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Plasticity of ageing in burying beetles

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Contents

List of Tables and figures

Abstract

Ageing is the progressive physiological deterioration and decline in performance with advancing age. Patterns of ageing are incredibly diverse both across and within species, with pronounced differences in how much and how quickly mortality, fertility and performance deteriorate over age. While the drivers of this variation are not yet fully understood, it is clear they have profound implications for our understanding of the evolutionary processes shaping ageing.

For example, early-life conditions may broadly be expected to shape adult phenotypes, with consequences for adult life-histories and patterns of ageing. However, life-history theories differ in their predictions for the late-life consequences of early hardship. This is particularly the case for important phenotypic traits that can underpin survival and reproduction, which are often overlooked in ageing studies.

Further, a great deal of our understanding of ageing has come from research on laboratory invertebrate populations. However, ageing is a highly plastic trait that is sensitive to biotic and abiotic environmental change and can be ecologically dependent. Natural populations face more hostile environments and are exposed to predation, starvation, immune challenge, competition, and a whole host of abiotic stresses from which their laboratory counterparts are protected. Despite broad acknowledgement that the environment can shape ageing, very few species have been studied in both laboratory and natural contexts.

In this thesis, I set out to explore drivers of variation in patterns of ageing, attempting to address the gaps in the current literature with respect to early-life environmental effects on patterns of actuarial, reproductive, and functional ageing, and the effect of broad shifts in environment (from the laboratory to the wild) on patterns of demographic and physiological ageing.

To this end, in Chapter 2, I examined the role of early-life food abundance in shaping ageing in flight performance in a laboratory population of burying beetles. In Chapter 3, I investigated the effect of parental loss on the developing phenotype and subsequent patterns of actuarial and reproductive ageing. In Chapter 4, I explored the effect that broad shifts in environment (the laboratory versus natural conditions) had on patterns of actuarial and physiological ageing. In Chapter 5, I outlined a workflow and python pipeline I developed to facilitate photographic record-based identification of individuals in mark recapture studies. This chapter explains how individually-unique patterns present on the elytra of burying beetles can be leveraged as a means of individual recognition.

I found that while early-life food abundance can have age-independent effects on flight performance, there was no evidence that early-life conditions shaped ageing in these traits. Further, I found that the presence or absence of maternal care during development played a role in shaping patterns of ageing and lifespan. In assessing in wild- and laboratory-living

populations I found overall mortality was higher in the wild but that age-specific increases in mortality were only apparent in the laboratory-maintained population.

Lay summary

Ageing is a complex process characterized by a gradual decline in survival and performance over time. This process varies significantly among and within species, with noticeable differences in the rate and extent of declines in survival and other traits over time. Understanding why this is the case, and how the environment might be involved in shaping these differences is a key challenge in ageing research.

Much of our current understanding of ageing comes from studies on insect populations in laboratories. However, the process of ageing is very sensitive to changes in the physical or social environment. Populations in the wild face harsher conditions and challenges such as predation, starvation, and competition, unlike populations protected in the laboratory. Despite the widespread recognition that the environment can influence ageing, very few species have been studied in both laboratory and natural contexts.

Here I addressed this question of the effects environmental variation and ageing, first in the laboratory, and then across both laboratory and natural conditions. First, I conducted a laboratory experiment, exploring how conditions experienced in early life (the abundance of food) affected ageing in an ecologically-important trait – flight. Then, I looked at how the receipt or loss of maternal care shaped ageing in survival and in the laboratory. Finally, I tracked individual beetles living in the laboratory and the wild to measure how survival and body mass changed with age in both environments. The species of burying beetle I worked with are particularly suitable for studying in the wild because they have unique patterns on their wing casings that can be used to identify individuals without the need for applying cumbersome or harmful marks.

I found that while early-life food abundance can improve the average level of some flight traits, there was no evidence that early-life conditions shaped ageing in these traits. The presence or absence of maternal care during development did play a role in shaping patterns of ageing and lifespan. Finally found that overall survival was lower in the wild, but age-specific declines in survival were only apparent in the laboratory-maintained population.

Declaration

The work described in this thesis was carried out by myself with guidance from my supervisor, unless otherwise stated and detailed below. I declare that the thesis has been composed by myself and that the work has not be submitted for any other degree or professional qualification.

I undertook the statistical analyses and writing in Chapters 2,3,4, and 5 of this thesis, with guidance from my supervisors.

For Chapter 2, data collection was performed by Jude Stone, Callum Donaldson and Kali Wilson, in addition to myself.

For Chapter 3, Jacob Moorad conceived the ideas and designed the methodology. Ashleigh Whiffin and Ben Whitaker performed the data collection.

For Chapter 4, data collection was assisted by Theo Atkinson, Arianna Berbeglia, and Katie Smith.

For Chapter 5, data collection was assisted by Arianna Berbeglia, and Katie Smith. Emmet Delaney assisted in developing aspects of the Python pipeline.

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I once, while doing fieldwork in Blackford, dropped a bag of raw chicken and dead mice down a hill. I then immediately fell down that same hill, rolling through an indescribable mess. Unfortunately, work still needed doing and traps still needed bait, so I ended up combing through the undergrowth I had just flattened in the world's worst treasure hunt. There is nothing else I have experienced that has more exemplified the process of doing a PhD.

Nevertheless, there have been many people who have joined me on this strange journey, and I owe them thanks.

To Jacob, my supervisor, for reading every word in this thesis more than was good for his sanity. I have enjoyed our random talks when we were both avoiding writing.

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There are many more I could mention but, true to form, here I am on the last day of my PhD, moving graphs, rounding estimates to three decimal places, and trying to remove all the instances of "()" from my thesis where I forgot to put in references. To you, thank you.

1 General introduction

1.1 Overview

Ageing, or senescence, is the gradual decline in physiological function with advancing age, characterized by increased mortality rates (actuarial ageing; Kowald, 2002) and diminished reproductive or functional abilities (reproductive or functional ageing, respectively; (Rose, 1994; Grotewiel *et al.*, 2005; Bretman & Fricke, 2019). The terms 'senescence' and 'ageing' are often used interchangeably but are sometimes reserved to describe deleterious age-related changes in the first case, or more general age-related changes in the latter (Monaghan *et al.*, 2008). In this thesis, I treat these terms broadly synonymously but establish, where relevant, the theory and observed patterns that specifically consider senescence or ageing in their stricter definitions.

The majority of studies in this field concentrate on the decrease in Age-specific reproduction and survival as these are the characteristics most directly associated with an individual's fitness (Bouwhuis *et al.*, 2012; Kowald & Kirkwood, 2015; Lemaître & Gaillard, 2017; Cooper & Kruuk, 2018). A multifarious, widespread phenomenon across multicellular life (Finch, 1994; Hughes & Reynolds, 2005), ageing exhibits significant variation in its patterns and the age at which it begins. This variation can be seen within individuals, populations, and species, and even at the level of specific traits (Lemaître *et al.*, 2013; Jones *et al.*, 2014; Moorad & Ravindran, 2022). Ageing is such a multi-faceted trait, shaped by shifting patterns of selection, trade-offs and environmental quality (Monaghan *et al.*, 2008; Flatt & Partridge, 2018; Maklakov & Chapman, 2019) that the proximate and ultimate drivers of this variation are poorly understood (Medvedev, 1990). Understanding these drivers is a key research focus of the evolution of ageing (Balbontín & Møller, 2015; Lemaître *et al.*, 2015; Cooper & Kruuk, 2018; Spagopoulou *et al.*, 2020; Moorad & Ravindran, 2022).

According to evolutionary theory, natural selection's influence on mortality and reproduction weakens after sexual maturation and as individuals age (Medawar, 1952; Williams, 1957; Hamilton, 1966). This results in a 'selection shadow' in later life stages, where the efficiency of selection in maintaining survival, reproduction, and bodily repair diminishes (Medawar, 1952; Williams, 1957; Kirkwood, 1977; Fabian & Flatt, 2011).

Our understanding of ageing primarily comes from a combination of observational studies in natural populations and experimental research in laboratory settings, both of which have informed and been informed by theoretical models of ageing (Abrams, 1993; Monaghan *et al.*, 2008; Nussey *et al.*, 2013; Lemaître *et al.*, 2015; Cooper & Kruuk, 2018; Zajitschek *et al.*, 2020; Moorad & Ravindran, 2022). Hypotheses about the roles of trade-offs between life-history traits, variation in the environment, and the genetic pathways and mechanisms that shape ageing were largely developed based on the latter (Reichard, 2016; Zajitschek *et al.*, 2020). Inferences from such laboratory studies are often assumed to be broadly

representative of, or applicable to, natural systems (Briga & Verhulst, 2015; Reichard, 2016; Zajitschek *et al.*, 2020). There are many reasons to question this assumption, however.

Firstly, there is a notable taxonomic bias in ageing research. Studies in natural populations often focus on large, long-lived vertebrates or birds, while laboratory-based research typically involves insects and short-lived vertebrates (Nussey *et al.*, 2013; Gaillard & Lemaître, 2020; Zajitschek *et al.*, 2020). This bias can limit the generalizability of our understanding of ageing, as life-history patterns and mechanisms may vary significantly across different taxa and environments (Harshman & Hoffmann, 2000; Vepsäläinen & Spence, 2000; Zajitschek *et al.*, 2020).

Secondly, the species most frequently studied in the laboratory, often chosen for their short generation times, high fecundity, and docility, may not be representative of their respective taxonomic groups (Minelli & Baedke, 2014; Griffith *et al.*, 2021). These 'outlier' model organisms may exhibit unique behaviours and life-histories that are not reflective of closely related species or broader taxa (Phifer-Rixey & Nachman, 2015; Griffith *et al.*, 2021).

Thirdly, genetic differences between laboratory-maintained populations and natural populations can further complicate the interpretation of research findings. Laboratory populations, often derived from a small number of founding individuals and maintained at low effective population sizes, can experience high levels of genetic drift and inbreeding (Lohr *et al.*, 2014; Maclean *et al.*, 2018). Genetic drift in small populations and inbreeding more generally can result in more rapid ageing, relative to ancestral populations (Lohr *et al.*, 2014; de Boer *et al.*, 2018). Additionally, artificial selection, whether intentional or otherwise, can exacerbate phenotypic and genetic differences between laboratory and natural populations (Sgrò *et al.*, 2000; Matos & Avelar, 2001; Gasch *et al.*, 2016).

Finally, phenotypic or behavioural plasticity can result in qualitatively different responses to stimuli across environmental contexts (Flatt *et al.*, 2013; Briga & Verhulst, 2015; Zajitschek *et al.*, 2020). This is most often studied in the context of manipulative experiments designed to exaggerate variation in environmental conditions (for example: Dudycha, 2003; Bauerfeind *et al.*, 2009; Zajitschek *et al.*, 2014; Jehan *et al.*, 2020), but even subtle differences in rearing conditions can have profound effects on ageing (Partridge & Gems, 2007). The quality of the adult environment can have direct effects on ageing, with benign conditions generally being associated with slower rates of ageing (Reichert *et al.*, 2010; Cooper & Kruuk, 2018). Further, the environmental conditions encountered during critical developmental stages can have life-long effects on the ageing phenotype (Monaghan, 2008).

A challenge for current research is therefore to connect our understanding of ageing in the laboratory to that in the wild, accounting for the challenges and nuances posed by plasticity, genetic differences, and taxonomic bias (Zajitschek et al., 2020; Reichard, 2016). While some work has compared patterns of ageing in captive or semi-natural populations of vertebrates to those of wild populations (Ricklefs & Scheuerlein, 2001; Lemaître *et al.*, 2013; Hämäläinen *et al.*, 2014; Tidière *et al.*, 2016), this would appear to not address genetic influences in ageing. Nor are populations maintained in zoos (Hämäläinen *et al.*, 2014; Tidière *et al.*, 2016), or for

the purposes of agriculture or forestry (Crawley *et al.*, 2020) exposed to homogenous environments that are characteristic of laboratory studies. Rather, the increasing number of studies of ageing in wild insects more directly tackles the issues outlined above.

Given that much of our understanding of ageing was derived from captive invertebrate populations, an intuitive next step is to consider the performance of these taxa in their natural environments. While this can be challenging - small, short-lived species can be difficult to track and identify in the wild (Zajitschek *et al.*, 2020) - recent research has characterised plasticity of ageing in a small number of wild insect populations in response to environmental conditions, population dynamics, and experimental dietary manipulation. Bonduriansky and Brassil (2002) investigated and observed actuarial ageing in wild male antler flies, *Protopiophila litigata*, almost two decades ago. Perhaps the most expansive dataset on invertebrate ageing in the wild is that describing a population of field crickets, *Gryllus campestris* (Rodríguez-Muñoz *et al.*, 2019a; b). This long-term study has both served to highlight the feasibility of using insects as a model for wild ageing, but also to describe high variability in the rate of ageing between years and plasticity in ageing in response to natural variation in sex ratios. Direct comparisons between laboratory and wild-living populations of insects remain rare, however (but see: Kawasaki *et al.*, 2008; Mautz *et al.*, 2019). Previous studies of ageing in wild insects have focussed on species that form stationary, persistent mating aggregations or systems that are largely sedentary, or spatially isolated (Nussey *et al.*, 2013; Zajitschek *et al.*, 2020). This minimises the consideration of a suite of traits (i.e., locomotion or flight) that might trade off against other life-history traits and contribute to variation in ageing rates (Zajitschek *et al.*, 2020). Greater diversity in models of ageing, which may exhibit unique life-histories, naturally facilitates a greater array of questions to be asked about how ageing may be shaped by the environment or by trade-offs in life-history traits.

1.2 Study species

The burying beetle, *Nicrophorus vespilloides* is a small-bodied, holometabolous insect that is common throughout the UK and much of Europe (Scott, 1998; Lane *et al.*, 2020). Burying beetles have a rather unique life-history for insects, playing a role as predator, grave-digger, and attentive parent. In nature, this species preys on dipteran larvae (Pukowski, 1933) and other small invertebrates, but may mostly feed on carrion (Steiger *et al.*, 2007). Further this species breeds and provisions their larvae from a rare, ephemeral resource – small vertebrate carcasses (Scott, 1998). Burying beetles locate these bonanza resources using chemoreceptors on their antennae (Ernst, 1972) which are sensitive enough to detect a carcass within a day of placement in the wild, over long distances (Petruška, 1975; Smith & Heese, 1995).

Naturally, there is high competition for such resources in wild communities (Hopwood *et al.*, 2016b), both between conspecifics and with myriad other vertebrate and invertebrate carrion-eating species. Further, as one of the smaller burying beetle species, *N. vespilloides* may be at a physical disadvantage to the often-larger *Nicrophorus* spp. with which it co-occurs

(Easton, 1979; Sun *et al.*, 2020). As such, reproductive events are expected to be rare in the wild (Eggert & Müller, 1997; Scott, 1998; Steiger *et al.*, 2009).

As the name suggests, burying beetles will bury such carcasses to avoid competition or predation (Scott, 1998; Royle *et al.*, 2013). The carcasses serve as competitive arenas for prospective parents, food sources, and nests for developing offspring (Scott, 1998). After securing a carcass, parents process and maintain the breeding resource by stripping it of fur and feathers, rolling it into a ball, creating a feeding cavity for larvae, and coating the carcass with anti-microbial secretions, facilitating feeding and growth of larvae (Scott, 1998; Royle *et al.*, 2013). Both parents can provide care to offspring for several days, either by directly regurgitating digested food for begging larvae, maintaining the carcass, or defending the brood from competitors. This type of extended, complex parental care is rare among invertebrates (Royle *et al.*, 2013), but equally impressive is the fact that in *N. vespilloides*, this care is facultative – receipt of this elaborate care is not strictly required for proper growth and development of larvae (Eggert *et al.*, 1998).

Previous work in *N. vespilloides* that has considered ageing or lifespan has predominantly focussed on aspects of reproduction, parental care, or competition (e.g., Lock *et al.*, 2007; Ward *et al.*, 2009; Cotter *et al.*, 2011; Benowitz *et al.*, 2013; Lee *et al.*, 2014; Pilakouta *et al.*, 2015; Ivimey-Cook & Moorad, 2018; Houslay *et al.*, 2020; Cope *et al.*, 2022). Particularly, there has been significant attention given to parental age effects on caregiving and offspring outcomes (Lock *et al.*, 2007; Ward *et al.*, 2009; Cotter *et al.*, 2011; Benowitz *et al.*, 2013; Ivimey-Cook & Moorad, 2018; Houslay *et al.*, 2020; Cope *et al.*, 2022). However, studies examining ageing in functional traits not directly associated with sexual competition or parental care have been scarce. Reavey et al. (2015) stands out as a rare example. Here, they investigated the effects of reproduction on immuno-senescence in *N. vespilloides*. Their findings suggested that different components of the immune system were maintained, declined, or even increased with age, and in response to reproductive history.

Figure 1-1: A female Nicrophorus vespilloides exploring a fresh mouse carcass in the laboratory.

Despite this body of research into ageing in this species, demographic analyses focusing on actuarial ageing in *N. vespilloides* have been notably absent. This is particularly interesting, as previously reported mean and maximum lifespans seem to differ widely between studies. Many studies report survival and lifespan data differently, complicating direct comparisons. Nevertheless, Pilakouta et al. (2015) reported mean lifespans of 30-40 days in outbred individuals; Bartlett (1988) reported median lifespans of approximately 60 days. Ivimey-Cook and Moorad (2018) reported only 26% cumulative survival rates to the latter age group (60 days old). Benowitz et al. (2013) described low overall mortality to ages 28-35 days, followed by a noticeable onset of mortality. This suggests a degree of variation in mean mortality or ageing rates (or both) as a result of environmental differences or other undefined variation between research groups and founding populations. Similarly, studies conducted under natural or semi-natural conditions are limited (but see: Easton, 1979; Hopwood *et al.*, 2016b). Notably, none of these studies have explored the role of age in individual condition or survival.

1.3 Aims of this thesis

In this thesis, I set out to investigate the role of early-life and adult environments in shaping patterns of ageing in the burying beetle, *Nicrophorus vespilloides*. I explored plasticity of ageing not only in terms of mortality and reproduction but also in functional traits – flight and body mass.

In Chapter 2, I considered the effects of early-life resource availability (i.e., food restriction or abundance during a critical period of development) and age on flight performance in adult burying beetles. Here, I experimentally manipulated food-availability in

developing larvae, altering the quantity of resources available for forming the adult body. I subsequently assessed within-individual trends in flight performance on a custom flight-mill apparatus, from early adulthood through to late life (approximately 90 days old). Further, to address the potential that longitudinal trends in flight performance were influenced by individual experience, I assessed flight performance in aged individuals with varying prior experience in flight.

In Chapter 3, I considered the effects of maternal loss in the same critical developmental window (i.e., at the larval stage) on age-specific patterns of mortality and reproduction. I conducted a large-scale demographic analysis on an existing dataset describing cohorts of burying beetles that developed in the presence or absence of maternal care.

In Chapter 4, I investigated how broad shifts in environment (from benign laboratory conditions to natural, wild conditions) impacted patterns of actuarial and body-mass ageing. In this study, I attempted to develop *N. vespilloides* as a system for exploring ageing in wild populations of insects. To this end, I engaged in an intensive schedule of mark-recapture efforts in two local populations of burying beetles. Using naturally-occurring carapace markings, I identified and tracked individuals, either sourced from our laboratory or natural populations, over space and time to estimate actuarial ageing rates, and characterise the population dynamics of the wild populations.

In Chapter 5, I outline a workflow and computer-vision pipeline I developed that facilitated the efficient, effective use of individual markings to identify individual *N. vespilloides* from a large-scale photographic record.

In summary, this thesis aims to investigate plasticity in ageing in response to variation in juvenile environments. In an extension of this question, I tackle the broader question of plasticity in ageing across the more dramatic shift of laboratory to natural environments.

2 Early-life effects, prior experience, and ageing in flight performance

2.1 Introduction

Early-life conditions can shape the development of an organism and have long-lasting effects on adult phenotypes (Lindström, 1999; Monaghan, 2008). Variation in the quality (e.g., resource availability, temperature, population density) of early-life environments experienced by individuals can underpin the variation in phenotypic traits and fitness across a population (Descamps *et al.*, 2008; Hopwood *et al.*, 2014; Spagopoulou *et al.*, 2020; Sanghvi *et al.*, 2021, 2022). Individuals from high-quality early-life environments are often of improved physiological condition, exhibit greater performance across diverse phenotypic traits, and have longer lifespans compared to individuals that experienced poor early-life conditions (the "silver spoon" hypothesis: Grafen, 1988; Monaghan *et al.*, 2008). However, poor quality early-life conditions need not result in strictly negative outcomes for their survivors. Indeed, silver-spoon effects are not observed for all traits that determine fitness (Sanghvi *et al.*, 2021). Individuals may adjust their phenotype in response to unfavourable conditions experienced early in life to maximise later-life fitness (Nettle & Bateson, 2015). Adverse early-life conditions have been associated with increased dispersal ability (Applebaum & Heifetz, 1999), competitive ability (Stockley & Seal, 2001), and starvation resistance (Rehman & Varghese, 2021) compared to individuals that developed under benign conditions. Furthermore, harsh early-life conditions can act as "early mortality filters" that remove poor-quality individuals from the population (Chen & Maklakov, 2012). Survivors may then represent a subset of the population comprised of robust individuals who perform well in adulthood (Garratt *et al.*, 2015). This non-random removal of poor performers may minimise apparent differences between cohorts that experienced adverse or benign juvenile backgrounds. Evidence for silver spoon effects is mixed, and our knowledge of the diverse strategies individuals may adopt to cope with harsh environmental conditions early in life is limited (Cooper & Kruuk, 2018; Spagopoulou *et al.*, 2020). Understanding the impact of early-life adversity on phenotypic traits expressed across adulthood can shed light on how context-dependent these silver spoon effects are and how they manifest.

If early-life conditions matter, then their effects on fitness traits may be age-independent or age-dependent; the latter may influence how traits change with advancing age (Pigeon *et al.*, 2019). Senescence, physiological deterioration with advancing age resulting in reduced fertility and survival in later life, evolves because of declining forces of selection with age (Hamilton, 1966), but the direction of the effects of early-life adversity on ageing is unclear. Organisms raised in good-quality early-life environments may experience slower rates of age-related physiological decline (Hayward *et al.*, 2013; Cooper & Kruuk, 2018) because of

increased resources masking or lessening trade-offs in investment into survival or other traits (Hooper *et al.*, 2017). Alternatively, individuals may invest heavily in growth and early-life reproductive traits, resulting in accelerated senescence in late-life (Preston *et al.*, 2011; Lemaître *et al.*, 2015). Empirical studies in captive and wild populations have found ample evidence for each of these outcomes across a variety of traits – age-independence of early-life adversity (Bouwhuis *et al.*, 2010; Millon *et al.*, 2011; Pigeon *et al.*, 2021), increased ageing in response to early-life adversity (Nussey *et al.*, 2007; Sanghvi *et al.*, 2021, 2022), and increased ageing in response to benign developmental conditions (Hunt *et al.*, 2004; Marshall *et al.*, 2017; Spagopoulou *et al.*, 2020). Because different models make opposing predictions, and outcomes of empirical studies vary greatly, it is important to continue to investigate how age-independent and age-dependent effects align with the predictions for early-life effects.

Here, we investigated the role of early life conditions (food availability) on age-specific flight performance in a species of burying beetle, *Nicrophorus vespilloides*. In this species, like all holometabolous species, the adult body is constructed entirely from resources acquired during the larval stage (Hopwood *et al.*, 2013; Bladon *et al.*, 2020). Consequently, larvae that receive more food develop into larger adults (Jarrett *et al.*, 2017). Body size, in many species, is an important determinant of fitness and can pose a constraint for aspects of lifetime performance or reproduction (Kaufmann *et al.*, 2013; Steiger, 2013; Richardson & Smiseth, 2019). Flight, in turn, plays a vital role in dispersal, resource acquisition, and mate selection in many insect species (Attisano & Kilner, 2015; Brown *et al.*, 2017; Sanghvi *et al.*, 2021), yet its relationship with early-life conditions, and particularly associations between early-life conditions, senescence and flight, has received relatively little attention (but see: Sanghvi et al., 2021). Burying beetles rely on rare and ephemeral resources, small vertebrate carcasses, for reproduction (Scott, 1998). These carcasses serve as competitive arenas for prospective parents and provide both nesting sites and food sources for developing offspring. Accessing these resources in a timely manner is crucial for reproductive success in natural environments and requires flight (Eggert, 1992; Attisano & Kilner, 2015). Flight is, therefore, likely to be an important component of adult reproductive success and survival. Further, flight is a key trait through which to explore the long-term effects of developmental conditions.

In this study, we manipulated food availability during larval development to investigate the impact of early-life conditions on the age-specific flight ability of *N. vespilloides* adults. Previous work suggests that early-life hardship, in the form of parental loss, negatively impacted adult flight performance, regardless of body size (Attisano & Kilner, 2015). Studies in other insect species also highlight the detrimental impact of low food availability on adult phenotypic traits (Barrett *et al.*, 2009; Zajitschek *et al.*, 2009b). Food restriction during development may result in worse overall adult flight performance if the surviving individuals are of worse quality. However, if beetles respond to limited resources by increasing investment in flight, differences in flight performance between early-life treatments may be minor or may even favour individuals that experienced adverse early-life conditions.

Additionally, we were interested if the rate of change in flight performance with age was influenced by early-life food availability. Early-life food availability can have pronounced effects on body size, which is known to mediate early-life effects in several traits (Tigreros *et al.*, 2013; Bladon *et al.*, 2020; Grula *et al.*, 2021), and it may play a role in mediating age-dependent or independent effects on flight performance. Benign early-life conditions may act to maximise early-life performance with diminishing effects in later life or expose trade-offs between early- and late-life performance which may manifest as increased rates of performance decline. Alternatively, individuals from food-abundant backgrounds may be of higher condition and maintain a level of flight performance across ages, consistent with silver spoon ageing. Age-specific declines may also be independent of early-life food availability, suggesting other aspects of the adult environment may be more influential in shaping ageing.

2.2 Methods

2.2.1 Study species

We used beetles from our outbred laboratory stock population maintained at the University of Edinburgh. This population descended from beetles originally collected in the wild at the Hermitage of Braid (55°55’25”N, −3°16’16”E), Edinburgh, UK. Genetic diversity has since been maintained by annual additions of wild beetles trapped from the same site. All beetles were kept under 16:8 light:dark conditions and at 20 °C. Adult beetles were housed individually in plastic containers (twelve cm × eight cm × two cm) filled with moist soil and fed organic beef twice a week. We conducted two experiments aimed at understanding how ageing affects flight performance in *N. vespilloides*. In the first experiment, we explored how early-life food availability influenced the decline in flight performance within individuals. In the second experiment, we investigated whether previous flight experience played a role in age-specific flight trends.

