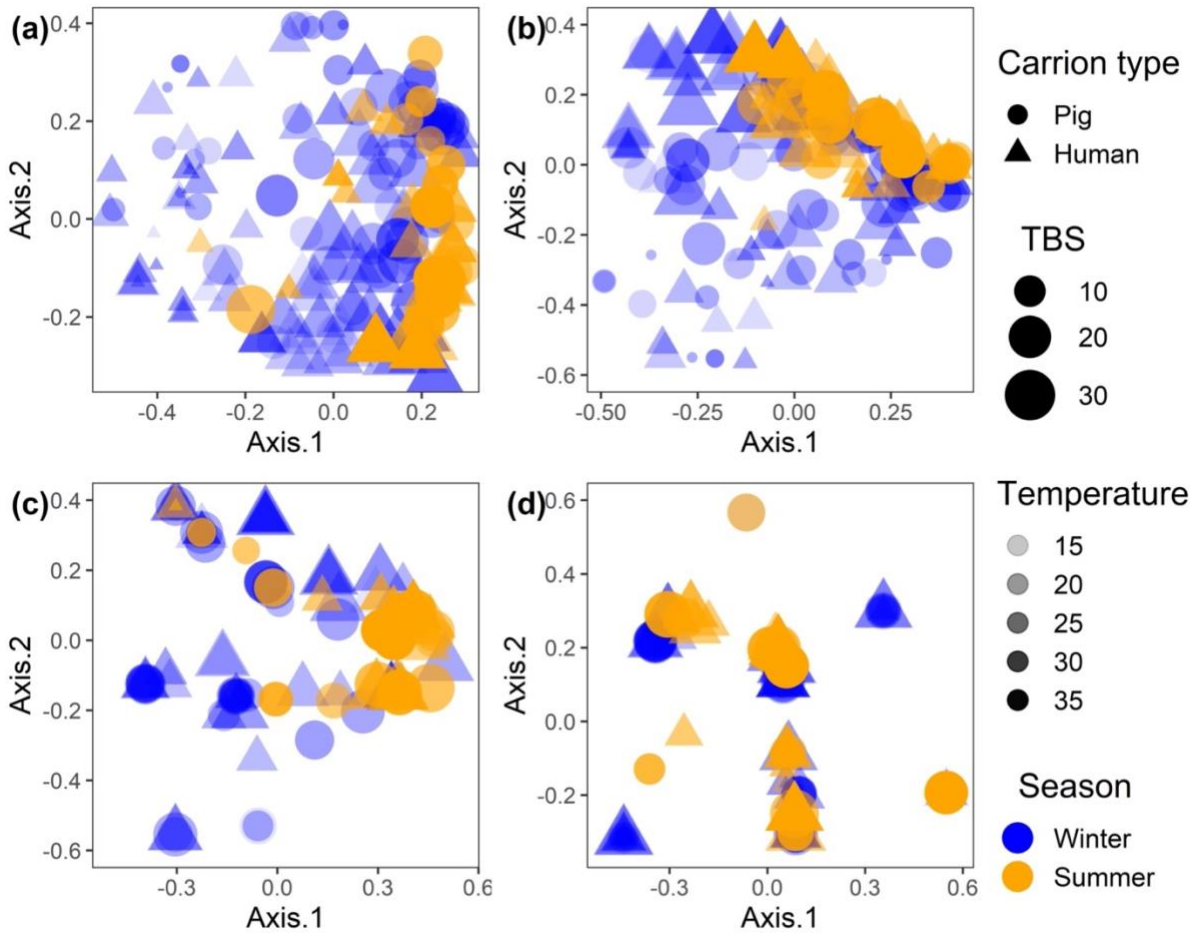

Figure 16 Principal coordinate analysis ordination plots of carrion insect communities: (a) all insect species, (b) flies, (c) beetles, and (d) ants. Shapes denote carrion type; shape size denotes decomposition progression (TBS); alpha denotes ambient temperature; shading indicates season.

We next examined insect community composition statistically, and found significant effects of TBS on all taxonomic species groups (Fig. 17). We found that carrion type had a significant effect on the community composition of flies and beetles. The initial carrion mass had no effect on any taxonomic species group. Season had a significant effect on the community composition of beetles only. Lastly, we found that ambient temperature only had a significant effect on flies.

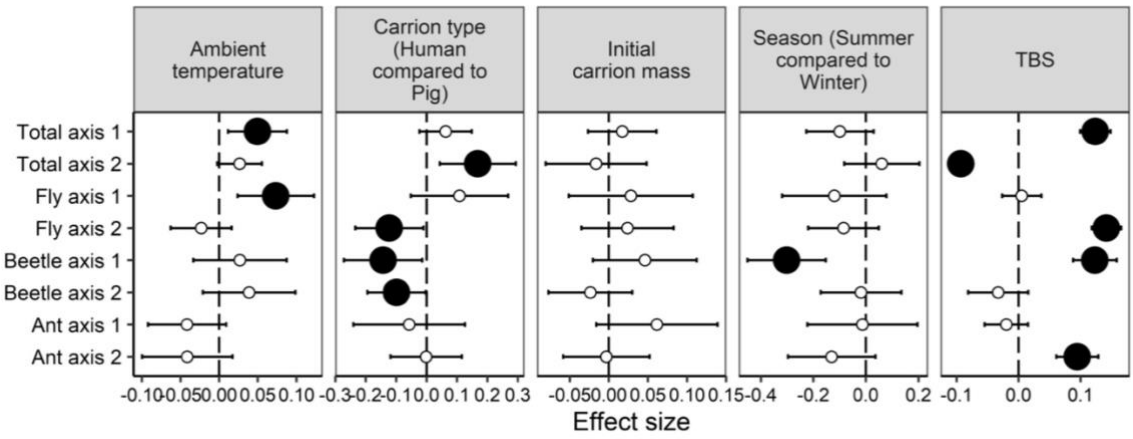

Figure 17 Effects of ambient temperature, carrion type (human compared to pig), initial weight, season (summer compared to winter), and TBS on insect community composition. Significant effects are denoted by 95% CIs that do not cross zero (shown in bold). Effect sizes are derived from linear mixed models and model selection of ordination axis scores.

5.5 Discussion

We set out to determine how factors relating to carrion resource change (TBS, initial carrion mass, and carrion type) and associated abiotic factors (ambient temperature and season) were driving changes in species richness and community composition of carrion insects. We found that most factors relating to carrion resource change had some kind of effect on the species richness or composition of the insect community. As hypothesized, TBS was the most influential driver of species community change and carrion type affected the species richness and composition of flies and necrophages, while surprisingly, carrion mass had no influence on any species groups. These results are similar to other studies that have examined carrion insect change with discrete decay stages (Matuszewski et al. 2008, Voss et al. 2009, Benbow et al. 2013). However, our results provide new information, because we found that, when examining decomposition rate and carrion age as a continuum, species richness increased with increasing TBS for all species except for flies and ants. Community composition also changed for all species as TBS changed.

5.5.1 TBS as a measure of resource change driving species change

We used TBS as the main proxy for resource change in our study, with the results unsurprising for ants as they are active throughout the decomposition process and have a generalist feeding strategy, enabling them to feed either on the remains or other carrion insects (Evans et al. 2020). Although the species richness of ants did not change in our study with TBS, previous studies have found that ant abundance is significantly higher at the start of decomposition, demonstrating their rapid colonization ability and potentially important role in shaping community assemblages of carrion insects (Barton et al. 2017). The non-significant effect reported in our study may also be due to the small number of ants recorded resulting in a lack of statistical power. Of all the taxonomic groups in our experiments, only species richness of flies was unaffected by TBS, but, in contrast, community composition changed with TBS. This suggests that specific communities of flies are adapted and specialized to feed or oviposit on carrion at different decomposition ages, such as Piophilidae, which prefer to colonize older carrion (Matuszewski et al. 2008, Evans et al. 2020). Despite the change in community composition, the species richness remained constant. A possible explanation for this is that the carrion can support a particular number of species at any one time as resources and space on the carrion are limited. Therefore, only those species that exhibit a competitive advantage during a particular phase of decomposition will be present on the carrion. Interspecific competition for resources or oviposition sites is likely to be one of the main mechanisms shaping carrion communities. Although species richness did not change, it is possible the relative abundance of individual taxa did change with TBS. Though not analyzed here, modeling the abundance of taxa may provide further insight into the finer details of carrion ecology and species interactions on carrion.

Beetles were the second most speciose taxonomic group, and unlike flies, showed the largest increase in species richness as TBS increased. This suggests that beetle assemblages on carrion are shaped and driven by different factors and mechanisms compared to flies. The decomposition age of the resource is a dominant factor driving beetle richness and composition, as most carrion beetles are adapted to consuming older and drier remains. Few other taxa feed on remains in the later stages of decay, resulting in competition being relatively low, and allowing for less constraint on species richness numbers. Other studies have observed this increase in beetle taxa as decomposition progresses, though the richness has been shown to decrease after active/advanced decay (Kočárek 2003, e Castro et al. 2013). Some carrion beetles also act as facultative predators on other carrion insects, particularly flies. It is therefore

expected to see an increase in beetle species richness as decomposition progresses, as more food resources become available to the beetles (Dekeirsschieter et al. 2011; Dekeirsschieter et al. 2013). This is reinforced by our findings that species richness increased with changes in TBS for all trophic groups, highlighting their dependency on the carrion decomposition age for feeding and/or breeding. This predatory pressure is also another mechanism working in conjunction with competition to structure fly species richness on carrion, preventing any species from completely dominating.

5.5.2 Other aspects of resource change and environmental influence on carrion insect succession

We found TBS, the main proxy for resource change, to be a key driver of species richness and community composition, while other factors pertaining to resource change were less influential. In particular, we found the initial carrion mass of the remains to have no effect on the species richness or community composition of any taxonomic or trophic group. This result is surprising as previous studies have documented more diverse assemblages of insects on larger carrion because more biomass is available for consumption and there is a greater surface area, leading to reduced levels of interspecific competition (Matuszewski et al. 2016, Wang et al. 2017). The insects in our study showed no preference for body mass, possibly due to lack of relatively small-sized remains in our experiment, with the majority being above 50 kg. There may be a threshold size of carrion above which there is little difference in the number of species able to be maintained, meaning that the biomass differences in our study may not have been large enough to detect any differences in insect activity. Other studies observed this pattern, with all guilds of insects colonizing carcasses above 35 kg, while carcasses below this threshold did not support all guilds of carrion insects (Matuszewski et al. 2016).

Another aspect of resource change that was investigated in our study was carrion type, as it encompasses the physiological and microbial structure of the remains. We found that flies and necrophagous species displayed higher species richness on pig remains, while omnivores had higher species richness on human remains, with carrion type having no influence on the other species groups. The community composition of flies and beetles was significantly affected by carrion type, while ants remained unaffected. Immediately after death, internal tissues begin to breakdown and be decomposed by microorganisms and bacteria, resulting in the release of VOCs, and the carrion becoming bloated (Forbes and Perrault 2014). These

VOCs are what attract insects to carrion, particularly flies that use highly specialized chemoreceptors (Brodie et al. 2016). Pigs and humans have been found to release different VOCs at the onset of decay, which could account for the differences in flies and other necrophagous species observed between pigs and humans (Knobel et al. 2019, Dawson et al. 2020). Pigs may be producing more attractive VOCs, particularly for carrion-breeding flies, whose larvae might have a greater fitness and/or survival if oviposited on remains with more attractive VOCs. Other factors that might influence the species richness and community composition differences observed in our study could be the perimortem treatment and the physiological structure of the pigs and humans (Dawson et al. 2020).

Species richness increased with ambient temperature, with seasonal differences in species richness observed for beetles and ants, and more species found in summer. No species group showed higher species richness in cooler temperatures or winter, which is not unsurprising given that insects may be both ectothermic and heterothermic, requiring warmer temperatures for optimal metabolism and other physiological processes (Colinet et al. 2015). Greater species richness at higher temperatures may also be a sampling artifact simply due to more insects being caught on warmer days because they are more active and abundant (Lutz et al. 2019). Although there were also community composition differences in relation to temperature, some species, such as flies of the genus *Calliphora*, are cold-adapted and better able to utilize carrion at temperatures suboptimal for other species (Norris 1959, O'Flynn 1983). Because ambient temperature rather than TBS influenced the species richness of flies, ADD models may be more accurate for predicting fly species richness changes on carrion, rather than models incorporating resource change. Overall, consideration of ambient temperature is critically important when analyzing insect species richness and community composition on carrion.

5.5.3 Implications and conclusions

Our study reinforces the vital role of resource change as a driver of carrion insect succession, with TBS being a valuable indicator of species turnover. Our research also shows the value of incorporating a continuous measure of resource change when analyzing carrion insect succession, rather than relying solely on temporal measures, such as number of days, to reveal species change over time. Temporal measures implicitly take into account the change in resource and act as a proxy for decay progression, but can be confounded by temperature.

Using TBS as an alternative approach to analyzing insect assemblages enables comparisons among seasons, and for succession to be examined in finer detail along the continuum of change on carrion, which is more difficult when using discrete and coarse assessment of decay stage. By incorporating TBS into PMI calculations, models will be able to take into account decomposition changes occurring on a cadaver, and when combined with temporal measures, may create more accurate PMI estimations. We also present here an approach of presenting effect sizes to investigate the importance of the variables on the insect communities. This approach is new in the field of forensic entomology and will be useful for future studies to consider. Modeling of species communities using TBS may also be of value to forensic investigators and improve the accuracy of PMI-based estimates where community composition data are involved. Other variables should continue to be considered in conjunction with resource change, however, to allow for the most accurate and precise modeling possible of the dynamics of carrion insect communities.

5.6 Acknowledgements

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Chapter 6. Insect abundance patterns on vertebrate remains reveal carrion resource quality variation

6.1 Abstract

Resource quality is a key driver of species abundance and community structure. Carrion is unique among resources due to its very high nutritional quality, rapidly changing nature, and the diverse and dynamic community of organisms it supports. Yet the role resource quality plays in driving variation in abundance patterns of carrion-associated species remains poorly studied. Here we investigate how species abundances change with an objective measure of resource change, and interpret these findings to determine how species differ in their association with carrion that changes in quality over time. We conducted three field succession experiments using pig carcasses and human cadavers over two winters and one summer. We quantified the effect of total body score (TBS), an objective measure of resource change, on adult insect abundance using generalised additive models. For each species, phases of increasing abundance likely indicated attraction to a high quality resource, and length of abundance maxima indicated optimal oviposition and feeding time. Some species such as the beetle *Necrobia rufipes* had a rapid spike in abundance, suggesting a narrow window of opportunity for carrion resource exploitation, while species like the wasp *Nasonia vitripennis* had a gradual change in abundance, indicating a wide window of resource exploitation. Different abundance patterns were also observed between species occurring on pigs and humans, suggesting cadaver type is an important aspect of resource quality. Our findings show that species abundances, unlike species occurrences, can reveal greater nuance about species exploitation of carrion and provide new details about how resource quality may drive competition and variation in insect community succession.

6.2 Introduction

Resource quality is a key driver of species abundance and diversity, often having a direct influence on competition and predation within ecological communities (Lucas *et al.* 2008; Marcarelli *et al.* 2011; Ochoa-Hueso *et al.* 2019). For consumption-related resources such as food, resource quality is usually defined by the nutritional output provided by the resource (Hladyz et al. 2009). Resources of higher nutritional value often attract a higher abundance and diversity of species; a result driven by individuals seeking greater metabolic return on foraging investment, and leading to enhanced survival and reproduction outcomes (Ober & Hayes, 2008; Price *et al.* 2016; Vaudo *et al.* 2018). The influence of resource quality and its effects on species abundances have been studied extensively in numerous ecological communities such as pollinator and leaf litter communities (Rantalainen *et al.* 2004; Sileshi & Mafongoya, 2007; Fowler *et al.* 2016). But there has been little study of resource quality and its effect on carrion communities, despite the implicit acknowledgement of the rapid change in carrion quality underpinning the very well-documented patterns in succession (Michaud *et al.* 2015; Barton & Evans, 2017; Evans *et al.* 2020; Dawson *et al.* 2021a).

Carrion is unique among resources due to its very high nutritional quality and its ephemeral nature (Barton *et al.* 2013; Barton *et al.* 2019). Carrion is able to support a wide range of species including vertebrate apex and meso-scavengers, and an enormous diversity of invertebrates and microorganisms, all coexisting on a shared resource and collectively forming the 'necrobiome' (Benbow *et al.* 2019). Both interspecific and intraspecific competition for resources on carrion is intense due the high number of species exploiting the remains (Charabidze *et al.* 2021). One of the key mechanisms allowing co-existence of carrion species is temporal partitioning, where species will utilise carrion at different times over the course of decomposition, which leads to successive waves of carrion use and colonisation (Hanski, 1987; Ives, 1991). Carrion-dependent species are generally highly specialised and adapted to locating decomposing remains and determining when carrion resource quality is optimal for feeding or ovipositing behaviour (Evans *et al.* 2020).

The quality of carrion as a resource is difficult to define due to its dynamic and continuously changing state, which includes changes in quality (nutritional output), digestibility, and quantity (biomass) (Barton *et al.* 2019; Benbow *et al.* 2019). The objective quality of carrion varies greatly from the onset of fresh decay through to dried remains, with optimal nutritional quality differing among species and depending on their adaptations and timing of colonising the remains (Dawson *et al.* 2021a). For some arthropods, such as primary

colonising flies that require carrion in this condition for their larvae to feed on, fresh carrion is preferred as a high quality resource (Evans *et al.* 2020). By contrast, carrion at this initial stage is likely undesirable and is poor quality for necrophagous beetles that specialise on dried remains for feeding (Battán Horenstein & Linhares, 2011; Caballero & León-Cortés, 2014). The resource quality of carrion is therefore a species-specific concept and likely a key driver of the dynamics of carrion insect community succession, diversity and abundance.

Recent research on carrion insects has demonstrated that changes in carrion resource, as indicated by total body score (TBS), is an important factor driving insect species richness and community turnover (Dawson *et al.* 2021a). Total body score is an ideal measure of resource change as it is a measure of decomposition and incorporates both the physical and chemical changes occurring on carrion. Importantly, using TBS allows researchers to incorporate the continuous and dynamic change occurring of carrion, thereby enabling more complex carrion insect community succession models to be developed. (Schoenly & Reid, 1987; Michaud *et al.* 2015). However, to determine how resource quality is influencing carrion insects, individual species abundance patterns need to be examined. This is because resource quality is a species-centric and species-specific concept, and assessment of abundance patterns against an external and objective measure of resource change provides a way to assess the quality of the resource as preferred by different species.

Abundance dynamics, as opposed to species occurrence data, may also reveal further details of the time window of preferred resource quality by species, and provide insight into coexistence mechanisms occurring on carrion by showing non-overlapping abundances (Barton & Evans, 2017). For example, we suggest a phase of increasing abundance might indicate preferred high resource quality, whereas a phase of decreasing abundance might indicate undesirable and waning resource quality and reduced oviposition or feeding potential. This type of information is potentially lost when analysing only species richness or occurrence data. Studies of insect species abundance tend to focus on the forensic applications through minimum post-mortem interval (mPMI) estimations by examining species patterns in relation to time (Matuszewski *et al.* 2010), or they assess abundance changes in relation to thermal summation models such as accumulated degree days (ADD) (Michaud & Moreau, 2009). The few studies that have examined species abundances within an ecological context have shown abundances varying among seasons and habitat types (Benbow *et al.* 2013; Barton *et al.* 2017; Engasser *et al.* 2021). Using an objective measure of resource change, such as TBS, allows comparisons of abundance patterns to be made among habitats, seasons, and potentially carrion types.

The aim of this study was to identify how different species of the carrion insect community perceive the change in carrion as it decomposes as a shift in resource quality over time. To do this, we examined the relationship between adult insect species abundance patterns and an objective measure of carrion resource change using the total body score (TBS) metric. Total body score is a semi-quantitative measure of resource change that incorporates both the physical and chemical changes occurring on carrion, and therefore the change in nutritional value of the remains (Megyesi *et al.* 2015). We focused on examining abundance patterns of the most common species of three dominant insect taxa found at carrion: Diptera, Coleoptera and Hymenoptera. We tested the following hypotheses:

1. Abundance of Diptera (flies) species will be high at low TBS values. This is because fresh carrion would likely be of higher quality to primary colonising species therefore driving high abundance numbers (Frederickx *et al.* 2012).
2. Coleoptera (beetles) species will exhibit high abundance at high TBS values. This is either because some beetles prey upon other carrion insects, and therefore will not arrive until prey has colonised carrion, while other beetles feed on dried remains and will not arrive until late in the decomposition process (Watson & Carlton, 2005).
3. Hymenoptera (ants and wasps) abundance will have no association with TBS. This is because ants are generalist and opportunistic species that are present throughout decomposition (Eubanks *et al.* 2019), while wasps are parasitic and dependent on host abundance (Voss *et al.* 2009).