2.2.2 Age and early life environment

To generate our experimental beetles, we randomly paired unrelated virgin males and females in large transparent plastic containers (17 cm x 12 cm x 26 cm). These containers were supplied with a one cm bed of moist soil and a freshly thawed mouse carcass (Livefoods Direct Ltd) of known size (range: 19 – 29 grams). The parents then prepared the carcass, and females laid eggs in the soil.

After approximately 60 hours, at which point eggs had been laid but prior to larvae hatching, we moved the female and her carcass into a new container supplied with a bed of moist soil. The male was discarded because males occasionally eat eggs and because male care is redundant under laboratory conditions (Smiseth *et al.*, 2005). Once the eggs started hatching, the larvae were gathered in holding containers and assigned to new foster mothers in broods of 20, approximately the average brood size for *N. vespilloides* (21 larvae; Smiseth

& Moore, 2002). Foster broods were only allocated to a female after her own eggs had started hatching since females use temporal kin recognition and kill larvae that arrive at the carcass before their own eggs have begun to hatch (Müller & Eggert, 1990).

These cross-fostered broods were left undisturbed for approximately 48 hours or until the mean larval weight of a brood reached 150 mg (± 50 mg). This weight range is broadly consistent with the food-restricted treatment employed by Richardson & Smiseth (2019) to investigate the role of early-life acquisition of resources on life-history traits (100 – 150 mg). Upon reaching this threshold, the number of surviving larvae in each brood was recorded, and the mothers were discarded. The remaining larvae in each brood were divided randomly between two treatments: they either remained on the carcass (food-abundant early-life environment) or they were transferred to an identical box lacking food resources (food-restricted early-life environment). Initial maternal presence was intended to maximise larval survival at the earliest stages of development (Eggert *et al.*, 1998). However, as the focus of our experimental manipulation was early-life access to food and not maternal care, mothers were subsequently removed to maintain parity between treatments. At the time of dispersal, we recorded average larval mass and the proportion of larvae that survived to dispersal and placed them in boxes where they eclosed after approximately 21 days.

Adult beetles were kept in individual plastic boxes and maintained in our standard laboratory conditions. They were unmated, fed organic beef and checked for death twice a week, and flight-tested (described below) at ages 10-20 days (young), 60-70 days (old), and again at ages 90+ days (very old). These ages represent cumulative survival rates, in our experimental population, of ~99%, ~64%, and ~31%, respectively. These ages also match or exceed those usually included in studies of ageing in this species (see: Cotter *et al.*, 2011; Lee *et al.*, 2014; Ivimey-Cook & Moorad, 2018; Houslay *et al.*, 2020; Cope *et al.*, 2022).

2.2.3 Age and prior experience

To generate experimental beetles, we randomly paired unrelated virgin males and females in large transparent plastic containers (17 cm x 12 cm x 26 cm). These containers were supplied in the same manner as described before. The parents prepared the carcass, and females laid eggs in the soil and remained undisturbed for several days until larvae were observed dispersing from the carcass. Parents were discarded, and dispersing larvae were transferred to a large eclosion box until emergence as adults.

Experimental adults were maintained under standard laboratory conditions throughout the experiment. This population was divided into three cohorts: those flying for the first time whilst 10-20 days old (young-naïve), those flying for the first time whilst 60-70 days old (old-naïve), and beetles gaining flight experience whilst 40-50 days old and performing again at age 60-70 (old-experienced). Young and old ages were chosen to be broadly consistent with the previous experiment. Experience was gained between ages 40-50 days so that each individual would have at least 10 days to recover following mounting on the mill and potentially strenuous flight activity. As we were assessing within-individual declines

in performance in experiment one, age was confounded with flight experience. While this is likely a common association in natural populations, we nevertheless wanted to account for any role of repeated measurements and the timing of flight assays on age-specific behaviours. Engaging in flight can accelerate subsequent declines in flight performance due to physical demands and potential physiological deterioration, while restricting flight can contribute to muscle atrophy and the deterioration of flight-related skills; either could lead to faster declines in flight performance (Lane *et al.*, 2014). Additionally, flight mills provide several unnatural stimuli (handling, imposed absence of tarsal support, restricted movement/orientation) to which beetles may become more or less sensitive to over time. Our intention was to tease apart effects of age and prior flight experience on performance whilst avoiding any effects of short-term exhaustion. Pronotum width was recorded, as this is one proxy for size (Müller *et al.*, 1990; Creighton, 2005; Richardson *et al.*, 2020), and beetles were weighed before and after each flight trial to capture the weight change associated with flight activity.

2.2.4 Measuring flight performance

Flight assays were performed using a flight mill design adapted from Attisano et al. (2015). Each flight mill consisted of seven chambers (31.5 cm x 32.5 cm x 16 cm; Figure 1). Supports extending from the centre of the ceiling and floor of each chamber bore neodymium magnets that acted as a low-friction axle for a freely spinning rotating arm. This arm consisted of a four cm upright steel needle and brass tube (30 cm height x 0.5 cm radius) joined with epoxy resin. The arm was mounted asymmetrically, such that the radius of a complete rotation of flight was 14cm, yielding a travel circumference of ~0.88m. Beetles' pronota were attached to entomological pins with the aid of a skin-safe cyanoacrylate adhesives (Chemence Ltd, Corby, U.K.) and adhesive putty (Bostik White-Tak; Attisano & Kilner, 2015). This combination of adhesives allowed beetles to be firmly attached to the mill arm for several hours but was also removable in moments with little to no apparent residue. This pin was then inserted into the rotating arm such that the beetle was positioned below the arm and banked at an angle of approximately 30 degrees. A banked flight angle can increase flight efficiency and performance of insects mounted on a fixed mill (Ribak *et al.*, 2017). At the opposite end of the arm, an approximately eight cm strip of card was placed to act as a counterweight and trigger for a wide-slotted optical sensor (OPB315, OPTEK Technology, Carrollton, TX, USA; OPB300, OPTEK Technology, Carrollton, TX, USA).

Experiment one involved one mill with seven chambers, all recording flight activity simultaneously to a Raspberry Pi 2B (www.raspberrypi.org) configured as a datalogger. Experiment two involved two mills, each with seven chambers. A Raspberry Pi 2B and Raspberry Pi 4B, identically configured as dataloggers, were connected to each mill, respectively. Due to logistic constraints, flight assays lasted four hours for experiment one and three hours for experiment two. The number of individuals and total number of flight trials involved in each experiment determined this difference in assay timing (experiment one: 325 individuals, 651 trials; experiment two: 1234 individuals, 1486 trials). In both cases, assays

were conducted under constant laboratory lighting and temperature conditions. Flight activity by a mounted beetle drove the rotating arm, with each revolution recorded when the card trigger broke the IR beam of the mill sensor. Flight behaviours were recorded as a time-series of discrete events (revolutions) from which periods of activity could be identified and characterised. Measures of momentary speed, average speed, duration and distance of flight, as well as the distribution of discrete periods of flight activity within an assay were calculated in this manner.

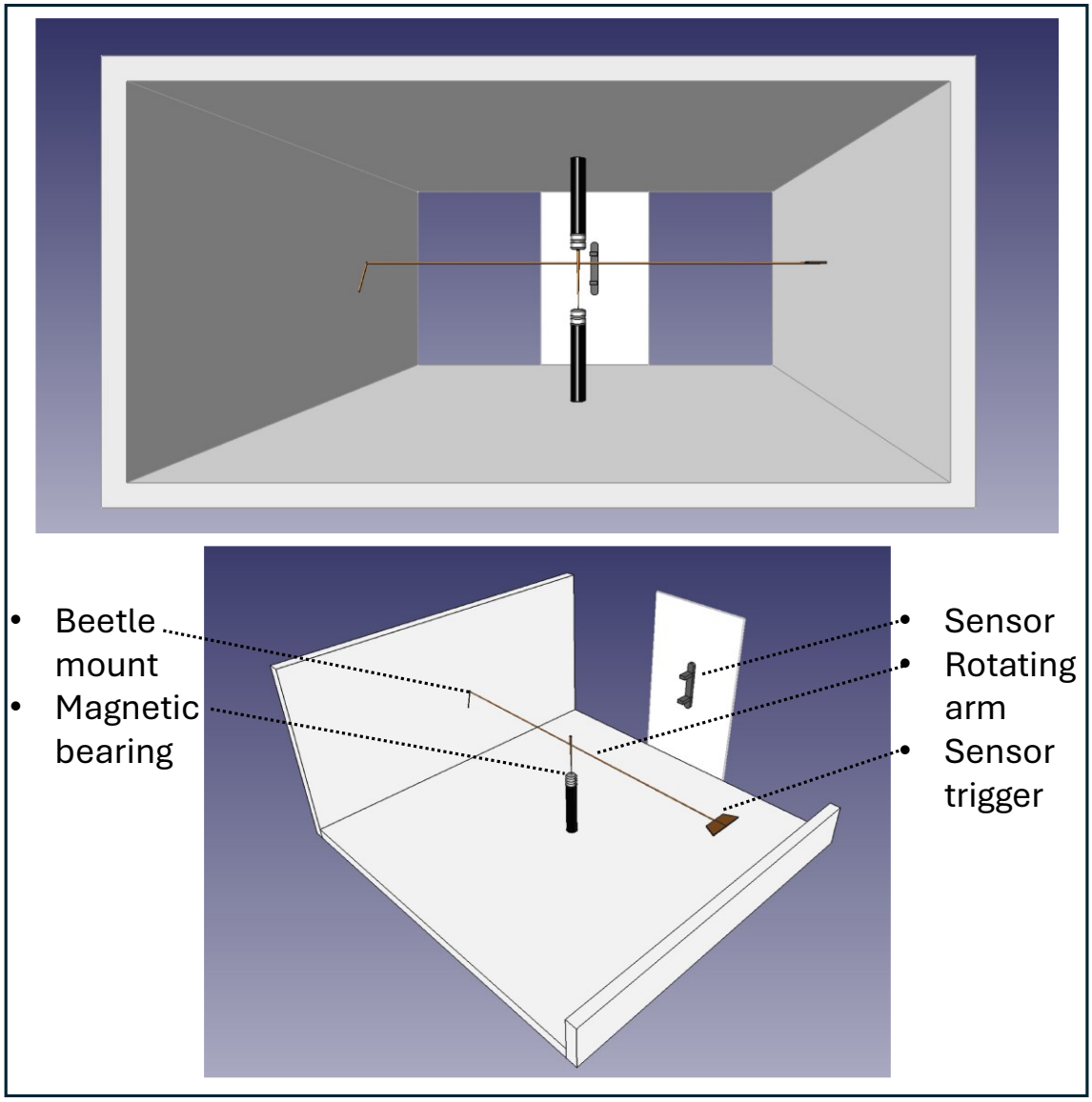

Figure 2-1: A scale representation of a flight mill chamber employed in this experiment. A wide-slotted optical switch was mounted at the rear of each chamber. Beetles were mounted at one end of a rotating arm, with a card trigger attached at the other end. A series of magnets held this rotating arm stable, facilitating low-friction rotation. As beetles engaged in flight, the arm rotated anti-clockwise, with the sensor-trigger tripping the optical sensor on each rotation. Data was recorded on a Raspberry Pi 2B or 4B.

Data recorded from each flight mill were processed prior to analyses as follows: flight events were characterised as periods of activity exceeding three rotations (2.64 metres) in which each rotation occurred within nine seconds of the last (reflecting thresholding below). Periods of flight were considered as discrete events if they were separated by at least 14 seconds. Momentary speed (the time between rotations scaled by the circumference of the mill path) was used as an upper threshold to exclude mill activity associated with malfunction or voltage spikes in the data loggers or other interference patterns. A lower threshold of momentary speed was used to exclude periods of "flight" that were associated with weak, intermittent activity by a beetle that may cause movement unrelated to flight, or slowing rotations associated with continuing and lessening momentum following a period of flight activity. The values of these thresholds were based on personal observations of typical flight speeds (lower threshold = 0.1 m/s; upper threshold = 3 m/s). Measures of propensity to fly, distance flown etc. were calculated following this thresholding. Thus, for the purposes of our analyses, distance flown was taken to be number of rotations associated with appropriate speeds, scaled by the circumference of the mill path. Peak flight speed was, by definition, bounded between 0.1 and 3 m/s. Across both experiments, and 1151 trials in which flight occurred, only 3.15% of beetles exceeded flight speeds of 2.75 m/s. As such, we are confident that these bounds were not overly restrictive nor likely to unduly impact our measures of flight performance. Our approach was also broadly consistent with that employed by Attisano et al. (2015).

2.2.5 Statistical analysis

All statistical analyses were conducted using R version 4.2.2 with the packages car 3.1-1 (Fox & Weisberg, 2019), glmmTMB 1.1.5 (Brooks *et al.*, 2017) and emmeans 1.8.4-1 (Lenth, 2024). Model fit was assessed using DHARMa 0.4.6 (Hartig, 2022) and overall significance was tested using type III ANOVAs. Where necessary, we conducted post-hoc pairwise comparisons using a Tukey's test with Bonferroni correction for multiple testing.

We used binomial models in our analyses on propensity to fly (0/1) within an assay and linear models to analyse continuous performance traits (peak flight speed, duration, distance, etc.) conditional on having flown. Time to initiate flight was calculated as the interval between the start of a given assay and the first recorded flight activity of the individual in the mill. These data were analysed according to a gamma error distribution, which is usually appropriate for wait-time data (Dagpunar, 2019). Distance flown within a four-hour assay was calculated as the number of complete rotations associated with beetle activity, scaled by the circumference of the mill. Therefore, these rotation data were analysed according to a negative binomial error distribution, with results presented on the transformed distance scale (metres). Peak flight speed reached within an assay was analysed according to a Student-t distribution.

2.2.5.1 Age and early life environment

In each case, we included mean-centred age at flight as a linear covariate, treatment (benign early life environment vs food-restricted early life environment), and an interaction between these two effects in our models. Individual ID was included as a random effect to account for pseudo-replication. Experimental block, describing the week in which an experimental brood eclosed, and foster brood were also included as random effects to account for cohort, shared parental care, and carcass quality effects. Due to unforeseen circumstances, the duration of recorded data for some trials did not encompass the full conducted time. In order to account for this, an offset term of "recorded trial length" was included in models for distance. For both distance flown and peak flight speed, there was evidence that both the trait mean and variance varied with age. Therefore, we included mean-centred age at flight as a dispersion parameter using the function 'dispformula'. Finally, in order to account for selective disappearance that might mask a signal of ageing (van de Pol & Verhulst, 2006), we included age interval at death as a factor (Ivimey-Cook & Moorad, 2018). This factor had three levels: died between first and second flight trial (~10 – 60 days old), died between second and third flight trial (~60 – 90 days old) and died after last flight trial (90+ days old).

2.2.5.2 Age and prior experience

In each case, we included flight assay (10-20 days old, 60-70 days old), prior experience (naïve, experienced), and an interaction between these two effects in our models. We did not account for selective disappearance in analyses of age-specific performance in this experiment. This was due to logistic constraints associated with maintaining the 1234 individuals assayed on the flight mill for significant periods of time following assessment. However, our analyses of the prior experiment suggested no evidence for selective disappearance by age 60 days. A signal of selective disappearance was only apparent in experiment one in a single trait (time to initiate flight) related to individuals surviving past age 90 days, with no significant effect between individuals dying before, or after, age 60 days. A fixed effect of mill was included to account for any potential discrepancies between the two flight mills' performance. This experiment involved a cross-sectional assessment of flight performance, so we did not included ID as a random effect. However, experimental block (the week of eclosion), and natural brood (family) were included as random effects to account for shared genetic, environmental, and cohort effects. An offset term of "recorded trial length" was included in models of distance flown, and we included flight assay as a dispersion parameter in models of distance flown and peak flight speed.

2.3 Results

2.3.1 Age and early life environment

2.3.1.1 Propensity to fly

Propensity to fly within an assay declined with increasing age. There was a significant, negative direct effect of age on the proportion of beetles engaging in flight (Table 1; Fig. 2A). At young ages (15 days old) nearly all beetles engaged in flight (food-abundant treatment: 0.962, 95% CI [0.917, 0.983]; food-restricted treatment: 0.960 [0.909, 0.983]), while by age 90 days, only half of beetles flew within a 4-hour trial (food-abundant treatment: 0.598 [0.411, 0.761]; food-restricted treatment: 0.414 [0.265, 0.582]). There was no significant effect of the early-life environmental conditions on propensity to fly (Table 1, Fig. 2A). Age-related declines in propensity to fly were also independent of early-life conditions, as suggested by a non-significant interaction between these two terms (Odds Ratio = 0.991 [0.973, 1.008]). There was no significant effect of selective disappearance in this trait (Table 1).

2.3.1.2 Time to initiate flight

Among beetles that engaged in flight, time to initiate flight following introduction to the mill increased with advancing age (Table 1). There was no statistical support for either a main effect of early-life conditions on time to initiate flight nor for an interaction between age and early-life conditions (Table 1; Fig. 2B). A significant effect of selective disappearance suggested that those beetles that were longest lived (death post age 90 days) were slower to engage in flight overall (Ratio = 2.778 [1.238, 6.234]) relative to those individuals that survived only past the first assay. Beetles that ultimately died in the age interval of 60 to 90 days were not significantly slower to engage in flight than short-lived counterparts (Table 1), nor did they significantly differ from their longest-living companions (Ratio = 0.639 [0.331, 1.231]).

Table 2-1: Effects of age and early-life environment on propensity to fly and time to initiate first flight (conditional on engaging in flight). Age (days) was centred on mean age at flight for the population (n = 651, u = 43.51 days). The intercept of 'time to initiate flight' is the average time in seconds after which individuals began flying. Subsequent terms are described by scalars (values > 1 referring to slower instigation of flight; values < 1 referring to faster instigation of flight).

	Propensity to fly				**Time to initiate flight (s)**			
Predictors	*Odds Ratios*	*CI*	*Statistic*	*p*	*Estimates*	*CI*	*Statistic*	*p*
(Intercept)	6.448	2.282 – 18.224	3.516	**4.4e−04**	240.506	116.223 – 497.691	14.777	**<2e-16**
Age [days]	0.963	0.949 – 0.977	-5.076	**3.85e-07**	1.026	1.016 – 1.036	5.242	**1.59e-07**
Early-life conditions [food-restricted]	0.740	0.405 – 1.355	-0.975	0.330	0.883	0.540 – 1.445	-0.495	0.621
Selective disappearance [death between ages 60 – 90 days]	1.110	0.372 – 3.313	0.186	0.852	1.774	0.816 – 3.854	1.448	0.148
Selective disappearance [death post 90 days]	2.100	0.647 – 6.817	1.235	0.217	2.778	1.238 – 6.234	2.478	**0.013**
Age [days] * Treatment [food-restricted]	0.991	0.973 – 1.008	-1.052	0.293	0.998	0.985 – 1.012	-0.272	0.786
Random Effects								
Variance	0.92 $_{ID}$				1.81 $_{ID}$			
	0.22 $_{Family}$				0.00 $_{Family}$			
	0.00 $_{Block}$				0.00 $_{Block}$			
N	325 $_{ID}$				315 $_{ID}$			
	42 $_{Family}$				42 $_{Family}$			
	5 $_{Block}$				5 $_{Block}$			
Observations	651				526			

2.3.1.3 Distance flown

Distance flown within a 4-hour trial did not vary significantly between ages (Table 2), although there was a weakly negative trend effect of increased age (Fig. 2C). At young ages, 15 days old, beetles flew approximately 3.5 kilometres (food-abundant treatment: 3.87km [3.15, 4.74]; food-restricted treatment: 3.52km [2.80, 4.42]). By age 90 days, mean distance flown was closer to 3 kilometres (food-abundant treatment: 3.53km [2.32, 5.35]; food-restricted treatment: 2.64km [1.75, 4.00]). Individuals that experienced benign early-life conditions did not have a significant advantage in distance flown over their counterparts from harsh early-life conditions (Table 2). There was no evidence for a significant interaction between age and early-life conditions, nor any apparent effect of selective disappearance (Table 2).

Table 2-2: Effects of age and early-life environment on distance flown and peak speed achieved in an assay (conditional on engaging in flight). Age (days) was centred on mean age at flight for the population (n = 651, μ = 43.51 days). Distance flown was analysed as total number of rotations associated with flight activity per assay.

	Distance flown (rotations)				**Peak flight speed (m/s)**			
Predictors	*Log-mean*	*CI*	*Statistic*	*p*	*Estimates*	*CI*	*Statistic*	*p*
(Intercept)	3.017	2.677 – 3.358	17.375	**<2e-16**	1.708	1.585 – 1.831	27.237	**<2.22e-16**
Age [days]	-0.001	-0.007 – 0.005	-0.383	0.702	-0.004	-0.006 – -0.002	-3.379	**7.27e-04**
Early-life conditions [Harsh]	-0.169	-0.408 – 0.069	-1.390	0.164	-0.240	-0.325 – -0.155	-5.545	**2.95e-08**
Selective disappearance [death between ages 60 – 90 days]	-0.208	-0.560 – 0.145	-1.154	0.249	-0.085	-0.196 – 0.026	-1.507	0.132
Selective disappearance [death post 90 days]	-0.161	-0.532 – 0.211	-0.849	0.396	-0.077	-0.203 – 0.048	-1.204	0.229
Age [days] * Treatment [Harsh]	-0.003	-0.011 – 0.006	-0.611	0.541	0.001	-0.002 – 0.004	0.880	0.379
Dispersion Parameter								
(Intercept)	-0.449	-0.555 – -0.343	-8.271	**<2.22e-16**	-0.985	-1.103 – -0.867	-16.355	**<2.22e-16**
Age [days]	-0.008	-0.012 – -0.004	-4.392	**1.12e-05**	0.008	0.005 – 0.011	5.482	**4.21e-08**
Random Effects								
Variance	0.00 $_{ID}$				0.00 $_{ID}$			
	0.09 $_{Family}$				0.01 $_{Family}$			
	0.00 $_{Block}$				0.00 $_{Block}$			
N	315 $_{ID}$				315 $_{ID}$			
	42 $_{Family}$				42 $_{Family}$			
	5 $_{Block}$				5 $_{Block}$			
Observations	526				526			

2.3.1.4 Peak flight speed

Peak speed achieved declined across ages (Table 2). Individuals from benign early-life environments reached greater peak speeds than beetles from the harsh environmental

treatment (food-abundant treatment at age 15 days: peak speed = 1.77 m/s [1.70, 1.83]; food-restricted treatment at age 15 days: peak speed = 1.49 m/s [1.42, 1.56]). We found no support for an interaction between age and early-life environment. In broad terms, this indicates that individuals from benign early life environments had advantage in engaging in faster flight, for a significant portion of their lifespan, over their deprived counterparts. Furthermore, individuals from both backgrounds declined in ability at largely similar rates with respect to age (Table 2; Fig. 2D).

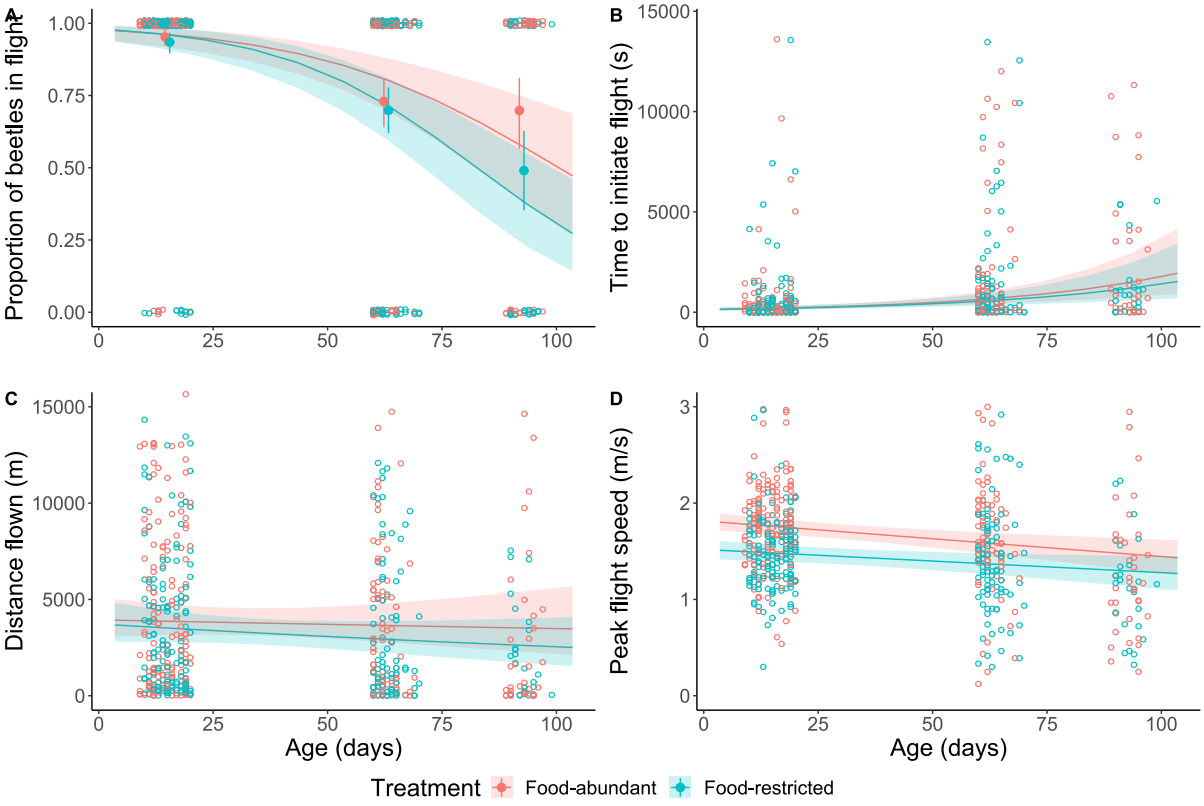

Figure 2-2: Mean age- and treatment-related changes in performance in four aspects of flight: A – Proportion of beetles in flight, B – time to initiate flight, C – total distance flown, D – Peak flight speed. For clarity, raw group means and bootstrapped 95% confidence intervals were included for binomial (0/1) data modelled in A.

2.3.2 Age and prior experience

2.3.2.1 Propensity to fly

Propensity to fly within a 3-hour trial declined sharply between young and old beetles (Table 3). Assuming a linear decline in propensity to fly with respect to age, which appears consistent with our results from experiment 1, this equates to a 4% decline in probability of engaging in flight per day (Odds ratio = 0.963 [0.949, 0.976]). Approximately 86% of young beetles, aged 10 – 20 days, engaged in flight [0.759, 0.918]; this declined to approximately half of beetles assayed between 60 and 70 days old (Old-Naïve: 0.502 [0.350, 0.654]; Old-Experienced: 0.579

[0.430, 0.715]; Fig. 3A). Propensity to fly was positively associated with body size, measured by pronotum width (Odds ratio = 3.326 [2.084, 5.309]).

2.3.2.2 Time to initiate flight

Among beetles that engaged in flight, time to first flight activity increased 3-fold between young and old ages (Estimate = 3.228 [1.690, 6.168]; Fig. 3B). There was no significant difference between naïve flyers and experienced fliers in time to initiate flight (Table 3). Larger beetles were quicker to start flying once mounted on a mill (Estimate = 0.535 [0.335, 0.854]

Table 2-3: Effects of age and prior flight experience on propensity to fly and time to initiate first flight (conditional on engaging in flight). Body size (mm) was centred on mean pronotum width for the population (n = 888, u = 5.29 mm). The intercept of 'time to initiate flight' is the average time in seconds after which individuals began flying. Subsequent terms are described by scalars (values > 1 referring to slower instigation of flight; values < 1 referring to faster instigation of flight).

	Propensity to fly				**Time to initiate flight**			
Predictors	*Odds Ratios*	*CI*	*Statistic*	*p*	*Estimates*	*CI*	*Statistic*	*p*
(Intercept)	5.225	3.099 – 8.809	6.203	**5.53e-10**	245.477	156.157 – 385.888	23.845	**<2.22e-16**
Age [60-70 days]	0.170	0.089 – 0.325	-5.358	**8.42e-08**	3.228	1.690 – 6.168	3.548	3.89e-4
Flight experience [Experienced]	1.363	0.886 – 2.097	1.408	0.159	1.263	0.735 – 2.168	0.845	0.398
Body size	3.326	2.084 – 5.309	5.037	**4.72e-07**	0.535	0.335 – 0.854	-2.618	**0.009**
Flight mill	1.294	0.939 – 1.783	1.577	0.115	1.192	0.836 – 1.697	0.870	0.332
Random Effects								
Variance	0.22 $_{Family}$				0.41 $_{Family}$			
	0.12 $_{Block}$				0.24 $_{Block}$			
N	189 $_{Family}$				178 $_{Family}$			
	13 $_{Block}$				13 $_{Block}$			
Observations	888				625			

2.3.2.3 Distance flown

Older beetles flew significantly less far than their younger counterparts (Incidence rate ratio = 0.625 [0.462 – 0.845]; Fig. 3C). This translates to young beetles flying approximately 5km [4.008 – 6.183] on average during a three-hour assay while old beetles flew on average less than 4 km (Old-Naïve = 3.112 km [2.177 – 4.449]; Old-Experienced = 3.860 km [2.790 – 5.339]). There was little evidence for an association between either prior flight experience or body size and total distance flown in three hours (Table 4).

Table 2-4: Effects of age and on distance flown and peak speed achieved in an assay (conditional on engaging in flight). Body size (mm) was centred on mean pronotum width for the population (n = 888, u = 5.29 mm). Distance flown was analysed as total number of rotations associated with flight activity per assay. Distance flown in metres is recovered as point estimate x 2πr (r = .14 metres).

	Distance flown (rotations)				**Peak flight speed (m/s)**			
Predictors	*Log-Mean*	*CI*	*Statistic*	*p*	*Estimates*	*CI*	*Statistic*	*p*
(Intercept)	34.435	28.364 – 41.804	35.766	**<2.22e-16**	1.788	1.681 – 1.895	32.781	**<2.22e-16**
Age [60-70 days]	0.625	0.462 – 0.845	-3.055	**0.002**	-0.279	-0.419 – -0.139	-3.903	**6.29e-05**
Flight experience [Experienced]	1.240	0.898 – 1.712	1.308	0.191	0.062	-0.064 – 0.188	0.972	0.317
Body size	0.967	0.718 – 1.304	-0.218	0.827	0.293	0.179 – 0.406	5.053	**4.58e-07**
Flight mill	0.837	0.698 – 1.003	-1.928	0.054	-0.061	-0.134 – 0.012	-1.632	0.141
Dispersion Parameters								
(Intercept)	0.832	0.735 – 0.942	-2.897	**0.004**	1.788	1.681 – 1.895	32.781	**<2.22e-16**
Age [60-70 days]	0.785	0.645 – 0.954	-2.434	**0.015**	-0.279	-0.419 – -0.139	-3.903	**0.002**
Random Effects								
Variance	0.00 $_{Family}$				0.01 $_{Family}$			
	0.02 $_{Block}$				0.01 $_{Block}$			
N	178 $_{Family}$				178 $_{Family}$			
	13 $_{Block}$				13 $_{Block}$			
Observations	625				625			

2.3.2.4 Peak flight speed

Older beetles flew slower than their younger counterparts (Estimate = -0.279 m/s [-0.419, -0.139]). The average peak speed of flight among young beetles was 1.78 m/s [1.66,1.90], while old naïve flyers reached speeds of 1.49 m/s [1.32, 1.65], and old experienced beetles flew at 1.56 m/s [1.41, 1.71]. There was no significant difference between flight speeds of the experienced and naïve groups (Estimate = 0.062 m/s [-0.064, 0.188]). However, larger beetles flew significantly faster than smaller beetles (Table 4). A 1 mm increase in body size (measured as the width of the pronotum) was associated with a 0.293 m/s [0.179, 0.406] increase in peak speed (Table 4).

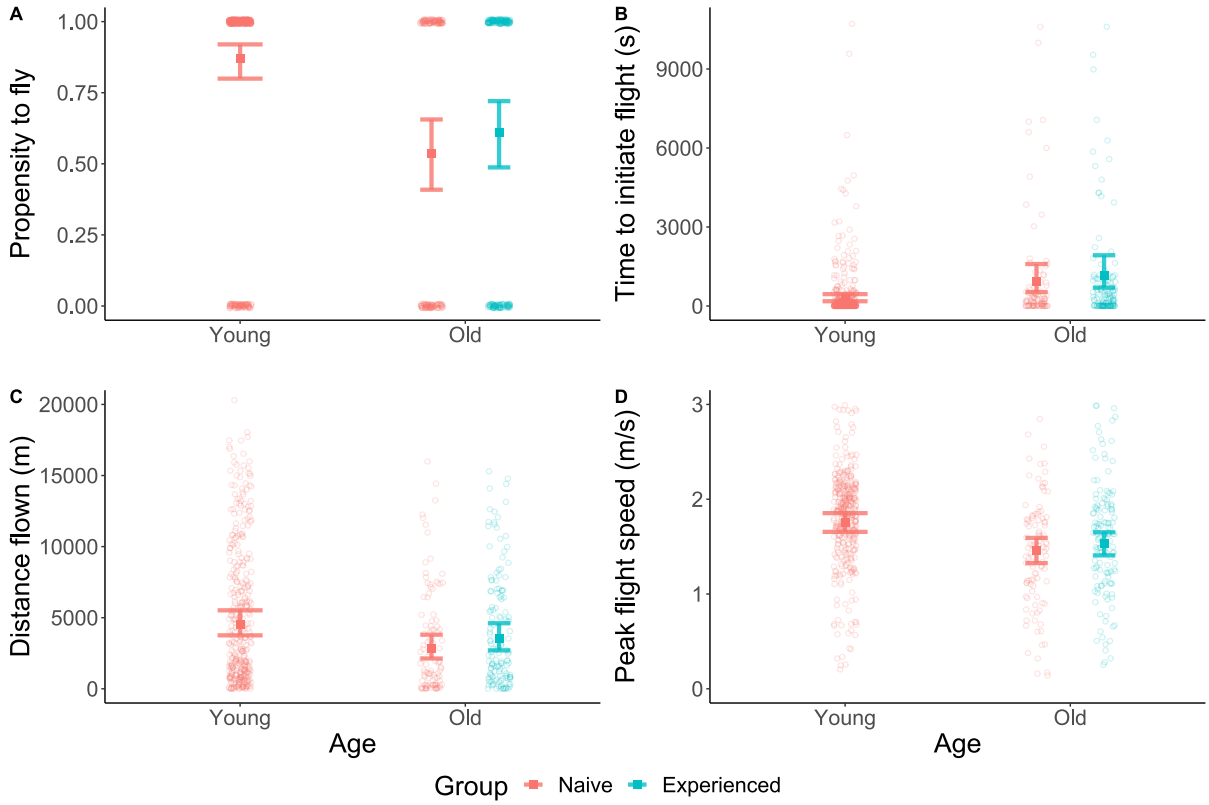

Figure 2-3: Mean age- and experience-related changes in performance in four aspects of flight: A – Proportion of beetles in flight, B – time to initiate flight, C – total distance flown, D – Peak flight speed.

2.4 Discussion

2.4.1 Overview

The aim of this study was to investigate the role of early life food availability in shaping age-specific patterns of flight performance. To achieve this, we manipulated access to food during a key developmental stage (Hopwood *et al.*, 2013; Wong & Kölliker, 2014) in mixed broods of *N. vespilloides* larvae. we subsequently assayed within-individual trends in adult flight performance across ages 10 days to 95 days (Early-life manipulation). Additionally, we separately investigated the role of prior flight experience on age-specific flight behaviours (ages 10 to 70 days) in beetles that developed under more typical laboratory conditions (natural broods, parental care, access to carcass).

Beetles from food-restricted backgrounds flew slower than their well-fed counterparts. However, there was no apparent main effect of early-life food availability on propensity to fly, time to initiate flight, or distance flown. Individuals from food-abundant backgrounds were larger, and we found positive effects of body size on propensity to fly and peak flight speed, but not on time to initiate flight or distance flown (Supplementary Information). A positive effect of body size on these flight traits was consistent across both experiments. This may suggest that body size is important in mediating otherwise subtle early-life effects on performance (Supplementary Information). We found that several flight traits

declined with age: propensity to fly; time to engage in first flight; and peak flight speed. There was no evidence of an effect of age on distance flown in our early-life experiment but a significant negative relationship with age in our experience experiment (discussed below). The rate of decline in flight ability appeared to be independent of early-life food availability. Finally, prior experience on the flight mill did not appear to affect flight performance, suggesting that repeated assessment of individuals did not contribute to the age-specific patterns we characterised.

2.4.2 Main effects of early-life resource availability on flight performance

Early-life conditions are known to affect aspects of adult flight performance in many insect and bird species (Saastamoinen & Rantala, 2013; Attisano & Kilner, 2015; Brown *et al.*, 2017; Reim *et al.*, 2019; Grula *et al.*, 2021; Niitepõld & Boggs, 2022). In some instances, early-life food deprivation or hardship is associated with improved flight or dispersal ability (Evenden *et al.*, 2015; Brown *et al.*, 2017; Grula *et al.*, 2021). Patterns such as these may arise through either increased early-life mortality filtering out the lowest-quality individuals in the population (Chen & Maklakov, 2012) or adaptive shifts in resource allocation towards key traits in response to internal or external environmental cues (Evenden *et al.*, 2015; Brown *et al.*, 2017) (viability selection or adaptive response hypotheses). In other instances, lack of resources during development simply results in impaired adult performance ("silver spoon"; Reim *et al.*, 2019; Niitepõld & Boggs, 2022). Peak flight speed may be reflective of the quantity of resources available, or the rate at which resources can be consumed, in aid of flight (Attisano & Kilner, 2015). Individuals from food-restricted backgrounds were significantly smaller than well-fed counterparts and likely had smaller flight muscles or less stored resources to fuel intensive flight (Kaufmann *et al.*, 2013; Attisano & Kilner, 2015). While, in many species, larger insects tend to have smaller flight structures relative to body size (Angelo & Slansky, 1984; Grula *et al.*, 2021) and may face greater relative and absolute metabolic costs engaging in flight, larger individuals in many species also tend to have the advantage in flight speed and dispersal ability (Kaufmann *et al.*, 2013; Crall *et al.*, 2015). Increased body size may come with disadvantages, however, in terms of increased costs of taking flight (supporting greater body mass), reduced power available for flight (Grula *et al.*, 2021), or decreased manoeuvrability (Crall *et al.*, 2015).

Peak flight speed may be of particular importance in natural contexts. Burying beetles rely on rare and widely distributed carcasses for breeding, and gaining timely access to these resources may contribute to reproductive success (Scott, 1998; Attisano & Kilner, 2015). Arriving before conspecific or heterospecific competitors may confer benefits to survival or reproduction. Also, during attacks or escapes, animals may move at maximum speed (Terlau *et al.*, 2023). It is unclear if peak flight speeds, as measured here, reflect realistic escape speeds generated by individuals under stress. However, it would be relatively straightforward to assess individual's responses to manual stimulation or relevant predator cues under tethered- or free-flight conditions.

There was no evidence of a relationship between the other aspects of flight we considered (propensity to fly, time to initiate flight, and distance flown) and early-life food availability. It may be the case that engaging in minimal flight behaviours (measured by propensity or time to initiate flight) in the context of a tethered flight mill is relatively easier to achieve than free flight (Attisano & Kilner, 2015). Possibly, individuals that experienced resource restriction during development may still have had sufficient resources to perform well. This is unlikely to be the case for distance flown, as prolonged activity on flight mills is known to impose significant body mass loss and metabolic costs (Ribak *et al.*, 2017). Our observation of similar performance across various traits in both early-life treatments may reflect complementary or opposing effects of adult resource acquisition, selective disappearance, or adaptive responses.

Early-life food availability influences body size and larval energy reserves (Brown *et al.*, 2017; Reim *et al.*, 2019), and manipulations like parental loss affect wing development in burying beetles (Attisano & Kilner, 2015). However, the importance of these factors for flight performance may diminish in comparison to resources obtained through adult feeding (Brown *et al.*, 2017). This means that favourable developmental conditions might provide a short-lived advantage for newly-eclosed adults in flight, which diminishes as they continue to feed as adults. This would be consistent with the environmental saturation hypothesis, which suggests that under favourable adult conditions, all individuals will perform well, regardless of their early-life environment (Engqvist & Reinhold, 2016; Pigeon *et al.*, 2019). It is worth noting that our assay schedule included several ad libitum feeding periods before an individual's first flight, and these potentially missed the initial post-eclosion stage. It would be illuminating to assess how early-life and adult conditions might interact to shape adult performance. Under the “environmental matching” or “predictive adaptive response” hypotheses (Nettle & Bateson, 2015; Pigeon *et al.*, 2019), individuals might be expected to display enhanced performance where juvenile and adult conditions are similar. For example, individuals developing under food restriction can exhibit increased starvation resistance during later life (Wang *et al.*, 2016; Rehman & Varghese, 2021). Larger individuals from benign early-life conditions may incur greater absolute metabolic costs that can become increasingly detrimental to condition, performance, and survival under adult resource restriction (Blanckenhorn *et al.*, 1995; Reim *et al.*, 2006). There may simply be additive effects of early-life hardship, with resource restriction at multiple life stages compounding to result in severely reduced performance (Wong & Kölliker, 2014; Engqvist & Reinhold, 2016). Manipulating both early-life and adult resource availability would be an interesting next step in teasing apart the relative importance of early-life and adult environmental effects and understanding the nature of potential adaptive developmental responses or constraints in the face of environmental cues.

Additionally, food restriction during development may have acted as an early mortality filter, removing low-quality individuals from this experimental group (Chen & Maklakov, 2012). Survivors of this early-life food restriction may then represent a subset of relatively high quality, high performing, individuals; such viability selection could weaken associations

between adult performance and early life conditions (Sanghvi *et al.*, 2022). As this hypothetical mortality must have occurred prior to any assessments of flight performance, we cannot link larval death to flight ability or quality. However, there were clear differences in early-life mortality between the two treatments, which may have increased the potential for selective disappearance to affect outcomes. Among larvae that survived to be split into experimental groups, almost all (91.4% of 326 larvae) of those in the food abundant treatment survived to eclosion. Of those removed from the carcass prematurely, only 76.1% (of 331 larvae) survived to eclosion.

The development and maintenance of flight mechanisms incur significant energetic costs (Marden, 2000; Grula *et al.*, 2021). Despite this, when environmental conditions are poor or resources are low, some insects allocate growth to flight-related structures (Angelo & Slansky, 1984). This can result in relative increases in the size of the thorax, where flight muscles develop (Saastamoinen *et al.*, 2010), or wing size, which can lead to increased flight performance (Angelo & Slansky, 1984). This differential resource allocation may allow for the production of highly dispersive morphs (Braendle *et al.*, 2006; Evenden *et al.*, 2015; Han, 2020) or for individuals to compensate for poor early-life food availability and minimise performance deficits (Gluckman *et al.*, 2019; Lu *et al.*, 2019) to individuals that have abundant larval resources to invest in body formation, reproduction, and flight. Previous work that explored the effect of parental loss on adult flight performance found that smaller, parent-less *N. vespilloides* beetles had disproportionately large wings for their body size (Attisano & Kilner, 2015), in line with this kind of resource allocation. In this instance, larvae from all experimental groups developed in similar nutritional environments, and parental loss was not associated with any changes in body size, nor was body size associated with flight performance. However, individuals that received no post-hatching parental care engaged in fewer flights, flew less far, and flew slower than beetles that received parental care. Less efficient food consumption by larvae developing without parents present, or some other response to developmental stress may have contributed to this effect (Attisano & Kilner, 2015). This is consistent with the idea that adaptive resource allocation into flight doesn't eliminate developmental constraints but may instead help to mitigate the effect of poor developmental conditions (Gluckman *et al.*, 2019; Lu *et al.*, 2019). Nevertheless, this type of adaptive response is expected to involve trade-offs of investing dwindling resources into competing traits (Monaghan, 2008), which we did not explore in our experiments. In some insect species, investment in flight structures come at the expense of reproduction (Chang *et al.*, 2021; Guo *et al.*, 2023), while other species appear to be able to maintain both adequate flight and reproductive performance (Niitepõld & Boggs, 2015), even in the face of early-life resource restriction (Niitepõld, 2019). In some cases, flight muscle is resorbed and redistributed towards reproduction following the typical period early-adult of dispersal (Johnson, 1969; Shiga *et al.*, 1991). Assessing only flight performance and not aspects of sexual competition, reproductive success, or other measures of condition likely provides an incomplete picture of the broader life history impacts of early-life adversity. It would be interesting to investigate the relationship between flight and other life-history traits. Trade-offs between flight and life history traits such as reproductive rates

might be expected if the resources necessary for both functions are shared, and both traits compete for this shared pool (Zera & Harshman, 2001). Negative associations between flight and reproduction that would indicate such trade-offs are common, but recent work has raised questions of how widespread this case is (Tigreros & Davidowitz, 2019). Positive correlations between flight and other traits have been found in many insect species (reviewed in: Tigreros & Davidowitz, 2019). If positive associations are brought about or shaped by the quality of the developmental environment (Grafen, 1988), then this might provide stronger evidence for a more pronounced, general silver spoon effect.

2.4.3 Main effects of age on flight performance

Flight is a costly behaviour, both in terms of its high metabolic budget and in oxidative stress and cellular damage (Marden, 2000; Lane *et al.*, 2014; Margotta *et al.*, 2018). Across many insect species, peak flight capacity is both brief and appears to senesce (Grotewiel *et al.*, 2005; Vance *et al.*, 2009; Sanghvi *et al.*, 2021). General declines in late-life flight performance have been described in many insect species – flight metabolic rate in the Mormon fritillary butterfly, *Speyeria mormonia* (Niitepõld & Boggs, 2022); propensity to fly in seed beetles, *Callosobruchus maculata* (Sanghvi *et al.*, 2021); wing beat frequency and duration in free flight in *Drosophila* (Leffelaar & Grigliatti, 1983; Lane *et al.*, 2014). Here, we observed age-specific declines in propensity to fly, time to initiate flight, and peak flight speed, and mixed evidence for age-related declines in distance flown.

Age-specific declines in flight performance may be attributed to deterioration of flight muscle, either through oxidative stress or cellular damage (Ridgel & Ritzmann, 2005; Miller *et al.*, 2008) or reallocation of resources to reproduction or other functions (Shiga *et al.*, 1991; Zera & Brink, 2000). Insect wings are sensitive structures that accrue physical wear over time, and they cannot be repaired (Hayes & Wall, 1999; Mountcastle & Combes, 2014). Individuals may be able to compensate partially for the loss of a certain amount of wing area. For example, *Drosophila* can engage in active flight even when missing up to 34% of wing area, while bumblebees can lose more than a fifth of wing area, with only a 9% reduction in acceleration (Wehmann *et al.*, 2022). However, progressive erosion of wings is likely to play a role in declines in flight performance. Finally, sensory or neurological declines may decrease individuals' sensitivity to the stimuli that prompt flight (e.g. lack of metatarsal contact with a surface, or certain scents/ volatile compounds), or impair the processing of received cues, resulting in reduced flight activity (Ridgel & Ritzmann, 2005; Ribak *et al.*, 2017).

Age-specific declines in propensity, time to initiate flight, and speed were broadly consistent across both experiments. However, the effect of age on distance flown differed between our early-life manipulation experiment and our experience experiment. In the first experiment, flight did not appear to change with age. Early-age flight was improved in the second experiment, but late-age flight appeared similar in both cases. As a result, flight appeared to senesce only within the second experiment.

One possibility is that different developmental experiences between the two experiments contributed to different levels of performance in early adulthood, with this effect dissipating with time or adult feeding (Brown et al., 2017). Our early-life experiment involved generating and then splitting cross-fostered broods, and parental removal occurred very early in development, while our experience experiment involved largely un-manipulated natural broods receiving parental care. Previous work in this species found that parental removal alone, without any pronounced effects on body, could impair subsequent adult flight performance (Attisano & Kilner, 2015). It is possible our early-life manipulation in the first experiment resulted in a short-term deficit in flight ability, which attenuated over time, obscuring the signal of ageing. Additionally, our early life experiment was conducted across spring/summer of 2021 while the experience experiment was carried out in winter/spring 2020, thus stochastic cohort or seasonal effects may have contributed to this difference.

We accounted for selective disappearance, the non-random mortality causing only individuals with certain trait values to survive to old ages (Vaupel & Yashin, 1985; van de Pol & Verhulst, 2006; Hämäläinen *et al.*, 2014; Zhang *et al.*, 2015), in all flight performance traits in our early-life manipulation experiment. There was no signal of selective disappearance in most aspects of flight measured (propensity to fly, distance flow, or peak flight speed). However delayed flyers appeared to live longer than quick flyers, and this led to the appearance of more rapid senescence in the population than is experienced by individuals. It is unclear why this would be the case. Perhaps individuals that expended less energy or were slower in processing the external stimuli that prompted flight had more energy to devote to somatic maintenance.

While we describe linear relationships between age and flight performance, many studies describe initial improvements in flight, as individuals mature or gain resources through adult feeding, followed by late life declines (Dingle, 1972; Vance *et al.*, 2009; Sanghvi *et al.*, 2021). In our case, we considered only linear relationships with age, as the schedule of flight windows (10-20 days, 60-70 days, and 90+ days old) did not include assays of sexually immature or teneral adults or periods of mid-life. We instead attempted to maximise the contrast (and statistical power) between young and old performance by assessing age classes that corresponded to the youngest sexually mature beetles, and those at or exceeding ages commonly assessed in this species (see: Cotter *et al.*, 2011; Lee *et al.*, 2014; Ivimey-Cook & Moorad, 2018; Houslay *et al.*, 2020). As such, it remains unclear whether burying beetles improve in flight ability during early adulthood, or whether the ages included in our youngest assessment interval represent the period of peak flight ability in this species. Assessing flight at more ages would be a useful next step in characterising potential improvements in flight ability through early adulthood and assessing ages for peak performance and senescent declines.

In any case, the observed age-specific declines in flight performance are likely to have consequences for fitness in natural contexts. As outlined above, flight performance has consequences for gaining access to breeding resources (Scott, 1998; Attisano & Kilner, 2015). Additionally, flight ability is also likely important for predator avoidance and escaping

disadvantageous antagonistic interactions with sexual competitors or other carrion consumers that may be competing for the same resources (Terlau *et al.*, 2023). Thus, declining flight ability may underpin patterns of both age-specific reproductive success and mortality.

2.4.4 Interactions between early-life conditions and age

There is great interest in understanding variation in the pattern and onset of ageing within populations (Monaghan *et al.*, 2008). While early-life conditions can clearly be influential in shaping morphology and mean adult performance (Braendle *et al.*, 2006; Reim *et al.*, 2006; Attisano & Kilner, 2015; Brown *et al.*, 2017; Grula *et al.*, 2021), how these early-life effects may shape rates of decline across ages is unclear. Under the silver spoon hypothesis, ageing can be slower in individuals that experienced high-quality early-life conditions (Cooper & Kruuk, 2018). Conversely, individuals that have abundant resources to invest in growth and reproduction may maximise early-life growth and performance at the expense of late-life condition and survival if they trigger early:late trade-offs (Adler *et al.*, 2016; Hooper *et al.*, 2017). In this study, the rate at which flight performance declined appeared to be independent of early-life food availability (i.e., there was an absence of an effect of the interaction between early-life conditions and age). Previous work in field crickets (Zajitschek *et al.*, 2009b), bighorn sheep (Pigeon *et al.*, 2021), and seed beetles (Sanghvi *et al.*, 2021) have suggested that adult, rather than juvenile environments, are more important in shaping ageing in several demographic or physiological traits. Early-life hardship can have trait-specific effects on senescence, with some traits being impacted while others appear to remain unaffected (Cooper & Kruuk, 2018; Sanghvi *et al.*, 2021; Niitepõld & Boggs, 2022); flight traits may fall into the latter category. This could potentially stem from adaptive physiological mechanisms during development that conserve some survival-enhancing traits over competing concerns (i.e., reproductive traits; Cooper & Kruuk, 2018). Alternatively, abundant food availability during adulthood may have relaxed the potential costs associated with early-life food restriction during development, minimising differences between experimental groups. All adult beetles were provided the same abundant diet throughout their lives. It Is possible that adult flight was less dependent on resources available during development, but rather on energy reserves that were acquired during adult feeding (Brown *et al.*, 2017). Similar to mean adult performance (discussed above), this lack of a clear interaction between age and early-life conditions may reflect viability selection among developing larvae that removed the lowest quality individuals from the food-restricted developmental group before they could be assessed. In much the same vein as mean adult performance, it would be interesting to assess potential effects of the interaction of early-life and adult environmental conditions on age-specific performance.