6.3 Material and methods

6.3.1 Study site

We conducted three field insect community succession experiments using human cadavers and pig carcasses over two Austral winters ('Winter A': 30th May – 15th October 2018 & 'Winter B': 8th May – 2nd October 2019) and one summer ('Summer': 9th November – 16th December 2019). We aimed to replicate seasons to account for yearly variation but unfortunately a second summer experiment could not be conducted in 2018 due to donor unavailability. Experiments were completed once no noticeable decomposition changes were occurring and insect activity was minimal, resulting in the disparity in length between the winter and summer experiments as decomposition was slower during winter. All experiments took place at the Australian Facility for Taphonomic Experimental Research (AFTER), operated by the University of

Technology Sydney (UTS). The site is 4.86 hectares and surrounded by dry sclerophyll *Eucalyptus* forest with scattered rural housing in the nearby vicinity. We recorded on site measurements of ambient temperature and humidity every fifteen minutes using a HOBO MX2302 Ext temperature and relative humidity data logger protected by a solar radiation shield.

6.3.2 Human and pig set-up and experimental design

We used six human cadavers and five pig carcasses (see Dawson *et al.* 2021a for human and pig details). Our sample size is consistent with other studies using human cadavers as sourcing cadavers in high numbers is logistically challenging (Knobel *et al.* 2019). The cadavers were donated to AFTER through the UTS Body Donation Program, approved by the UTS Human Research Ethics Committee Program Approval (UTS HREC REF NO. ETH15– 0029). Domestic pigs (*Sus scrofa*) were purchased post-mortem from a licensed abattoir, therefore requiring no ethics approval in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (2004). Pigs were killed by the abattoir using a captive head bolt and were transported to AFTER within one hour of death. All human cadavers were kept refrigerated after death and delivered to AFTER within 48 hours.

Once at AFTER, 5m x 5m plots were selected within the facility and human cadavers were placed on their backs within the plots. Due to licencing agreements, pigs were placed along the outside of the facility on their sides. To maximise entomological independence between replicates, we placed pigs a minimum of 20 metres apart from one another, and at least 100 metres away from any human cadavers. Entomological independence within the facility among human cadavers was difficult to achieve; therefore cadavers were placed closest to other cadavers that had already skeletonised or become desiccated, with little or no observable entomological activity. All pigs and humans were placed on a metal mesh on top of the soil, apart from pigs and humans in Winter A which were placed directly on the soil. Pigs and humans placed on metal mesh were infrequently lifted off the ground for a few seconds throughout the decomposition process to collect weight-loss data for another study. We selected semi-shaded areas for pig and human placement and protected them from vertebrate scavenging animals by enclosing the pigs and humans in scavenger proof metal cages. We placed four pitfall traps filled with a mix of propylene glycol and 70% ethanol evenly around each pig and human following the layout described by Dawson *et al.* (2020).

On each sampling day we collected both adult and larval carrion insects using a combination of standardised methods. These methods included timed sweep netting and manual sampling, as well as pitfall traps. We sampled between 11:00 and 13:00 h and documented decomposition progress and photographed the remains using a Canon EOS 70D camera with a 18-55mm STM lens. We sampled once every day for the first week, once every second day for the following three weeks, and then gradually reduced our sampling frequency depending on the progress of decomposition and level of insect activity. All adult insects and larvae were identified to species level where possible using morphological keys and molecular techniques, as described by Dawson *et al.* (2020).

6.3.3 Data analysis

To determine how abundance patterns differed with resource change for common carrion insects, we used total body score (TBS) as a proxy for resource change, using the method by Megyesi *et al.* (2015). TBS is a commonly used and objective measure of decomposition state and indicator of resource change (Nawrocka *et al.* 2016; Connor *et al.* 2017; Roberts *et al.* 2017; Dawson *et al.* 2021a). A separate numeric value based on the physical decomposition of the remains was applied to each body region (head, torso and limbs). These values were combined together to provide an overall score of the decomposition rate. Across the 11 cadavers (six humans and five pigs), 336 TBS points were recorded and used for analysis. We used those Diptera, Coleoptera and Hymenoptera species that had a total abundance of > 200 across all experiments as these species were deemed the most common carrion insects, and modelling of species requires a minimum abundance for statistically rigorous models to be constructed. Exceptions to this were four *Calliphora* species (*Calliphora augur*, *Calliphora hilli hilli*, *Calliphora ochracea* and *Calliphora stygia*), which we grouped together into one '*Calliphora'* species group for modelling. We grouped these species due to their importance as early colonisers. Phorids were also grouped together into one 'Phoridae' species group as identifying them to genus and species level was difficult due to limited taxonomic resources available for this family. In total, 18 species and species groups were used for analysis, comprising 22,780 insects, including nine groups of Diptera (11,974), five of Coleoptera (5,445) and four of Hymenoptera (5,361).

To analyse abundance patterns, we fitted generalised additive models (GAMs), using TBS as our predictor variable and species abundance as our response variable. We used GAMs

because the species abundance relationships over the decomposition process of carrion were most likely to be non-linear (Moreau, 2021). All abundance data from each experiment were pooled together and GAMs fitted for each individual species or species group. Each GAM used species abundance as the response variable and TBS as the smoothed predictor variable with cadaver type (pig versus human) as a multiplier in the smoothed term. We assumed a Poisson error distribution with a logarithmic link function for each model, unless a fit was over dispersed, in which case we assumed a negative binomial error distribution. Only TBS values in the range 3 – 24 were used for modelling as not all pigs and humans reached a TBS above 24. By truncating our data to within this range we were able to provide a full set of comparisons among all cadavers in winter and summer. We also measured humidity and rainfall, but these factors were not included in our analysis as they did not vary between experiments. We also recorded ambient temperature and accumulated degree days (ADD), which did vary at each TBS value though this variation was consistent between pigs and humans (Appendix Fig. S2). There was also seasonal variation in ambient temperature (See Dawson *et al.* 2021a). Temperature does influence decomposition progress, but TBS is a standardised decomposition metric, which can account, in part, for seasonal and temperature differences (Dawson *et al.* 2021b). We conducted all modelling and graphing using the R software program (R Core Team, 2019) using the mgcv (Wood, 2017) and ggplot2 (Wickham, 2016) packages.

6.4 Results

6.4.1 Diptera

For species in the family Calliphoridae, GAM results showed that most species were significantly associated with TBS on humans, except for the *Calliphora* species group (Appendix Table S8). Similarly, most species' abundances were significantly associated with TBS on pigs, except for *Chrysomya rufifacies*. The percent deviance explained by the models varied between 18.6-45.2%, with *Chrysomya nigripes* having the highest. The abundance patterns of *Ch. nigripes* and *Chrysomya incisuralis* were similar, with both displaying a large spike in adult abundance during mid decomposition on pigs only, with few adults present on humans (Fig. 18). Larvae of *Ch. nigripes* were present on pigs after the initial spike, while larvae of *Ch. incisuralis* were present slightly before the spike (Fig. 18).

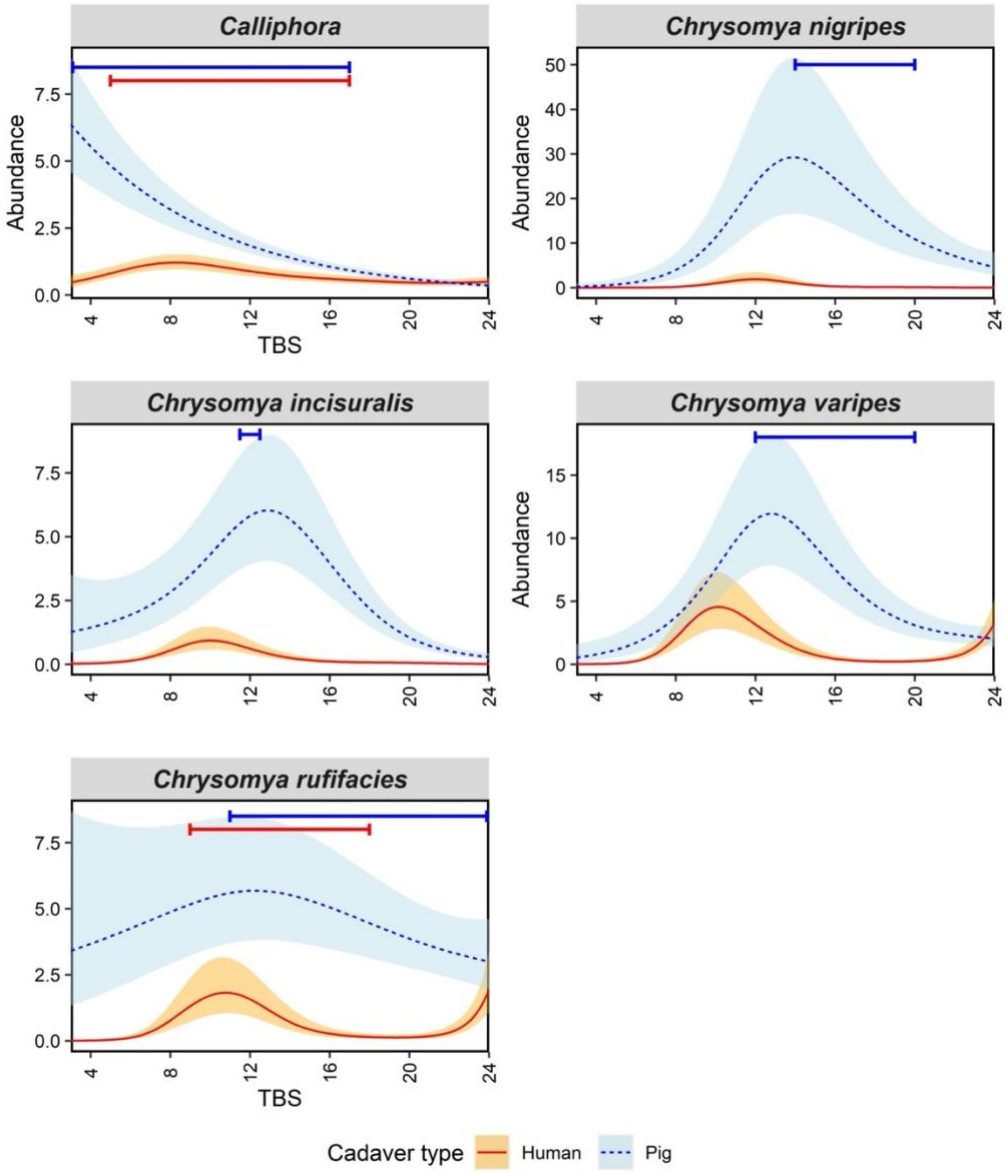

Figure 18 Generalised additive model plots modelling mean adult calliphorid abundance over total body score (TBS). Blue dotted lines represent mean adult abundance for pigs and red solid lines for humans. Shaded error bands represent standard errors of adult means. Coloured bars along the top of the plots display the minimum and maximum TBS when larvae of that species were collected on pigs (blue, above) and humans (red, below).

Adults of *Chrysomya varipes* and *Ch. rufifacies* also shared similar patterns, with a small spike in adult abundance occurring early in decomposition for humans, and a larger spike occurring on pigs slightly later in decomposition (Fig. 18). The abundance spike was more prolonged for *Ch. rufifacies* than *Ch. varipes*. Adult abundance on humans for both species increased again towards the higher TBS values in late decomposition. Larvae on pigs and humans were first recorded for both species during the first abundance spike, except for *Ch. varipes* where no larvae were recorded on humans. The *Calliphora* species group, unlike the

other calliphorids in this study, showed no mid decomposition spike in adult abundance (Fig. 18). Instead, the abundance of this group on pigs peaked during initial decomposition and slowly decreased, while on humans it displayed low adult abundance throughout decomposition. Despite this group's low adult abundance, larvae were present from the beginning of decomposition until mid to late decomposition.

For all other Diptera, the models revealed that all species' abundances on pigs were significantly associated with TBS, while almost all species' abundances on humans were significantly associated with TBS, with the exception of *Dichaetomyia* sp. (Muscidae) (Appendix Table S8). The percent deviance explained by the models varied between 20.1-35.7%, with *Australophyra rostrata* (Muscidae) having the highest. *Piophila casei* (Piophilidae) showed a difference in adult abundance patterns between pigs and humans as the pigs had one abundance peak and the humans had two, with neither peak occurring at the same TBS values (Fig. 19). Despite adult differences, the presence of larvae was similar between pigs and humans, with larvae first recorded well after adult abundances first peaked. The Phoridae species group also displayed different patterns of adult abundance between pigs and humans, with the group on pigs exhibiting one drawn out spike, while on humans the group displayed a linear increase in abundance as TBS increased (Fig. 19).

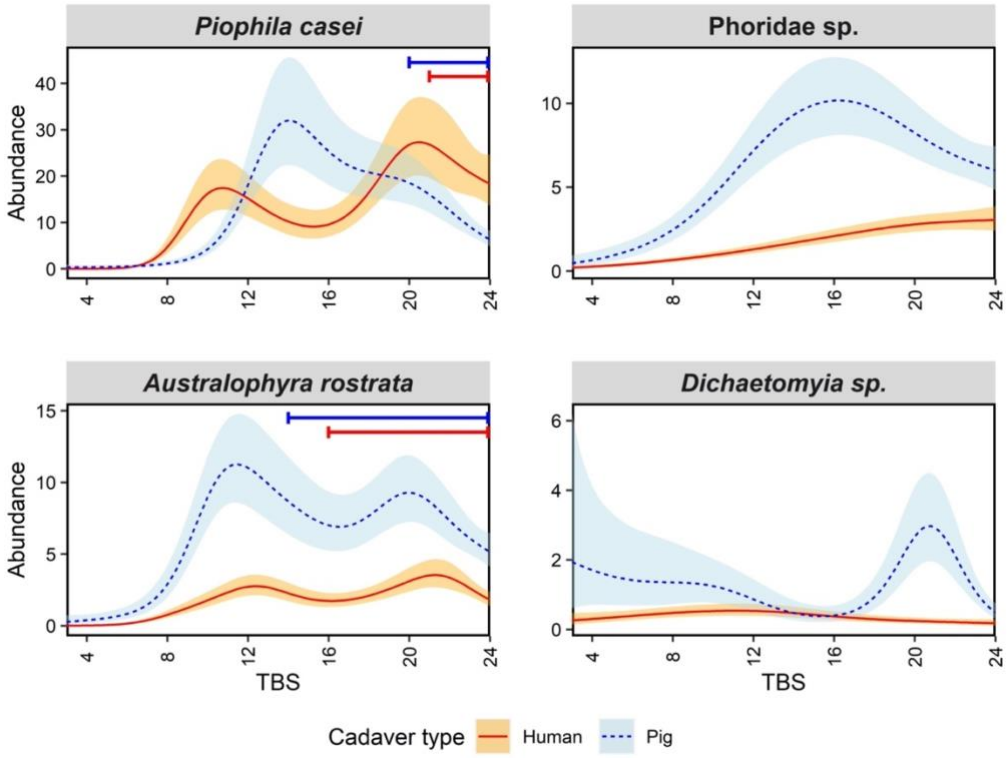

Figure 19 Generalised additive model plots modelling mean adult Diptera (excluding calliphorids) abundance over total body score (TBS). Blue dotted lines represent mean adult abundance for pigs and red solid lines for humans. Shaded error bands represent standard errors of adult means. Coloured bars along the top of the plots display the minimum and maximum TBS when larvae of that species were collected on pigs (blue, above) and humans (red, below).

Australophyra rostrata also had two distinct spikes of abundance on pigs but on humans had a more flattened abundance relationship with TBS (Fig. 19). The larval activity of *A. rostrata* was similar between carcass types despite the adult abundance differences, with larvae not observed until after the first abundance spike. For *Dichaetomyia* sp., pigs displayed varied abundance throughout decomposition with a spike in abundance at higher TBS values, while the species was absent from humans (Fig. 19).

6.4.2 Coleoptera

For species within the order Coleoptera, we found almost all species' abundances to be significantly associated with TBS on both pigs and humans, with the exception of *Necrobia rufipes* (Cleridae) on pigs (Appendix Table S8). The percent deviance explained by the models varied between 37-64.3% with *N. rufipes* having the highest. The models for all species are similar in that there were no early spikes in adult abundance, with adults not arriving until

around 11 TBS on both pigs and humans. *Necrobia rufipes* was found to have a large spike in adult abundance on pigs towards the end of decomposition, while they were mostly absent on pigs (Fig. 20). Conversely, *Creophilus lanio* (Staphylinidae) displayed a large adult abundance spike on pigs, but limited abundance on humans (Fig. 20). We found *Saprinus cyaneus cyaneus* (Histeridae) and *Omorgus quadrinrodosus* (Trogidae) to share similar adult abundance patterns between pigs and humans, as both gradually increased as TBS increased, with initial adult arrival occurring at the same time (Fig. 20). By contrast, for *S. cyaneus caynaues*, abundance continued to rise on humans but gradually decreased on pigs. *Creophilus erythrocephalus* displayed the most volatile adult abundance patterns with multiple spikes occurring on both pigs and humans, but at different TBS values (Fig. 20).

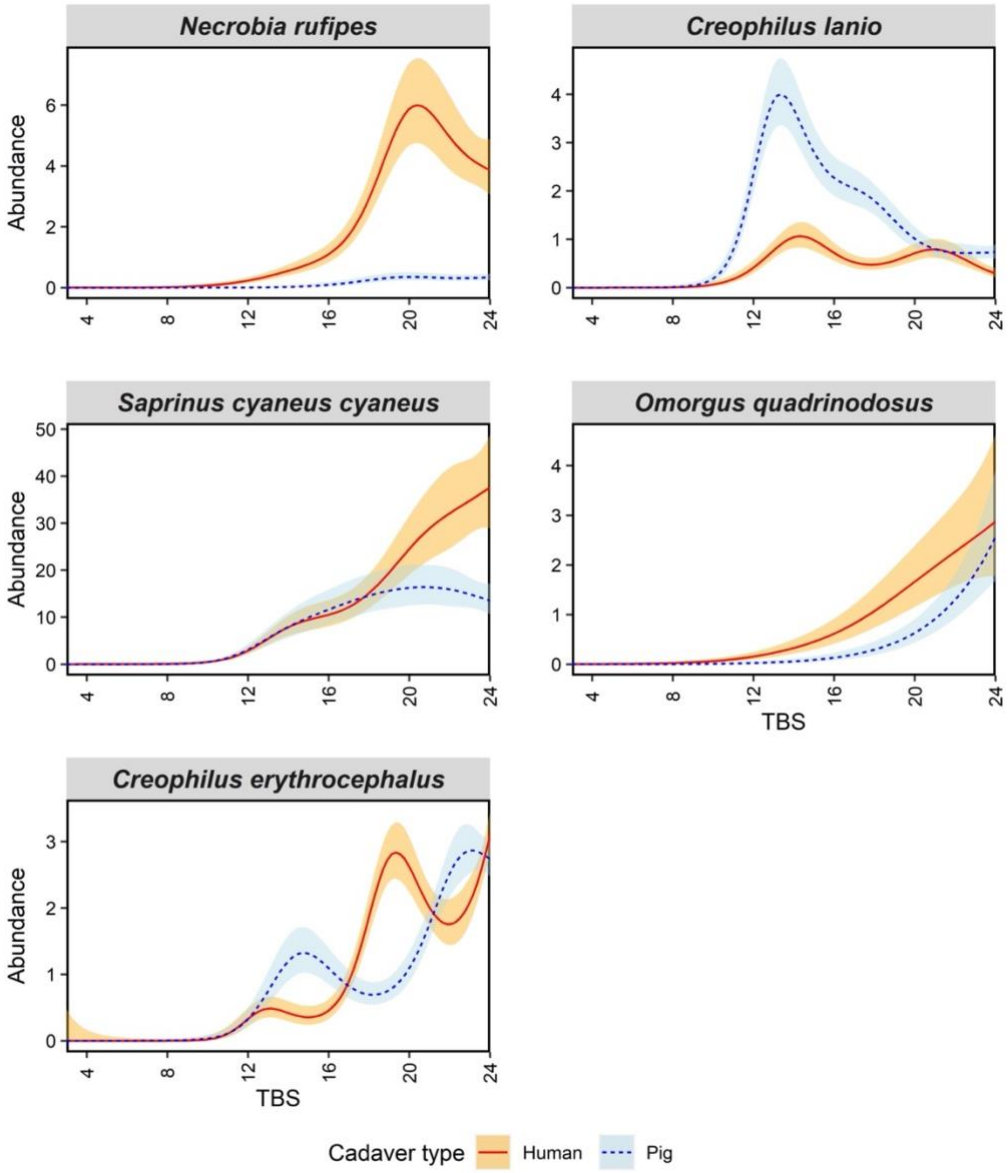

Figure 20 Generalised additive model plots modelling mean adult Coleoptera abundance over total body score (TBS). Blue dotted lines represent mean adult abundance for pigs and red solid lines for humans. Shaded error bands represent standard errors of adult means. No Coleoptera larvae were collected.