Early-life conditions can independently affect average trait expression, as well as the onset and slope of age-related declines (Pigeon *et al.*, 2019). As we considered only linear effects of age, we cannot account for potential differences in the timing of the onset of ageing in flight performance between experimental groups. In insects, flight is often lowest in sexually

immature adults, increasing in early adulthood and declining after the reproductive peak (Dingle, 1972). Early-life conditions may act on the pace of development, sexual maturation, or timing of reproduction in adults (Angelo & Slansky, 1984; Angell *et al.*, 2020), with knock-on effects on flight performance, or age-of-onset of flight senescence may be independently sensitive to early-life conditions. Again, assessing flight across a more comprehensive set of ages would be useful in identifying the timing of peak flight activity, and how sensitive this timing may be to early-life manipulations. Exploring a reaction norm of flight performance across age would allow for a more nuanced consideration of early-life effects on the onset and rate of decline of flight capacity.

Additionally, we did not assess trade-offs within flight traits or between flight and lifespan or reproduction, thus we may have underestimated early-life effects on ageing. Engaging in flight is costly, generating oxidative stress and associated cellular damage, which may impair future performance and reduce lifespan (Lane *et al.*, 2014; Chang *et al.*, 2021). A further step would be to consider if elevated flight activity in early adulthood has negative impacts on later-life flight performance or lifespan. Larger beetles, as may have experienced food abundance during development, are less thermally efficient in flight than smaller individuals (Merrick & Smith, 2004), which may contribute to increased heat stress in addition to the elevated metabolic stress associated with flight (Kaufmann *et al.*, 2013), offering a clear mechanism by which we might expect early-life manipulations to influence age-specific relationships.

Furthermore, it is possible that food restriction during development exacerbates trade-offs between competing traits (Zera & Harshman, 2001). Insect flight is fuelled initially by carbohydrates but sustained flight is primarily reliant on lipid metabolism (Canavoso *et al.*, 2003). *N. vespilloides* also consume stored lipids when performing the early stages of parental care (Benowitz *et al.*, 2017), suggesting that these care and flight may compete for the same limited pool of resources. Assessing paired performance of age-specific reproductive and flight ability might shed light on how trade-offs between these two traits resolve across ages.

2.4.5 Conclusion

Our study provides support for the presence of a silver spoon effect of early-life food abundance on aspects of flight performance in our study species, *N. vespilloides*. It is generally found that larger individuals grown under food abundant larval conditions are more successful in direct intrasexual competition (Otronen, 1988). In addition, other studies have found that larger females can produce more and larger eggs during reproductive events (Steiger, 2013; Lee *et al.*, 2014; Hopwood *et al.*, 2016b). Early-life conditions can have pronounced effects on individual fitness and performance (Lindström, 1999; Monaghan, 2008; Cooper & Kruuk, 2018), but it remains to be seen how the costs of flight might affect investment in, or the expression of, other traits such as reproduction or lifespan (Sanghvi *et al.*, 2021). Flight is an important precursor to reproduction in natural contexts (Scott, 1998; Attisano & Kilner, 2015), but it has been largely overlooked in prior studies of reproductive success. Given the breadth

of developmental and behavioural responses to early-life adversity observed across species and traits and the relatively subtle effects observed here, it is clearly warranted to consider broader trends in suites of important fitness-related traits (Sanghvi *et al.*, 2021).

There was no apparent difference in the rate of ageing in flight performance in individuals from either developmental background (i.e., better fed larvae performed better overall as adults and maintained these advantages into late life), perhaps due to an abundance of food available to adult fliers from our two treatments. An important factor for age-specific expression of flight behaviours is the adult environment, which has a direct effect on senescence (Cooper & Kruuk, 2018). This may include manipulations of the adult environment to match or contrast with environmental conditions experienced during development (i.e., predictive adaptive response; Nettle & Bateson, 2015; Pigeon *et al.*, 2019) and environmental mismatch hypotheses (Gluckman *et al.*, 2019). It is also important to consider how more complex environmental shifts may shape ageing. Further work may also focus on flight capacity of wild-living individuals, who are subjected to more challenging environments and energetic demands than their laboratory counterparts. Assessing flight under natural schedules of activity alongside the suite of nutritional and reproductive challenges faced by wild-living individuals could shed more light on the ecology of this species and clarify how age-specific flight performance may affect fitness. This system is particularly suited to addressing this question. Firstly, flight mills offer a non-destructive method to repeatedly sample individuals. Secondly, the use of mark-recapture studies with *N. vespilloides* allows for repeated contact with known individuals, which is valuable for studying age-specific physiological performance (see Chapter 5). Studies of age-specific physiological performance in wild insects are relatively uncommon (Zajitschek *et al.*, 2020), but these are important in understanding phenotypic selection and trade-offs between traits.

3 The effect of maternal loss on ageing rates

3.1 Introduction

Ageing, a progressive physiological deterioration and decline in performance with advancing age, evolves because the strength of natural selection declines after sexual maturation and with advancing age (Medawar, 1952; Williams, 1957; Hamilton, 1966; Moorad *et al.*, 2019). Mutations with late-acting deleterious effects are, therefore, less efficiently removed by selection than those with similar effects manifested early in life (Mutation accumulation – Medawar, 1952). The declining strength of selection also favours alleles or patterns of resource investment that maximise early-life reproduction at the cost of somatic maintenance and late-life negative effects (Antagonistic pleiotropy – Williams, 1957; Disposable soma – Kirkwood, 1977). Most research in the field focusses on declines in age-specific survival and reproductive success (actuarial and reproductive ageing, respectively), as they are the traits most directly linked to individual fitness (Bouwhuis *et al.*, 2012; Kowald & Kirkwood, 2015; Lemaître & Gaillard, 2017; Cooper & Kruuk, 2018). While ageing is a near-ubiquitous phenomenon across the tree of life (Hughes & Reynolds, 2005), its patterns and age-of-onset can vary greatly within (at a trait-specific level) and between individuals, populations, and species (Lemaître *et al.*, 2013; Jones *et al.*, 2014; Moorad & Ravindran, 2022). The drivers of this variation are still poorly understood; understanding these mechanisms is a key research focus of the evolution of senescence (Balbontín & Møller, 2015; Lemaître *et al.*, 2015; Cooper & Kruuk, 2018; Spagopoulou *et al.*, 2020; Moorad & Ravindran, 2022).

Early-life environmental conditions can have pronounced effects on developing phenotypes, with consequences for adult life-histories and ageing rates (Monaghan, 2008; Cooper & Kruuk, 2018). How these early-life effects manifest can vary greatly across traits and species. Individuals developing under benign conditions often exhibit age-independent positive effects on size (Steiger, 2013), condition (Crosland *et al.*, 2022), and performance (Adler *et al.*, 2016); these are termed "silver-spoon" effects (Grafen, 1988). However, challenging early-life conditions may also act as a cue or constraint that alters the developing phenotype, yielding context-dependent benefits to performance (Bateson *et al.*, 2014; Nettle & Bateson, 2015). Improved conditions in early life may result in slower rates of ageing (Nussey *et al.*, 2007; Cooper & Kruuk, 2018) if individuals have greater resources or can more efficiently allocate acquired resources to both somatic maintenance or survival, and growth or reproduction (Hooper *et al.*, 2017). However, the opposite is sometimes found: increased early investment in growth and reproduction can lead to more rapid declines in condition (Adler *et al.*, 2016; Crosland *et al.*, 2022) and survival (Spagopoulou *et al.*, 2020). Early-life adversity may also act to remove frail individuals (i.e., selective disappearance; Chen & Maklakov, 2012; Payo-Payo *et al.*, 2023), resulting in a surviving cohort of robust, high-performing individuals that exhibit improved performance or lower apparent rates of ageing. Parental care has evolved to improve fitness (Royle *et al.*, 2012) by providing more benign

conditions in the young, which may have consequences for adult offspring (Attisano & Kilner, 2015; Pilakouta *et al.*, 2015). This investment in the well-being of offspring during their early stages of development can potentially have lasting consequences for the performance and outcomes of adult offspring (Thesing *et al.*, 2015).

Variation in the quality or duration of care provided to offspring can underpin variation in offspring survival, growth, and development (Rauter & Moore, 2002; Head *et al.*, 2012). Greater rates of provisioning can improve offspring growth (Sofaer *et al.*, 2018), and in Seychelles warblers, *Acrocephalus sechellensis*, a species with alloparental care, greater levels of care result in greater survival to adulthood and lower adult mortality of offspring (Brouwer *et al.*, 2012). The impacts on offspring resulting from variations in the quality and provision of care have been extensively investigated in relation to gradual alterations in maternal traits, including age, body size, and diet (Ozanne & Hales, 2004; Steiger, 2013; Ivimey-Cook & Moorad, 2018; Rodríguez-González *et al.*, 2019). Parental loss constitutes an extreme exaggeration in difference in care, with potentially severely deleterious fitness consequences for offspring (Thesing *et al.*, 2015; Stanton *et al.*, 2020). The lifetime effects of parental loss or removal have predominantly been explored in vertebrates with altricial young (Thesing *et al.*, 2015). In chimpanzees, *Pan troglodytes*, maternal loss during sexual immaturity negatively affected survival but showed no long-term effects on stress responses (Stanton *et al.*, 2020; Girard-Buttoz *et al.*, 2021). Andres *et al.* (2013) found that the survival cost of maternal loss is pronounced in both male and female juvenile red deer, *Cervus elaphus*. Morrison *et al.* (2021) found no effect of maternal loss in infants or sub-adults on survival in mountain gorillas, *Gorilla beringei beringei*. In killer whales, *Orcinus orca*, males orphaned at any age, even post-30 years old, exhibit reduced survival (Foster *et al.*, 2012). The late-life effects of maternal loss in invertebrate systems, which can include precocial young with only facultative reliance on maternal care, have received less attention (Thesing *et al.*, 2015). Instead, invertebrate studies have focussed usually on short-term survival to adulthood or other early-life performance (for example: Monteith *et al.*, 2012; Attisano & Kilner, 2015; Capodeanu-Nägler *et al.*, 2016; Kramer *et al.*, 2017; Grew *et al.*, 2019). Pilakouta *et al.* (2015) considered the lifespan costs associated with maternal loss in burying beetles, *Nicrophorus vespilloides*, finding costs in inbred but not outbred offspring. However, mean differences in lifespan alone can tell us little about ageing (Kowald, 2002; Lemaître *et al.*, 2020b; Keshavarz *et al.*, 2023), as these may arise from either changes in age-independent and/or age-dependent rates of mortality (Kowald, 2002), and an understanding of changes in actuarial senescence require that both components of mortality are estimated. How maternal loss may impact rates of ageing in the context of precocious young with facultative maternal care remains unexplored.

In this study, we investigated the role that maternal presence or absence plays in shaping actuarial and reproductive ageing in a species of burying beetles, *N. vespilloides*. This species engages in complex, facultative, bi-parental care: larvae can be protected and tended by both parents, a single mother or father, or develop in the absence of care entirely (Eggert *et al.*, 1998). Pre-hatching parental care involves the burial of a small vertebrate carcass (which serves as the sole food source for developing larvae) and the subsequent removal of

any hair, scales, or feathers and opening of an abdominal cavity for larvae to enter upon hatching (Eggert *et al.*, 1998; Scott, 1998). Post-hatching care involves the spreading of anti-microbial secretions on the carcass, guarding the developing offspring from invertebrate challenges, and directly provisioning offspring via the regurgitation of consumed carcass (Scott, 1998).

Here, we focussed on the contribution of post-hatching care, in which mothers can actively engage with offspring via provisioning or other typical care behaviours (Bartlett, 1987; Eggert & Müller, 1997), to patterns of age-specific mortality and reproduction. Prior work in this species has found inconsistent effects of maternal loss on offspring performance across studies and traits. Total larval growth appears to generally improve in the receipt of maternal care (Eggert *et al.*, 1998; Smiseth *et al.*, 2007; Pilakouta *et al.*, 2015; Capodeanu-Nägler *et al.*, 2016; Grew *et al.*, 2019), but development time has been alternatively shown to be shortened in the presence of maternal care (Pilakouta *et al.*, 2015) and to be independent of maternal care (Grew *et al.*, 2019). Adult body size, which may have consequences for future reproductive ability (Steiger 2013) and competitive success (Lee *et al.*, 2014; Hopwood *et al.*, 2016a), has been found to be independent of the presence or absence of post-hatching care (Attisano & Kilner, 2015). Further, Capodeanu-Nagler *et al.* (2016) found that adult *N. vespilloides* that had received post-hatching care were the same size as those that received no care at all, while individuals that received only pre-hatching care were the smallest of all. Survival to adulthood may increase in the receipt of maternal care, though this effect may be greater in inbred than outbred larvae (Pilakouta *et al.*, 2015) or obvious only at certain temperatures (Grew *et al.*, 2019). Capodeanu-Nagler *et al.* (2016) found high rates of survival to adulthood among larvae from either maternal care background. Mothers in this species engage in filial cannibalism, removing surplus larvae from the carcass, enhancing the growth and survival of the remaining brood (Bartlett, 1987; Klug & Bonsall, 2007; Capodeanu-Nägler *et al.*, 2016). However, maternal care may exacerbate competitive asymmetries within a brood by interacting with and favouring faster-developing or older larvae over their slower-developing or younger siblings (Smiseth *et al.*, 2007). As a result, it is uncertain whether individuals that develop under benign conditions (i.e., in this case the presence of care) will exhibit slower rates of ageing as they could be of higher overall condition (van Noordwijk & de Jong, 1986; Hayward *et al.*, 2013; Hooper *et al.*, 2017; Cooper & Kruuk, 2018) or exhibit accelerated ageing as high condition individuals may heavily invest in early growth and reproduction (Hughes & Reynolds, 2005; Adler *et al.*, 2016; Crosland *et al.*, 2022). Alternatively, stage-specific patterns of selective disappearance may affect apparent age-specific patterns of mortality and reproductive performance. Differing levels of juvenile mortality in cared-for and uncared-for broods may reflect different strengths of selective disappearance (Chen & Maklakov, 2012), resulting in overall differences in quality of surviving offspring, with consequences for rates of actuarial or reproductive ageing. Selective disappearance may further act across adulthood, exacerbating or mitigating patterns of age-specific reproduction.

3.2 Methods

3.2.1 Study species

This study used beetles generated in 2014 and 2015 from an outbred laboratory stock population maintained at the University of Edinburgh. This population descended from beetles originally derived from a colony in the Netherlands in 2013. Genetic diversity was subsequently maintained by annual additions of wild beetles trapped from natural populations around Edinburgh. All beetles were kept under 16:8 light:dark conditions and at 21 °C. Adult beetles were housed individually in plastic containers (twelve cm × eight cm × two cm) filled with moist soil and fed organic beef twice a week.

3.2.2 Maternal loss manipulation

To generate our experimental beetles, we randomly paired unrelated virgin males and females in large, transparent plastic containers (17 cm x 12 cm x 26 cm). These containers were supplied with a one cm bed of moist soil and a freshly thawed mouse carcass (Livefoods Direct Ltd). The parents then prepared the carcass, and females laid eggs in the soil. After approximately 60 hours, at which point eggs had been laid but prior to larvae hatching, males were removed from all mating chambers and discarded. This was done in part because males occasionally eat eggs, because male care is redundant under laboratory conditions (Smiseth *et al.*, 2005), and to simplify our focus on the effects of maternal care on offspring lifespan and ageing. At this stage, mothers were also removed from half of all active broods to form our maternal loss treatment. In this experiment, all larvae benefitted from bi-parental prehatching care (carcass preparation, depilation etc.), with our control group retaining post-hatching maternal care throughout development, and our maternal loss group developing in the absence of any post-hatching care. At the time of dispersal, the remaining larvae of each brood were transferred to large boxes full of moist soil, in which they pupated and eventually eclosed as adults after approximately 21 days.

Eclosed experimental beetles from both treatments (905 individuals from 53 families exposed to the maternal loss treatment, 1268 individuals from 68 families exposed to the maternal care treatment) were kept in individual plastic boxes and maintained in our standard laboratory conditions. Individuals were sexed and size was recorded using digital callipers (CD-6” CSX, Mitutoyo Corp.; accuracy: +/-0.02mm). Measurements of the length of the pronotum were made in millimetres to the nearest 0.01 mm (Maternal loss: N = 887, mean = 3.74 mm, sd = 0.33 mm; Maternal care: N = 1213, mean = 4.03 mm, sd = 0.40 mm; (Beeler *et al.*, 1999; Walling *et al.*, 2009; Potticary *et al.*, 2023). The majority (1996 of 2173 individuals) remained unmated and were fed organic beef and checked for death twice a week.

Burying beetles are sexually immature for a further ten days post-eclosion. Therefore, we considered beetles as sexually immature adults prior to age ten days old and as sexually mature adults after this threshold. Our analyses of adult survival were divided into pre- and post-age ten days to reflect this shift in maturation state.

To assess the effect of maternal loss on age-specific reproduction, a small number of females from each treatment (89 from the maternal loss group, 88 from the control group) were removed at certain ages at random and paired with a young virgin male and freshly thawed mouse carcass of known size (range: 19.15 g to 23.84 g). Each mother was provided only one opportunity to mate, which occurred between the ages of 12 days and 93 days old. The production of any larvae was scored as a measure of mating success (no production of larvae was scored as "0", and production of any larvae was scored as "1"), and the resulting descendent larvae surviving to dispersal were counted and weighed. This process was conducted in five experimental blocks of varying size (block one: N = 727; block two: N = 190; block three: N = 97; block four: N = 607; block five: N = 552) over two consecutive years. Both maternal care treatments were present in all blocks but, due to relatively small sample sizes in our third replicate ("block 3"), reproductive data were only gathered in blocks one, two, four and five.

3.2.3 Statistical analyses

All statistical analyses were conducted using R version 4.3.1 (R Core Team, 2023). We assessed model fits with the package DHARMa v0.4.6 (Hartig, 2022) and tested overall significance using type III ANOVAs.

Survival from eclosion to sexual maturity (ten days post-eclosion) was analysed with a generalised linear mixed model, using the package glmmTMB v1.1.8 (Brooks *et al.*, 2017), assuming a binomial error distribution. We included receipt of maternal care as a two-level factor, and experimental block and brood ID as random effects to account for cohort, shared maternal care, or carcass quality effects.

Group-specific survival curves of sexually mature adults (post ten-days old) were visualised with Kaplan-Meier plots and analysed using non-parametric log-rank tests from the package survival v3.5-5 (Therneau, 2023).

The effects of the receipt of maternal care on age-specific mortality were initially explored using accelerated failure time (AFT) models according to several common distributions (Weibull, Gompertz, Lognormal, Log-logistic, and Exponential) using the packages survival (Therneau, 2023) and flexsurv v2.2.2 (Jackson, 2016). AFTs are suitable for analysing censored lifespan data (Wei, 1992), where the ages at death are not known or available for all individuals, either due to individuals remaining alive at the end of the observation period, removal from the population due to changes in status (here, removed for mating), or to loss. These models can account flexibly for differences in overall mortality rates between groups, as well as the rates of increase in age-specific mortality (i.e., rate of senescence) and potential decelerations of mortality rates depend on the appropriate distribution chosen. Exploratory analyses of population-, treatment-, and block-level effects (as in: Mautz et al., 2019) revealed that different blocks were most appropriately modelled by different distributions (determined by AIC; experimental block one: Gompertz, block two: Gompertz; block three: long-normal; block four: log-logistic; block five: log-logistic; Table 1).

Table 3-1: Summary or AIC comparison within blocks for appropriate AFT model distributions. The best fitting distribution for each block is highlighted in bold.

Distribution		**Block 1**		**Block 2**		**Block 3**		**Block 4**		**Block 5**	
	d.f.	*N*=707	AIC	*N*=167	AIC	*N*=91	AIC	*N*=603	AIC	*N*=550	AIC
Exponential	3		6318.33		1484.92		881.30		5681.46		4767.58
Gompertz	5		**5457.72**		**1171.86**		762.07		4905.35		3964.28
Weibull	5		5506.06		1180.22		733.50		4770.55		3800.44
Log-logistic	5		5685.88		1220.45		707.29		**4760.49**		**3662.34**
Log-normal	5		5753.70		1231.82		**705.10**		4812.10		3679.71

Survival data were analysed using generalised additive mixed models (GAMMs; similar to: Barks *et al.*, 2018; Pietrzak *et al.*, 2020; Tully, 2023) using mgcv v1.8-41 (Wood, 2011, 2017). This approach allows modelling of non-linear relationships between covariates and outcomes where the shape of these relationships varies between specified grouping levels (Pedersen *et al.*, 2019), which was block in this study (Figure 1B). We used an event-history model where survival was analysed as a binomial trait, with each day scoring an individual as 0 for alive or 1 for dead. Censored individuals, either removed from the population at a certain age for mating, or lost with no known age at death, were included as a series of only 0s, terminating at the age of removal or loss. We included receipt of maternal care as a two-level factor, age as a smooth term (i.e., a curve), and an interaction between age and an ordered factor of treatment (yielding a "difference smooth" interpreted as age-specific difference in mortality across treatments over and above that captured by mean difference in intercept). We also included an age-by-block factor-smooth term, accounting for block-specific differences described above. Individual ID was included as a random effect to account for repeated measures of individuals. Experimental block and brood ID were also included as random effects to account for cohort, shared parental care, and possible variation in carcass quality. All GAMMs were fit using cubic regression basis splines and, to avoid overfitting (Barks *et al.*, 2018), the number of basis functions, or maximum complexity, of all smooths was restricted to five. All models were fit using maximum likelihood, and model selection was performed by adding an extra penalty to each smooth term so that it could be penalized to zero (Marra & Wood, 2011).

We further investigated the components of the fitted GAMM, namely, the age-specific slope of mortality of our reference group (the maternal loss treatment), the age-specific slope of the "difference smooth" describing the difference in mortality across ages relative to the reference, and the overall difference in mortality experienced across ages by individuals from the maternal care treatment relative to the maternal loss group. This post-hoc analysis, conducted using the packages gratia v0.8.1 (Simpson, 2023) and itsadug v2.4.1 (van Rij *et al.*, 2022), allowed us to be explicit about how the apparent rate of ageing and overall mortality differed across adult ages and maternal care backgrounds. This post-hoc approach involved recalculation of confidence intervals around these model components via simulation to account for multiple comparisons. The confidence intervals generated under model fitting by

mgcv (Wood, 2011) have the property of being 95% “across the function” intervals (Marra & Wood, 2012): under repeated sampling of the population, the true function will be contained within the confidence interval over 95% of the x-axis (Marra & Wood, 2012; Sørensen *et al.*, 2021). Simultaneous confidence intervals fully contain the complete function 95% of the time (Marra & Wood, 2012; Sorensen *et al.*, 2021) and are generally wider than “across the function” intervals but more appropriate for considering smooths across multiple points (i.e., multiple comparisons).

We conducted path analyses to further explore and visualise the relationships between the receipt of maternal care, subsequent adult body size, and lifespan. Path analyses allow for the explicit testing of assumed causal relationships between variables according to some defined model (Lefcheck, 2016). These models are often visualised with a path diagram and can include variables that are both predictors and responses (Lefcheck, 2016). This allows for the testing of indirect effects that might be missed by other statistical approaches (Grace *et al.*, 2007). Further, path analyses can render model results as standardised path coefficients; these are dimensionless effect sizes, scaled by mean and variance (Lefcheck, 2016) that allow for the direct comparison of the direction and magnitude of effects, including mediated or indirect effects, of one variable on another, controlling for prior variables (Lleras, 2005). These analyses were conducted using the packages piecewiseSEM v2.3.0 (Lefcheck, 2016) and lme4 v1.1-35.1 (Bates *et al.*, 2015), with standardised direct and indirect path estimates being derived from the bootstrap method of the package semEff v0.6.1 (Murphy, 2022). The model structure specified in our path analysis was determined a priori, based on previous experience with this study species, and patterns established in previous research (Bartlett & Ashworth, 1988; Pilakouta *et al.*, 2015). Initial analyses and visual comparison of diagnostic plots of lifespan and body size suggested that, in both cases, the data were most appropriately modelled assuming a student-t error distribution. However, this precluded the estimation of standardised path coefficients in either the piecewiseSEM or semEff packages. Therefore, all component models were modelled assuming a Gaussian error distribution. Raw model estimates in either case were qualitatively and quantitively similar. Furthermore, the confidence intervals of the Gaussian models were approximately equal to, or wider than those of models fit to Student-t distributions, meaning our path analyses may be considered a conservative estimation of the effects of maternal care and body size on lifespan. These models also included random effects of experimental block and brood ID.

Finally, age-specific reproductive traits were analysed with generalised linear mixed models using the package glmmTMB v1.1.8 (Brooks *et al.*, 2017), according to the appropriate error distributions. Mating success (measured as whether larvae were produced or not; 0 or 1, respectively) was analysed with a binomial model and number of dispersing larvae as zero-inflated negative binomial. Mean weight at dispersal was initially modelled assuming a normal distribution, but diagnostic plots generated with DHARMa (Hartig, 2022) suggested this model was mis-specified and a Student-t error distribution was chosen to be more appropriate. All models included age at reproduction as a continuous covariate, receipt of maternal care as a two-level factor, and an interaction between these two terms. To address selective

disappearance that could potentially obscure signals of aging in functional traits (van de Pol & Verhulst, 2006) and to consider the distribution of mating opportunities within discrete age ranges throughout typical burying beetle lifespans, we incorporated age interval at death as a factor (Ivimey-Cook & Moorad, 2018). This factor had four levels: death between first mating and the second mating window (12 - 30 days old), death between second and third windows (30 - 50 days old), death between second and third windows (50 - 70 days old), death during or after the final mating window (70+ days old). For traits other than survival, the effects of selective disappearance can be quantified, and the true rate of ageing can be estimated. However, while individual heterogeneity can contribute to population patterns of mortality (Vaupel & Yashin, 1985; McDonald *et al.*, 1996; Horiuchi & Wilmoth, 1998; Service, 2000), this method cannot be applied to assessing actuarial senescence. These models also included random effects of experimental block and brood ID.

3.3 Results

3.3.1 Survival, lifespan, and age-specific mortality

Receipt of maternal care during development significantly affected survival from eclosion to sexual maturity at 10 days old (Risk ratio = 1.468, 95% CI [1.097, 1.964], z = 2.584, p = 0.010). The magnitude of this effect was minor: individuals that received maternal care survived at only slightly higher rates (0.995 [0.966, 1.000]) than their uncared-for counterparts (0.972 [0.910, 0.995]).

Receipt of maternal care also significantly affected adult survival post age 10 days (Kaplan-Meier log-rank test: χ^2_1 = 35.36, p = 2.74e-09); individuals that did not receive maternal care exhibited higher survival than those that did not (Fig 1A). Individuals that did not receive maternal care had a mean survival time of 53.877 days [52.758, 54.997], while individuals that received maternal care had a mean survival time of 48.359 days [47.388, 49.329].

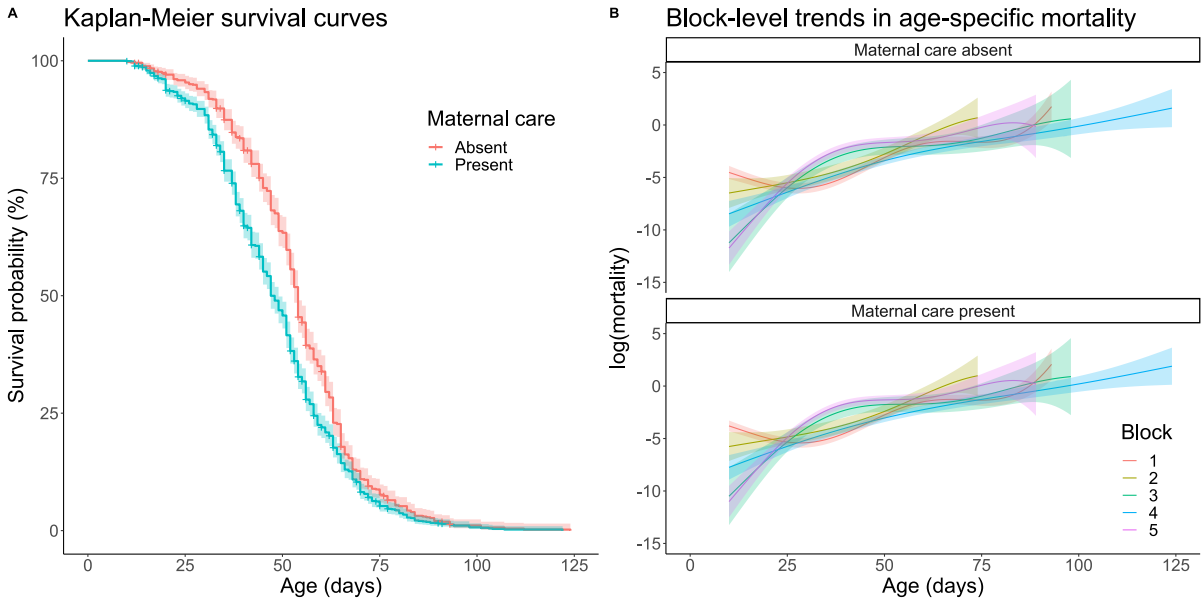

Figure 3-1: Patterns of survivorship (A) and age-specific mortality, presented at the level of experimental block (B) in response to the presence or absence of maternal care. Plot A describes survivorship of 2118 individuals (Maternal care present: N = 1250, censored events = 100; Maternal care absent: N = 868, censored events = 94). Plot B describes predictions of block-level trends from GAMM (Table 2), including the effect of random age-by-block factor smooths, from age of sexual maturity to the maximum age recorded for each block. Ribbons denote 95% confidence intervals.

Baseline mortality (i.e., difference in intercept) was significantly higher among individuals that received maternal care (Table 2; B = 0.391 [0.181, 0.600], χ^2_1 = 13.38, p = 3.0e-4). The pattern of age-specific mortality deviated significantly from a linear trend (Table 2; Figure 2): increasing across early adulthood and slowing down at late ages. A post-hoc evaluation of the age-specific slope of this smooth (i.e., rate of ageing) using simulated simultaneous confidence intervals approximately 1.31 times wider than confidence intervals of raw model estimates (critical value of 2.57 standard errors vs typical value of 1.96) suggested that mortality was initially low and increased in an approximately linear fashion from age 26 to 47 days old, at which point mortality appeared to decelerate (Figure 2; Figure 3A). The shape of the relationship between age and mortality also differed between cared-for and uncared-for individuals (i.e., a significant interaction effect between age and the receipt of maternal care; Table 2). The rate of increase in mortality (i.e., rate of ageing) was generally lower across early adulthood in individuals that received maternal care. However, post-hoc analyses of the slope of this "difference smooth" using simulated simultaneous confidence intervals approximately 1.39 times wider than model-estimated confidence intervals (critical value of 2.74) suggested no significant difference in age-specific slopes between cared-for and uncared for individuals assessed across ages 10 to 100 days of age (Figure 3B). The combined effect of this difference in baseline mortality and age-specific pattern, accounting for post-hoc simultaneous confidence intervals 1.32 times wider than model estimates (critical value of 2.59), was for individuals that received maternal care to suffer higher overall levels of mortality from the age

of sexual maturity to approximately age 47 days, after which both groups faced similar levels of mortality (Figure 3C).

Table 3-2: The effects of maternal care on age-specific mortality. Estimates from a generalised additive model fit with a binomial error distribution, on the complementary log-log scale. Smooth term 's(Age)' is interpreted as the function of age-specific mortality among individuals that did not receive maternal care during development. Smooth term 's(Age:Maternal care (present))' is interpreted as the difference in the shape of ageing in our cared-for group, relative to the 's(Age)' curve.

		Mortality		
Predictors	*Log-Odds*	*CI*	*z*	*p*
(Intercept)	-4.646	-5.134 – -4.158	-18.66	**< 2e-16**
Maternal care (present) [Linear]	0.391	0.181 – 0.600	3.66	**2.55e-4**
Smooths	*Estimated d.f.*	*Reference d.f.*	*χ2*	*p*
s(Age)	1.672	4	41.79	**<2e-16**
s(Age:Maternal care (present))	1.800	4	20.24	**0.028**
Random effects	*Estimated d.f.*	*Reference d.f.*	*χ2*	*p*
s(ID)	478.203	2112	705.05	**<2e-16**
s(Family)	84.985	119	793.29	**<2e-16**
S(Age:Block)	19.495	24	4741.54	**<2e-16**
Observations	77728 days			
Adjusted R^2 / Deviance explained	0.088 / 0.243			

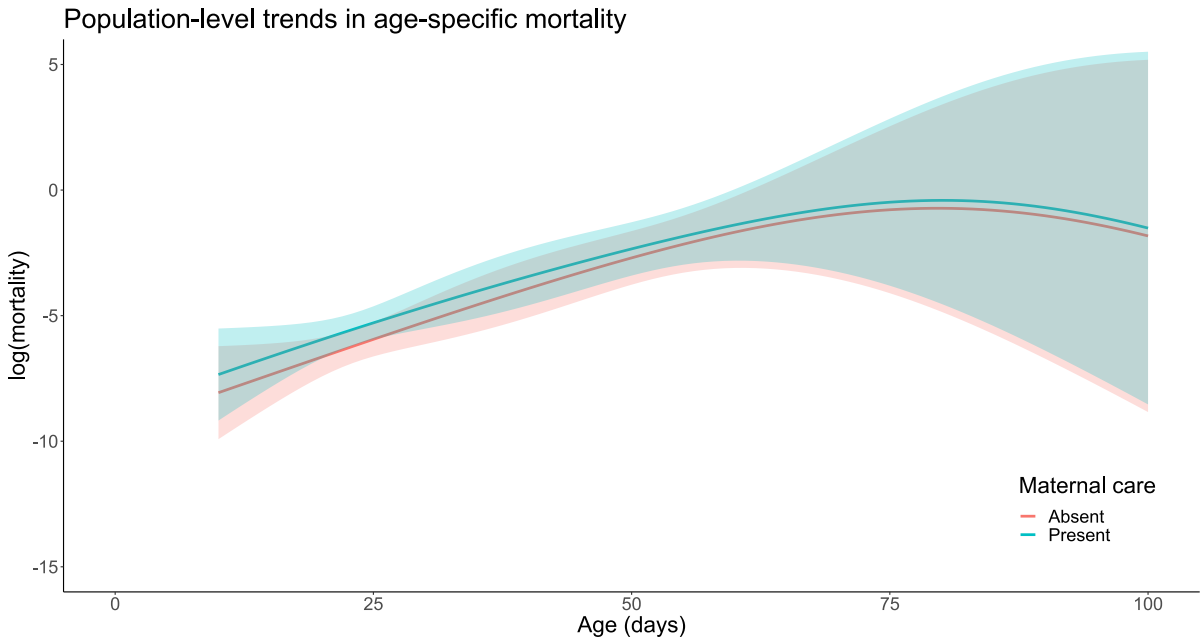

Figure 3-2: Global, or population-level, patterns of age-specific mortality in response to the presence or absence of maternal care. This plot describes predictions from GAMM (Table 2), excluding the effect of random age-by-block factor smooths, restricted to age 10 to 100 days. Model predictions were restricted to this range because only 11 individuals remained alive to generate mortality estimates post age 100 days. Rapidly widening confidence intervals reflect, in part, diminishing sample sizes at late ages. Ribbons denote 95% confidence intervals.

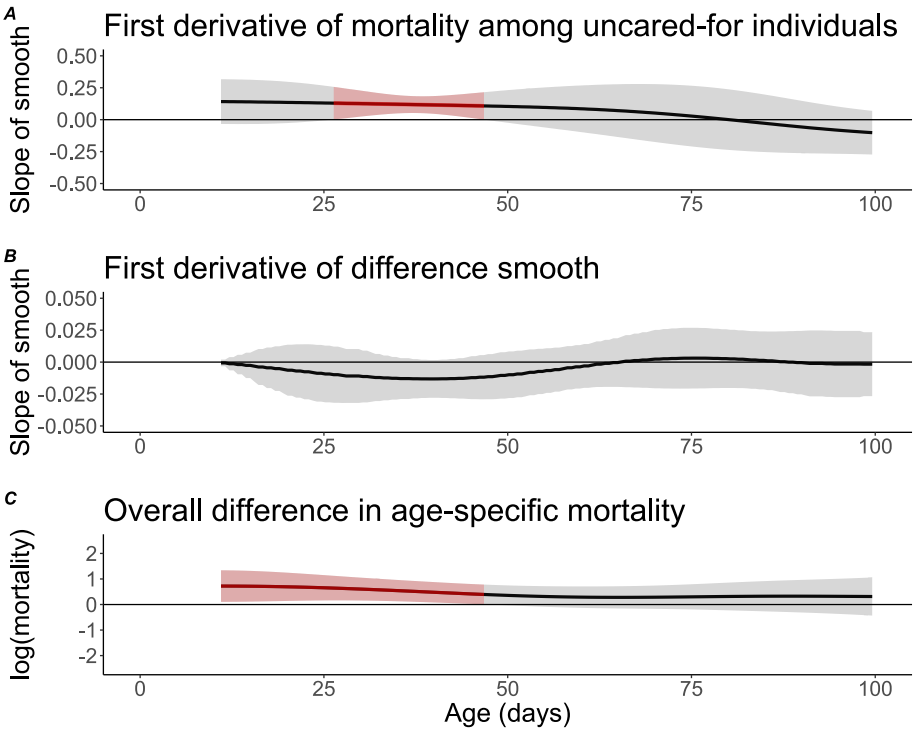

Figure 3-3: The exact interpretation of highlighted colours differs between plots A-C (described below). In general, red indicates higher mortality or faster ageing compared to a reference level. A) First derivative of the smooth describing age-specific mortality among individuals that did not receive maternal care (Figure 1B). Regions highlighted red denote ages across which the slope was positive and confidence intervals did not overlap zero (i.e., mortality increased with age). Regions in grey denote a slope not significantly different from zero, or plateaus in mortality. B) First derivative of the smooth describing the difference in age-specific mortality between cared-for and uncared-for individuals. Grey regions denote ages where rates of increase in mortality (ageing) are similar between experimental groups. C) Overall difference in age-specific mortality reflects both the difference in baseline mortality (i.e., mean difference between groups; Table 2: 'Maternal care (present)'), and the pattern of mortality across ages (i.e., the difference between fitted curves; Table 2: 's(Age:Maternal care (present))'). The highlighted region (red) denotes the range of ages where mortality among individuals that received maternal care is significantly higher than that in individuals that did not receive maternal care. Ribbons denote 95% confidence intervals.

Our path analysis involved a simple plausible causal model that related maternal care to lifespan, based on prior experience with the study species. This model suggested that there was little direct effect of the receipt of maternal care on adult lifespan (Figure 3B; standardised estimate =-0.052 [-0.130, 0.004]). However, maternal care was positively associated with body size (as measured by pronotum length), which in turn had negative effects on adult lifespan (Figure 4; Table 3). This resulted in a significant, negative indirect effect of maternal care on lifespan, mediated via body size (standardised estimate = -0.065 [-0.081, -0.040]).

Table 3-3: Bootstrapped standardised path coefficients and confidence intervals from structural equation model. Indirect effects are calculated as the product of path coefficients maternal care -> body size and body size -> lifespan. Unstandardised and standardised direct effects are visualised in Figure 4 (A, B). Statistically significant associations or pathways are in bold.

	Path analysis	
	Standardised path coefficient	*Standardised CI*
Direct effects on body size		
Maternal care (present)	**0.348**	0.280 – 0.423
Direct effects on lifespan		
Maternal care	-0.052	-0.130 – 0.004
Body size	**-0.187**	-0.256 – -0.138
Indirect effects on lifespan		
Maternal care via body size	**-0.065**	-0.081 – -0.040
Total effects on lifespan		
Maternal care	**-0.117**	-0.191 – -0.073
Observations	1914	

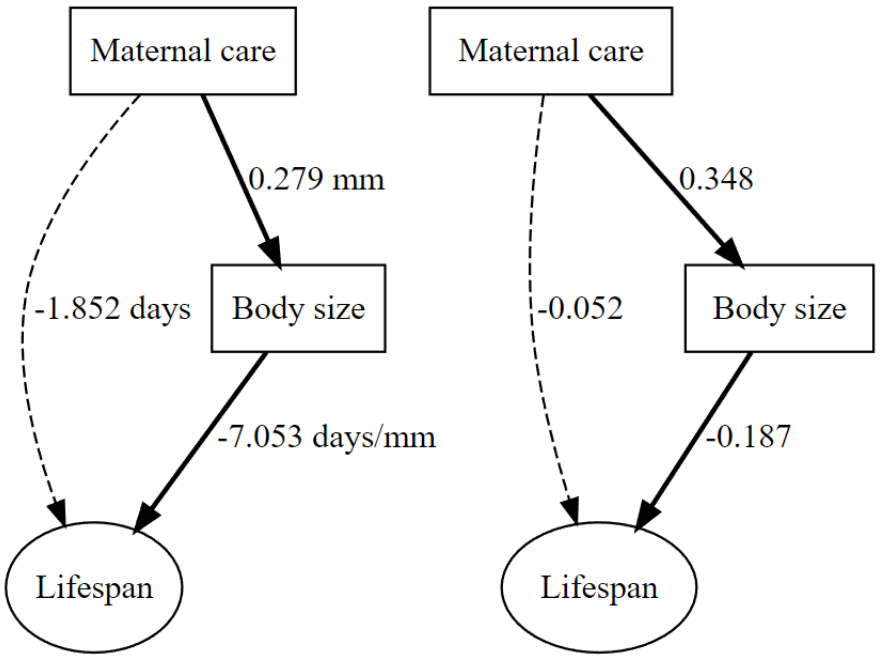

Figure 3-4:Path diagrams showing the direct and indirect effects of the receipt of parental care, body size (as measured by pronotum length), and mating status on lifespan. Statistically significant path coefficients are represented by solid arrows, non-significant paths are indicated by dashed arrows) Path diagram A presents unstandardised path coefficients in clear units (millimetres (mm), days, and days/mm). Path diagram B presents standardised path coefficients. Mediated effects (i.e., the effect of maternal care on lifespan via body size) are calculated as the product of path coefficients in the mediation chain. The standardised mediated path coefficient = -0.05306 [-.06716, -.03941]. The total effect of maternal care on lifespan is the sum of direct and mediated effects = -0.10302 [-0.18849, -.05864].

3.3.2 Reproduction

There was no evidence for an effect of age at reproduction on mating success, number of successfully dispersing larvae, or mean larval mass at dispersal (Table 4,5). There was no evidence for an effect of maternal care, nor for an interaction between receipt of maternal care and age at reproduction on any reproductive trait measured (Table 4,5). Finally, there was no strong signal for selective disappearance at the adult stage (accounted for by the inclusion of lifespan in all models) in any reproductive trait measured (Table 4,5). However, with respect to mating success, our results appear consistent, though tenuously so, with poor maters dying earlier than successful performers. Individuals that survived until at least 50 days old appeared to be more successful producing larvae than individuals that died prior to age 50 days old (Risk ratio = 2.346 [1.034, 5.324], z = 2.039, p = 0.041; Table 5). This effect size and width of confidence interval were echoed in individuals surviving post age 70 day, though was marginally non-significant (Risk Ratio = 2.388 [0.987, 5.781], z = 1.930, p = 0.054).

Table 3-4: ANOVAs of mixed-models exploring the effects of the receipt of maternal care and age at reproduction on reproductive traits.

	Mating success			**No. of dispersed larvae**			**Mean larval mass**		
Predictors	*χ2*	*df*	*p*	*χ2*	*df*	*p*	*χ2*	*df*	*p*
Age at reproduction	0.067	1	0.795	0.437	1	0.512	0.462	1	0.497
Maternal care (present)	2.942	1	0.086	0.200	1	0.718	0.504	1	0.478
Selective disappearance	4.457	2	0.108	0.317	2	0.700	0.857	2	0.652
Age at reproduction × Maternal care (present)	1.551	1	0.213	0.580	1	0.117	0.343	1	0.558
Observations	165			144			144		

Table 3-5: The effects of age and receipt of maternal care on reproductive ability, as measured by success in producing a brood (mating success), number of offspring surviving to dispersal, and mean mass of dispersing broods.

	Mating success				**No. of dispersed larvae**				**Mean larval mass**			
Predictors	*Risk Ratios*	*CI*	*z*	*p*	*Incidence Rate Ratios*	*CI*	*z*	*p*	*Estimates*	*CI*	*z*	*p*
Intercept	0.909	0.351 – 2.358	-0.195	0.845	14.383	8.312 – 24.889	9.529	**1.58e-21**	0.202	0.180 – 0.224	17.935	**<2.22e-16**
Age at reproduction	0.998	0.982 – 1.014	-0.259	0.795	0.998	0.991 – 1.005	-0.661	0.508	1.4e-4	-1.8e-4 – 4.5e-4	0.842	0.39976
Maternal care (present)	2.226	0.892 – 5.554	1.715	0.086	1.088	0.752 – 1.575	0.447	0.655	0.002	-0.017 – 0.020	0.177	0.860
Selective disappearance (death between ages 50-70 days)	2.346	1.034 – 5.324	2.039	**0.041**	1.154	0.700 – 1.903	0.561	0.575	-0.010	-0.034 – 0.014	-0.788	0.431
Selective disappearance (death post age 70 days)	2.388	0.987 – 5.781	1.930	0.054	1.139	0.669 – 1.941	0.480	0.631	-0.001	-0.034 – 0.014	-0.788	0.431
Age at reproduction × Maternal care (present)	0.987	0.967 – 1.007	-1.245	0.213	0.996	0.987 – 1.006	-0.762	0.446	-1.4e-04	-5.9e-4 – 3.2e-4	-0.586	0.558
Zero-Inflated Model												
Intercept					0.063	0.031 – 0.129	-7.551	**4.32e-14**				
Random Effects												
σ^2	1.645				0.305				0.019			
τ_{00}	0.040 $_{Block}$				0.001 $_{Block}$				6.81e-6 $_{Block}$			
	1.8e-8 $_{Family}$				4.10e-8 $_{Family}$				1.92e-5 $_{Family}$			
N	4 $_{Block}$				4 $_{Block}$				4 $_{Block}$			
	88 $_{Family}$				83 $_{Family}$				83 $_{Family}$			
Observations	165				144				144			

3.4 Discussion

3.4.1 Overview

The aim of this study was to investigate how maternal loss during development influences reproductive and actuarial ageing in a species with facultative maternal care, *N. vespilloides*. To that end, we established broods that either received post-hatching maternal care or lacked it. By recording the age at death of the resulting adults, we assessed age-specific mortality patterns. Furthermore, we investigated the connections between the presence/absence of maternal care, body size, and lifespan. Additionally, we evaluated the age-specific reproductive success of a subset of virgin females aged 12 to 93 days for each maternal care treatment.

Mortality from eclosion to sexual maturity was low among all beetles, but slightly lower among individuals that received post-hatching maternal care. After the onset of sexual maturation, individuals that developed with maternal care exhibited higher mean mortality and a slightly different pattern of age-specific mortality than individuals from our maternal loss treatment. In considering lifespan, our path analyses suggested that this maternal loss effect may be mediated via effects on body size. Individuals that received care were larger than their care-deprived counterparts, and larger beetles, in turn, had shorter lifespans (Figure 4). When accounting for this mediated effect, there was little direct association between the presence or absence of maternal care and lifespan. Finally, there were no apparent effects of maternal loss or age on reproduction in primiparous females.

3.4.2 Maternal loss, lifespan, and actuarial ageing

Parental care is expected to confer some fitness benefit to offspring, such that the loss or removal of care is predicted to incur pronounced costs to offspring (Royle *et al.*, 2012; Thesing *et al.*, 2015; Munch *et al.*, 2018). In line with this expectation, maternal loss in vertebrate systems has often been associated with reduced adult survival and lifespan (Andres *et al.*, 2013; Nakamura *et al.*, 2014; Tung *et al.*, 2016; Stanton *et al.*, 2020), though these studies do not assess rates of ageing. The long-term consequences of maternal loss in invertebrates have received relatively less attention (Thesing *et al.*, 2015). The present work is the first to explicitly tease apart how the effects of maternal loss manifest on ageing rates and lifespan.

The effects of body size on lifespan and ageing are mixed within studies of invertebrates (e.g., Hirose *et al.*, 2003; McCulloch & Gems, 2003; Khazaeli *et al.*, 2005). For example, there may be generally positive relationships between body size and lifespan in speckled cockroaches, *Nauphotea cineria*, and antler flies, *Protopiophila litigata*, while the relationship between body size and lifespan can vary among different strains and mutant of *D. melanogaster* or *C. elegans* (Khazaeli *et al.*, 2005; Angell *et al.*, 2020; Badwan & Harper, 2021a). Further, the interplay between early-life growth and somatic maintenance can underpin the trade-offs influencing aging and lifespan in many species (Inness & Metcalfe, 2008; Monaghan, 2008; Monaghan *et al.*, 2009; Lee *et al.*, 2011; Angell *et al.*, 2020).

Organisms must allocate resources to either fuel growth and reproduction or prioritize somatic maintenance and repair functions (Stearns, 1976; Kirkwood, 1977). Individuals developing under benign conditions (e.g., receiving maternal care) may have greater access to resources to invest in growth that either reduces the investment in somatic maintenance or elevates the cost associated with maintaining somatic functions. For example, in the neriid fly, *Telostylinus angusticollis*, high-condition, larger males were more susceptible to somatic damage from conspecifics or experimental mechanical intervention than smaller, low condition males (Adler *et al.*, 2016). Further, residual lifespan was negatively associated with the degree of somatic damage, meaning the fragility of high condition may have negated previously established survival benefits of increased body size (Adler *et al.*, 2016). Large body size may also come with increased energetic costs (Hooper *et al.*, 2017), either through general increased metabolic demands, or greater costs of locomotion and feeding (Kotiaho *et al.*, 1999; Basolo & Alcaraz, 2003; Kaufmann *et al.*, 2013). These metabolic costs, or increased costs of somatic maintenance may have contributed to the reduced lifespan of larger individuals.

This demographic analysis suggests that the observed lifespan effect was primarily driven by an age-independent increase in mortality rather than a change in the rate of ageing. However, it should be noted that individuals who received care exhibited generally lower ageing rates in early adulthood compared to those who did not (Fig 3B). It is possible we simply did not have had the power to detect statistically significant changes in ageing rates at the stringent 99.69% confidence level of our post-hoc test. Nevertheless, these reduced rates of ageing align with a convergence in mortality levels between maternal care backgrounds in later life (Figure 3C). This trend may be indicative of diminishing power to detect differences between our experimental groups but is also consistent with a diminishing survival advantage of small body size among those without maternal care. Further, this trend is consistent with a signal of selective disappearance at the juvenile stage. In *N. vespilloides*, the presence of maternal care can reduce the rate of total brood failure (all hatchlings dying before dispersal) and improve overall brood survival to dispersal (Pilakouta *et al.*, 2015; Capodeanu-Nägler *et al.*, 2016; Grew *et al.*, 2019). The patterns of age-specific mortality we described may, in part, be an outcome of the non-random loss of low-quality individuals at the juvenile stage (Chen & Maklakov, 2012; Chen *et al.*, 2013). Lower adult mortality (Figure 3C) among uncared-for individuals may have reflected the trend of a high-quality subset of surviving individuals exposed to the maternal loss treatment. A convergence in overall mortality rates later in life may then be the result of the progressive removal in early adulthood of lower-quality individuals in the maternal care treatment that were buffered from environmental hardship and survived to eclosion. In the maternal loss cohort, low-quality individuals may have been removed as larvae or sexually immature adults. In the maternal care cohort, mothers may have buffered these low-quality individuals, increasing survival to eclosion and delaying death until early adulthood.

Actuarial ageing is often described by the rate exponential increase in age-specific mortality rates with age (e.g., Gompertz ageing; Ricklefs & Scheuerlein, 2001, 2002; Gaillard

et al., 2004; Kirkwood, 2015; Ronget & Gaillard, 2020). However, the occurrence of a decelerating force of mortality, or mortality plateaus, at advanced ages is a common trend observed across various taxa (Carey *et al.*, 1992; Charlesworth & Partridge, 1997; Khazaeli *et al.*, 1998; Vaupel *et al.*, 1998). The cause for these late-life plateaus is a lingering question in ageing research with two non-exclusive mechanisms commonly offered to explain this phenomenon: population heterogeneity (Vaupel & Yashin, 1985) and individual risk (Curtsinger *et al.*, 1992; Horiuchi & Wilmoth, 1998).

Individuals in a population may differ in their robustness or frailty – their intrinsic baseline mortality risk or rate of increase with age (Vaupel & Yashin, 1985; Chen *et al.*, 2013). Late-life mortality plateaus, or even declines, may result from the removal of frail (relatively higher mortality risk) individuals at early ages, leading to mortality rates in later life reflecting those of a surviving subset of robust (relatively lower mortality risk) individuals (Vaupel & Yashin, 1985). Population-level trends in mortality in this sense then reflect changing ratios between frail and robust individuals (Vaupel & Yashin, 1985; McDonald *et al.*, 1996). The individual-risk theory suggests that mortality deceleration occurs at the individual level (Carey *et al.*, 1992; Curtsinger *et al.*, 1992; Horiuchi & Wilmoth, 1998). This may arise if investment in somatic repair at old ages can compensate for previously accrued damage (Carey *et al.*, 1992) or if the' rate of living' – activity levels, investment in reproduction or metabolic rate – declines with age, and the rate of ageing follows suit (Horiuchi & Wilmoth, 1998).