6.4.3 Hymenoptera

For Hymenoptera we found that some species abundances were significantly associated with TBS, except for *Rhytidoponera metallica* (Formicidae) and *Nasonia vitripennis* (Pteromelidae) on pigs and *R. metallica* and *Aphaenogaster longiceps* (Formicidae) on humans (Appendix Table S8). The percent deviance explained by the models varied between 6.6-38.2% with *Crematogaster* sp. (Formicidae) having the highest. The hymenopteran models are mostly

different from those for Diptera and Coleoptera models in that they lack spikes in adult abundance; rather, they either gradually increase or decrease with TBS in a linear nature. The exception to this is *N. vitripennis* which displayed a gradual spike during mid decomposition at the same time on both pigs and humans (Fig. 21). *Aphaenogaster longiceps* adult abundance started high then decreased quickly on pigs, while on humans the adult abundance stayed relatively stable (Fig. 21). *Rhytidoponera metallica* displayed the opposite pattern with a steady abundance increase on humans but relatively stable abundance on pigs. Lastly, we found *Crematogaster* sp. adult abundance patterns to be very similar between pigs and humans with a rapid increase in abundance as TBS increased.

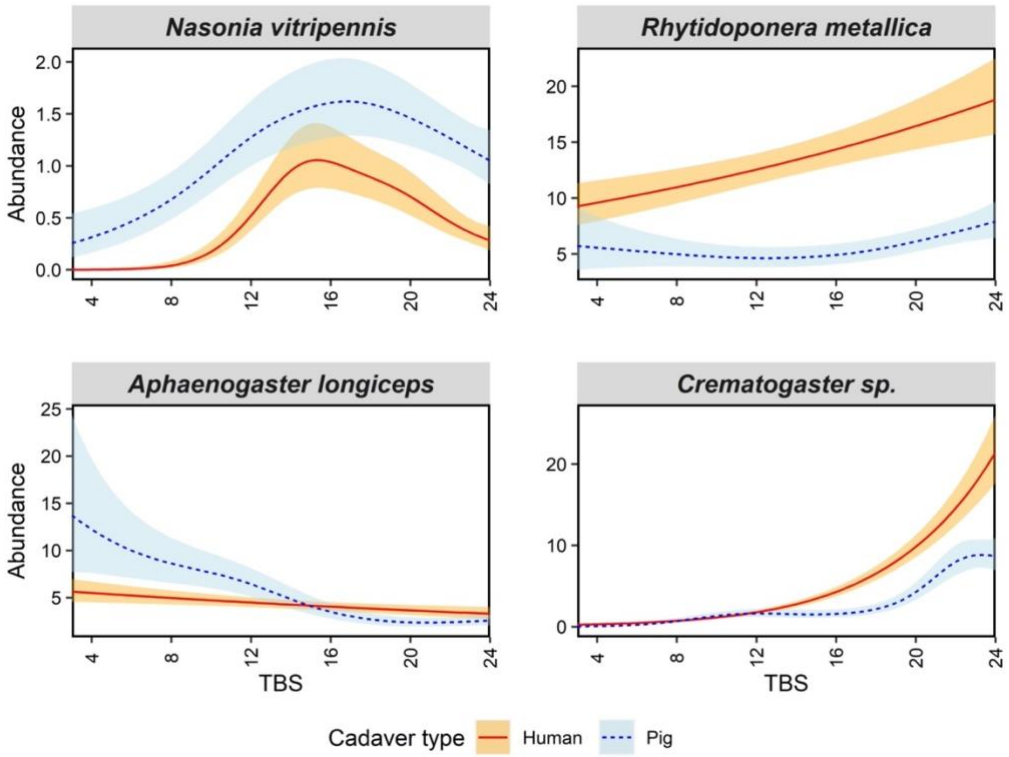

Figure 21 Generalised additive model plots modelling mean adult Hymenoptera abundance over total body score (TBS). Blue dotted lines represent mean adult abundance for pigs and red solid lines for humans. Shaded error bands represent standard errors of adult means. No Hymenoptera larvae were collected.

6.4.4 Relative abundance patterns

Clear successional patterns emerged when viewing the abundance of individual species and species groups across the whole community (Fig. 22). These patterns were most obvious for Diptera and Coleoptera with *Calliphora* species arriving first, followed by other Diptera

species, and Coleoptera species arriving last. Most Diptera arrived in large numbers in association with mid TBS scores, although some arrived earlier in lower abundance, particularly on pigs. The Coleoptera displayed greatest abundances at higher TBS scores with almost none present during low TBS scores. Hymenoptera species displayed no clear successional pattern as they arrived first in high numbers on both pigs and humans and remained in relatively stable populations throughout decomposition. In general, most species were more abundant and arrived earlier on pigs than humans, as seen with the *Chrysomya* and the other mid-late stage Diptera species.

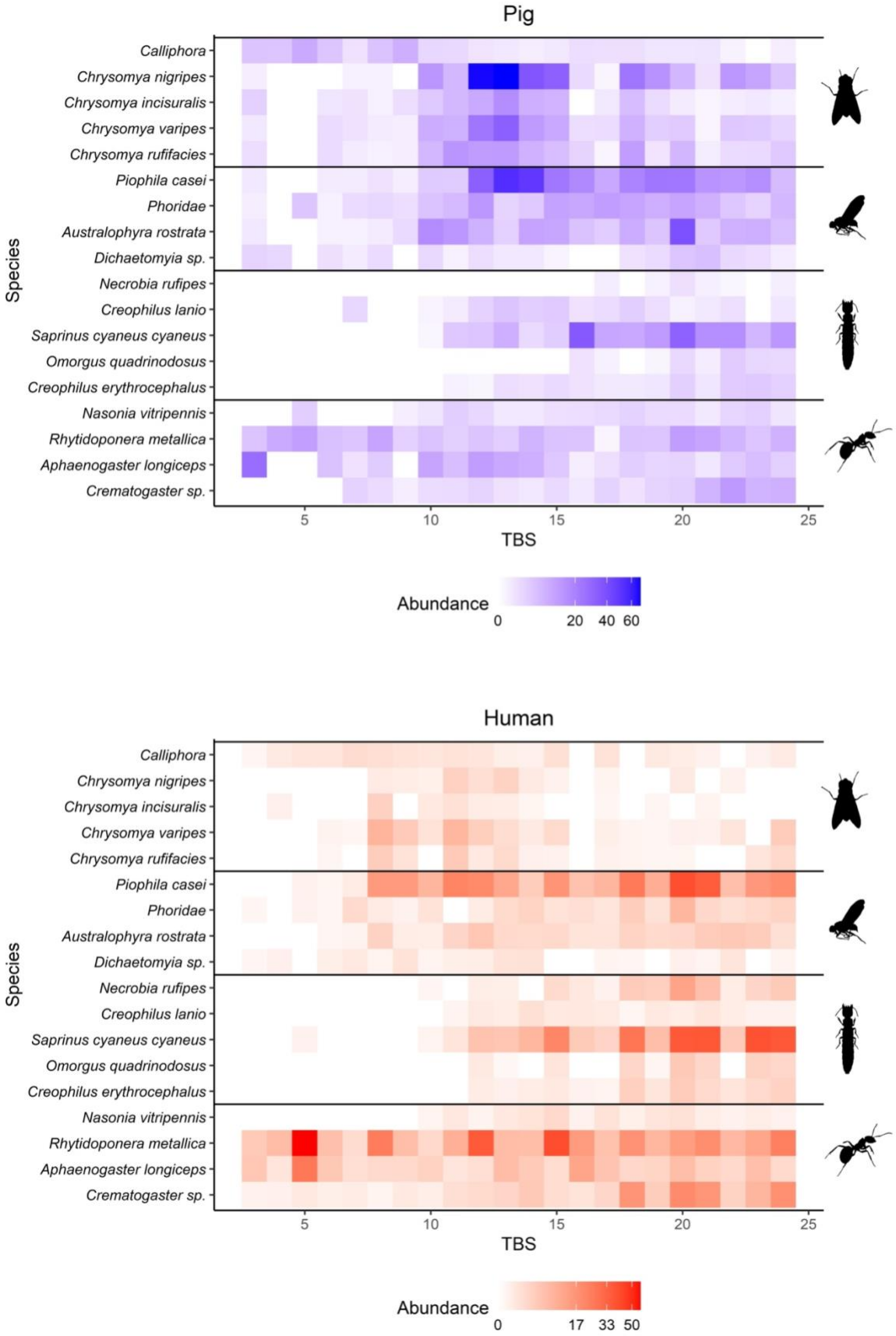

Figure 22 Heat map depicting changes in mean adult abundance with increasing TBS for each species and species group. Blue represents pigs and red for humans.

6.5 Discussion

We aimed to determine how species of carrion insects perceive resource change as a shift in resource quality over time by examining adult abundance patterns on vertebrate remains. We found that the abundance of insects was significantly associated with resource change for almost all insect species. Our results provide insight into how each species responds to carrion resource change, as some species displayed gradual changes in abundance, indicating large windows of preferred high resource quality. By contrast, other species displayed rapid peaks in abundance indicating short windows of preferred high resource quality. Below we discuss how each taxonomic group responds to resource change and how cadaver type relates to resource quality.

6.5.1 Adult abundances reveal timing of high resource quality

As hypothesised, Coleoptera species did not arrive on either pigs or humans until mid TBS values and either had a rapid peak in abundance upon initial arrival, or a gradual increase in abundance. A rapid increase and decrease was observed for the omnivorous clerid *N. rufipes* and the predatory staphylinid *C. lanio*, which suggests that these species have a narrow window of optimal carrion exploitation and preferred resource quality. This narrow window of optimal resource quality suggests that these species might be under strong intraspecific competitive pressure as they are constrained to a narrow niche (Kocárek, 2001). This contrasts with other Coleoptera species such as the predatory histerid *S. cyaneus cyaneus* and necrophagous trogid *O. quadrinodosus*, which displayed a sustained, gradual increase in abundance after initial arrival on carrion. This behaviour indicates that these species have a larger window of optimal resource quality for colonising carrion, where carrion quality is high for a longer period. This gradual colonisation behaviour suggests competition for resources is less intense compared with those species that display rapid peaks in abundance (Braack, 1987). This is because the resource used by these species, whether it be the carrion or prey species, is optimal during decomposition for a longer period, therefore likely leading to less competition. For example, *Dermestes maculantus* has a narrow feeding preference, consuming skin and fleshy tissue, while other species such as *Trox* spp. avoid interspecific competition by having a wider feeding preference (Braack, 1987). Our results are similar to other studies that examined Coleoptera arrival patterns in relation to coarser decay stages, finding that most species delay arriving until

active decay (Sharanowski *et al.* 2008; Battán Horenstein & Linhares, 2011; e Castro *et al.* 2013; Weithmann *et al.* 2021). The narrow window of peak abundance of the clerid *N. rufipes*, and its strong association with resource change, suggest that this species could provide more accurate PMI estimations than species with peak abundance spread over a wider window.

The Diptera displayed a mixed association with resource change, with most species arriving as predicted, such as *Calliphora* species arriving first, followed by *Chrysomya* species. The larvae of *Chrysomya* species were first recorded during the phase of decrease after adult abundances peaked. Oviposition, therefore, likely occurred around peak adult abundance and not when adults first arrived at carrion. Adults therefore arrived at carrion when resource quality was less than optimal, and wait until resource quality is high before ovipositing. Being secondary colonisers, *Chrysomya* larvae likely prefer carrion to be in a particular state of decomposition to allow for optimal growth and development. This is facilitated by modification of the carrion resource by primary colonising flies such as *Calliphora* species. In this case, the resource quality required by the larvae might reflect both the nutritional output as well as the digestibility of the carrion. However, previous research has suggested that the process of facilitation does not adequately apply to carrion succession (Michaud & Moreau, 2017). Alternatively, delayed oviposition may be the result of priority effects, as some *Chrysomya* larvae such as *Ch. rufifacies* are facultative predators of other Diptera larvae (Brundage *et al.* 2014). Therefore, *Chrysomya* larvae may gain additional fitness benefits if colonising carrion after other Diptera species as the *Chrysomya* larvae gain an additional resource for consumption. Delayed oviposition may also relate to one or more other factors such as lack of optimal oviposition sites (Archer & Elgar, 2003), requirement of a protein meal before ovary development in females (Avancini & Linhares, 1988), density requirements of larvae for optimal maggot mass growth (Charabidze *et al.* 2011; Johnson & Wallman, 2014), or selective mating behaviour (Beehler & Foster, 1988; Jones *et al.* 2014).

The other Diptera families generally displayed complex series of both increasing and decreasing phases of abundance during carrion decomposition, with some species displaying multiple peaks in abundance. This complex abundance pattern was most evident for the muscid *A. rostrata* and piophilid *P. casei*, with larvae first observed towards the end of decomposition, well after initial adult arrival. Carrion resource quality for these species, unlike others, may have multiple points where quality is high, rather than a single point as observed in the calliphorids and Coleoptera. Despite being known late stage colonisers, *A. rostrata* and *P. casei* arrived relatively early at low TBS values, indicating that they are likely able to locate carrion using chemical cues similar to those employed by primary and secondary colonising flies

(Frederickx, *et al.* 2012). Previous studies have reported that these species generally do not arrive until competition for resources is less intense, or the remains are in an optimal condition for larvae to feed upon. For some piophilids, the latter may occur when fatty acids are present (Martín-Vega, 2011). However, like in our study, other studies have observed these species arriving early in decomposition (Barton *et al.* 2017) and arriving in successive waves, with oviposition not recorded until later waves (Voss *et al.* 2011). These results highlight the complex species-resource interactions occurring on carrion and the role resource quality plays in carrion insect community succession.

Hymenoptera species either gradually decreased or increased in abundance in a relatively linear relationship as decomposition progressed, with only the parasitic pteromalid *N. vitripennis* displaying a gradual spike during decomposition. This gradual spike indicates a large window of optimal resource quality for *N. vitripennis*, which, rather than feeding directly on the remains, parasitises Diptera larvae (Steiner *et al.* 2006). The other Hymenoptera in our study are generalist Formicidae (ant) species that were present in the local environment and likely using carrion to either prey on other insects around the remains, exploit the moisture present in decomposition fluids, or feed on the epidermal skin layer of the carrion (Barton *et al.* 2017; Eubanks *et al.* 2019; Evans *et al.* 2020). Resource quality is likely not a specific factor in driving the adult abundance of Hymenoptera. This abundance is probably more influenced by hymenopteran populations and the proximity of nests in the local environment.

6.5.2 Cadaver type influence on species abundance

Adult abundance for most insect species generally varied in two distinct ways, which suggests species are responding differently to the resource quality of the two cadaver types. First, species were observed arriving at the same time, but at different magnitudes, with pigs generally having higher abundance (e.g. *C. lanio*). This suggests that preferred resource quality of pigs is higher overall for those species, thereby them appearing more attractive for colonisation. This is potentially due microbial activity and the associated volatile organic compounds being released during decomposition. Microbes are the first species to colonise carrion and have a dynamic relationship with insects as VOCs released by microbes are used by insects to detect carrion (Frederickx *et al.* 2012). The microbial community is inherently part of the carrion resource and a large aspect of the resource quality of carrion. As insects arrive on carrion, they also introduce additional microbes to the carrion, driving a shift in the VOCs released, thereby

altering the attractiveness of the carrion (Tomberlin *et al.* 2011). Volatile organic compounds released during decomposition have been shown to be different between pigs and humans, suggesting the microbial communities may vary between these cadaver types, which would alter the resource quality. (Knobel *et al.* 2019; Dawson *et al.* 2020). This difference is particularly true for donated human cadavers which are often sourced from hospitals and have been treated with numerous antemortem chemicals (Matuszewski *et al.* 2019; Dawson *et al.* 2020). More attractive VOCs may indicate higher nutritional output or digestibility of the carrion due to the microbial communities (Stavert *et al.* 2014; Kotzé *et al.* 2021).

The second distinct difference between pigs and humans was abundance peaking at different TBS values (e.g. *P. casei*). If abundance is similar, but timing of phases of increase and decrease vary, then this may indicate that the resource quality changes at different rates, with the optimal resource quality state for each species therefore varying between pigs and humans. The varying rates of resource quality are most likely due to the different decomposition rate exhibited by pigs and humans, with pigs often decomposing faster (Connor *et al.* 2017; Dautartas *et al.* 2018; Dawson *et al.* 2020). With quicker decomposition, pigs move through decay stages faster, giving associated species less time on pigs when resource quality is optimal.

6.5.3 Conclusions and implications

Our study revealed how adult abundance patterns on carrion can provide insight into how species perceive carrion resource quality. Species may be arriving in similar abundances but at different times. This temporal partitioning likely leads to coexistence of species on carrion as species are adapted to exploiting carrion at different time or for different purposes. Contrastingly, species may be arriving at the same time but differing in magnitude of abundance. In this case, coexistence is likely achieved as species are using the carrion resource for different purposes (feeding, oviposition, etc.). If all species used carrion for the same purposes, then a single species would be unable to dominate and outcompete others. Abundance patterns also display the window of optimal resource quality for carrion insect species. Species with a short abundance peak suggest a narrow window of opportunity when resource quality is optimal and intraspecific competition is likely to be strong. In a forensic context, a species with a narrow window may also be useful for PMI estimations. Species with a long abundance peak suggest a wide window of opportunity when resource quality is optimal.

These species persist for longer and likely under lower levels of intraspecific competition. Our study highlights the many ways in which abundance data, as opposed to occurrence data, provide additional information relating to species ecological patterns. By analysing abundance patterns we can investigate species-resource interactions and the mechanisms allowing for coexistence of species on carrion. Our study only takes into consideration how insects respond directly to the carrion resource, and does not examine other aspects of the necrobiome. To improve upon our models, we suggest considering other variables such as ambient temperature, vertebrate scavengers and microbial communities to further disentangle carrion resource quality and its effect on insect community succession. To further test these coexistence mechanisms, manipulation experiments could be conducted to remove or add key species at different time points to determine how coexistence is affected. In addition, experiments altering the nutritional output or digestibility of the carrion resource may reveal additional information about how carrion insect might perceive resource quality. Future research needs to examine how all aspects of the necrobiome responds to carrion resource quality to gain a more complete understanding of the complex interactions occurring on carrion.

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Chapter 7. Priority effects and density promote coexistence between the facultative predator *Chrysomya rufifacies* and its competitor *Calliphora stygia*

7.1 Abstract

Highly competitive ephemeral resources like carrion tend to support much greater diversity relative to longer-lived resources. The co-existence of diverse communities on short-lived carrion is a delicate balance, maintained by several processes including competition. Despite this balance, few studies have investigated the effect of competition on carrion, limiting our understanding of how competition influences co-existing species. We investigated how priority effects and larval density influence co-existence between two blowfly species, the facultative predator *Chrysomya rufifacies* and its competitor *Calliphora stygia*, which occupy broadly similar niches but differ in their ecological strategies for exploiting carrion. We examined how adult oviposition, larval survival, developmental duration, and adult fitness were affected by the presence of differently aged heterospecific larval masses, and how these measures varied under three larval densities. We found *Ch. rufifacies* larvae struggled to survive in conspecific masses at low larval densities. In heterospecific masses, survival increased, particularly at high larval density, with priority effects having minimal effect, suggesting a dependency on collective exodigestion. For *C. stygia*, we found survival to be constant across larval densities in a conspecific mass. In heterospecific masses, survival decreased drastically when *Ch. rufifacies* arrived first, regardless of larval density, suggesting *C. stygia* is temporally constrained to avoid competition with *Ch. rufifacies*. Neither species appeared to completely outcompete the other, as they were either constrained by density requirements (*Ch. rufifacies*) or priority effects (*C. stygia*). Our results provide mechanistic insight into the ecological processes allowing for co-existence on a competitively intense, ephemeral resource such as carrion.