As the outcomes of population heterogeneity and decelerating individual risk are largely indistinguishable in the general case, our results are consistent with either. We identified at least one environmental factor (maternal care), and possibly body size, which could contribute to inter-individual differences in baseline mortality. For example, were we to consider our experimental population as a whole, failing to account for maternal care background (and by proxy, body size) this source of heterogeneity would lead to an under-estimation of the rate of ageing. However, there may be many sources of such heterogeneity, and we considered only a limited number; one cannot know if a factor contributes to cohort-level effects unless one measures that factor. Further, we likely did not have the power to assess more cohorts or classes of individual with respect to population heterogeneity. Previous work testing the contribution of population heterogeneity to mortality plateaus have achieved mixed results (Kirkwood, 2015) using sample sizes orders of magnitude greater than those presented here (Khazaeli *et al.*, 1998; Wu *et al.*, 2006). Obvious candidates for sources of individual heterogeneity that may be assessed in the laboratory are natural variation in body size, basal metabolic rate, immune function or inbreeding status (Keller *et al.*, 2008; Hamel *et al.*, 2017; Froy *et al.*, 2019).

We described striking differences in the age of onset of ageing, age-specific rates of ageing, and presence of late-life mortality plateaus all varied across experimental blocks. This was unexpected, as each block of the experiment was conducted under relatively constant conditions (temperature, light/dark cycle, and food source). Given the apparent homogeneity of laboratory conditions, the source of this variation is unclear. Recent work in the springtail, *Folsomia candida*, characterised age-specific trends in mortality using a broadly similar

statistical approach to those described here, finding similar striking differences in pattern between two genetically distinct clades of their study species (Tully, 2023). This work found that both genetic background and food availability during adulthood contributed to diverging trajectories of actuarial ageing (Tully, 2023). As our work was conducted over two consecutive years, it is possible that the genetic characteristics of our experimental population varied over time, contributing to the patterns we observed. This possibility would appear to be supported by proximate blocks (which may be comprised of more closely-related or genetically similar individuals) adhering to similar parametric functions of ageing (blocks one and two: Gompertz; blocks four and five: Log-logistic; Table 1). Rueppel *et al.* (2007) suggested that the overall trajectory (i.e., Gompertz or Logistic functions) of ageing in honey bees, *Apis mellifera*, may depend on transitions between hive tasks and foraging activity, suggesting different age-dependent patterns of activity or somatic damage may play a role. However, ageing is a highly labile trait that can be sensitive to even minor perturbations in environmental conditions (Partridge & Gems, 2007; Flatt *et al.*, 2013), so there may be myriad sources of this variation that we have not accounted for. It is also possible that ageing trajectories in more complicated environments (e.g., natural populations) may be more sensitive to these kinds of effects. For example, environmental conditions change more dramatically between seasons and years in the wild than in the laboratory. Much attention has been given to how ageing functions (e.g., Gompertz, Weibull etc.) may differ between species (Ronget *et al.*, 2020; Ronget & Gaillard, 2020), their implications for the biological causes of demographic aging (Ricklefs & Scheuerlein, 2002). However, it appears less attention has been paid to how environmental effects may shape underlying patterns of ageing (e.g., Figure 1B) within species (but see: (Barks *et al.*, 2018; Pietrzak *et al.*, 2020; Tully, 2023). Our analyses suggest that variation in ageing patterns may be at once greater and more ephemeral that than described in recent studies (Jones *et al.*, 2014; Ronget *et al.*, 2020). For natural populations for which appropriate data - reasonable coverage of known dates of birth and death – are available, it may be feasible to re-analyse age-specific survival trajectories with the current GAMM approach. This could help evaluate whether general age-specific trends in mortality, or any phenotypic trait, are relatively robust with respect to temporal or environmental variation, or if patterns of ageing are more plastic than indicated here.

3.4.3 Maternal loss, age, and reproduction

Larger *N. vespilloides* adults enjoy intra-sexual competitive advantages in breeding resource contests (Lee *et al.*, 2014; Hopwood *et al.*, 2016a) and positive outcomes for several non-competitive aspects of reproduction (Steiger, 2013; Richardson & Smiseth, 2019; Bladon *et al.*, 2020). However, in our reproduction assessment that excluded competitive factors, a mothers own maternal care background had no discernible impact on their subsequent reproductive performance. While individuals with greater access to food during development tend to be larger and lay larger eggs (Steiger, 2013; Richardson & Smiseth, 2019), the benefits of egg size to offspring growth can be mitigated in the presence of maternal care (Monteith

et al., 2012). Our assays of reproductive performance involved maternal care, which would likely mask body- or egg-size effect on larval performance. Previous work has also suggested that resources acquired during development may be less important to number of offspring produced than resources acquired at the start of breeding (i.e., the carcass: Steiger, 2013; Richardson & Smiseth, 2019). This is consistent with our observation that number of offspring surviving to dispersal did not differ between maternal care backgrounds. In a cross-fostering experiment, Steiger (2013) found that, regardless of the size of biological mothers, large foster mothers raised larger offspring than small foster mothers, an effect not echoed in our case. However, the previous study induced exaggerated differences in body size by manipulating early-life food availability (Steiger, 2013), while in our case, differences in body size arose from individuals experiencing similar nutritional environments (as in: Attisano & Kilner, 2015) but varying in maternal presence. This distinction in the source of body size variation, as well as less pronounced differences between experimental groups, might account for the disparities observed in the influence of females' body size on offspring growth (Crosland *et al.*, 2022).

In many species, advancing maternal age is associated with decreased fertility and increasingly negative outcomes for offspring fitness or life-history (Fox *et al.*, 2003; Schroeder *et al.*, 2015; Ivimey-Cook & Moorad, 2020). Nevertheless, previous work in Nicrophorus sp. has yielded only mixed support for these ageing-related declines (Trumbo, 2009; Cotter *et al.*, 2011; Ivimey-Cook & Moorad, 2020; Cope *et al.*, 2022) and we observed no such declines here. Of the reproductive traits considered here, an absence of an effect of age is consistent with prior work in this species (Cotter *et al.*, 2011; Ivimey-Cook & Moorad, 2018; Cope *et al.*, 2022). Further, we accounted for selective disappearance – the association between trait values, or performance, and lifespan – to assess the true rate of ageing in reproductive traits (similar to: Ivimey-Cook & Moorad, 2018; Cope *et al.*, 2022). The weak trend of poor performers (in terms of mating success) dying earlier that we described would act to disguise the apparent effects of advancing age as assessed by population or cohort-level assessments of ageing. Those surviving to be assayed at late ages would be expected to be above-average performers. While we accounted for, and found no signal of, selective disappearance in adulthood on adult reproductive traits, we could not account for disappearance at the juvenile stage that may have affected adult patterns of reproduction or mortality. The developmental trajectory of *N. vespilloides* makes it difficult to account for individual survival across several stages of development. Hatchlings grow an order of magnitude in size in days, while consuming the interior of a breeding resource, followed by pupation buried in soil (Scott, 1998). Accounting for individual progression between each stage, and subsequent adult performance is often unfeasible. However, it would be possible to account for overall differences in early life mortality between maternal care backgrounds to outline the potential for selective disappearance at the juvenile stage to affect adult performance. The current study used natural broods that varied in number of larvae, obscuring the juvenile mortality rate. Establishing mixed broods, of known size, is a common practice in *N. vespilloides* studies (e.g., Ivimey-Cook & Moorad, 2018; Cope *et al.*, 2022); differences in potential for selective disappearance between treatments could therefore be determined from the proportion of

successfully eclosing adults from each early-life background. Future work that addresses stage-specific selective disappearance on reproductive ageing may be important to fully describe the fitness costs of maternal loss.

The absence of competitive influences may explain, in part, the limited effects of maternal care background or age on reproduction we described. The threshold for reproductive performance in the laboratory is, presumably, artificially low – lacking the search costs associated with finding carcasses, environmental variation, or biotic challenge. In the absence of direct intrasexual competition, carcass resources are perhaps sufficient to produce offspring, masking many effects of maternal quality. Previous work in *N. orbicollis* has suggested that aspects of competitive behaviour may improve with age, potentially because a reproductive event holds increased value for older primiparous females compared to their younger counterparts (Trumbo, 2009). In *N. vespilloides*, Lee *et al.* (2014) found no evidence of age affecting competitive success between two individuals newly introduced to a carcass. These studies have similar limitations, as they compared two distinct age groups, and these groups may not have included individuals old enough to capture the expected declines associated with ageing (Trumbo, 2009; Lee *et al.*, 2014). Where only two age classes are considered, only linear effects of age can be estimated, whereas true ageing functions may be much more complicated. Further, the oldest age classes in either case fell before the mean lifespan typical in either species (*N. orbicollis*: 110 days in virgins – Smith *et al.*, 2015; *N. vespilloides*: 50 days in virgins – current study). Assessing competitive ability across a range of ages representing the extremes of typical lifespan, where the effects of ageing may be most pronounced, as in our exploration of reproductive traits in this study (ages 12 – 93 days), would provide us insight into how age effects may manifest in a trait important to fitness.

The fitness cost of small body size may also vary across environments. Laboratory work has emphasized the importance of body size in determining reproductive success via improving competitive ability (Otronen, 1988; Hopwood *et al.*, 2013; Lee *et al.*, 2014). The present study suggests maternal care confers a conspicuous body size benefit to offspring. However, in the wild, smaller males may be more successful in attracting females and securing monogamous breeding associations than larger males (Hopwood *et al.*, 2016a). Small males may avoid intrasexual competition, thus mitigating the apparent disadvantage of smaller size (Hopwood *et al.*, 2016b). This suggests that the outcomes of maternal presence or absence may be nuanced across environments. Exploring the effects of age and individual quality, potentially originating from early life conditions, on performance across different environments would contribute to a better understanding of their roles.

3.4.4 Conclusion

Our study presents the first large-scale demographic analysis of actuarial ageing in the burying beetle, *N. vespilloides*. Further, our results suggest that even where adult conditions are shared, interventions in early life (maternal presence or absence) can have long term consequences on age-specific patterns of mortality. Our work highlights and echoes the

findings of previous studies in this system, in which the effects of ageing on reproductive traits appears minimal or absent. Future work may consider pre-reproductive behaviours (e.g., flight; Chapter 2) or competition in exploring the wider fitness consequences of effects mediated by early life conditions or age. Finally, trends apparent in the laboratory may not offer a holistic picture of outcomes in natural settings (Hopwood *et al.*, 2016b; Zajitschek *et al.*, 2020). For robust predictions of the consequences of variation in individual quality and ageing, these trends must be explored in wild populations.

4 Ageing across laboratory and natural environments

4.1 Introduction

The gap between studies conducted in laboratory settings vs those in natural populations is an ongoing concern in evolutionary ecology (Matos *et al.*, 2000; Reichard, 2016; Maclean *et al.*, 2018). Studies conducted in the lab assume that patterns or responses identified in the laboratory are representative of those found in natural populations (Briga & Verhulst, 2015; Reichard, 2016; Zajitschek *et al.*, 2020). However, there are several reasons to question this assumption. First, there is a taxonomic bias in what kinds of animals get studied in different environmental contexts. Studies of wild populations often focus on large, long-lived vertebrates or birds (Nussey *et al.*, 2013; Dochtermann *et al.*, 2019), while laboratory-based studies involve insects and short-lived vertebrates (Partridge, 2010; Gems & Partridge, 2013; Promislow *et al.*, 2022), and it may be difficult to generalize life-history patterns and mechanisms across both taxa and environment (Harshman & Hoffmann, 2000; Vepsäläinen & Spence, 2000; Zajitschek *et al.*, 2020). From the most basic level, differences in sensitivity to environmental change of ectotherms versus endotherms (Burraco *et al.*, 2020; Reinke *et al.*, 2022), differences in reproductive scheduling, physiology, diapause (Guo *et al.*, 2020), and selection on late-life performance on long lived, large animals versus short-lived, small animals make such comparisons problematic (Briga & Verhulst, 2015; Zajitschek *et al.*, 2020).

Second, the species most often studied in the laboratory might not be good representatives of their respective taxonomic groups (Griffith *et al.*, 2021). Characteristics typically associated with laboratory models – short generation time, high fecundity, and ease of manipulation – might distinguish these models from even closely related species (Minelli & Baedke, 2014; Griffith *et al.*, 2021). For example, model systems such as the zebra finch, *Taeniopygia guttata*, and the house mouse, *Mus musculus*, may be outliers among their genera or broader taxa due to breeding and phenological traits or adaptations associated with commensalism that may set them apart from other passerine birds or rodents (Phifer-Rixey & Nachman, 2015; Griffith *et al.*, 2021).

Third, the populations maintained in the laboratory can be genetically distinct from natural populations. Laboratory populations may be derived from small number of founding individuals and maintained at low effective population sizes, resulting in genetic drift and inbreeding (Lohr *et al.*, 2014; Maclean *et al.*, 2018). Further, artificial selection, imposed intentionally or otherwise in the laboratory, may exacerbate phenotypic and genetic differences between natural and laboratory-maintained populations (Sgrò *et al.*, 2000; Matos & Avelar, 2001; Gasch *et al.*, 2016).

Finally, even among genetically similar populations, phenotypic or behavioural plasticity can result in qualitatively different responses to stimuli across environmental contexts (Flatt *et al.*, 2013; Briga & Verhulst, 2015; Zajitschek *et al.*, 2020). For example, strains

of the house mouse, *M. musculus*, that are resistant to nematode infections under laboratory conditions may be highly susceptible to infection in natural environments (Leung *et al.*, 2018). Similarly, long-lived mutants that are successful in laboratories may struggle to compete with wild-type conspecifics in natural settings (Briga & Verhulst, 2015).

All of these concerns are applicable to studies of ageing - the progressive deterioration of physiological function with increasing age – which manifests as age-related increases in mortality (actuarial ageing) and declines in reproductive or functional performance (reproductive or functional ageing, respectively; Rose, 1994). The evolutionary theory of ageing maintains that the strength of natural selection on mortality and fecundity declines after sexual maturation and with advancing age (Medawar, 1952; Hamilton, 1966). Consequently, there exists a "selection shadow" at later ages, where selection is less efficient at maintaining survival, reproduction, and somatic repair (Medawar, 1952; Williams, 1957; Kirkwood, 1977; Fabian & Flatt, 2011). While functional declines have received less attention in theoretical and empirical studies than demographic measures (Grotewiel *et al.*, 2005; Burger & Promislow, 2006), this selection shadow is believed to manifest on functional traits, albeit to a lesser degree (Moorad & Ravindran, 2022).

Ageing is a highly plastic trait, sensitive to minor changes in proximate or early-life environmental conditions (Kawasaki *et al.*, 2008; Monaghan *et al.*, 2008; Flatt, 2014). Laboratory studies, which form the body of empirical work on the mechanisms underpinning ageing, often deliberately minimize or homogenize environmental influences on mortality and ageing to focus on intrinsic aspects of ageing (i.e., deteriorative changes that cause mortality regardless of environment; Williams *et al.*, 2006; Zajitschek *et al.*, 2009a; Roach, 2012). This reductionist approach may reduce the generalizability of trends discovered in the laboratory to more natural contexts. For example, the removal of some external sources of mortality (e.g., predation) in laboratory settings might interact with individual condition or age (i.e., condition- or density-dependent effects), and this may lead to underestimating the role of individual quality in shaping ageing rates (Zajitschek *et al.*, 2009a, 2020; Roach, 2012; Hämäläinen *et al.*, 2014). Similarly, mutations or interventions that prolong lifespan or slow aging under benign laboratory conditions may have lesser, or even opposite, effects on survival in more challenging natural environments (Briga & Verhulst, 2015; Zajitschek *et al.*, 2023). Moreover, ageing patterns vary significantly among different species, and the taxonomic disconnect between species commonly studied in laboratories (i.e., insects) and those studied in the wild (i.e., mammals and birds) further complicates generalizations across both taxonomic and environmental divides (Mautz *et al.*, 2019). Recent work has begun to investigate patterns and drivers of ageing in natural insect populations, addressing, in part, this taxonomic bias (Zajitschek *et al.*, 2009a; Sherratt *et al.*, 2010; Carroll & Sherratt, 2017; Mautz *et al.*, 2019; Rodríguez-Muñoz *et al.*, 2019a; b). However, the effect of broad shifts in environment on ageing, from the laboratory to the wild, has remained largely unexplored (Kawasaki *et al.*, 2008; Mautz *et al.*, 2019; Zajitschek *et al.*, 2020).

Only two studies have directly addressed the question of plasticity in ageing across laboratory and natural environments in insects by comparing genetically similar populations of insects across both environmental contexts. Kawasaki *et al.* (2008) compared actuarial ageing and lifespan in wild and captive cohorts of a population of neriid flies, *Teleostylinus angusticollis*, using mark-release-recapture methods. This study found that wild-living males exhibited greater rates of both overall mortality and actuarial ageing than captive males. However, while wild-living females also exhibited higher mean levels of mortality than captive counterparts, mortality was only observed to increase significantly with age in the captive cohort. Higher overall mortality rates in wild-living females than wild-living males may have eroded power to detect ageing (Kawasaki *et al.*, 2008). Alternatively, theory predicts that sexual selection can shape optimal sex-specific patterns in investment into reproductive traits and somatic maintenance, often favouring more rapid ageing in males than females (reviewed in: Bonduriansky *et al.*, 2008). Greater apparent ageing in wild-living males than females may have reflected greater investment into intra-sexual competition and sexually-selected traits at the expense of somatic maintenance and late-life survival (Vinogradov, 1998; Bonduriansky *et al.*, 2008; Hooper *et al.*, 2017). In any case, ageing rates appeared greater in laboratory-living females than wild-living females.

More recently, the role of diet in actuarial and reproductive ageing was investigated in wild and captive antler flies, *Protopiophila litigata*, representing the first experiment in insects to consider the effect of a dietary manipulation across laboratory and wild contexts (Mautz *et al.*, 2019). While there were some spatial and dietary inconsistencies between replicates of this study, the general trend favoured higher mortality in the wild than in the laboratory, but the rate of actuarial ageing was either lower or similar in the wild than the laboratory (Mautz *et al.*, 2019). This apparent lessening of the rate of ageing in the wild compared to the lab might reflect selective disappearance, wherein low-quality individuals experience higher mean levels of mortality than high-quality individuals, leading to high-quality individuals with greater survival increasing in proportion in later ages (Vaupel & Yashin, 1985; Chen *et al.*, 2013; Mautz *et al.*, 2019). This pattern of selective disappearance acts to reduce apparent population-level ageing rates, masking the true effect of ageing in the wild population (Vaupel & Yashin, 1985; Nussey *et al.*, 2008; Fay *et al.*, 2018; Hamel *et al.*, 2018). Further, selective disappearance of this manner may have contributed to masking apparent ageing rates in wild females of *T. angusticollis* (Kawasaki *et al.*, 2008).

Both studies relied on the continuous release of marked, lab-born individuals to be tracked in the wild. However, this approach fails to account for possible early-life effects experienced in the lab that might shape patterns of ageing differently from wild populations. For example, in *P. litigata*, the quality of early life diet can accelerate or delay development, which can in turn increase or slow the rate of ageing, respectively (Angell *et al.*, 2020). Further, these study systems involved highly philopatric species, both forming mating aggregations on discrete media – rotting tree trunks in the case of *T. angusticollis* (Bonduriansky, 2006; Kawasaki *et al.*, 2008) and discarded moose and deer antlers in the case of *P. litigata* (Bonduriansky, 1995; Mautz *et al.*, 2019). Many studies that consider ageing only in wild

insects are similarly conducted in philopatric or geographically restricted populations (e.g., Bonduriansky & Brassil, 2002; Zajitschek *et al.*, 2009a; Carroll & Sherratt, 2017; Rodríguez-Muñoz *et al.*, 2019a; b; Sielezniew *et al.*, 2020; Pásztor *et al.*, 2022). Patterns of ageing can vary at very small geographic scales, perhaps in response to environmental quality or shifts in the trade-offs underpinning ageing (Tully *et al.*, 2020). Populations studied at small scales may be subject to relatively homogenous conditions owing to exposure to similar micro-habitat or micro-climate conditions over time (Tully *et al.*, 2020); this may underestimate the true scale of variability in ageing under natural conditions, particularly in philopatric systems. Less is known about ageing and ageing in natural populations of highly dispersive or motile insects (Zajitschek *et al.*, 2020). Trade-offs between investment in dispersal and somatic maintenance or condition-dependent selection related to motility or migration may be more relevant here than in philopatric or sedentary species (Zajitschek *et al.*, 2020). There is a need to develop more highly mobile invertebrate study systems that can be studied at relatively greater spatial scales, so as to incorporate the effects of greater environmental variation.

This study makes the first attempt to characterise age-specific changes in mortality in wild-living populations of the burying beetle, *Nicrophorus vespilloides*, using mark-recapture methods. More generally, this is also the first attempt to study ageing in a natural population of a highly dispersive insect species. Our study differs from previous work by sampling wild-living populations of a highly dispersive species (Attisano & Kilner, 2015; Chapter 2) at relatively greater spatial scales. Further, we assessed ageing in laboratory- and wild-born individuals (of known and estimated age, respectively) living in the wild, as well as in laboratory-born populations maintained in a standard laboratory environment. This allowed us to assess whether initial exposure of laboratory-born cohorts to benign conditions had long-term effects on condition or performance in the wild, relative to wild-born cohorts. Mean lifespan may be expected to be shorter, and baseline mortality greater, in wild-living populations due to greater exposure to environmental challenges, competition, predation, and disease (Kawasaki *et al.*, 2008; Tidière *et al.*, 2016). However, some species perform poorly in captivity, exhibiting reduced lifespans, or overall poorer condition, perhaps due to inadequate diet, housing, or social environments (reviewed in: Mason, 2010; Tidière *et al.*, 2016). Further, ageing rates in wild populations may be greater than in captive populations, due to increased rate of accumulation of somatic damage. Wild-living populations are likely exposed to a greater array of harms (environmental stress, predation, competition, disease) than laboratory-maintained populations, resulting in more rapid somatic deterioration and ageing rates (Kawasaki *et al.*, 2008). Alternatively, ageing rates may be reduced, due to stronger condition-dependent mortality in natural settings selecting for slower rates of ageing (i.e., selective disappearance; Vaupel & Yashin, 1985; Chen & Maklakov, 2012; Chen *et al.*, 2013; Fay *et al.*, 2018). Greater heterogeneity in individual quality or condition in the wild versus the laboratory may act to appear to increase early mortality and supress ageing rates (Brunet-Rossinni & Austad, 2005). We aimed to assess patterns of ageing among wild-living

populations of *N. vespilloides* and how these patterns in mortality differed from those of laboratory-maintained populations.

A secondary aim of this study was to characterise other key population dynamics of natural populations of *N. vespilloides* as well as age-specific declines in body mass in wild-living individuals. Records on many wild insect species are sparse (Montgomery *et al.*, 2020), with little known about population ecology or dynamics (Zajitschek *et al.*, 2020) or within-individual phenotypic changes with age in wild-living populations (Zajitschek *et al.*, 2020; Pásztor *et al.*, 2022). This is concerning, as the ecology of even well-developed laboratory models are often poorly understood (e.g., Félix & Braendle, 2010; Alfred & Baldwin, 2015; Parichy, 2015). Population characteristics such as population size or sex ratio may fluctuate within and between years. This may have consequences for levels of intra-sexual and intra-specific competition, and ageing rates (Mysterud *et al.*, 2005; Adler & Bonduriansky, 2011; Rodríguez-Muñoz *et al.*, 2019a). Typical laboratory methods of population maintenance and husbandry for *N. vespilloides* and other species often fail to account for such population (as described in: Smiseth *et al.*, 2006; Reavey *et al.*, 2014; Gray *et al.*, 2018; Lambert & Smiseth, 2024). Rather, population dynamics inherently simulated in laboratory populations (i.e., equal sex ratio, low population sizes) likely differ from those of wild populations. Further, functional or somatic declines (i.e., performance in some trait, or body condition) may underpin patterns of mortality, and assessing such declines may shed light on variation in life-history strategies within populations (Réale *et al.*, 2010; Reichard, 2016; Fisher *et al.*, 2018). Body mass is an indicator of general condition in many invertebrate species, and it is positively correlated with flight ability, immune response, cold tolerance, and over-winter survival (Stoehr, 2007; Boggs, 2009; Halle *et al.*, 2015; Knapp & Řeřicha, 2020). Among *Nicrophorus* spp., increased body mass can have positive effects upon competitive performance and aspects of reproduction and flight in the laboratory (Merrick & Smith, 2004; Hopwood *et al.*, 2013; Richardson & Smiseth, 2019) that may also be relevant to patterns of ageing in the wild. Through our mark-recapture efforts, we collected supplementary data –longitudinal changes in body mass in multiply-encountered individuals, general occurrence data, and relative abundances of males and females. This enabled us to estimate age-dependent changes in body mass and temporal or spatial variation in population size and sex ratio to gain important ecological and demographic insights into wild-living populations and to contextualise our estimates of actuarial ageing in wild-living cohorts.

4.2 Methods

4.2.1 Study species

This study used individuals sourced from two natural populations of *N. vespilloides*. Burying beetles are small-bodied, highly motile insects that breed and feed on small carrion (Scott, 1998), and they are particularly suitable for mark-recapture studies that aim to gain longitudinal data on individuals (Zajitschek *et al.*, 2020). Nicrophorinae locate carrion with

chemoreceptors on their antennae (Ernst, 1972), which are sensitive enough to detect a carcass within one day of placement or death (Smith & Heese, 1995) from several kilometres away (Petruska, 1975). Therefore, populations can be sampled over a large area using traps baited with carrion with relatively little difficulty. Further, *N. vespilloides* have conspicuous orange patterns on their elytra that are unique to individuals (J. Moorad, pers. comm.; Chapter 5). Similar patterns are present in the closely related species *N. orbicollis* and *N. americanus* and can serve as "fingerprints" with which to identify individuals in a population (Quinby *et al.*, 2021). These patterns have been used in a short-term mark-recapture study (several days) to estimate population abundances in this species (Quinby *et al.*, 2021; methods described below) but not, until now, to facilitate longer term efforts focussed on tracking individuals over several months. Based on unique natural patterns, after an initial encounter or release, individuals can be identified over time and space with high fidelity. Previous attempts to conduct mark-recapture studies in *Nicrophorus* spp. have largely relied on applying various permanent and temporary markings that were either could not resolve individual identity (Trumbo & Thomas, 2018; Kim *et al.*, 2020), were suitable only for short-terms studies (Butler *et al.*, 2012), or had negative effects on individual behaviour and survival (Butler *et al.*, 2012; Hall *et al.*, 2015; Jenkins *et al.*, 2016). Relying on natural elytral patterns reduces the risk of harm to individuals due to marking and is robust to a high degree of environmentally-acquired cosmetic damage.