7.2 Introduction

Interspecific competition is a common biotic mechanism that shapes and drives species community structure (Goldberg & Barton, 1992). On ephemeral resources, those resources that are limited, patchy and unpredictable, interspecific competition can be particularly intense owing to the fact that the resource can only host a finite number of species (Atkinson & Shorrocks, 1981; Kneidel, 1985). This is most evident on carrion, the decomposing remains of dead animals, which host a diverse range of eukaryotic and prokaryotic species, collectively known as the 'necrobiome' (Kneidel, 1984; DeVault *et al.* 2011; Benbow *et al.* 2019). Many of these species, particularly insects, display a predictable sequence of arrival on carrion (Payne, 1965). This temporal partitioning is a common outcome of interspecific competition, and allows species that share broadly similar niches to co-exist (Trumbo & Bloch, 2002; Pronk *et al.* 2007; Hood *et al.* 2021). The carrion insect succession process has been extensively studied at a community level, with studies determining the influence of a range of biotic and abiotic factors such as temperature and season on the insect community (Sharanowski *et al.* 2008; Matuszewski *et al.* 2016; Dawson *et al.* 2021b). However, few studies have examined the finer details of succession at the species level to determine the role competition and between-species interactions have in shaping the successional process (Ito, 2020).

The carrion insect community comprises a diverse assemblage of species including necrophages, omnivores, predators, and parasites, which all form a complex trophic web (Villet, 2011). Interspecific competition among insects is intense on carrion, particularly among necrophages, which consume the decomposing tissues (Charabidze *et al.* 2021). At high temperatures, necrophagous insects can remove a significant amount of carrion biomass over a short period (Payne, 1965; Barton & Evans, 2017). Among necrophagous insects, the most abundant taxon are Diptera, the larvae of which are responsible for the majority of carrion biomass consumption (Payne, 1965; Archer, 2004). Diptera larvae aggregate together forming large maggot masses, often consisting of thousands of individuals, which can be composed of multiple heterospecific (organism belonging to a different species) larvae (Fouche *et al.* 2018). This aggregation behaviour among carrion blowflies on patchy resources such as carrion can lead to co-existence among heterospecific species (Ives, 1991). Despite being in direct competition for resources in a heterospecific maggot mass, the benefits of increased growth and development from thermal dynamics outweigh the detrimental effects of competition (Ives, 1991; Rivers *et al.* 2011; Barton *et al.* 2021; Charabidze *et al.* 2021). Heterospecific maggot masses are generally dominated by blowflies (Calliphoridae), but can also consist of flesh flies

(Sarcophagidae), house flies (Muscidae), cheese flies (Piophilidae) and scuttle flies (Phoridae) (Levot, 2003).

Not all blowflies display this interspecific social behaviour and aggregate together. For example, 'smooth' maggot species of *Calliphora* and *Sarcophaga* avoid aggregating with 'hairy' maggots, such as *Chrysomya rufifacies* (Fuller, 1934; Yang & Shiao, 2012; Pimsler *et al.* 2019). This is because *Ch. rufifacies* larvae are facultative predators of other blowflies, with predation thought to occur mostly when resources are limiting and competition is high, with cannibalism also reported to occur, although less frequently (Baumgartner, 1993). The species forms extremely large maggot masses and is considered a secondary coloniser of carrion, but has also been documented as a primary coloniser (Baumgartner, 1993). Due to this predatory behaviour, the larvae of *Ch. rufifacies* have a large competitive advantage over the larvae of other blowfly species, which have little defence against predation (Appendix Movie S1). In North America, where *Ch. rufifacies* is invasive, it has been suggested that the species could even outcompete native species like *Cochliomyia macellaria*, which may eventually lead to the eradication of *C. macellaria* from the wild (Wells & Greenberg, 1992). *Chrysomya rufifacies* has the ability to shape the successional process and community composition as other species will be outcompeted or avoid ovipositing entirely (Yang & Shiao, 2012). This is of particular importance to forensic entomology, as *Ch. rufifacies* might also influence the development time of other species, thereby impacting post-mortem interval (PMI) estimates derived from larval development rates (Swiger *et al.* 2014; Carmo *et al.* 2018).

Despite *Ch. rufifacies* displaying strong competitive behaviours, other blowfly species native to Australia have been able to colonise and co-exist on carrion using numerous physiological and behavioural adaptations (Arias-Robledo *et al.* 2019). A key adaptation displayed by some blowfly species is their ability to locate and colonise carrion within minutes of the death of the animal, enabling them to exploit the resource before other species such as *Ch. rufifacies* arrive (Frederickx *et al.* 2012; Evans *et al.* 2020). Species arriving earlier than *Ch. rufifacies* may have time for their larvae to develop and avoid predation by *Ch. rufifacies* if they can reach a developmental optimum, at which point *Ch. rufifacies* cannot feed upon them. *Chrysomya rufifacies* will gain an additional resource to feed upon if arriving shortly after a heterospecific blowfly species (Brundage *et al.* 2014). However, what remains unknown is how the precise timing of *Ch rufifacies* arrival, and the impact of larval densities on these priority effects, influence *Ch. rufifacies* survival and predation rate (Carmo *et al.* 2018). Importantly, what degree of temporal advantage do other blowfly species need to survive on a resource and successfully co-exist with a facultative predator like *Ch. rufifacies*? To date, no

study has examined these questions or tested the role of priority effects on competition between *Ch. rufifacies* and other blowfly species under different larval densities.

To address these questions, we conducted a series of manipulative laboratory experiments analysing the role of priority effects and larval density on competition between *Chrysomya rufifacies* and another Australian species, *Calliphora stygia*. *Calliphora stygia* shares an overlapping geographic distribution and arrives at carrion at similar times to *Ch. rufifacies*. Despite this, these species co-exist frequently in nature, with the exact mechanisms allowing for co-existence currently unknown. To unravel these mechanisms, we tested two hypotheses:

1. That adult *C. stygia* will reduce oviposition on a resource that has already been colonised by *Ch. rufifacies* larvae. In contrast, adult *Ch. rufifacies* will increase oviposition on a resource that has already been colonised by *C. stygia* larvae.
2. That *C. stygia* larval survival rate and adult fitness would decrease, while development time would increase if this species arrived on a resource that was already colonised by *Ch. rufifacies* larvae, particularly at higher larval densities where competition is predicted to be more intense. In contrast, *Ch. rufifacies* larval survival and adult fitness would increase, while development time would decrease if arriving at a resource after *C. stygia* larvae, regardless of larval density.

7.3 Material and methods

7.3.1 Insect colonies and maintenance

To establish laboratory colonies, we purchased *C. stygia* pupae from a commercial supplier (Sheldon's Bait). Adults *Ch. rufifacies* were collected from wild populations around the University of Wollongong (UOW), Australia, and provided with kangaroo mince for oviposition. Once adults emerged from the pupae, they were transferred to a large plastic colony cage (300 x 500 x 250mm) with a fly screen lid and provided with a constant supply of granulated raw sugar and water. A small amount of kangaroo mince (~20g) was provided to recently emerged adults to act as a protein meal, which females need for ovarian development (Cook, 1991). We kept the two blowfly species in separate colony cages at all times. To establish a new generation, we provided adults with ~50g of kangaroo mince in a weigh boat, with cotton wool placed on top of the mince to replicate mammalian fur. Once oviposition

occurred, the mince was removed from the colony cage and placed in a small plastic rearing container (130 x 190 x 70mm) with the bottom of the container layered with wheaten chaff to act as a pupation substrate. Once hatched, the larvae were provided with a constant supply of kangaroo mince until pupation to ensure food was not limiting. Upon adult emergence, we placed the new generation into a clean large colony cage (300 x 500 x 250mm). All colonies were maintained in a temperature-controlled room at 24 °C (±1 °C) with a 12:12 h light/dark cycle.

7.3.2 Adult oviposition experiment

To examine adult oviposition preference, we provided adults with kangaroo mince that either had different ages of heterospecific larvae feeding on it (2 or 4 day old larvae) or no larvae present on the mince (control) (Appendix Fig. S3). To attain heterospecific larvae, adults from the stock laboratory colonies were placed in a small plastic rearing container with a weigh boat containing kangaroo mince and a layer of cotton wool on top of the mince. We then transplanted these heterospecific larvae onto fresh (less than 1 day old) kangaroo mince (50 ± 1g) 30 minutes prior to the addition of adults to create the three different treatments (+2 day old, +4 day old and control). For each replicate, 10 adult flies (5 males and 5 females) and 20 heterospecific larvae (except for the control, which had no larvae) were used, for a total of 12 replicates per treatment. We conducted the experiment twice, once using adult *Ch. rufifacies* laying on mince with different age *C. stygia* larvae present, and a second time with the roles reversed using adult *C. stygia* and *Ch. rufifacies* larvae. All adults were sourced from laboratory stock colonies with adults being at least 9 days old (to ensure they were sexually mature (Cook, 1991)) and had not previously laid. We placed treatments in temperature cabinets for a period of 4 hours set at 25 ± 0.5 °C and 50% ± 10% humidity. Only one treatment was in a cabinet at any one time to avoid chemical cues from other treatments influencing adult oviposition behaviour. After the 4 hour period, we removed treatments from the cabinets and counted the number of eggs laid by manually separating them from the mince using a damp paint brush.

7.3.3 Larval experiment

To examine the role of priority effects and larval density on competition, we placed larvae of

Ch. rufifacies and *C. stygia* of different ages into mixed maggot masses on kangaroo mince (Appendix Fig. S3). Adult flies from the stock laboratory culture were provided with kangaroo mince for oviposition. Once eggs had hatched, larvae were provided with a constant supply of mince to ensure food was not limiting. We then removed the larvae and transplanted onto new fresh (less than 1 day old) kangaroo mince (50 ± 1g) in a plastic weigh boat once they had reached the desired age, depending on the heterospecific priority effect treatment. We used five heterospecific priority effect treatments, which consisted of: 0 day old *Ch. rufifacies* + 0 day old *C. stygia* (0R + 0S), 2 day old *Ch. rufifacies* + 0 day old *C. stygia* (2R + 0S), 4 day old *Ch. rufifacies* + 0 day old *C. stygia* (4R + 0S), 0 day old *Ch. rufifacies* + 2 day old *C. stygia* (0R + 2S) and 0 day old *Ch. rufifacies* + 4 day old *C. stygia* (0R + 4S). We placed the kangaroo mince with the heterospecific treatments in a small plastic rearing container, with the bottom of the container layered with chaff. Two conspecific larval treatments were also created consisting of just 0 day *Ch. rufifacies* or just 0 day old *C. stygia* larvae.

We conducted the larvae priority effect treatments under three larval densities: 25 of each species (50 total), 50 of each species (100 total) and 75 (150 total) of each species. To standardise density in the conspecific treatments, the larval density matched the total density of the priority effect treatments. For example, 50 larvae were used for the priority effect conspecific control to match the density of the 25 larvae of each species in that treatment. Within each larval density, we used six replicates for each priory effect treatment (6 x 0R + 0S within the '25 each' larval density). A total of 12,600 larvae were used across the experiment (6,300 per species). We placed treatments into temperature cabinets for a period of four weeks set at 25 ± 0.5 °C and 50% ± 10% humidity with a 12:12 h light/dark cycle. We visibly assessed treatments daily and time until adult eclosion was recorded. After the four-week period, treatments were removed from the temperature cabinets and the total number of flies that reached the adult life stage were counted. The dry weight of each adult fly was weighed using a Mettler Toledo ML204 Newclassic ml Analytical Balance. We converted the weight in grams (g) to milligrams (mg) by multiplying by 1000. We also recorded the sex of all individuals that survived to adulthood.

7.3.4 Data analysis

To assess adult oviposition preference in the first experiment, we compared the number of eggs laid for each species between heterospecific larvae age treatments (no larvae, 2 day old and 4

day old larvae). We used two generalised linear mixed models (GLMMs), one for each species, with larvae age treatment as a fixed, categorical predictor variable (3 levels) and the number of eggs laid as a continuous response variable. We assumed a Poisson error distribution and a logarithmic link function for both GLMMs, unless the data were over dispersed, in which case we assumed a negative binomial error distribution and a log link function.

For the second experiment, to determine the effect of priority effects and larval density on species competition, we conducted a series of GLMMs on multiple response variables. The three response variables included: number survived to adult life stage, time until adult eclosion (days) and adult fitness (mass (mg)). For each species, we conducted three separate GLMMs, one for each of the three response variables, totalling six GLMMs. For each GLMM, the predictor variables were priority effect (6 levels) and larval density (3 levels) which were treated as fixed, categorical factors. The GLMMs compared the priority effect treatments to a control within each larval density treatment. For the control, we used the 0+0 treatment group as this represented no priority effect in a heterospecific mass. In the same analysis, we also compared the conspecific treatment group with the control to determine the effect of adding heterospecifics. To control for density in the survival analyses, we halved the survival data for the conspecific treatments, as this treatment had double the number of individuals, enabling us to compare to the control (0+0) treatment.

Again, for the count of emerged adults (survival), we assumed a Poisson (or negative binomial) error distribution. For the models using continuous time until adult eclosion (days) and adult fitness (mass) variables we assumed a Gaussian error distribution. We plotted the GLMM estimates as effect sizes and interpreted their effects as significant if their 95% confidence intervals did not cross the zero-effect line (du Prel *et al.* 2009). We conducted all GLMMs using R (3.6.0) (R Core Team, 2019), and the package glmmTMB (Brooks *et al.* 2017). All plots were created using ggplot2 (Wickham, 2016).

7.4 Results

7.4.1 Adult oviposition preference

For *Ch. rufifacies*, the presence of both 2 and 4 day old *C. stygia* larvae on a resource had no effect on the number of eggs laid by adults when compared to a resource with no *C. stygia* present (2 day GLM: coefficient = 0.8722, t = 1.435, P = 0.1606; 4 day GLM: coefficient = 1.1297, t = 1.861, P = 0.0717). A non-significant trend of increasing number of eggs laid when older heterospecific larvae were present on the resource can be observed (Fig. 23a). For *C. stygia*, we also found that the presence of both 2 and 4 day old *Ch. rufifacies* larvae on a resource had a non-significant effect on the number of eggs laid by adults when compared to a resource with no *Ch. rufifacies* (2 day GLM: coefficient = -0.525, t = -1.168, P = 0.251; 4 day GLM: coefficient = -0.4633, t = -1.031, P = 0.31). Compared to *Ch. rufifacies* oviposition, the opposite trend was observed with *C. stygia*, as adults laid more eggs on a resource without *Ch. rufifacies* larvae present (Fig. 23b).

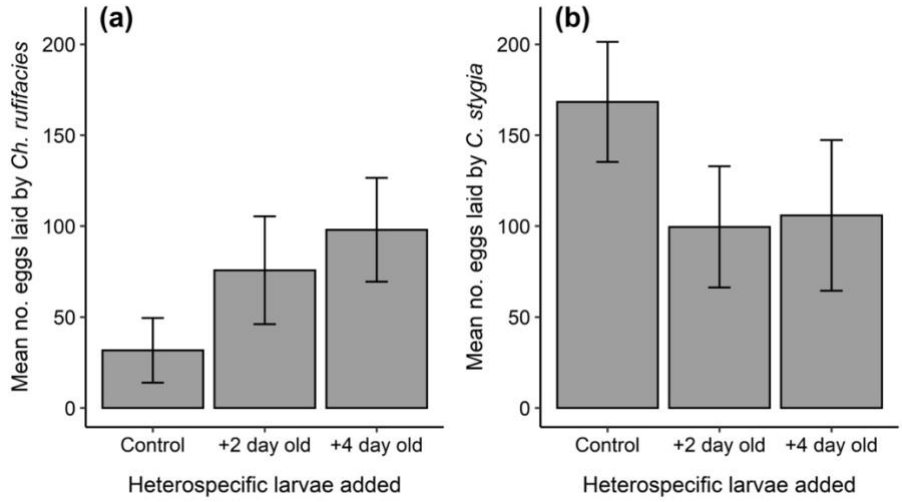

Figure 23 Bar plot of mean (± S.E.) number of eggs laid by adult (a) *Chrysomya rufifacies* and (b) *Calliphora stygia* on kangaroo mince with no larvae present (control), 2 day old heterospecific larvae present (+2 day old) or 4 day old heterospecific larvae present (+4 day old).

7.4.2 Priority effects and larval density: survival to adulthood

For the priority effect, survival was not significantly different from the control for *Ch. rufifacies* within the '25 each' larval density (Fig. 24a). However, for the '50 each' and '75

each larval densities, almost all priority effects had significantly higher survival than the control, except for 0R + 2S within the '75 each' larval density, which was not significantly different from the control (Fig. 24a). Survival in a conspecific mass compared to the control (0R only) had a strong association with larval density. The conspecific mass had significantly lower survival in the '25 each' larval density, was not significantly different in the '50 each' larval density and significantly higher survival in the '75 each' larval density (Fig. 24a). For *C. stygia,* we found the 4R + 0S and 2R + 0S priority effects had significantly lower survival than the control for all three larval densities (Fig. 24b). The other priority effects (0R + 2S and 0R + 4S) both had significantly higher survival than the control in the '25 each' larval density but were not significantly different in the '75 each' larval density. Conspecific survival was also not significantly different from the control in any of the three larval densities (Fig. 24b).

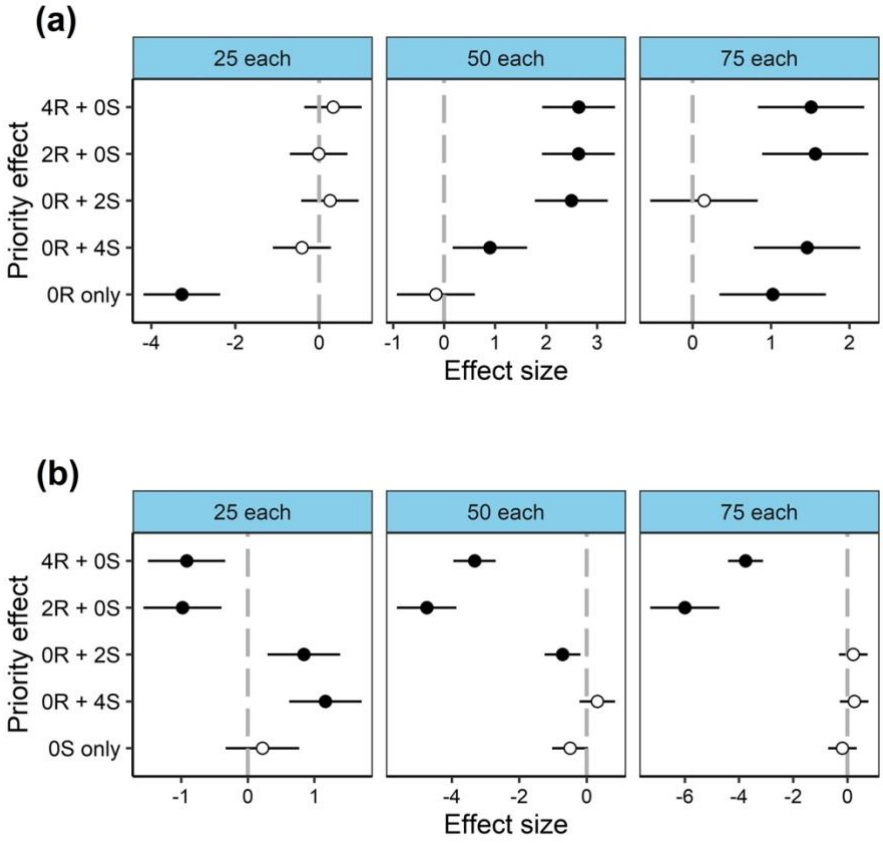

Figure 24 Effects of priority effect treatments on larval survival to adulthood relative to the control (0R + 0S; no priority effect in a heterospecific mass) within three different larval densities for (a) *Chrysomya rufifacies* and (b) *Calliphora stygia*. Priority effect treatments within heterospecific masses is on the y-axis, with numbers representing age of larvae (0, 2 or 4 day old) and letters representing species (R = *Ch. rufifacies* and S = *C. stygia*). Conspecific mass consisting of only one species is the bottom tick of the y-axis (0R only for *Ch. rufifacies* conspecific mass and 0S only for *C. stygia* conspecific mass). Significant effects (shown in bold) are denoted by 95% CIs that do not cross zero, which represents the control for priority effect (grey dotted line). Effect sizes are derived from GLMMs.

7.4.3 Priority effects and larval density: development time until adult eclosion

For *Ch. rufifacies* within the '25 each' larval density, development time was significantly longer than the control for the 0R + 2S and 0R + 4S priority effects, but not significantly different for 4R + 0S and 2R + 0S (Fig. 25a). The '50 each' and '75 each' larval densities displayed similar results for *Ch. rufifacies*, with development time significantly shorter for 4R + 0S, and significantly longer for 0R + 2S. In the same larval densities, 2R + 0S displayed no significant difference in development time compared to the control, while 0R + 4S was not significantly different in the '50 each' larval density but was significantly shorter in the '75

each' larval density. The conspecific mass had significantly longer development times compared to the control for the '25 each' and '50 each' larval densities, but significantly shorter development time in the '75 each' larval density (Fig. 25a). For *C. stygia* development time, almost all priority effects were significantly longer than the control, except for 0R + 2S in the '75 each' larval density, which was found to be not significantly different from the control (Fig. 25b). The conspecific mass was also found to have significantly longer development time compared to the control for all larval densities.

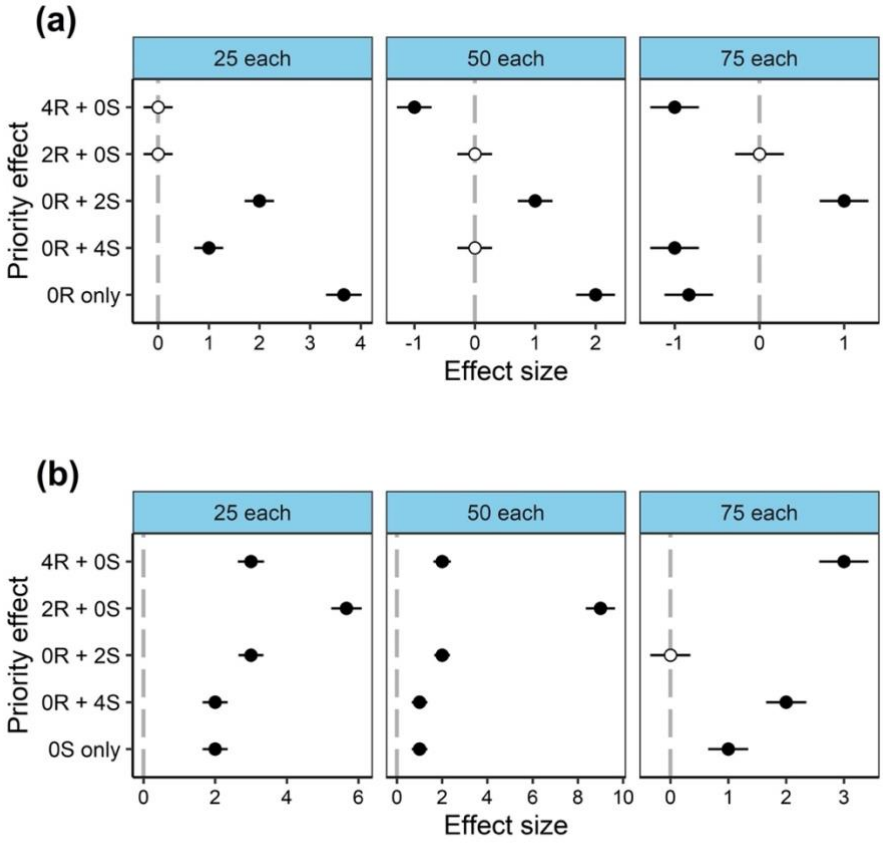

Figure 25 Effects of priority effect treatments on development time to adult eclosion relative to the control (0R + 0S; no priority effect in a heterospecific mass) within three different larval densities for (a) *Chrysomya rufifacies* and (b) *Calliphora stygia*. Priority effect treatments within heterospecific masses is on the y-axis, with numbers representing age of larvae (0, 2 or 4 day old) and letters representing species (R = *Ch. rufifacies* and S = *C. stygia*). Conspecific mass consisting of only one species is the bottom tick of the y-axis (0R only for *Ch. rufifacies* conspecific mass and 0S only for *C. stygia* conspecific mass). Significant effects (shown in bold) are denoted by 95% CIs that do not cross zero, which represents the control for priority effect (grey dotted line). Effect sizes are derived from GLMMs.

7.4.4 Priority effects and larval density: adult fitness

For adult fitness (body mass), *Ch. rufifacies* had significantly lower fitness than the control for 4R + 0S and 2R + 0S priority effects in the '25 each' larval density (Fig. 26a). In the same larval density, 0R + 2S was not significantly different from the control, while 0R + 4S had significantly higher fitness. The '50 each' and '75 each' larval densities shared similar results, with fitness found to be significantly lower than the control in 4R + 0S but not significantly different in 2R + 0S. The larval density did differ however in some priority effects, as 0R + 2S had significantly higher fitness in the '25 each' larval density, but not significantly different in the '75 each' larval density. By contrast, 0R + 4S had significantly higher fitness in the '50 each' larval density but significantly lower fitness than the control in the '75 each' larval density. The *Ch. rufifacies* conspecific mass was not significantly different from the control, except for the '50 each' larval density, where it was significantly higher (Fig. 26a). For *C. stygia* fitness, almost all priority effects were found to be significantly lower than the control, particularly for the '50 each' larval density. The exception to this was 2R + 0S and 0R + 2S in the '25 each' larval density and 4R + 0S in the '75 each' larval density which were all found to be not significantly different from the control. 4R + 0S in the '25 each' larval density was the only priority effect found to have significantly higher fitness than the control. The conspecific mass when compared to the control displayed mixed results. It was found to be significantly higher in the '25 each' larval density, significantly lower in the '50 each' larval density and not significantly different in the '75 each' larval density (Fig. 26b).

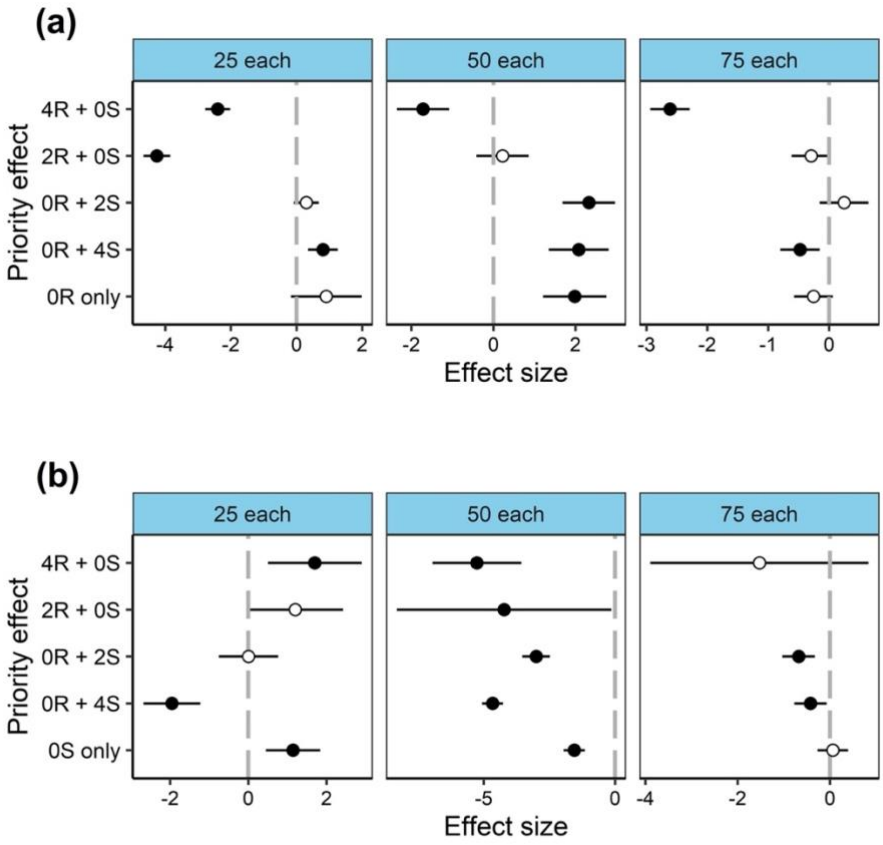

Figure 26 Effects of priority effect treatments on adult fitness (body mass (mg)) relative to the control (0R + 0S; no priority effect in a heterospecific mass) within three different larval densities for (a) *Chrysomya rufifacies* and (b) *Calliphora stygia*. Priority effect treatments within heterospecific masses is on the y-axis, with numbers representing age of larvae (0, 2 or 4 day old) and letters representing species (R = *Ch. rufifacies* and S = *C. stygia*). Conspecific mass consisting of only one species is the bottom tick of the y-axis (0R only for *Ch. rufifacies* conspecific mass and 0S only for *C. stygia* conspecific mass). Significant effects (shown in bold) are denoted by 95% CIs that do not cross zero, which represents the control for priority effect (grey dotted line). Effect sizes are derived from GLMMs.

7.4.5 Priority effects and larval density: overall comparison

When combining all the larval analyses, we can see *Ch. rufifacies* had low survival and slow development speed in low larval density conspecific masses, with survival and development speed increased as larval density increased (Fig. 27a). This trend was not observed in *C. stygia* conspecific masses as survival and development time did not change as larval density increased (Fig. 27b). In heterospecific masses where *Ch. rufifacies* arrived first, *C. stygia* survival was greatly reduced regardless of larval density (Fig 27c & 27e). Survival rate of *C. stygia* only increased or was similar to the control (heterospecific mass with no priority effect) when *C.*

stygia arrived first (Fig. 27d & 27f). *Chrysomya rufifacies* on the other hand only had increased survival in high densities, regardless of priority effect (Fig. 27e & 27f), with no effect observed in low density masses (Fig. 27c & 27d).

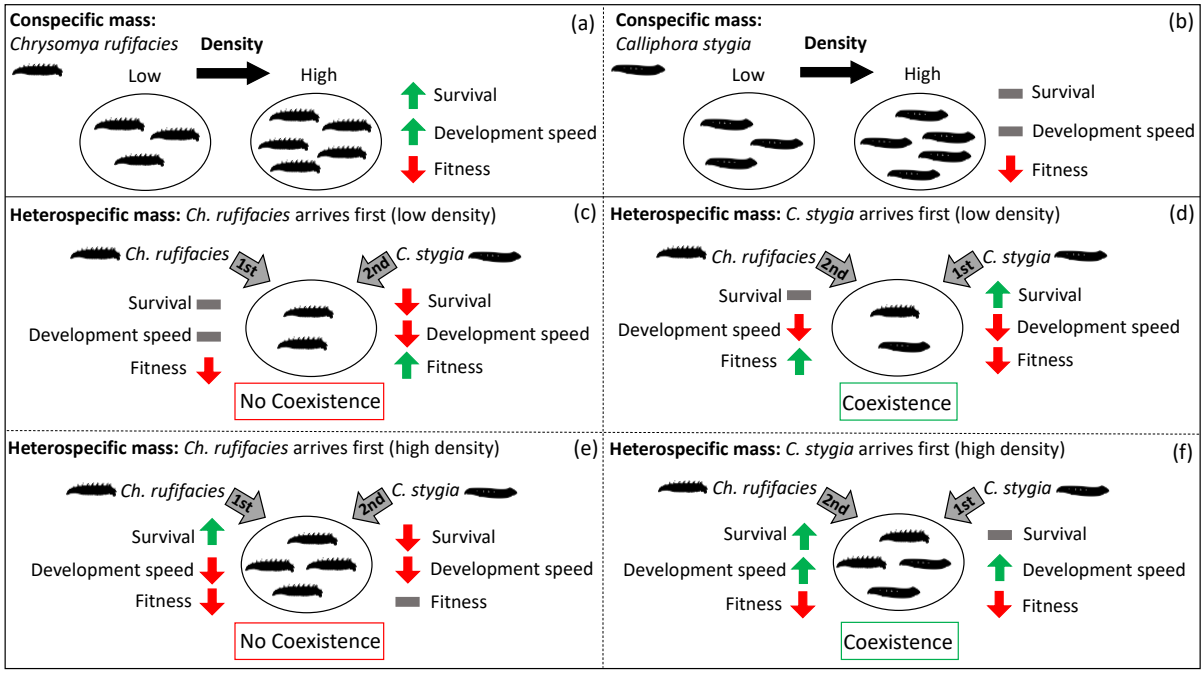

Figure 27 Conceptual diagram of larval density effects on conspecific masses of (a) *Ch. rufifacies* and (b) *C. stygi*a larvae. The effect of different combinations of priority effects and larval density effects is also displayed for heterospecific masses: (c) *Ch. rufifacies* arriving first at low larval densities, (d) *C. stygia* arriving first at low larval densities, (e) *Ch. rufifacies* arriving first at high larval densities and (f) *C. stygia* arriving first at high larval densities. Coloured arrows represent changes relative to the 0 + 0 control for survival (survival to adulthood), development speed and fitness (body mass). Green up arrows = increased survival rate, faster development speed (quicker) and increased fitness, and vice versa for red down red arrows. Grey dashed lines represent no effect.

7.5 Discussion

We conducted a series of laboratory experiments to determine how competition between *Chrysomya rufifacies* and *Calliphora stygia* is influenced by priority effects and larval density. Our first hypothesis was not supported statistically as we found the presence of different aged heterospecific larvae on a resource had no significant effect on adult oviposition for either *Ch. rufifacies* or *C. stygia*. Our second hypothesis was supported, in part, with results showing *C. stygia* unable to survive when arriving after *Ch. rufifacies*, regardless of density. *Chrysomya rufifacies* survival increased when in high density heterospecific masses, regardless of priority effect. Together, our findings indicate there are a complex array of outcomes resulting from

competitive interactions between *Ch. rufifacies* and *C. stygia*. We discuss these findings below in relation to co-existence among species with different strategies for carrion resource exploitation.

7.5.1 Adults of both species displayed no oviposition preference

Our results suggest that neither species displays a strong oviposition preference between a resource that had heterospecific larvae and one without. These results are unexpected as previous research had found *Ch. rufifacies* lay significantly more eggs on a resource with larvae of the blowfly *Chrysomya megacephala* present than one without (Yang and Shiao, 2012). Conversely, *Ch. megacephala* was found to lay significantly more eggs on a resource without larvae of *Ch. rufifacies* (Yang and Shiao, 2012). Several other studies have also demonstrated how egg laying behaviour of blowflies can be influenced by the presence of heterospecifics (Giao & Godoy, 2007; Spindola *et al.* 2017). Though our results were non-significant, we did observe a similar trend in egg laying behaviour as previous studies with adult *Ch. rufifacies* laying more eggs in the presence of *C. stygia* larvae, while *C. stygia* displayed the opposite trend, laying fewer eggs in the presence of *Ch. rufifacies* larvae. However, if our results reflect what would happen under natural settings, then it may be the case that adult *C. stygia* are not able to detect the presence of *Ch. rufifacies* larvae via visual or chemical cues on a resource as effectively as other species (Brundage *et al.* 2017). Similarly, adult *Ch. rufifacies* may not display an obvious preference for a resource with or without *C. stygia* as the fitness benefits from the heterospecific treatments may not differ considerably from a resource without heterospecific larvae present. *Chrysomya rufifacies* larvae may only become predatory when the carrion resource is limited. Therefore, if the resource is plentiful, adults may display no oviposition preference for the presence of heterospecific prey (Gomes *et al.* 2007; Pimsler *et al.* 2019). Alternatively, the number of heterospecific larvae on the resource in our experiments might not have been large enough to elicit a significant response from either species, or the 30-minute interval for feeding was insufficient to produce heterospecific volatiles detectable by adults. It is possible that further replication may have increased the trends we observed and towards a significant result – so modification of oviposition behaviour should not be ruled out as an adaptation in either species.

7.5.2 Chrysomya rufifacies is dependent on larval mass size

Our findings indicate that while in low-density conspecific larval masses, *Ch. rufifacies* survival rate was low, and development time for surviving maggots was high. In fact, almost no individuals survived in conspecific masses, unless at the highest larval density, at which point survival rate increased significantly. This is a surprising result, as in nature, high levels of interspecific density generally lead to increased interspecific competition and higher mortality levels (Agnew *et al.* 2002). The enhanced survival of *Ch. rufifacies* in high densities may be explained by the fact that *Ch. rufifacies*, like many other blowflies, likely feeds on carrion resources via exodigestion mechanisms – which are where adult and larval flies excrete enzymes to breakdown food particles to a liquid state before ingestion (Scanvion *et al.* 2018). Exodigestion is made more efficient when larval mass size is increased and enzyme production is greater due to collective gregarious behaviour (Scanvion *et al.* 2018; Charabidze *et al.* 2021). For example, research has found larval *Lucilia sericata* had high mortality in low density masses on a fresh resource (Scanvion *et al.* 2018). But when the same resource was altered to be more digestible, mortality rate was decreased in low density masses, while high density masses had low mortality rates on either resource (Scanvion *et al.* 2018). This suggests that *L. sericata* requires a minimal larval density for effective exodigestion on a fresh resource. *Chrysomya rufifacies* is likely under similar larval mass density requirements to successfully feed on a fresh resource that has not yet been broken down by bacteria or other species secreting enzymes. Higher temperatures associated with higher larval densities may have also influenced survival rates, although this effect is likely to be limited as temperatures are likely to have been increased only a few degrees above ambient due to the limited mass sizes used in our experiments (Rivers *et al.* 2010; Heaton *et al.* 2014; Scanvion *et al.* 2018).

This requirement of collective gregarious behaviour may also explain why *Ch. rufifacies* survival rate increased in heterospecific larval masses at high larval densities regardless of the priority effect treatment. The resource may be more effectively broken down by the heterospecific larvae, enabling *Ch. rufifacies* to successfully feed on the fresh resource and thereby enhance its survival and reduce development time (Komo *et al.* 2019; Charabidze *et al.* 2021). This effect is increased in high density larval masses as the resource was collectively broken down at a faster rate due to the high density (Goodbrod & Goff, 1990). Alternatively, *Ch. rufifacies* may use the heterospecific mass as an alternative food source. However, because predatory behaviour only occurs during second and third instar stages, predation is likely only occurring in the age treatments where *Ch. rufifacies* was older

(Baumgartner, 1993). In these treatments, survival was high, suggesting that *Ch. rufifacies* benefit from arriving at a fresh resource after a heterospecific – regardless of their age. The requirements of minimum mass size for exodigestion or presence of heterospecific larvae are likely reasons why *Ch. rufifacies* generally acts as a secondary coloniser – laying larvae later in the decomposition process, even though adult flies arrive at carrion relatively early (Dawson *et al.* 2021a). It currently remains unknown what morphological or physiological factors limit *Ch. rufifacies* exodigestion capabilities on fresh carrion, but these might relate to the larval mouthparts or the type of enzymes produced (Shiao & Yeh, 2008). Surprisingly, co-existence with a heterospecific in this case is beneficial for *Ch. rufifacies* survival and life history traits, despite the detrimental effects of interspecific competition.

Fitness of *Ch. rufifacies* generally decreased when in heterospecific masses with younger *C. stygia* regardless of larval density. A reduction in fitness is likely due to the increased survival rate in the same priority effect treatments. *Chrysomya rufifacies* survival was increased in these conditions, resulting in more conspecifics on the resource and subsequently higher levels of interspecific competition, leading to reduced fitness of individuals (Peters & Barbosa, 1977). In nature, there are likely trade off decisions that adults must make as they either lay in high densities where individuals are more likely to survive but have reduced fitness, or risk laying in low densities were survival is reduced, but those that do survive will be more fit (Raitanen *et al.* 2013). With survival so low in conspecific masses, *Ch. rufifacies* has likely evolved to favour ovipositing in high density masses due to their potential reliance on collective exodigestion. The facultative predatory behaviour of *Ch. rufifacies* may be an additional adaptation to allow them to cope better in high density larval masses and thereby reduce reliance on the carrion resource directly (Polis, 1981).

7.5.3 Calliphora stygia is dependent on priority effects

Calliphora stygia displayed the opposite pattern to *Ch. rufifacies*, with *C. stygia* having higher survival rates in conspecific masses regardless of larval density. When in heterospecific masses and arriving after *Ch. rufifacies*, *C. stygia* survival was reduced, and in some treatments, no *C. stygia* survived. This result demonstrates the likelihood of *C. stygia* larvae surviving to be substantially reduced when they arrive after *Ch. rufifacies*. Survival of *C. stygia* and co-existence with *Ch. rufifacies* on a resource is only likely when they arrive at the same time or earlier than *Ch. rufifacies*. In these situations, *C. stygia* can feed and grow before *Ch. rufifacies*

is able to reach a developmental stage at which they can display predatory behaviour (Brundage *et al.* 2014). Therefore *C. stygia* survival on carrion can be mediated by the species arriving before *Ch. rufifacies*, thereby displaying temporal partitioning as a response to interspecific competition (Brundage *et al.* 2014). *Calliphora stygia* also had superior survival rates than *Ch. rufifacies* in low larval densities. *Calliphora stygia* likely has more effective feeding capabilities on a fresh resource potentially due to mouthpart morphology or type of enzymes produced, and does not have a large reliance on collective exodigestion nor a lower threshold of larval mass size (Goodbrod & Goff, 1990; Scanvion *et al.* 2018). This adaptation to feeding on fresh remains that are less digestible to *Ch. rufifacies* has also likely led to the emergence of temporal partitioning behaviour on carrion (Barton *et al.* 2019; Benbow *et al.* 2019). *Calliphora stygia*, along with other Diptera and bacterial species alter the biochemistry of carrion, making the remains better suited to species like *Ch. rufifacies* (Tomberlin *et al.* 2017).