4.2.2 Field sites

Our study area was located in Edinburgh, Scotland, United Kingdom, and encompasses observations of individual burying beetles from two wild populations located in the Hermitage of Braid and Blackford Hill (c. 31 hectares; 55° 55' 26.25"N, 3° 11' 38.76"W), and Corstorphine Hill (c. 42 hectares; 55° 56' 51.66" N, 3° 15' 49.83"W) Local Nature Reserves (Figure 1). Both sites are characterised by semi-natural woodlands and areas of scrubland and grassland. In addition, we measured lifespan in a laboratory-maintained population that was derived from the natural Blackford Hill population. Genetic diversity following founding has been maintained by annual additions of wild beetles gathered from the Blackford Hill field site. Our assessments of ageing in wild-living and laboratory-maintained populations were conducted concurrently over two field seasons: 2021 and 2022. Data was collected in the laboratory and Blackford Hill field site in both years, but the Corstorphine Hill population was only sampled in 2022.

Figure 4-1: Outline of field sites and locations of traps in 2021 and 2022. Sampling was only conducted in Corstorphine in 2022. Black dots denote trap locations. Solid light-green regions denote woodlands, grasslands, and meadows encompassed within Local Nature Reserves. Striped regions indicate managed areas associated with golf courses. Waterways are in blue. Regions in white include buildings and other infrastructure, or urban/sub-urban areas outwith the Local Nature Reserves. The two field sites are approximately five kilometres apart.

4.2.3 Field sampling

Previous studies and citizen science-sourced occurrence data suggests that *N. vespilloides* are active in the wild from late-April to early-November (Easton, 1979; Sun *et al.*, 2020; National Biodiversity Network, 2024). We therefore focused on conducting sampling sessions at weekly intervals (N = 27 sampling sessions per site) between early May and late October.

Hanging funnel traps (Uni-Trap, International Pheromone Systems; Japanese beetle trap, Trécé Inc.) were suspended in trees and bushes approximately three feet above the ground. The number of trapping locations per site differed between years (2021: 14 trap locations in Blackford Hill; 2022: 16 trap locations per field site). Trap placement considered vegetation density and distance from frequently visited areas within the reserves. In Blackford Hill, traps were placed along either side of a wooded gorge, with a mean distance between consecutive traps of 78.81 meters (ranging from 35.52 to 144.92 meters) in 2021 and 66.91m (range: 31.75m – 155.16m) in 2022. Corstorphine Hill Nature Reserve afforded more uniform trap placement due to larger expanses of wooded areas removed from paths or human activity, resulting in a more linear transect of traps with a mean spacing of 43.64 meters (range: 34.20m – 53.37m; Figure 1).

Adult *N. vespilloides* can differ in their preference for carrion at different stages of decomposition (von Hoermann *et al.*, 2013). Therefore, traps were baited with small fillets of salmon or chicken or mouse carcasses that became putrescent over time. Across much of the field season, different traps contained bait at varying levels of decay, providing some variation for individual preferences. We monitored traps weekly, replacing bait that was infested with blowfly larvae or was otherwise consumed or desiccated, and checking for occupancy.

Encountered beetles were placed in large 50ml screw cap tubes (Sarstedt, UK) and transferred to the laboratory for assessment. Individuals were held overnight, and the location of encounter was noted, as well as sex and body mass. Pronotum width, a proxy of body size (Müller *et al.*, 1990; Creighton, 2005; Richardson *et al.*, 2020), was recorded using a digital calliper (CD-6" CSX, Mitutoyo Corp.; accuracy: +/-0.02mm).

4.2.4 Identifying individuals

In 2021, individuals' elytral patterns were imaged under standard laboratory lighting (approximately 3000K – 4000K fluorescent bulbs) using a tripod-mounted digital single lens reflex camera (DSLR Canon EOS 500D) in a fixed orientation approximately perpendicular to the subject. In 2022, more rigorous photography methods were employed to improve image quality to increase the efficiency of identifying individuals (Chapter 5) and extract data on colouration for future analyses. Images were taken under fixed lighting (5500 Kelvin, 1000 lumen, LED panels), focal length (60mm) and approximate orientation (perpendicular to subject). Images were further colour-standardised against a grey card (SpyderCheckr 24, Datacolor UK Ltd) in the open-source image-processing program *darktable* (v4.6.1), which corrects for the temperature of ambient lighting that may change between recording sessions. To identify recaptures, images of a given individual's elytral pattern were compared to the entire preceding photographic record, and any matching patterns were noted (see Chapter 5 for a detailed explanation of the identification methodology). For example, an individual captured on the second sampling occasion was compared only to those present in the first sampling occasion, while an individual captured on the tenth occasion was compared to all nine preceding occasions.

Once processing was complete, individuals were released back into the nature reserve the day following capture between 5 and 10 metres from the previous trapping location.

4.2.5 Laboratory component

To generate our laboratory stock beetles, we randomly paired unrelated virgin males and females in large, transparent plastic containers (26 cm x 17 cm x 12 cm) containing a bed of moist soil and a freshly thawed mouse carcass (Livefoods Direct Ltd). Parents laid eggs and provided care to offspring for approximately ten days until larval dispersal. Offspring were then removed to a large box filled with moist soil to pupate for approximately 21 days, after which eclosing adults were collected and maintained under standard laboratory conditions.

All individuals housed in the lab, either permanently or temporarily, were kept under 16:8 light:dark conditions and at 21 °C, housed individually in plastic containers (twelve cm × eight cm × two cm) filled with moist soil, and fed organic beef twice a week for the duration of their stay (i.e., until release into the wild or death).

Shortly after emerging as adults, laboratory-born individuals were divided at random into groups that were maintained in the laboratory and those that were released into the Blackford Hill field site. Pronotum width and body mass was recorded for all individuals, and elytral patterns imaged as described above in respective years. Laboratory-born, wild-living individuals were randomly assigned to a trap (1-14 in 2021; 1-16 in 2022) for release and were introduced to the wild alongside wild-encountered beetles captured that week. All laboratory-born individuals were descended from Blackford Hill beetles, these were not released into the Corstorphine field-site to maintain the natural rates of gene-flow between the two wild-living populations. In 2021, a total of 167 laboratory-born individuals were released in three waves between the 17th June and the 21st August, with 141 individuals retained in the laboratory to supplement our attempts to assess actuarial ageing. In 2022, 349 individuals were released in nine waves between the 20th May and the 18th August (Table 1), with 325 individuals retained in the laboratory.

4.2.6 Statistical methods

4.2.6.1 Phenotypic and behavioural traits

To identify the nature and consequences of trait differences between laboratory- and wild-born individuals, we used generalised linear models in the package glmmTMB v1.1.8 (Brooks *et al.*, 2017) in R v4.3.1 (R Core Team, 2023). We assessed model fits with the package DHARMa v0.4.6 (Hartig, 2022) and tested overall significance using type III ANOVAs from the package car v3.1-2 (Fox & Weisberg, 2019). Where necessary, post-hoc pairwise comparisons were facilitated by the package emmeans v1.10.0 (Lenth, 2024).

Pronotum width and body mass were analysed according to a Student's t-distribution, as supported by diagnostic plots generated with DHARMa (Hartig, 2022). Both models included origin (fitted as a two-level factor: lab-born vs wild-born), recapture status (fitted as a two-level factor: ever re-encountered vs encountered only once), year (fitted as a two-level factor: 2021 vs 2022). We also considered all two- and three-way interactions between these terms. These interactions were included to interrogate whether there were consistent relationships between trait values and recapture status across both time and origin.

The proportion of individuals recaptured (ever recaptured vs never recaptured; 1 or 0, respectively) was analysed with a binomial model using a logit link function. Time present in the study (conditional on being recaptured at least once) was analysed initially according to a lognormal distribution error distribution, as supported by diagnostic plots generated with DHARMa (Hartig, 2022). Both models included a fixed effect of origin, year, and an interaction between these two terms.

Minimum straight-line distance travelled between consecutive captures was deemed to be most appropriately modelled by a zero-inflated gamma model according to measures of model fit generated by DHARMa (Hartig, 2022). The distance model included repeated measures of individuals sampled over multiple sampling occasions. Therefore, the continuous portion of the model included a random effect of individual ID and fixed effects of origin, year, and an interaction between these two terms. The zero-inflation portion of this model included an intercept only.

4.2.6.2 General mark recapture models

Survival rates were estimated from encounter histories using the open-population Cormack-Jolly-Seber model (CJS; Cormack, 1964; Jolly, 1965; Seber, 1965). Open-population models allow population sizes to fluctuate over time due to birth, death, and migration processes (Pollock *et al.*, 2005). The population at the beginning of each season is composed of individuals that presumably survived overwintering as adults (Easton, 1979), with numbers likely increasing as breeding occurs during the active season (Easton, 1979; Scott, 1998) and changing in response to environmental conditions and carcass availability. Further, our field sites were nested in, and contiguous with, larger woodlands and semi-natural grassland and meadow habitats, and migration to and from our sampling area from these areas was likely ongoing throughout each sampling period.

CJS models condition on first capture and use forward-time modelling to estimate two parameters from encounter histories of individuals: apparent survival rate (φ) and recapture probability (ρ; Sandercock *et al.*, 2020). Apparent survival rate is the probability that a marked individual sampled in one period survives to the next and it does not emigrate from the sampling area (Cooch & White, 2023). This is the product of the rate of survival and the complement of the emigration rate. Recapture probability is the probability of being encountered in a sampling period, conditional on being alive and present in the sampling area (Cooch & White, 2023). Failing to adequately account for imperfect detection of individuals can lead to biased estimates of apparent survival (Sandercock *et al.*, 2020; Cooch & White, 2023). Apparent survival rate applies to sampling intervals, while recapture probability applies to sampling occasions (Table 1; Sielezniew *et al.*, 2020), and either parameter can vary independently of the other with respect to time, age, or other grouping factor.

Table 4-1: Example of CJS model parameters (φi, ρi) in a constant or time-/age-/cohort-varying model. φi denotes apparent survival from occasion i to occasion i+1. ρi denotes recapture probability of known individuals alive during the sampling occasion i.

Constant parameters							
Sampling occasion	1	→	2	→	3	→	4
		φ_1		φ_1		φ_1	
			ρ_2		ρ_2		ρ_2
Varying parameters							
Sampling occasion	1	→	2	→	3	→	4
		φ_1		φ_2		φ_3	
			ρ_2		ρ_3		ρ_4

CJS models rely on several key assumptions (Pledger *et al.*, 2003; Pollock *et al.*, 2005; Cubaynes *et al.*, 2021; Cooch & White, 2023):

1. Every marked individual of an identifiable group (age, cohort, sex, etc.) present in sampling period *i* has the same probability of recapture (ρ_i).
2. Every marked individual of an identifiable group (age, cohort, sex etc.) present immediately after sampling period *i* has the same probability of surviving to time *i+1* (φ_i).
3. Identifying marks are permanent and unique.
4. Sampling periods are instantaneous relative to the interval between period *i* and *i+1*.

The first two assumptions are explicitly assessed via goodness-of-fit testing during model fitting (Cooch & White, 2023). In our case, it is reasonable to assume that elytral marks, intrinsic and unique to each individual, were permanent in the wild, as is observed in the laboratory (Assumption 3). Traps were present in the sampling area throughout the season, but direct sampling was conducted over 24 hours every seven days. This schedule of sampling was intended to maximise capture and recapture rates of wild-living populations, and these sampling periods may be considered brief relative to sampling intervals (Assumption 4).

The size of wild-living populations was estimated using the 'POPAN' formulation of the Jolly-Seber model (JS; Jolly, 1965; Seber, 1965; Cooch & White, 2023). This JS model is an open-population model that is conditional on all encounters in the field site, rather than just recaptures as in the CJS model. JS models estimate two additional parameters: $\widehat{N}$ a "superpopulation" – the number of all individuals in the population available for capture during all of the sampling occasions, and b_i – the probability that an individual from the super-

population would enter the population between the occasion *i* and *i+1*. The assumptions of the JS model are similar to those of the CJS model, with the added assumptions that survival and capture probabilities are the same for all marked and unmarked individuals between sampling occasions and that the study area is constant (Cubaynes *et al.*, 2021; Cooch & White, 2023). As the JS model does not distinguish between marked and unmarked individuals, 'age' and 'cohort' models are not applicable (Sielezniew *et al.*, 2020), and this model is instead used to estimate abundance.

4.2.6.3 Mark-recapture analysis

Mark-recapture data were analysed using the program MARK v10.1 (White & Burnham, 1999) and the MARK interface package RMark v3.0.0 (Laake, 2013).

As there were minor differences in trapping effort and processing methodologies between field seasons, we analysed the 2021 and 2022 datasets separately. Further, given the different nature of data available for wild-living and laboratory-maintained cohorts, we analysed these two groups separately. Inferences of differences between populations and groups was taken from model estimates and confidence intervals. By nature, the following models provide estimates of age-specific survival, rather than age-specific mortality. Therefore, we describe model selection in terms of survival, but results are transformed and presented on the mortality scale for interpretability and relevance to similar studies (Gaillard *et al.*, 2004; Kawasaki *et al.*, 2008; Carroll & Sherratt, 2017; Mautz *et al.*, 2019; Rodríguez-Muñoz *et al.*, 2019a; Sielezniew *et al.*, 2020), where age-specific mortality is the negative natural logarithm of age-specific survival.

4.2.6.3.1 Analyses of ageing in wild-living individuals

We built several models independently testing the effects of 'age' and time in the study on apparent survival rate and recapture probability in wild-living cohorts using 'time since first capture/release' as our measure of 'age' (*sensu:* Crespin *et al.*, 2006b; Sielezniew *et al.*, 2020). As all laboratory-born individuals were approximately one week old at time of release, this simplifying assumption is reasonably made for this cohort. However, this is not likely to be the case for a proportion of wild-born individuals encountered throughout the study. There are currently no external phenotype-based age-grading methods developed for *N. vespilloides*. Newly-emerged, teneral adults may be identified in the hours and even days following emergence by overall paler colouration and softer exoskeleton. However, following this initial cue, individuals are phenotypically similar throughout their lifespans. Recent work in *N. americanus* has suggested the viability of elytral colour-based age grading of wild-living individuals (McMurry *et al.*, 2023), but this method has not yet been explored in our system.

Statistical methods can account for left-truncated (individuals of unknown age entering a study) or interval-censored (individuals of whom only 'time present in study' is known) mark-recapture data (e.g., BaSTA: Colchero *et al.*, 2012) However, these models do not currently allow for flexible modelling of recapture probabilities that may bias apparent

survival rates. Additionally, given sparse recapture data, as in our case, these methods yielded model convergence issues and poorly estimated model parameters. Further, models that account for heterogeneity in survival that may result from different initial ages at capture (e.g., mixture models or individual heterogeneity models; Gimenez *et al.*, 2018) faced convergence issues when applied to our data, or dropped parameters.

As a consequence of our simplified age assumption (i.e., time since first capture), middle-aged and old individuals might contribute to survival estimates at young ages. More simply, a death at one age is perceived as a death event at an earlier age. If survival truly decreases with age, the erroneous grouping of old individuals, with lower intrinsic survival rates, with young individuals acts to increase heterogeneity in apparent survival at young ages. Therefore, we expect our estimates of baseline survival to be biased downwards and increased uncertainty around age-specific estimates to cause ageing rates to be biased toward zero (McArdle, 2003; Crespin *et al.*, 2006b). In this manner, any estimates of ageing rates may be considered conservative (Crespin *et al.*, 2006a), and increasingly so, in proportion to both the number of individuals of incorrectly assigned ages and the magnitude of these errors.

In the candidate models built, apparent survival rate could be made constant or allowed to vary with respect to some function of 'age'. Survival analyses typically aim to characterise some baseline, age independent level of survival in a population, as well as a rate of decrease in age-specific survival (actuarial ageing rate; Ronget & Gaillard, 2020). In the simplest case, the exponential model holds the survival rate constant across ages (i.e., no actuarial ageing). Gompertz and Weibull models allow survival to decrease exponentially with age, or as a power function of age, respectively (Wilson, 1994; Gaillard *et al.*, 2004).

Exponential function:

$$mortality\ rate = \beta_{constant}$$

$$cumulative\ survival = e^{-\beta_{constant} \cdot a}$$

Gompertz function:

$$mortality\ rate = \beta_{rate} e^{\beta_{Gompertz} \cdot a}$$

$$cumulative\ survival = e^{\left(\frac{-\beta_{rate}}{\beta_{Gompertz}}\right)\left(e^{\beta_{Gompertz} \cdot a} - 1\right)}$$

Weibull function:

$$mortality\ rate = \frac{\beta_{Weibull}}{\beta_{scale}} \left(\frac{a}{\beta_{scale}}\right)^{\beta_{Weibull} - 1}$$

$$cumulative\ survival = e^{(\frac{-a}{\beta_{scale}})^{\beta_{Weibull}}}$$

Where $\beta_{constant}$, β_{rate}, β_{scale}, $\beta_{Gompertz}$, and $\beta_{Weibull}$ are estimated parameters, and *a* is age.

The exponential model is an intercept-only model, wherein survival rate is constant, $\beta_{constant}$. Under the Gompertz model, the rate parameter β_{rate} is a measure of baseline survival, and the shape parameter $\beta_{Gompertz}$ describes the rate (exponent) at which survival changes with age; values > 0 indicate decreasing survival. Under the Weibull model, the scale parameter (β_{scale}) is a measure of baseline survival and represents the age at which 63.2% of individuals are dead (Pasha *et al.*, 2006; Angell *et al.*, 2020). The shape parameter $\beta_{Weibull}$ describes the change in the age-specific survival rate; values > 1 indicate decreasing survival. Weibull and Gompertz models are the most commonly applied survival functions to survival data due to their simplicity (only two parameters are needed to describe baseline survival and ageing rate; i.e., an intercept and slope) and their ease of interpretation and comparison of derived ageing rates across populations (Wilson, 1994; Ricklefs & Scheuerlein, 2002; Ronget & Gaillard, 2020). Further, these survival functions describe patterns of adult human mortality very well (Juckett & Rosenberg, 1993; Hawkes *et al.*, 2012). On the log-log scale, linear effects of 'age' and the natural log of 'age' correspond to Gompertz and Weibull (Gaillard *et al.*, 2004) functions, respectively, and an Exponential model is parameterised with only an intercept.

Recapture probability was modelled as constant or allowed to vary linearly or quadratically with respect to time in the study. Occurrence records for this species suggest a broadly quadratic trend in activity between April and November (National Biodiversity Network, 2024), the extremes of our sampling period. Therefore, we considered that a quadratic effect of 'time in study' on recapture probability best reflects local population dynamics. Models of the 2022 field data included an additional main effect of site (two-level factor: Blackford versus Corstorphine) to account for overall differences in proportion of individuals recaptured between the two sites. This approach yielded nine models for 2021 (three 'apparent survival' rate parameterisations x three recapture probability parameterisations) and 24 models for 2022 (three 'apparent survival' rate parameterisations x eight recapture probability parameterisations), of which best models were chosen based on AICc values (Burnham & Anderson, 2002).

4.2.6.3.2 Analyses of ageing laboratory-maintained individuals

Analyses of laboratory-living individuals was broadly similar, but with simplified model parameters. Time of birth and death of laboratory-maintained individuals was converted to an encounter-history format, with a detection rate of one. This data format and perfect detection rate enabled us to model survival rate as a function of true age, keeping recapture probability as a fixed parameter equal to one. In this case, survival was modelled as constant, or varied with age according to the Gompertz, Weibull functions described above. Model selection was again conducted based on AICc values.

Further, to relate our analyses to previous work in this laboratory population, in which broad patterns of ageing rates differ across time (Chapter 3), lifespan data of laboratory-maintained individuals were analysed using accelerated failure time (AFT) models using the packages survival v3.5-7 (Therneau, 2023) and flexsurv v2.2.2 (Jackson, 2016). AFTs are appropriate for analysing censored lifespan data (Wei, 1992). They allow for fitting various survival functions that can flexibly describe baseline mortality rates, age-specific increases in mortality, as well as late-life decelerations in mortality. However, these models are not appropriate in cases where all individuals are censored (as is the case in our mark-recapture data), thus this approach was only applied to the laboratory data. We analysed our laboratory data according to Exponential, Gompertz, and Weibull functions, equivalent to models described above. Additionally, we considered Log-logistic and Log-normal survival functions that account for initial increases in mortality followed by decelerations at later ages. The program MARK does not allow for fitting demographic models as complex as Log-normal or Log-logistic (Sherratt *et al.*, 2010), but here we took advantage of more flexible model specification. Model selection was conducted via AIC (Burnham & Anderson, 2002), with the intent of identifying qualitative differences in patterns of ageing between years.

Log-logistic function:

$$mortality\ rate = \frac{\frac{\beta_{shape}}{\beta_{scale}}\left(\frac{a}{\beta_{scale}}\right)^{\beta_{shape}-1}}{1+\left(\frac{a}{\beta_{scale}}\right)^{\beta_{shape}}}$$

$$cumulative\ survival = \frac{1}{1+\left(\frac{a}{\beta_{scale}}\right)^{\beta_{shape}}}$$

Log-normal function:

$$mortality\ rate = \frac{\phi\left(\frac{\log(a)-\beta_{meanlog}}{\beta_{sdlog}}\right)}{\beta_{sdlog}.a\left[1-\Phi\left(\frac{\ln(a)-\beta_{meanlog}}{\beta_{sdlog}}\right)\right]}$$

$$cumulative\ survival = 1 - \Phi\left(\frac{\ln(a)-\beta_{meanlog}}{\beta_{sdlog}}\right)$$

Where β_{scale}, β_{shape}, $\beta_{meanlog}$, and β_{sdlog} are estimated parameters and a is age. ϕ and Φ denote the probability density function and cumulative distribution function of the normal distribution, respectively.

4.2.6.3.3 Estimating population dynamics

We built separate models for each year to estimate population sizes in our two field sites. JS models have four parameters (apparent survival rate, recapture probability, probability of entry, and 'super-population'; Schwarz & Arnason, 1996; Cooch & White, 2023). Our analyses of the 2021 data included a quadratic effect of 'time in study' and main effect of site on

recapture probability, but all other parameters were held constant. Our analyses of the 2022 data were similar but included a fixed effect of 'site' (Blackford or Corstorphine Hill) on the 'super-population' and recapture probability parameters. These models were chosen to be conservative with respect to the additional parameter 'probability of entry' which reflects birth and immigration processes, for which no prior data was available.

We estimated population sex ratios using generalised linear models in the package glmmTMB (Brooks *et al.*, 2017). For these analyses, sex ratios were generated directly from recapture histories, rather than estimates of super-populations (above), and excluded laboratory-born individuals that were introduced to the field sites. This reflected the proportion of wild-born females versus wild-born males captured across an entire season. The population sex ratio (male versus female; 0 or 1, respectively) was analysed with a binomial model using a logit link function and included a fixed effect of site (Blackford or Corstorphine) and year (2021 or 2022).

4.2.6.3.4 Age-specific phenotypic changes

We aimed to characterise age-specific changes in body mass in laboratory-maintained and wild-living individuals. We restricted these analyses to laboratory-born individuals of known age, as we likely had sufficient power to describe age-specific trends only in these groups (wild-living: N = 62 individuals; laboratory-maintained: N = 303 individuals). Given uncertainty around the true ages, including longitudinal data from the wild-born cohort (N = 229) may have obscured age-specific patterns, rather than increase our power to detect them. These data were analysed according to a Gaussian error distribution using the package glmmTMB (Brooks *et al.*, 2017).

We first analysed the effect of age on body mass in laboratory-maintained individuals, of whom complete lifespan and age of death data were available. This model included linear and quadratic terms of age, and an individual-level random effect was included to account for repeated measures of individuals. To account for selective disappearance that could act to mask signals of ageing (van de Pol & Verhulst, 2006), we included age-interval of death as a factor (Ivimey-Cook & Moorad, 2018). This factor had six levels (death in the first month after emerging as an adult, second month, and so on, until death in or after the sixth month of life) reflecting the interval over which body mass data were recorded.

Subsequently, we combined data from laboratory-maintained and wild-living, laboratory-born individuals, and ran a simplified model. This included linear and quadratic terms of age, a fixed effect of environment (fitted as a two-level factor: wild or laboratory), an interaction between this factor and both age terms, and an individual-level random effect. Selective disappearance could not be accounted for in this model, as accurate lifespan data were not available for wild-living individuals. However, presenting both sets of analyses did allow us to highlight how failing to include selective disappearance may affect age-specific estimates.

4.3 Results

4.3.1 Descriptive statistics and population dynamics

The number of captures increased from 2021 to 2022 (625 to 1856; Table 2). This may reflect the release of a greater number of lab-born individuals in 2022 than in 2021 (that may have bolstered the breeding population) and the addition of the Corstorphine field-site to trapping efforts in 2022. However, estimates of minimum population size (total number of individuals encountered over a season) in the Blackford site also increased between 2021 and 2022 (4434.909 95% CI [3317.536, 5928.621] to 6185.806 [5052.318, 7573.591]; Table 2). The population size estimated for the relatively larger field site of Corstorphine was 3296.6569 [2701.266 – 4023.279] (Table 2). The relatively tighter 95% confidence intervals around the estimate of super-population size in Corstorphine than Blackford (1322.013 vs 2521.273 and 2611.086) may reflect overall higher recapture rates Corstorphine than Blackford (Table 2), facilitating more precise estimation. As the effective sampling radius of our baited traps is unknown (discussed below), it is not clear if population densities were lower in the Corstorphine field site than in Blackford, or if differences in terrain, environment, or microhabitat affected our ability to sample the local population and thus affected our estimates of abundance. Nevertheless, at the estimated population sizes (above), and number of wild-born individuals known to be present (Table 2), we sampled approximately 10% the Blackford populations (2021: 0.09% [0.08 - 0.12]; 2022: 0.11% [0.09 – 0.14]) and 16% [0.13 – 0.19] of the Corstorphine population.