The presence of younger and older heterospecifics also increased development time of *C. stygia*. Slower development rates are likely due to increased pressure from interspecific competition or spending a greater amount of time exhibiting predator avoidance behaviour, thereby resulting in less optimal resource and nutritional uptake (Wells & Kurahashi, 1997). Altered development rates in heterospecific masses are an important consideration if larvae are used in forensic entomology for PMI estimates. Ideally, when estimating a PMI, forensic entomologists should not only consider the size of larval masses, but also whether they consist of one or more species.

Lower fitness levels were observed in *C. stygia* in higher larval densities, which also suggests that individuals were not feeding optimally, likely due to increased intra- and interspecific competition on a more densely packed resource. Although not analysed here, *C. stygia* larvae potentially leave the resource early and spend additional time in the post-feeding life stage, attempting to move away from *Ch. rufifacies*.

7.5.4 Implications and conclusions

Our results suggest that neither species can completely outcompete and dominate the other as they are constrained by density requirements (*Ch. rufifacies*) or priority effects (*C. stygia*). The two blowfly species use different morphological and behavioural adaptations to survive on carrion, particularly *Ch. rufifacies*, which likely has evolved facultative predatory behaviour to compensate for its reliance on high larval densities for survival. *Calliphora stygia* is reliant on

priority effects and temporal partitioning for survival as a consequence of the competitively superior *Ch. rufifacies*. Broadly, our study shows how priority effects enable co-existence to occur on a limiting and patchy resource such as carrion. The successional patterns observed on carrion is generally the outcome of intense competition and subsequent temporal partitioning, with each species employing a range of morphological and behavioural adaptations to survive. Examining these fine scale species interactions and outcomes of competition enables researchers to determine the exact drivers of succession and co-existence on an ephemeral resource.

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Chapter 8. General discussion and conclusions

8.1 Preamble

A greater knowledge of the ecological processes underpinning carrion insect succession may lead to more quantifiable and reliable approaches for determining the PMI. Despite this, few forensic studies have incorporated ecological-based methodology into research, with the extent of large sources of variation in the succession process currently recognised (e.g. carrion type) but remaining unresolved (Tomberlin *et al.*, 2011a; Tomberlin *et al.*, 2011b; Michaud *et al.*, 2012; Tomberlin *et al.*, 2012; Miles *et al.*, 2020). In my thesis, I aimed to address this issue by bridging carrion ecology methodology and analyses with forensic entomology to quantify sources of biological variation in the succession process to increase the reliability of succession data for forensic investigations.

To examine carrion insect succession, I conducted innovative field experiments over multiple seasons using several sampling techniques and approaches at the Australian Facility for Taphonomic Experimental Research (AFTER). Using this facility, I was able to employ both pig and human cadavers in carrion succession experiments, which has seldom been done. I found that insect succession and associated decomposition progress differed between pigs and humans. To analyse decomposition patterns in more detail, I used mass loss to model decomposition and compare between the cadaver types, a novel approach in forensic taphonomy. I found decomposition progress to be faster in pigs compared with humans, thus providing new insight into the applicability of pigs as models for humans in forensic research. I also used ecological based statistical modelling, new to the field of forensic entomology, to determine what factors are driving carrion insect succession, and importantly the role of the carrion resource in shaping insect community composition and species abundances on carrion. I also undertook innovative laboratory experiments and analyses to identify how priority effects and larval density promote coexistence among larvae of competing carrion-breeding Diptera. Overall, I demonstrated how complex the carrion insect succession process is and how variation can be quantified using ecological based approaches and applied to forensic research to improve PMI estimates derived from insect data.

8.2 Pigs as unreliable models for humans in forensic entomology and decomposition research

In Chapter 2 I conducted a preliminary study to determine whether broad decomposition and entomological patterns differed between pigs and humans. I found that pigs decomposed faster than humans, had higher insect species richness and significantly different insect community composition regardless of season. I hypothesised that these differences were likely attributed to the cadaver's mass, diet, physiology, medical histories and associated microbiomes, all of which may be altering the decomposition process and the timing of chemical cues produced for insect colonisation. Due to the challenges of using human cadavers, a number of systematic differences were unable to be controlled for, thereby making it difficult to pinpoint the exact reasons why differences were observed (Matuszewski *et al.*, 2020). Nevertheless, the results indicated the need to not only recognise intrinsic differences between humans and animal models, but also between humans. Cadavers sourced from donation programs may be different from those encountered in forensic casework, particularly in regard to their peri-mortem medical treatment (Miles *et al.*, 2020). The results suggest that pigs may not be ideal proxies for humans in forensic casework, and caution must be taken when applying data derived from pigs directly to casework. Though preliminary in nature, these findings highlight the need for further research into potential differences in decomposition and entomological activity between pigs and humans. The next step should be determining exactly how the two cadaver types vary and potentially developing correction or adjustment calculations to apply to data derived from pigs for PMI estimates on human cadavers. For any correction factor to be developed, a full account of the differences between pigs and humans is required, as well as the factors driving these differences. Therefore, from the findings of this study, I identified several important questions that warranted further exploration, such as addressing the forensic ramifications of using pig models and how to appropriately use human cadavers for forensically relevant research, as well as determining what biological factors are driving the entomological differences between pigs and humans.

Chapter 3 builds upon Chapter 2 by examining decomposition patterns of pigs and humans in more detail by using both direct and indirect decomposition metrics. Importantly, I examined mass loss differences between pigs and humans, which is seldom used as a measure of decomposition in forensic research, despite being a common metric in ecological studies (Chaloner *et al.*, 2002; Parmenter & MacMahon, 2009; Barton *et al.*, 2019). Mass loss can provide quantifiable information about the decomposition process that is potentially missed

when relying solely on indirect measures, such as total body score (TBS). As expected, pigs and humans differed in their decomposition progress, with mass loss and TBS displaying similar patterns at the start of decomposition as pigs decomposed faster from the onset of decay. The two metrics did, however, differ towards the end of decay as TBS stabilised at similar values on pigs and humans, but mass loss continued, with pigs losing a greater amount of mass by the end of the experiment, particularly during winter. The results indicated that mass loss can be a reliable metric to model decomposition and provide more quantifiable information about the decomposition progress that is not evident in other indirect metrics. Despite mass loss data being difficult to collect and the often unknown starting mass of cadavers in casework, it should still be considered an important tool for modelling decomposition, and when combined with TBS may produce more reliable and accurate PMI estimates (Maile *et al.*, 2017).

My research on mass loss also highlights the differences in the decomposition progress between pigs and humans, with pigs decomposing faster. Differences in mass loss between cadaver types is likely the result of increased insect activity on the pigs, which are one of the main drivers of mass loss (Payne, 1965). My findings add to the growing body of evidence suggesting that pigs differ in their decomposition progress to humans, strengthening the argument that pigs may be unreliable proxies for human cadavers (Connor *et al.*, 2018; Dautartas *et al.*, 2018; Knobel *et al.*, 2019). From my experiments, I have observed pigs always decomposing faster, with insects arriving more rapidly and in higher abundance. Before being applied to human casework, data derived from pigs should undergo a correction factor to account for the quicker decomposition and increased insect activity exhibited on pigs. Any such correction factor also needs to consider the local environment, particularly the temperature, season and climate. Ideally, a correction factor would be habitat specific, as my research conducted at AFTER cannot easily be applied to and compared with cadavers in other similar research facilities, such as in North America. To develop a correction factor, further research with high replication is needed comparing pigs and humans to reduce the unknown variability between cadaver types and account for systematic differences of human cadavers, such as body size, gender, age and peri-mortem medical treatment.

8.2.1 Validating research findings on human cadavers

Chapters 2 and 3 highlighted key differences between pigs and humans, thereby recognising the need to validate data derived from pigs before extrapolating results to forensic casework involving human cadavers. Therefore, in Chapter 4 I investigated carrion-associated Diptera colonisation patterns on human cadavers only. I determined which Diptera species are breeding on human cadavers and provided new ecological information, such as seasonal preference, distribution and importantly, their pre-appearance interval (PAI). When combining this ecological information with data derived from past casework, I was also able to identify some consistencies and gaps to determine how forensically useful species are to forensic investigations. A key finding from this work was the two-three day delay in oviposition I observed on human cadavers at AFTER for all carrion-breeding Diptera, with the delay more pronounced in winter. Other studies at AFTER have also reported similar delays in oviposition on human cadavers (e.g. Skopyk *et al.*, 2021). This delay in PAI is extremely important to PMI estimates derived from larval development data, as the longer the delay in oviposition, the further the estimated minPMI is from the actual time of death (Matuszewski *et al.*, 2014; Faris *et al*., 2016). It is important to recognise the high variability in the succession process, and how to consider this type of ecological information when determining which species are forensically useful. Few studies have used human cadavers to examine insect succession, with the work presented here being among the first to use human cadavers to provide ecologically relevant information for carrion-associated Diptera. This ecological information includes new season and distribution records for these flies, such as *Calliphora ochracea* and *Hemipyrellia fergusoni*, as well as new PAI information that could be applied to larval development data to reduce the gap between the minPMI and the actual time of death.

8.3 Carrion resource quality driving insect succession

As I had identified that entomological activity differed between pigs and humans, the next important question was to determine what factors were driving these differences. To do this, in Chapter 5 I investigated to what degree TBS, season, cadaver type, ambient temperature and initial starting mass had on driving changes in species richness and species composition. Total body score in this study is a semi-quantitative measure of resource change, representing morphological, and to some degree, microbial changes on carrion. I found TBS to be a key driver of species turnover and community succession, highlighting the role carrion change has in driving succession. Importantly, TBS is a continuous measure enabling succession to be examined as a gradient, rather than compared to discrete decay stages as is common in forensic

entomology (Michaud *et al.*, 2015). Recognising the continual changes occurring on carrion, and how species turnover is also likely to be continuous and fluctuating is important for providing more accurate and reliable models of succession (Schoenly & Reid, 1987). This is particularly true as forensic entomologists begin to move away from decay stage analyses and descriptive succession tables and move towards more quantifiable modelling approaches that incorporate carrion resource changes as a continuous variable. By using quantitative analyses, forensic entomologists could construct complex models that incorporate several key factors such as temperature and the carrion resource to predict succession more accurately, with the end goal of providing more reliable PMI estimates.

Analysing how resource change influences community composition patterns does not provide a complete picture of how succession progresses on carrion. Individual species patterns also need to be examined to determine how species perceive carrion resource change. In Chapter 6 I examined individual species abundance patterns in relation to TBS, a measure of resource change, and from the perspective of a species, a measure of resource quality, as resource quality is a species-specific concept. I interpreted phases of increasing abundance as perceived high resource quality, and the length of abundance peaks to indicate optimal oviposition and feeding time. Some species were abundant throughout decomposition, suggesting that they perceive resource quality as optimal during the whole decomposition process and therefore have a wide window of resource exploitation. For other species, abundance spiked over a narrow range of TBS values, suggesting that these species have a narrow window of opportunity on carrion where resource quality is optimal. As carrion is a competitively intense resource, species have undergone temporal partitioning to utilise carrion at different times (Hanski, 1987; Ives, 1991). By examining abundance patterns, temporal partitioning can be clearly observed to determine the exact ecological factors shaping the perceived niche of each species. Understanding how species respond to carrion resource change on a community and species level enables ecologists to determine the exact mechanisms shaping co-existence and driving species-resource interactions.

In Chapter 6 I also compared species abundance patterns between pigs and humans, finding that most species differed in their abundance patterns between the two cadaver types. Abundances were often higher on pigs, with species also arriving earlier, although this was not always the case as a few species displayed the opposite trend. Species likely perceive the two carrion types as either having different levels of carrion resource quality, or different rates of resource quality change. These differences are likely due to the complex relationship insects have with the necrobiome and carrion, particularly microbes that release the volatile organic

compounds (VOCs) that attract insects to carrion (Benbow *et al.*, 2019). Other workers have shown VOCs released during decomposition to differ between pigs and humans (Knobel *et al.*, 2019). My findings have important ramifications for forensic-based succession studies conducted on pigs, as I have found the intricacies of the succession process to differ between cadaver types. More research is needed to explore succession patterns on human cadavers if succession data are to be used for forensic investigations. Additionally, if we apply abundance modelling in a forensic setting, those species constrained to narrow windows of resource exploitation may be ideal for PMI modelling, as their arrival and departure times are more predictable (Michaud & Moreau, 2009). Therefore, continuing to integrate abundance data and species visitation models into forensic research may increase the reliability of PMI estimates derived from succession data. To integrate abundance data, again, more quantifiable models are needed that do not rely on occurrence data and can incorporate other factors to accurately predict species abundances. By predicting a range of species abundances, a more precise PMI could be determined by considering the abundance of all insects on a cadaver and inputting that data into a model. For this method to be feasible, replicated abundance patterns need to be investigated under different environmental conditions in order to produce reliable models.

8.4 Life history traits promote coexistence between competing carrion insects

For most of this thesis I focused on how the carrion resource is driving succession and species-resource interactions, but there are several other important factors responsible for succession that need to be taken into consideration, such as between-species interactions. In Chapter 7 I investigated the role of competition and between-species interaction in driving species diversity and coexistence on carrion. Specifically, I examined how priority effects and larval density promote co-existence between two carrion breeding flies, the facultative predator *Ch. rufifacies* and its competitor *C. stygia*. I found that *Ch. rufifacies* and *C. stygia* were able to co-exist despite *Ch. rufifacies* being a facultative predator and the two species sharing broadly similar niches. *Chrysomya rufifacies* was dependent on larval mass as the species needed a minimum larval density to survive, particularly in conspecific masses, suggesting a dependency on collective exodigestion (Scanvion *et al.*, 2018). Conversely, *C. stygia* could survive at low larval densities but was constrained by priority effects and unable to survive if arriving after *Ch. rufifacies* due to the predation pressure of *Ch. rufifacies* (Brundage *et al.*, 2014). These results highlight the mechanisms shaping carrion insect succession as species must develop different strategies to survive on a competitively intense resource such as carrion.

As displayed here, one such strategy enabling coexistence with other species is temporal partitioning, a common response to high competitive pressure. Species that are unable to compete directly will alter their temporal patterns to colonise a resource at times less favourable to other species that may be more competitively dominant. The sequential arrival patterns on carrion are unlikely caused solely by temporal partitioning, as other processes are simultaneously occurring, such as resource partitioning, which I explored in Chapter 6. Examining species interactions and outcomes of competition on carrion enables researchers to determine the exact drivers of succession and co-existence on a highly diverse, ephemeral resource. In this case, species have developed different morphological and behavioural adaptations to survive in a highly competitive environment, such as *Ch. rufifacies* evolving facultative predatory behaviour or the ability of *C. stygia* to digest fresh carrion efficiently without the need for large maggot masses.

From a forensic perspective, I found that development times were also impacted by priority effects and larval densities in heterospecific masses. As development time of Diptera larvae is a common method for PMI estimation, it is important to take into consideration all factors that might influence development rates (Slone & Gruner, 2007). From my results, development time generally increased in heterospecific masses, resulting in longer development. If a species is found on a cadaver in a heterospecific mass, then its development time may be slower compared to the development rate of the same species reared in laboratory conspecific masses. A minPMI could therefore be underestimated if comparing the two. Conversely, I found that development time decreased in *C. stygia* conspecific masses as mass size increased. If the mass size used in the laboratory development data differs to that of the cadaver, then the estimated PMI may also have large error margins. Therefore, to reduce error when estimating a PMI from development data, species composition and mass size should be taken into consideration.

8.5 Conclusion and future directions

Succession is complex, with numerous factors influencing the sequential arrival of species. I have found that, when combining biological factors such as cadaver type, carrion resource quality and species interactions all drive and shape the successional process, an approach that to my knowledge has not been taken before. Of these factors, cadaver type is the most important, needing to be addressed in the field of forensic entomology because of the current reliance on the pig model in research. Despite my findings, pigs are still attractive models for

forensic studies since they are easy to procure and permit high replication (Matuszewski *et al.*, 2020). But data derived from pigs need to be validated before their use in forensic investigations, with validation studies required to be conducted across different regions to account for habitat, climate and local species differences. With the increased number of 'body farms' being established, validation studies are becoming increasingly easier to undertake. Data derived from validation studies comparing pigs and humans may lead to correction or adjustment factors to reduce error in PMI estimates if applying pig derived data to casework, thereby improving confidence in the continued use of pigs in forensic research.

Several other important factors also play a role in succession but were not analysed in this thesis, including interactions among the necrobiome. Succession ideally needs to be examined in conjunction with the necrobiome, rather than as its own entity and process. Carrion insect succession is inherently linked to the necrobiome, particularly the microbes that are the first to break down the tissues and produce VOCs that initially attract insects (Benbow *et al.*, 2019). Furthermore, the role of large-bodied scavengers in inhibiting or accelerating carrion insect succession remains relatively unknown (Brundage, 2021). Carrion insect succession is just one cog in the greater necrobiome 'machine', and therefore the interconnected nature of this system needs to be taken into consideration when fully analysing carrion insect succession. In this thesis I have linked insect activity with key aspects of the necrobiome, such as the intrinsic quality of the carrion resource, the pool of species that drive decomposition, and the broader abiotic factors influencing it. I have demonstrated the general applicability of the necrobiome framework in uniting all aspects of carrion ecology together under one umbrella, thereby enabling interconnected research into the mechanisms driving ecological processes on carrion. I have also confirmed that aspects of the framework are highly useful for interpreting decomposition complexity and have shown carrion to be an ideal model for ecological research on trophic structure, niche separation, ecosystem function and co-existence.

A fundamental finding from many carrion ecological studies is the large amount of variation that exists in carrion succession. Currently succession data are difficult to incorporate into forensic investigations due to this variation; however if we can recognise the factors driving succession and associated variation, succession PMI methods may become more reliable. By borrowing from ecological approaches to analyse forensic entomology data, we can accurately model succession in a more quantitative manner, and move away from descriptive based approaches (Tomberlin *et al.*, 2011a). When the many sources of variation have been quantified, accurate succession models can be developed and employed in forensic casework. Currently, several sources of variation have already begun to be investigated, such

as the role of carrion type and mass. The next step will be to develop more complex models to incorporate these sources of variation, such as machine learning techniques, to reduce error in PMI estimations. This thesis represents an important step in bridging the gap between the fields of carrion ecology and forensic entomology, which I hope to become only better integrated as research into the fascinating world of carrion continues.

8.6 References

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Appendices

Appendix I: Supplementary material associated with Chapter 1

Table S1 Species list of all collected insects from AFTER experiments.

Order	Family	Genus	Species	Cadaver type	Season
Diptera	Calliphoridae	*Chrysomya*	*varipes*	both	both
			incisuralis	both	both
			rufifacies	both	both
			nigripes	both	both
			latifrons	both	both
			megacephala	both	both
		Hemipyrellia	*fergusoni*	both	both
		Calliphora	*ochracea*	both	both
			stygia	both	winter
			hilli hilli	both	both
			centralis	human	winter
			fulvicoxa	both	both
			augur	both	both
		Lucillia	*cuprina*	human	summer
		Amenia	*imperialis*	both	summer
	Sarcophagidae	*Sarcophaga*	*beta*	both	both
			furcata	both	summer
			aurifrons	both	summer
			impatiens	both	both
			africa	both	summer
			zeta	both	summer
	Muscidae	*Australophyra*	*rostrata*	both	both
		Hydrotaea	*chalcogaster*	both	both
			spinigera	both	both
		Musca	*vetustissima*	both	both
			domestica	both	summer
		Dichaetomyia	sp.	both	both

	Piophillidae	*Piophila*	*casei*	both	both
			australis	both	summer
		Piophilosoma	sp.	both	winter
	Phoridae		sp.	both	both
	Sepsidae	*Parapalaeosepsis*	*plebeia*	both	both
Coleoptera	Staphylinidae	*Creophilus*	*lanio*	both	both
			erythrocephalus	both	both
	Histeridae	*Saprinus*	*cyaneus cyaneus*	both	both
	Cleridae	*Necrobia*	*rufipes*	both	both
			ruficolies	both	both
	Silphidae	*Ptomaphila*	*perlata*	both	both
			lacrymosa	both	both
		Diamesus	*osculans*	both	both
	Dermestidae	*Dermestes*	*maculatus*	both	both
			ater	both	both
			frischii	both	both
	Trogidae	*Omorgus*	*quadrinodosus*	both	both
			suberosus	both	both
Hymenoptera	Formicidae	*Aphaenogaster*	*longiceps*	both	both
		Rhytidoponera	*metallica*	both	both
		Camponotus	*nigriceps*	both	both
			sp.	both	both
			aeneopilosus	both	both
		Crematogaster	sp.	both	both
		Iridomyrmex	sp.	both	winter
		Polyrhachis	sp.	both	both
		Mymecira	*urens*	pig	summer
			sp.	pig	winter
	Diapriidae	*Spilomicrus*	sp.	both	both
	Pteromalidae	*Nasonia*	*vitripennis*	both	both
	Braconidae	*Callibracon*	sp.	human	winter
	Sphecidae	*Prionyx*	sp.	both	summer
	Apidae	*Tetragonula*	*carbonaria*	human	winter
	Vespidae		sp.	human	summer
	Pompilidae		sp.	pig	summer

Appendix II: Supplementary material associated with Chapter 2

Table S2 Occurrence matrix for insect species attracted to the pig (black) and human (light grey) in the summer experiment. Species occurrence is reported in groupings of individual days (week 1), in two day intervals (weeks 2-3) and every week (weeks 4-9). Adults (a) represented by filled in lines and larvae (l) represented by dashed lines.

Order	Family	Genus	Species
Diptera	Calliphoridae	*Chrysomya*	*varipes* (a)
			varipes (l)
			incisuralis (a)
			incisuralis (l)
			rufifacies (a)
			rufifacies (l)
			nigripes (a)
			nigripes (l)
			megacephala (a)
			megacephala (l)
		Lucillia	*cuprina* (a)
			cuprina (l)
		Calliphora	*fulvicoxa* (a)
			augur (a)
			augur (l)
	Sarcophagidae	*Sarcophaga*	*beta* (a)
			furcata (a)
			aurifrons (a)
			impatiens (a)
			impatiens (l)
			zeta (a)
			africa (a)
	Muscidae	*Australophyra*	*rostrata* (a)
			rostrata (l)
		Hydrotaea	*chalcogaster* (a)
			spinigera (a)
			australis (a)
		Musca	*vetustissima* (a)
			domestica (a)
	Piophilidae	*Piophila*	*casei* (a)
			casei (l)
	Phoridae		sp. (a)
Coleoptera	Staphylinidae	*Creophilus*	*lanio* (a)
			erythrocephalus (a)
	Histeridae	*Saprinus*	*cyaneus* (a)
	Cleridae	*Necrobia*	*rufipes* (a)
			ruficolis (a)
	Silphidae	*Ptomaphila*	*perlata* (a)
			lacrymosa (a)
		Diamesus	*osculans* (a)
	Dermestidae	*Dermestes*	*maculatus* (a)
			ater (a)
			frischii (a)
	Trogidae	*Omorgus*	*quadrinodosus* (a)
			suberosus (a)

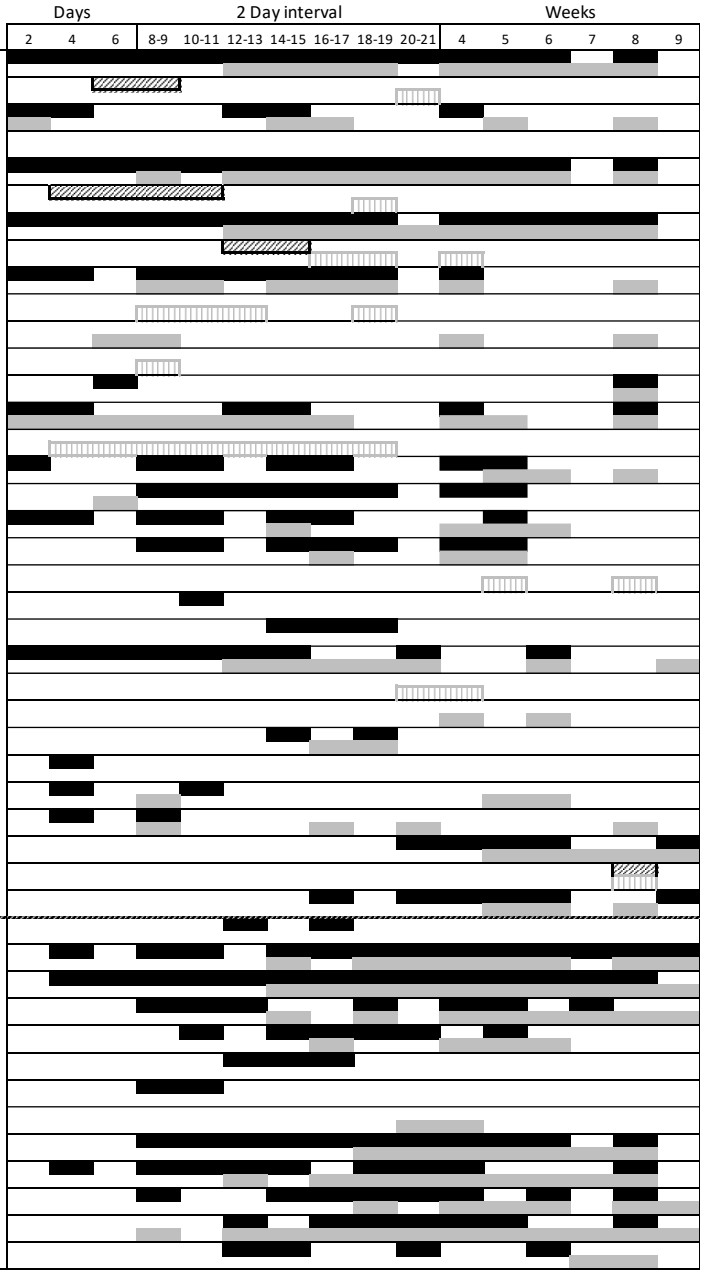
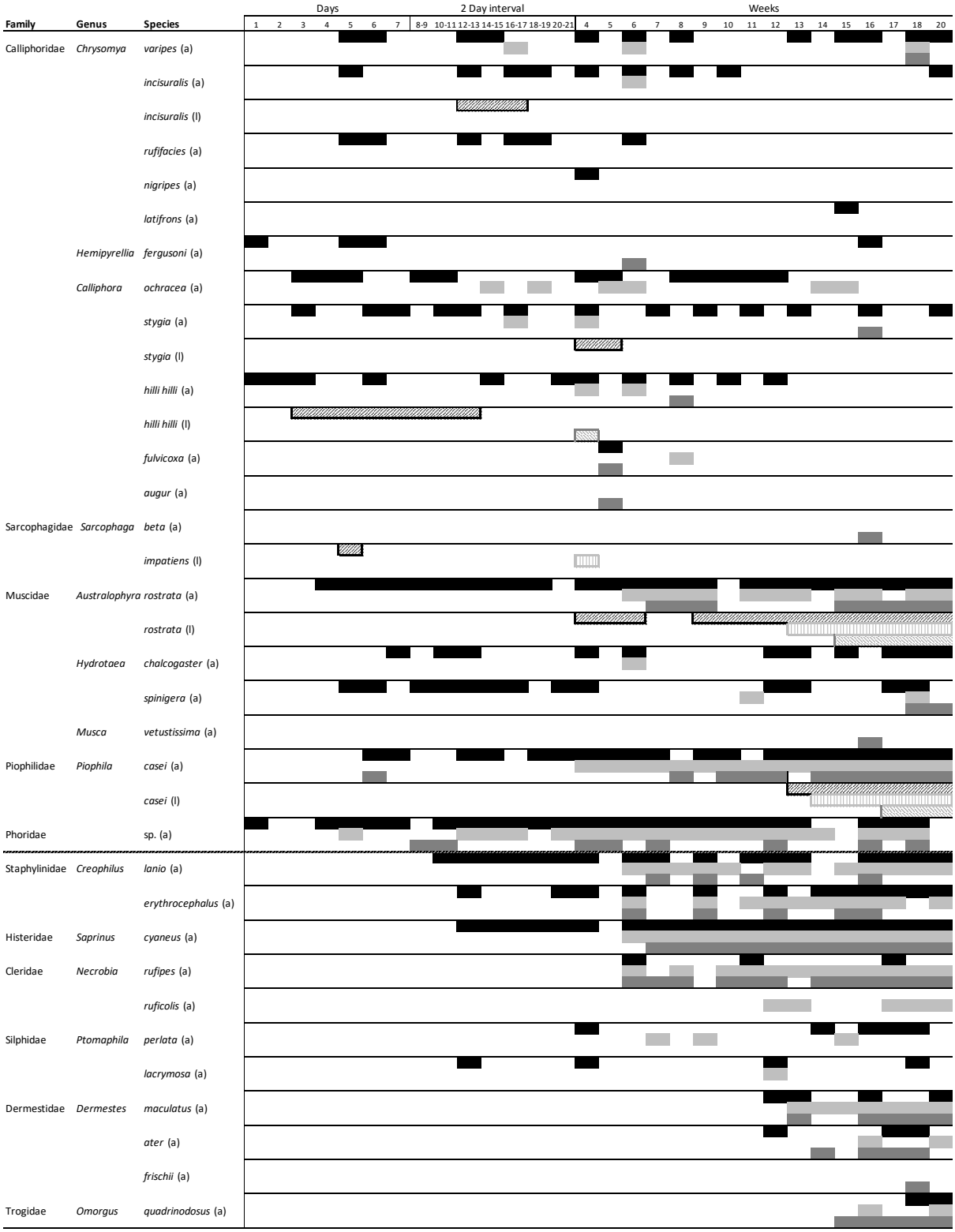


Table S3 Occurrence matrix for insect species attracted to the pig (black), human A (light grey) and human B (dark grey) in the summer experiment. Species occurrence is reported in groupings of individual days (week 1), in two day intervals (weeks 2-3) and every week (weeks 4-20). Adults (a) represented by filled in lines and larvae (l) represented by dashed lines.



Appendix III: Supplementary material associated with Chapter 4

Table S4 Details of human cadavers used in the succession experiments

Replicate	Experiment	Sex	Age (years)	Weight (kg)	Date of placement	Cause of death
Human 1	Winter A	Female	53	46.8	30/5/18	Metastatic malignancy of the lung
Human 2	Winter A	Female	86	80	2/6/18	Chronic obstructive pulmonary disease
Human 3	Winter B	Male	57	66	8/5/19	Cardiac arrest
Human 4	Winter B	Male	74	51.7	7/6/19	Cancer
Human 5	Summer	Female	82	60.5	9/11/19	Cancer / Aspiration pneumonia
Human 6	Summer	Female	97	46.8	14/11/19	Cardiac failure

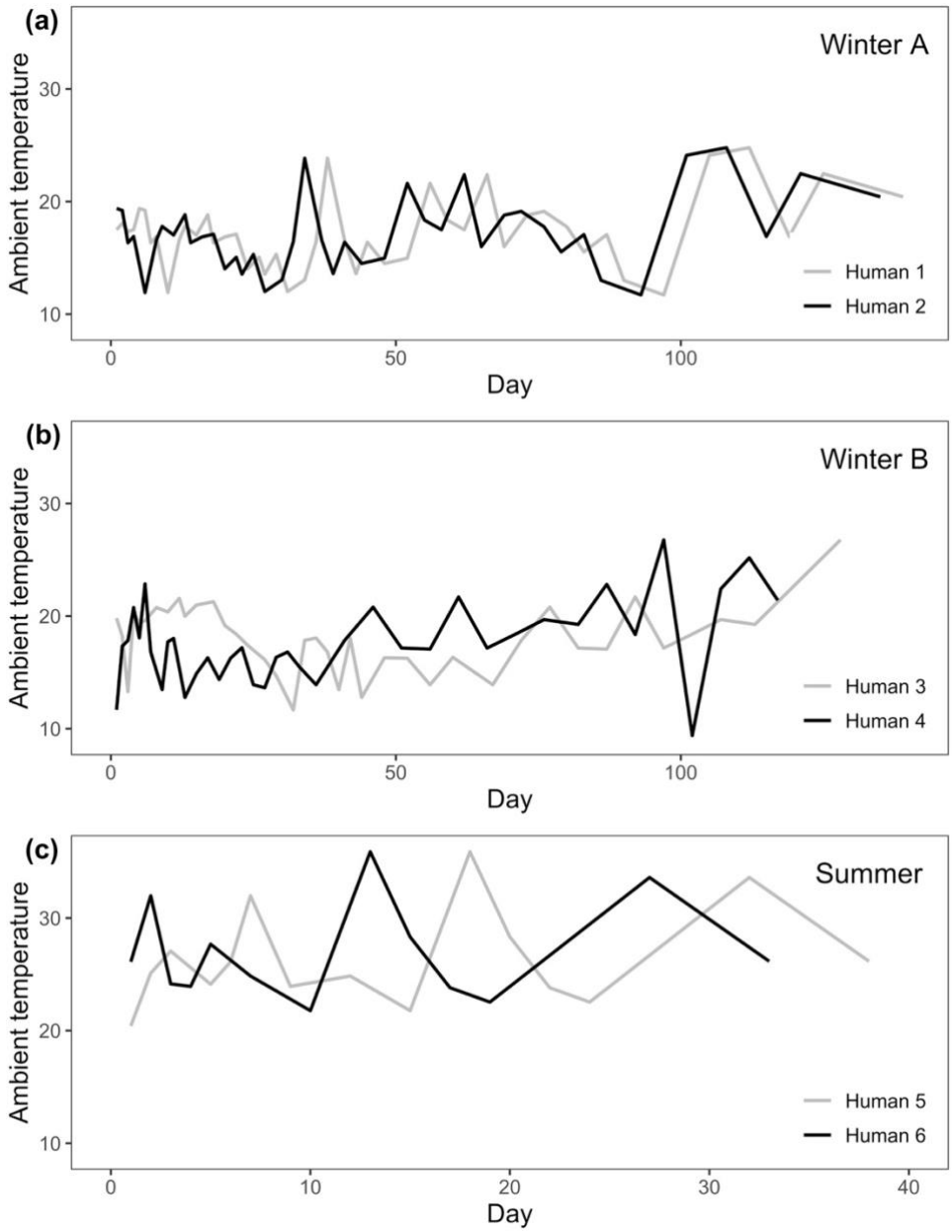


Figure 28 Ambient temperatures (°C) recorded in association with each cadaver using a single temperature logger during a) Winter A, b) Winter B and c) Summer succession experiments.

Table S5 List of references (n=25) that provide relevant information relating to the colonisation behaviour of flies collected in past casework and in our succession experiments.

Species	Colonisation behaviour	Reference
Chrysomya incisuralis	No information	
Chrysomya latifrons	No information	
Chrysomya megacephala	Primary	Badenhorst, R, Villet, MH (2018) The uses of *Chrysomya megacephala* (Fabricius, 1794) (Diptera: Calliphoridae) in forensic entomology. *Forensic Sciences Research* **3**, 2-15.
		Sebastião, M, e Castro, CP (2019) A preliminary study of carrion insects and their succession in Luanda, Angola. *Journal of Medical Entomology* **56**, 378-383.
		Silahuddin, SA, Latif, B, Kurahashi, H, Walter, DE, Heo, CC (2015) The importance of habitat in the ecology of decomposition on rabbit carcasses in Malaysia: Implications in forensic entomology. *Journal of Medical Entomology* **52**, 9-23.
		Voss, SC, Cook, DF, Dadour, IR (2011) Decomposition and insect succession of clothed and unclothed carcasses in Western Australia. *Forensic Science International* **211**, 67-75.
		Voss, SC, Spafford, H, Dadour, IR (2009) Annual and seasonal patterns of insects succession on decomposing remains at two locations in Western Australia. *Forensic Science International* **193**, 26-36.
		Wang, Y, Wang, J, Wang, Z, Tao, L (2017) Insect succession on pig carcasses using different exposure time – A preliminary study in Guangzhou, China. *Journal of Forensic and Legal* Medicine **52**, 24-29.
Chrysomya nigripes	Tertiary	O'Flynn, MA (1983) Notes on the biology of *Chrysomya nigripes* Aubertin (Diptera: Calliphoridae). *Australian Journal of Entomology* **22**, 341-342.
		O'Flynn, MA (1983) The succession and rate of development of blowflies in carrion in southern Queensland and the application of these data to forensic entomology. *Australian Journal of Entomology* **22**, 137-148.
		Wang, Y, Wang, J, Wang, Z, Tao, L (2017) Insect succession on pig carcasses using different exposure time – A preliminary study in Guangzhou, China. *Journal of Forensic and Legal* Medicine **52**, 24-29.
Chrysomya rufifacies	Primary	O'Flynn, MA (1983) The succession and rate of development of blowflies in carrion in southern Queensland and the application of these data to forensic

		entomology. *Australian Journal of Entomology* **22**, 137-148.
		O'Flynn, MA, Moorhouse, DE (1979) Species of *Chrysomya* as primary flies in carrion. *Australian Journal of Entomology* **18**, 31-32.
		Voss, SC, Cook, DF, Dadour, IR (2011) Decomposition and insect succession of clothed and unclothed carcasses in Western Australia. *Forensic Science International* **211**, 67-75.
		Wang, J, Li, Z, Chen, Y, Chen, Q, Yin, X (2008) The succession and development of insects on pig carcasses and their significance in estimating PMI in south China. *Forensic Science International* **179**, 11-18.
	Secondary	Eberhardt, TL, Elliot, DA (2008) A preliminary investigation of insect colonisation and succession on remains in New Zealand. *Forensic Science International* **176**, 217-223.
		Lang, MD, Allen, GR, Horton, BJ (2006) Blowfly succession from possum (*Trichosurus vulpecula*) carrion in sheep-farming zone. *Medical and Veterinary Entomology* **20**, 445-452.
		O'Flynn, MA, Moorhouse, DE (1979) Species of *Chrysomya* as primary flies in carrion. *Australian Journal of Entomology* **18**, 31-32.
		Voss, SC, Spafford, H, Dadour, IR (2009) Annual and seasonal patterns of insects succession on decomposing remains at two locations in Western Australia. *Forensic Science International* **193**, 26-36.
		Wang, Y, Wang, J, Wang, Z, Tao, L (2017) Insect succession on pig carcasses using different exposure time – A preliminary study in Guangzhou, China. *Journal of Forensic and Legal* Medicine **52**, 24-29.
Chrysomya saffranea	Primary	O'Flynn, MA (1983) The succession and rate of development of blowflies in carrion in southern Queensland and the application of these data to forensic entomology. *Australian Journal of Entomology* **22**, 137-148.
		O'Flynn, MA, Moorhouse, DE (1979) Species of *Chrysomya* as primary flies in carrion. *Australian Journal of Entomology* **18**, 31-32.
	Secondary	O'Flynn, MA (1983) The succession and rate of development of blowflies in carrion in southern Queensland and the application of these data to forensic entomology. *Australian Journal of Entomology* **22**, 137-148.
Chrysomya varipes	Primary	O'Flynn, MA (1983) The succession and rate of development of blowflies in carrion in southern Queensland and the application of these data to forensic entomology. *Australian Journal of Entomology* **22**, 137-148.

		O'Flynn, MA, Moorhouse, DE (1979) Species of *Chrysomya* as primary flies in carrion. *Australian Journal of Entomology* **18**, 31-32.
	Secondary	Lang, MD, Allen, GR, Horton, BJ (2006) Blowfly succession from possum (*Trichosurus vulpecula*) carrion in sheep-farming zone. *Medical and Veterinary Entomology* **20**, 445-452.
		O'Flynn, MA, Moorhouse, DE (1979) Species of *Chrysomya* as primary flies in carrion. *Australian Journal of Entomology* **18**, 31-32.
		Voss, SC, Cook, DF, Dadour, IR (2011) Decomposition and insect succession of clothed and unclothed carcasses in Western Australia. *Forensic Science International* **211**, 67-75.
		Voss, SC, Spafford, H, Dadour, IR (2009) Annual and seasonal patterns of insects succession on decomposing remains at two locations in Western Australia. *Forensic Science International* **193**, 26-36.
Calliphora augur	Primary	Archer, MS (2002) The ecology of invertebrate associations with vertebrate carrion in Victoria, with reference to forensic entomology, PhD thesis. *Department of Zoology, University of Melbourne.*
		O'Flynn, MA (1983) The succession and rate of development of blowflies in carrion in southern Queensland and the application of these data to forensic entomology. *Australian Journal of Entomology* **22**, 137-148.
Calliphora hilli hilli	Primary	Archer, MS (2002) The ecology of invertebrate associations with vertebrate carrion in Victoria, with reference to forensic entomology, PhD thesis. *Department of Zoology, University of Melbourne.*
		Lang, MD, Allen, GR, Horton, BJ (2006) Blowfly succession from possum (*Trichosurus vulpecula*) carrion in sheep-farming zone. *Medical and Veterinary Entomology* **20**, 445-452.
Calliphora ochracea	No information	
Calliphora nigrithorax	No information	
Calliphora stygia	Primary	Archer, MS (2002) The ecology of invertebrate associations with vertebrate carrion in Victoria, with reference to forensic entomology, PhD thesis. *Department of Zoology, University of Melbourne.*
		Eberhardt, TL, Elliot, DA (2008) A preliminary investigation of insect colonisation and succession on remains in New Zealand. *Forensic Science International* **176**, 217-223.
		Lang, MD, Allen, GR, Horton, BJ (2006) Blowfly succession from possum (*Trichosurus vulpecula*)

		carrion in sheep-farming zone. *Medical and Veterinary Entomology* **20**, 445-452.
Calliphora vicina	Primary	e Castro, CP, Serrano, A, da Silva, PM, García, MD (2012) Carrion flies of forensic interest: A study of seasonal community composition and succession in Lisbon, Portugal. *Medical and Veterinary Entomology* **26**, 417-431.
		Lang, MD, Allen, GR, Horton, BJ (2006) Blowfly succession from possum (*Trichosurus vulpecula*) carrion in sheep-farming zone. *Medical and Veterinary Entomology* **20**, 445-452.
		Matuszewski, S, Bajerlein, D, Konwerski, S, Szpila, K (2011) Insect succession and carrion decomposition in selected forests of Central Europe. Part 3: Succession of carrion fauna. *Forensic Science International* **207**, 150-163.
		Norris, KR (1959) The ecology of sheep blowflies in Australia. In: Keast A, Crocker RL, Christian CS (eds) Biogeography and Ecology in Australia. Monographiae Biologicae. Springer, Dordrecht, 514-544.
		Tantawi, TI, El-Kady, EM, Greenberg, B (1996) Arthropod succession on exposed rabbit carrion in Alexandria, Egypt. *Journal of Medical Entomology* **33**, 566-580.
Lucilia cuprina	Primary	Bornemissza, GF (1967) An analysis of Arthropod succession in carrion and the effect of its decomposition on the soil fauna. *Australian Journal of Zoology* **5**, 1-12.
		O'Flynn, MA (1983) The succession and rate of development of blowflies in carrion in southern Queensland and the application of these data to forensic entomology. *Australian Journal of Entomology* **22**, 137-148.
		Norris, KR (1959) The ecology of sheep blowflies in Australia. In: Keast A, Crocker RL, Christian CS (eds) Biogeography and Ecology in Australia. Monographiae Biologicae. Springer, Dordrecht, 514-544.
Lucilia sericata	Primary	Denno, RF, Cothran, WR (1975) Niche relationships of a guild of necrophagous flies. *Annals of Entomological Society of America* **68**, 741-754.
		Lang, MD, Allen, GR, Horton, BJ (2006) Blowfly succession from possum (*Trichosurus vulpecula*) carrion in sheep-farming zone. *Medical and Veterinary Entomology* **20**, 445-452.
		Voss, SC, Spafford, H, Dadour, IR (2009) Annual and seasonal patterns of insects succession on decomposing remains at two locations in Western Australia. *Forensic Science International* **193**, 26-36.
	Secondary	Voss, SC, Cook, DF, Dadour, IR (2011) Decomposition and insect succession of clothed and unclothed carcasses

		in Western Australia. *Forensic Science International* **211**, 67-75.
Hemipyrellia fergusoni	No information	
Sarcophaga africa	No information	
Sarcophaga crassipalpis	Primary	Farrell, JF, Whitington, AE, Zalucki, MP (2015) A review of necrophagous insects colonising human and animal cadavers in south-east Queensland, Australia. *Forensic Science International* **257**, 149-154.
Sarcophaga impatiens	Primary	Farrell, JF, Whitington, AE, Zalucki, MP (2015) A review of necrophagous insects colonising human and animal cadavers in south-east Queensland, Australia. *Forensic Science International* **257**, 149-154.
	Secondary	Farrell, JF, Whitington, AE, Zalucki, MP (2015) A review of necrophagous insects colonising human and animal cadavers in south-east Queensland, Australia. *Forensic Science International* **257**, 149-154.
Sarcophaga praedatrix	No information	
Sarcophaga zeta	No information	
Australophyra rostrata	Secondary	Archer, MS (2002) The ecology of invertebrate associations with vertebrate carrion in Victoria, with reference to forensic entomology, PhD thesis. *Department of Zoology, University of Melbourne.*
		Voss, SC, Cook, DF, Dadour, IR (2011) Decomposition and insect succession of clothed and unclothed carcasses in Western Australia. *Forensic Science International* **211**, 67-75.
	Tertiary	Archer, MS (2002) The ecology of invertebrate associations with vertebrate carrion in Victoria, with reference to forensic entomology, PhD thesis. *Department of Zoology, University of Melbourne.*
		Eberhardt, TL, Elliot, DA (2008) A preliminary investigation of insect colonisation and succession on remains in New Zealand. *Forensic Science International* **176**, 217-223.
		Voss, SC, Cook, DF, Dadour, IR (2011) Decomposition and insect succession of clothed and unclothed carcasses in Western Australia. *Forensic Science International* **211**, 67-75.
		Voss, SC, Spafford, H, Dadour, IR (2009) Annual and seasonal patterns of insects succession on decomposing remains at two locations in Western Australia. *Forensic Science International* **193**, 26-36.
Hydrotaea chalcogaster	Tertiary	Pont, AC (1973) Studies on Australian Muscidae (Diptera). IV. A revision of the subfamilies Muscinae and Stomoxyinae. *Australian Journal of Zoology Supplementary Series* **21**, 129-296.

Hydrotaea spinigera	Secondary	O'Flynn, MA (1983) The succession and rate of development of blowflies in carrion in southern Queensland and the application of these data to forensic entomology. *Australian Journal of Entomology* **22**, 137-148.
		Wang, Y, Ma, M, Jiang, X, Wang, J, Li, L, Yin, X, Wang, M, Lai, Y, Lao, L (2017) Insect succession on remains of human and animals in Shenzhen, China. *Forensic Science International* **271**, 75-86.
		Wang, Y, Wang, J, Wang, Z, Tao, L (2017) Insect succession on pig carcasses using different exposure time – A preliminary study in Guangzhou, China. *Journal of Forensic and Legal* Medicine **52**, 24-29.
	Tertiary	Pont, AC (1973) Studies on Australian Muscidae (Diptera). IV. A revision of the subfamilies Muscinae and Stomoxyinae. *Australian Journal of Zoology Supplementary Series* **21**, 129-296.
Synthesiomyia nudiseta	Secondary	Skidmore, P (1985) The biology of the Muscidae of the world. *Series Entomologica*. Dordrecht, The Netherlands.
Piophila australis	No information	
Piophila casei	Tertiary	Matuszewski, S, Bajerlein, D, Konwerski, S, Szpila, K (2011) Insect succession and carrion decomposition in selected forests of Central Europe. Part 3: Succession of carrion fauna. *Forensic Science International* **207**, 150-163.
		Smith, KGV (1986) A manual of forensic entomology. The Trustees, British Museum, London
		Voss, SC, Cook, DF, Dadour, IR (2011) Decomposition and insect succession of clothed and unclothed carcasses in Western Australia. *Forensic Science International* **211**, 67-75.
Parapalaeosepsis plebia	No information	

Appendix IV: Supplementary material associated with Chapter 5

Table S6 Details of humans and pigs used in the succession experiments.

Replicate	Experiment	Sex	Age	Weight (kg)	Cause of death
Human 1	Winter A	Female	53 years	46.8	Metastatic malignancy of the lung
Human 2	Winter A	Female	86 years	80	Chronic obstructive pulmonary disease
Pig 1	Winter A	Female	4-6 months	67	Head bolt
Human 3	Winter B	Male	57 years	66	Cardiac arrest
Human 4	Winter B	Male	74 years	51.7	Cancer
Pig 2	Winter B	Female	4-6 months	70.6	Head bolt
Pig 3	Winter B	Female	4-6 months	57.7	Head bolt
Human 5	Summer	Female	82 years	60.5	Cancer / Aspiration pneumonia
Human 6	Summer	Female	97 years	46.8	Cardiac failure
Pig 4	Summer	Female	4-6 months	102.9	Head bolt
Pig 5	Summer	Female	4-6 months	63.5	Head bolt

Table 6 Species list of all Diptera, Coleoptera and Hymenoptera collected from pigs and humans. Species in red were excluded from any statistical analysis due to their rarity.

Order	Family	Genus	Species	Trophic group
Diptera	Calliphoridae	*Chrysomya*	*varipes*	Necrophagous
			incisuralis	Necrophagous
			rufifacies	Necrophagous
			nigripes	Necrophagous
			latifrons	Necrophagous
			megacephala	Necrophagous
		Hemipyrellia	*fergusoni*	Necrophagous
		Calliphora	*ochracea*	Necrophagous
			stygia	Necrophagous
			hilli hilli	Necrophagous
			augur	Necrophagous
			fulvicoxa	Necrophagous
			centralis	Necrophagous
		Lucilia	*cuprina*	Necrophagous
		Amenia	*imperialis*	Adventive
	Sarcophagidae	*Sarcophaga*	*beta*	Necrophagous
			impatiens	Necrophagous
			africa	Necrophagous
			zeta	Necrophagous
	Muscidae	*Australophyra*	*rostrata*	Necrophagous
		Hydrotaea	*chalcogaster*	Necrophagous
			spinigera	Necrophagous
		Musca	*vetustissima*	Adventive
			domestica	Adventive
		Dichaetomyia	sp.	Adventive
	Piophilidae	*Piophila*	*casei*	Necrophagous
			australis	Necrophagous
		Piophilosoma	sp.	Necrophagous
	Phoridae		sp.	Necrophagous
	Sepsidae	*Parapalaeosepsis*	*plebeia*	Necrophagous

Coleoptera	Staphylinidae	*Creophilus*	*lanio*	Predator/Parasitoid
			erythrocephalus	Predator/Parasitoid
	Histeridae	*Saprinus*	*cyaneus cyaneus*	Predator/Parasitoid
	Cleridae	*Necrobia*	*rufipes*	Omnivore
			ruficolies	Omnivore
	Silphidae	*Ptomaphila*	*perlata*	Predator/Parasitoid
			lacrymosa	Predator/Parasitoid
		Diamesus	*osculans*	Predator/Parasitoid
	Dermestidae	*Dermestes*	*maculatus*	Necrophagous
			ater	Necrophagous
			frischii	Necrophagous
	Trogidae	*Omorgus*	*quadrinodosus*	Necrophagous
			suberosus	Necrophagous
Hymenoptera	Formicidae	*Aphaenogaster*	*longiceps*	Omnivore
		Rhytidoponera	*metallica*	Omnivore
		Camponotus	*nigriceps*	Predator/Parasitoid
			sp.	Predator/Parasitoid
			aenopilosus	Predator/Parasitoid
		Crematogaster	sp.	Omnivore
		Polyrhachis	sp.	Predator/Parasitoid
		Mymecira	*urens*	Predator/Parasitoid
			sp.	Predator/Parasitoid
	Diapriidae	*Spilomicrus*	sp.	Predator/Parasitoid
	Pteromalidae	*Nasonia*	*vitripennis*	Predator/Parasitoid
	Braconinae	*Callibrcon*	sp.	Predator/Parasitoid
	Sphecidae	*Pronyx*	sp.	Predator/Parasitoid
	Apidae	*Tetragonula*	*carbonaria*	Adventive
	Vespidae		sp.	Predator/Parasitoid
	Pompilidae		sp.	Predator/Parasitoid

Appendix V: Supplementary material associated with Chapter 6

Table S8 GAM results for each species model. Significance donated by <0.001 (***), <0.01 (**), <0.05 (*) and non-significance (-). Total abundance for each species on human and pig cadavers also displayed.

	Species	R-sq (adj)	Deviance (%)	Cadaver type	Human	Pig	Human abundance	Pig abundance
Diptera	*Piophila casei*	0.0851	31.5	-	***	***	2505	1896
	Chrysomya nigripes	0.0986	45.2	***	**	*	88	1880
	Australophyra rostrata	0.0496	35.7	***	***	***	344	1020
	Phoridae	0.183	28.4	***	***	***	337	954
	Chrysomya varipes	0.116	27.4	***	***	*	304	754
	Chrysomya rufifacies	0.0651	24.5	***	**	-	154	615
	Chrysomya incisuralis	0.142	44.1	***	*	***	508	27
	Dichaetomyia sp.	0.0463	20.1	***	-	*	48	346
	Calliphora	0.238	18.6	**	-	***	71	196
Coleoptera	*Saprinus cyaneus cyaneus*	0.163	52.9	-	***	***	2547	1437
	Creophilus erythrocephalus	0.163	37	-	***	***	211	194
	Necrobia rufipes	0.251	64.3	-	***	-	375	28
	Omorgus	0.095	38.1	-	***	***	175	121

	quadrinodosus							
	Creophilus lanio	0.256	39.3	-	**	***	188	50
Hymenoptera	*Rhytidoponera metallica*	0.0415	8.05	***	-	-	72	158
	Crematogaster sp.	0.164	38.3	**	***	***	2711	867
	Aphaenogaster longiceps	0.0357	6.6	-	-	***	1218	636
	Nasonia vitripennis	0.0845	26.5	***	**	-	836	630

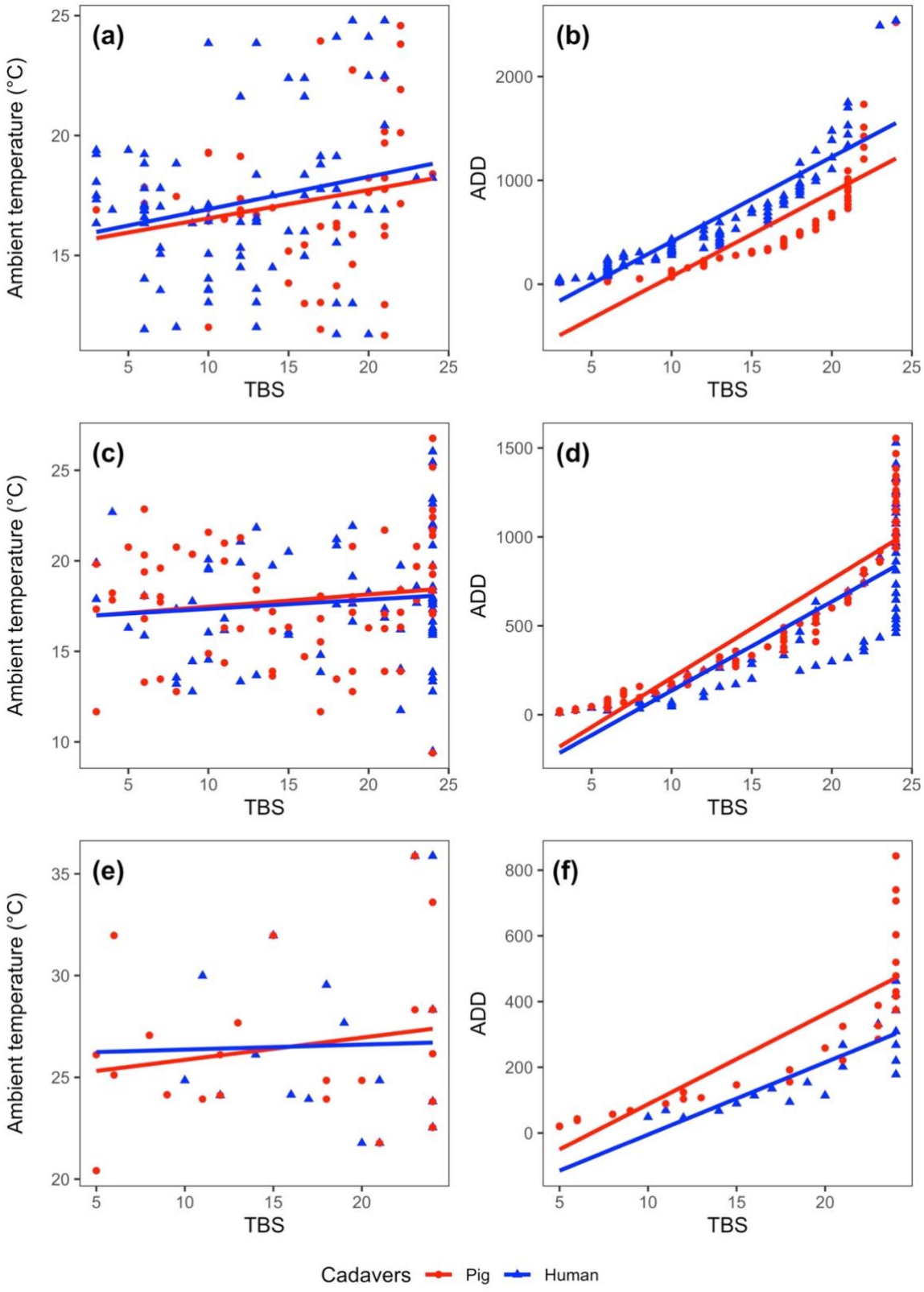


Figure 29 Temperature plots comparing Winter A (a) ambient temperature and (b) accumulated degree days (ADD) against total body score (TBS), Winter B (c) ambient temperature and (d) ADD against TBS and Summer (e) ambient temperature and (f) ADD against TBS for pig (red) and human (blue) cadavers.

Appendix VI: Supplementary material associated with Chapter 7

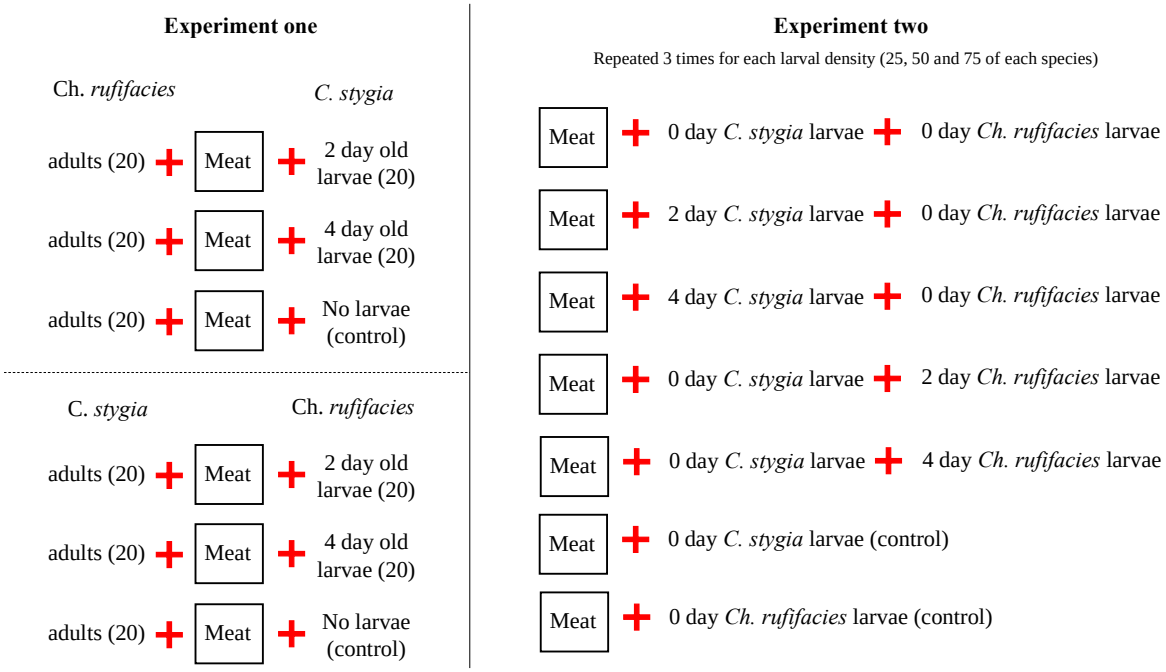


Figure S3 Treatments and experimental design for experiment one (adult ovipositional preference) and experiment two (priority effects and larval density).

Movie S1 Video displaying a mass of *Chrysomya rufifacies* preying upon a single *Calliphora stygia*.

https://www.youtube.com/watch?v=xpvqeRy_oTI