Population sex ratio varied significantly between field sites ($\chi^2 = 8.269$, df = 1, p = 0.004) but was consistent across years ($\chi^2 = 2.159$, df = 1, p = 0.142). In 2021, the wild-living population in Blackford showed a significant female bias (proportion of females: 0.562 [0.513, 0.610]). In 2022, a significant female bias was observed in Corstorphine (0.600 [0.557, 0.641]), while Blackford exhibited a non-significant female bias (0.516 [0.479, 0.553]).

Table 4-2: Summary statistics of mark-recapture efforts. Proportion recaptured is calculated as the number of individuals encountered (either a release of lab-born individuals or capture of an individual from the wild) more than once, divided by the total number of individuals known to exist in the study (calculated per group). Number of individuals is effectively the minimum bound of population size across a season at each field site.

	2021		**2022**	
Total number of encounter/release events	625		1856	
	No. of individuals		*No. of individuals*	
Number of wild-born individuals captured once/ lab-born individuals not encountered following release	514		1328	
Number of individuals recaptured	53		238	
	No. of individuals	*Proportion recaptured*	*No. of individuals*	*Proportion recaptured*
Lab-born individuals released into wild - Blackford	167	8.38%	346	13.87%
Wild-born individuals - Blackford	400	9.75%	703	12.80%
Wild-born individuals - Corstorphine			517	19.34%
Wild-born individuals - Total	400	9.75%	1220	15.57%
Total number of individuals	567	9.35%	1566	15.20%

We compared the fit of CJS models including constant, linear, and quadratic functions of time-in-season on recapture probability, in both years and sites of the study, by AIC (full table in Supplemental Information). We observed a significant, convex quadratic pattern of recapture probabilities across the field seasons (Figure 2). In both years, the last recorded recapture of a known individual occurred in the 19th week of study (i.e., the first week of September), resulting in near-zero estimates of recapture probability following this date. In 2021, peak recapture probabilities were apparent in the 12th week of field sampling (mid-July; recapture probability: 0.092 [0.047, 17.231]). In 2022, recapture probabilities were higher overall in the Corstorphine site (Estimate: 0.651 [0.390, 0.912]). Further, a peak in recaptures was observed slightly earlier in the 2022 season, in the 10th week of sampling (early July; Blackford peak recapture probability: 0.125 [0.095, 0.162]; Corstorphine: 0.069 [0.052, 0.092]). The wider confidence intervals around the estimates in 2021 likely reflect the much smaller sample size of captures and recaptures in that year.

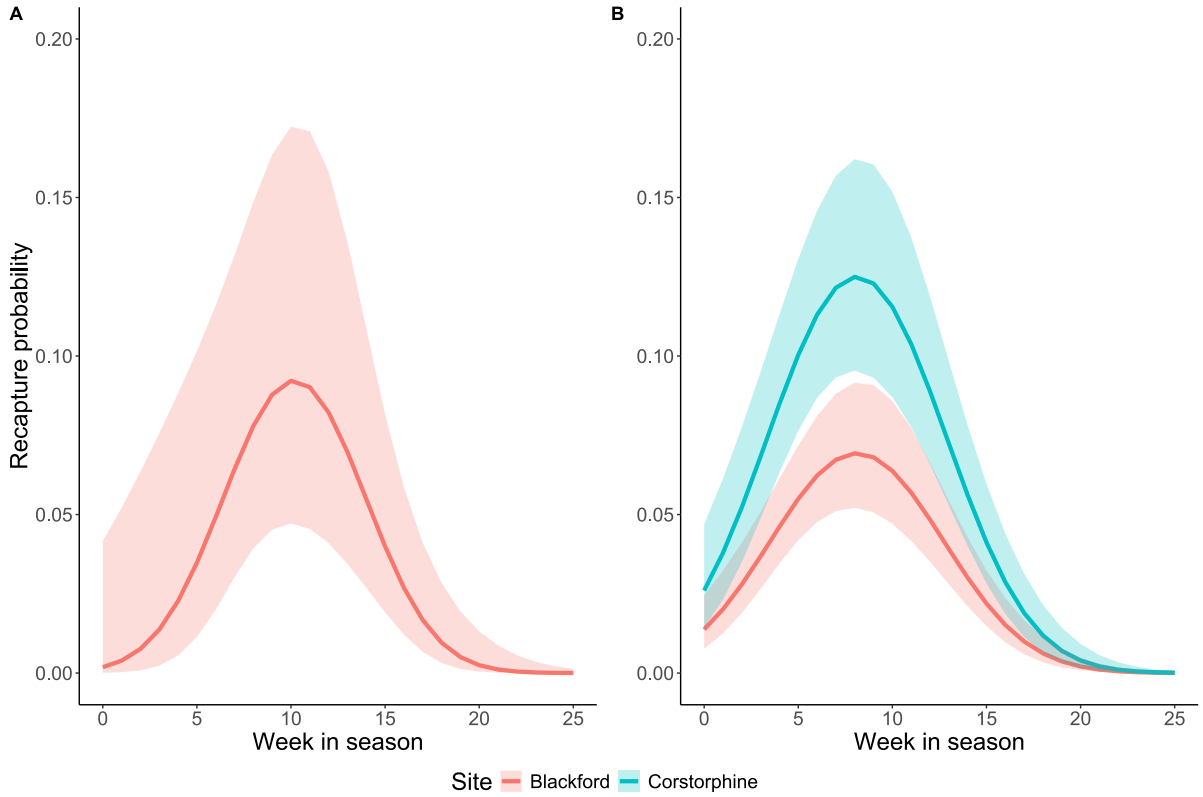

Figure 4-2: Recapture probabilities as a function of time in the season. A – the trend in recapture probabilities in 2021 in the sole field-site of Blackford. B – recapture probabilities on both field sites in 2022. Week 0 is the 1st week of May in both A and B. Ribbons denote 95% confidence intervals.

4.3.2 Phenotypic and behavioural traits of wild-living individuals

We conducted two and three-way ANOVAs to establish whether lab-born and wild-born beetles were phenotypically and behaviourally similar in several key traits (i.e., body size, body mass, likelihood of being recaptured, time present in the study, and movement between captures; Table 3,4). The interpretation of estimates from three-way interaction models can be complex; therefore, we present differences between years and wild-living groups from these models using pairwise comparisons of point estimates and confidence intervals (Table 5).

Lab-born beetles that were released into the Blackford field site were larger and heavier than their wild-born counterparts from the Blackford and Corstorphine populations (difference in pronotum width = 0.387 mm [0.300, 0.473]; difference in body mass = 0.035g [0.025, 0.045]; Tables 3, 5). A significant main effect that suggested body mass decreased between years (Estimate = -0.011g [-0.021, -0.001]; Table 3) was primarily driven by a reduction in the weight of lab-born individuals released into the wild in 2022 (Table 5). Otherwise, there were no significant differences (95% confidence intervals of point estimates overlapped) in mean body mass or pronotum width between individuals that were recaptured at least once, or never re-encountered, within or between years (Table 5).

Table 4-3: The relationships between origin (laboratory- versus wild-born), recapture status (ever recaptured versus never recaptured) and year (2021 versus 2022) and body size and mass. Both models were analysed according to a Student-t distribution, and estimates are presented on the identity scale (millimetres and grams).

	Pronotum width (mm)				**Body mass (g)**			
Predictors	*Estimates*	*CI*	*z*	*p*	*Estimates*	*CI*	*z*	*p*
(Intercept)	5.250	5.177 – 5.322	141.866	**<2e-16**	0.245	0.237 – 0.253	58.540	**<2.2e-16**
Origin [wild]	-0.387	-0.473 – -0.300	-8.738	**<2e-16**	-0.035	-0.045 – -0.025	-6.852	6.26e-11
Recapture status [Recaptured]	-0.036	-0.286 – 0.215	-0.278	0.781	0.005	-0.023 – 0.034	0.359	0.720
Year [2022]	-0.084	-0.174 – 0.006	-1.834	0.067	-0.011	-0.021 – -0.001	-2.085	**0.037**
Origin [wild] × Recapture status [Recaptured]	0.154	-0.139 – 0.446	1.030	0.303	-0.001	-0.035 – 0.032	-0.062	0.951
Origin [wild] × Year [2022]	0.126	0.020 – 0.232	2.329	**0.020**	0.008	-0.004 – 0.020	1.350	0.177
Recapture status [Recaptured] × Year [2022]	0.097	-0.189 – 0.383	0.664	0.507	0.012	-0.021 – 0.044	0.706	0.480
Origin [wild] × Recapture status [Recaptured] × Year [2022]	-0.191	-0.522 – 0.141	-1.127	0.260	-0.010	-0.048 – 0.028	-0.524	0.600
Observations	2117				2001			

The proportion of known individuals that were recaptured (i.e., wild-born individuals being captured at least twice, or lab-born beetles being re-encountered at least once following release) was similar in lab-born and wild-born beetles (Odds ratio: 1.181 [0.623, 2.237]; Table 4,5). Further, this proportion of recaptures was slightly, but non-significantly, higher in 2022 than 2021 (Odds ratio: 1.760 [0.941, 3.293]; Table 4,5). There was no significant interaction between year and origin suggesting that individuals were sampled in a consistent manner across years and backgrounds (Odds ratio: 0.970 [0.470, 2.003]; Table 4,5).

Lab- and wild-born individuals were present in the study (i.e., the interval between first capture/release and last capture) for similar periods of time (Estimate: 1.169 [0.878, 1.558]; Table 4,5). However, the overall time present in the study increased significantly in both groups in 2022 (1.506 [1.146, 1.980]; Table 4,5). In 2021, the maximum interval between first and last capture of an individual was eight weeks while, in 2022, the maximum interval was fourteen weeks. The lack of a significant interaction between origin and year indicated that laboratory-born individuals behaved similarly to wild born beetles in both years of the

study (0.769 [0.561, 1.054]; Table 4,5). This indicated that whilst environmental effects, behavioural differences in the study population, or methodological improvements between years may have affected our ability to sample the wild-living population, both wild- and lab-born beetles were similarly affected.

Individuals were equally mobile between captures across years (Estimate: 0.994 [0.650, 1.520]) and origin (wild- versus laboratory-born: 0.951 [0.615, 1.470]; Table 4,5). There was no significant indication of an interaction between origin and year (0.938 [0.579, 1.519]). The majority of recaptures (81.63% [0.754, 0.863])) involved individuals being found in traps different from their previous encounter, with a mean distance travelled between captures of approximately 180 metres (range: 0m – 529m). Additionally, if the mean distance travelled between captures is taken as an indication of the effective sampling radius of traps, it becomes straightforward to calculate a reasonable area over which trapping was conducted in each field site. At 180 metres radius, the Blackford site covered 41.271 ha in 2021 and 39.487 ha in 2021. Corstorphine, with more regularly spaced, densely packed traps encompassed 32.489 ha. This may be considered a conservative sampling area, given the sensitivity of *N. vespilloides* to sources of carrion. Given these estimated trapping areas, it is possible to estimate the annual population densities of our wild-living populations in 2021 (Blackford: 107 individuals/ha [80 – 144]) and 2022 (Blackford: 157 individuals/ha [128 - 192]; Corstorphine: 101 individuals/ha [83 - 124]). At the previously reported range of one kilometre or more (Petruška, 1975), the effective sampling area of our field sites would be an order of magnitude larger and apparent density estimates commensurately lower.

Table 4-4: The relationships between origin (laboratory- versus wild-born), year, and the proportion of individuals recaptured, the duration individuals were present in the study, and the minimum straight-line distance travelled between captures. These data were analysed according to a binomial, log-normal, and zero-inflated gamma distribution, respectively. Intercepts are presented on the identity scale (proportion, weeks, metres). Estimates are presented as odds ratios (values > 1 increasing probability relative to intercept), and scalars (values > 1 meaning longer intervals or greater distances relative to intercept).

	Proportion recaptured				**Time present in study (weeks)**				**Movement between captures (m)**			
Predictors	*Odds Ratios*	*CI*	*z*	*p*	*Estimates*	*CI*	*z*	*p*	*Estimates*	*CI*	*z*	*p*
(Intercept)	0.092	0.053 – 0.158	-8.564	**<2e-16**	2.551	1.969 – 3.303	7.096	**1.28e-12**	221.151	151.103 – 323.672	27.781	**<2e-16**
Origin [wild]	1.18065	0.623 – 2.237	0.509	0.611	1.169	0.878 – 1.558	1.068	0.285	0.951	0.615 – 1.469	-0.227	0.820
Year [2022]	1.760	0.941 – 3.293	1.769	0.077	1.506	1.146 – 1.980	2.934	**0.003**	0.994	0.650 – 1.520	-0.028	0.978
Origin [wild] x Year [2022]	0.970	0.470 – 2.003	-0.082	0.934	0.769	0.561 – 1.054	-1.634	0.102	0.938	0.579 – 1.519	-0.261	0.794
Zero-Inflated Model												
(Intercept)									0.184	0.137 – 0.246	-11.446	**<2.22e-16**
Random Effects												
σ^2									0.415			
τ_{00}									0.00000 $_{numeric\_id}$			
N									291 $_{numeric\_id}$			
Observations	2133				291				348			

Across all traits considered (body size, mass, likelihood of recapture, time present in study and movement between captures) differences between lab-born and wild-born individuals were relatively minor. Where present, phenotypic differences were unrelated to the likelihood of recapture or time present in the study.

Table 4-5: Model-predicted estimates of mean pronotum width, body mass, proportion of known individuals recaptured, time known to be present/alive, and movement between captures. 95% confidence intervals are in brackets.

	Pronotum width (mm)		Body mass (g)		Proportion recaptured (%)	Time present (weeks)	Movement between captures (m)
	Recaptured	Not recaptured	Recaptured	Not recaptured			
2021							
Lab-born	5.250	5.214	0.245	0.250	0.084	2.551	186.835
	[5.177-5.322]	[4.975-5.454]	[0.237-0.253]	[0.223-0.278]	[0.050-0.137]	[1.969-3.303]	[147.409-226.260]
Wild-born	4.863	4.981	0.210	0.214	0.098	2.982	177.633
	[4.815-4.911]	[4.837-5.125]	[0.205-0.216]	[0.198-0.231]	[0.072-0.131]	[2.537-3.506]	[106.426-248.840]
2022							
Lab-born	5.166	5.227	0.234	0.251	0.139	3.455	185.710
	[5.112-5.220]	[5.097-5.357]	[0.228-0.240]	[0.236-0.266]	[0.106-0.179]	[3.148-3.792]	[168.972-202.447]
Wild-born	4.905	4.929	0.208	0.214	0.156	3.842	165.587
	[4.877-4.933]	[4.864-4.995]	[0.204-0.211]	[0.206-0.221]	[0.137-0.177]	[3.371-4.378]	[130.408-200.767]

4.3.3 Age-specific mortality in wild- and lab-living populations

We compared the fit of several distributions that commonly describe age-specific mortality curves by AIC (Table 6) separately for each of wild-living and laboratory-maintained populations in both 2021 and 2022. In both years, patterns of ageing in laboratory-maintained populations were best described by a Weibull function, in which the log of mortality changes as a log function of age, while mortality in the wild-living populations was best described with an exponential function, indicating that mortality rate was constant across ages (Table 6; Fig 3).

Table 4-6: Results of AIC and AICc model selection for ageing functions in lab-maintained and wild-living populations in 2021 and 2022 using parametric AFTs and approximations of survival functions in MARK. Model selection was conducted separately for each population/year. MARK models of lab-maintained populations included recapture probability as a fixed parameter (known recapture rate = 1). All models of wild-living populations included a quadratic effect of recapture probability across each field season, resulting in three additional parameters (intercept, linear term, and quadratic terms) estimated in each case. Wild population models in 2022 included an additional fixed effect of site (Blackford vs Corstorphine) on recapture probability. Best fitting models by AIC and AICc are in bold; models within 2 units are considered equivalent and highlighted in bold.

		AFT Survival models			**MARK survival models**					
		Lab-maintained population			**Lab-maintained population**			**Wild-living populations**		
	Ageing function	*df*	*N*	*AIC*	*df*	*N*	*AICc*	*df*	*N*	*AICc*
2021	Exponential	1	141	1359.75	1	141	825.41	4	53	**557.27**
	Gompertz	2	141	1160.17	2	141	620.59	5	53	**559.17**
	Weibull	2	141	**1137.92**	2	141	**597.84**	5	53	**558.95**
	Log-logistic	2	141	**1136.59**						
	Log-normal	2	141	1184.87						
2022	Exponential	1	325	3358.92	1	325	2255.35	5	238	**2562.83**
	Gompertz	2	325	3166.13	2	325	2075.19	6	238	**2562.13**
	Weibull	2	325	**3140.28**	2	325	**2045.58**	6	238	**2562.08**
	Log-logistic	2	325	3170.84						
	Log-normal	2	325	3180.92						

There was little evidence that laboratory-maintained populations exhibited qualitatively different patterns of mortality between years (Table 6). Visual analyses of survival curves (Supplementary Information) describing the 2021 data suggested that the marginally improved fit of the Log-logistic function relative to the Weibull function was due to the latter overestimating early-life mortality. Both models were equivalent at modelling mortality between ages 5 and 11 weeks, with the Weibull function performing better than the Log-logistic at describing mortality at later ages.

As there was support for Weibull as an appropriate function in both laboratory-maintained populations, and all survival models in wild-living populations had approximately equivalent AICc scores (i.e., within two units), we directly compared apparent ageing rates between laboratory and wild-living conditions, within years, using this function (Table 7; Figure 3). Baseline mortality appeared significant higher in the wild-living populations than laboratory-maintained populations (Table 7; Figure 3). The rate of mortality increased but this rate decelerated with age in lab-maintained populations in both years but differed significantly between years (2021 Weibull shape parameter: -3.086 [-3.593, -2.579]; 2022: -1.270 [-1.470, -1.070]). Apparent mortality rate did not significantly change with either age or study year in wild-living individuals (Table 7), but rates appeared to decrease weakly and with some deceleration with age in 2021 and increase weakly with deceleration with age in 2022.

Table 4-7: Model-predicted estimates of the Weibull ageing function, fitted to years 2021 and 2022 separately. All models included a quadratic term for recapture probability across a field season, and a fixed parameter for recapture probability in the laboratory (known recapture rate = 1). Baseline mortality denotes ages at which 63.2% of individuals are dead/emigrated. Weibull shape is the rate at which apparent mortality changed with age (values > 1 denote increases in mortality; values = 1 denote constant mortality with respect to age).

	Weibull baseline mortality	**Weibull shape**
2021		
Lab-maintained	8.275 [6.457 – 10.605]	4.086 [3.579 – 4.593]
Wild-living	1.955 [0.488 – 7.830]	0.728 [0.105 – 1.560]
2022		
Lab-maintained	14.981 [12.026 – 18.664]	2.270 [2.070 – 2.470]
Wild-living	4.803 [2.924 – 7.888]	1.325 [0.911 – 1.739]

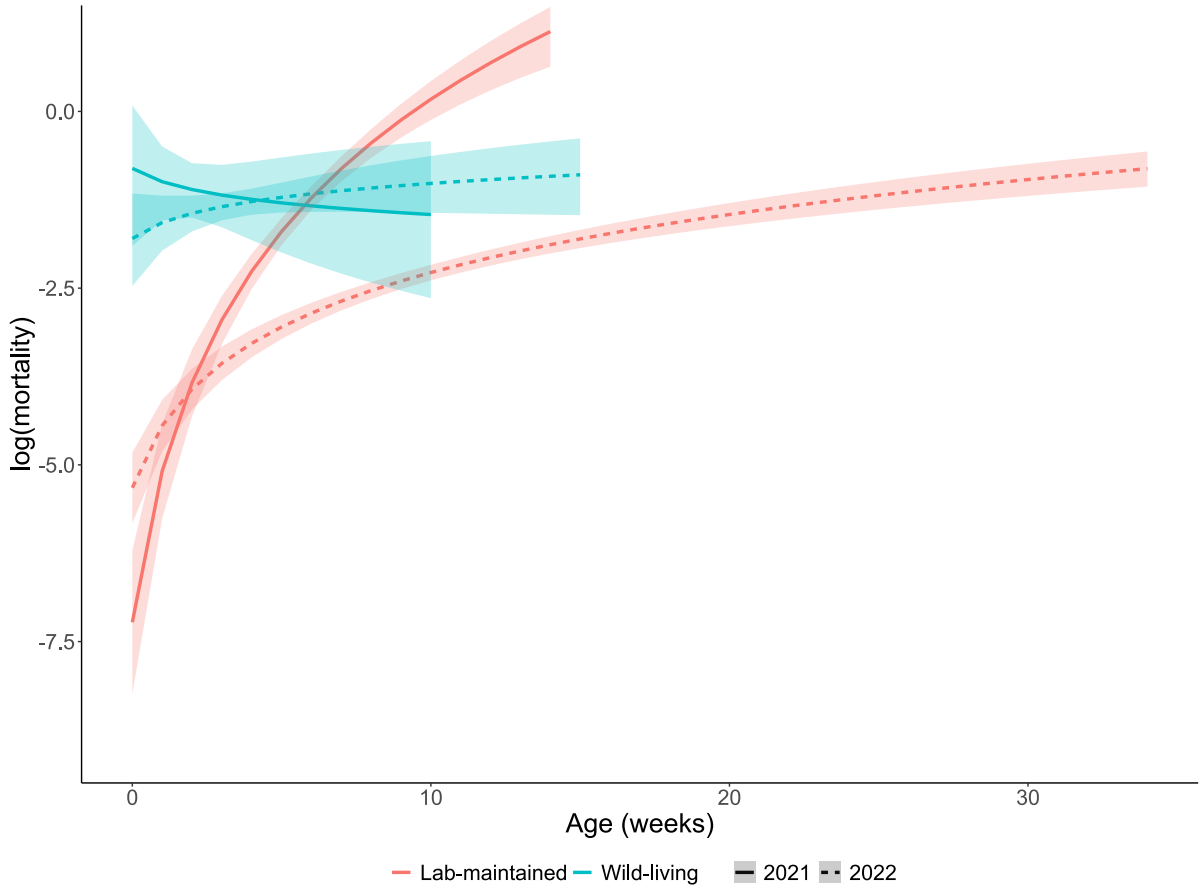

Figure 4-3: Patterns of age-specific mortality in wild-living and laboratory-maintained populations. Mortality curves fit to captive populations indicate true mortality rates. Curves fit to wild-living populations are "apparent" mortality rates - the product of death and dispersal from the field site. Ribbons denote 95% confidence intervals.

4.3.4 Age-specific trends in body mass in wild- and lab-living populations

Among laboratory-maintained individuals, mass increased significantly, and linearly, with age (0.0013 g/day [4.6e-4, 0.002]), with no significant quadratic effect (1.0e-5g/day^2 [-3e-5, 6e-5]). Further, there was a significant signal of selective disappearance (χ^2 = 26.20, df = 5, p = 8.17e-05) apparently driven by elevated mortality of the smallest individuals (mean mass: 0.163g [0.100, 0.226]) in the first month following emergence. Pairwise comparisons between the six levels (age-interval in which death occurred) of selective disappearance revealed a lack of a clear linear or monotonic pattern with respect to mass and lifespan following the early disappearance of the smallest individuals. Individuals dying in the second month following emergence (mean weight: 0.238g [0.217, 0.258]) and after the sixth month following emergence (0.255g [0.231, 0.278]) were the heaviest individuals, with those dying in the intervening months of apparent intermediate mass.

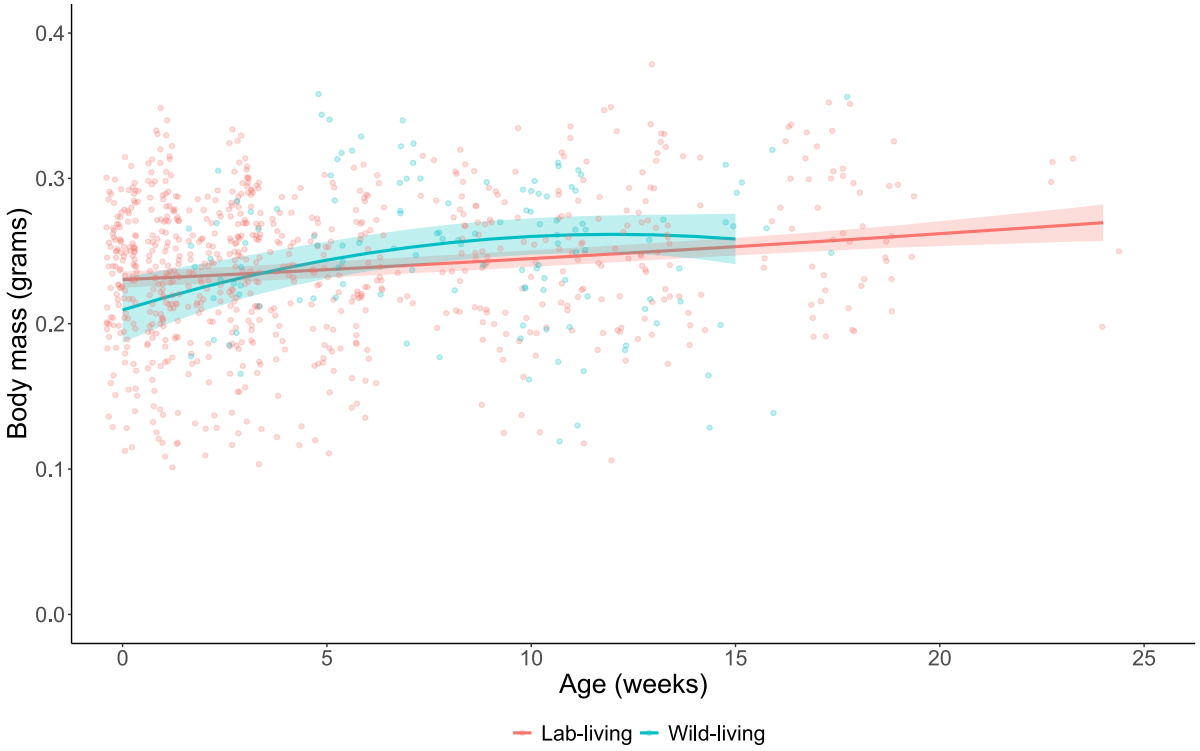

Figure 4-4: Age-specific trends in body mass among wild- and lab-living cohorts that were born in the laboratory. Estimates were derived from repeated measures taken of 62 wild-living individuals and 303 lab-living individuals. These estimates do not account for effects of selective disappearance. Ribbons denote 95% confidence intervals.

In combined analyses, excluding any effects of selective disappearance, linear and quadratic age effects on mass in lab-living individuals were quantitatively similar to above. Failing to account for selective disappearance in lab-living individuals resulted in an increase in intercept accounting for mean body mass, only. Wild-living, laboratory-born individuals were non-significantly lighter than their lab-living counterparts (-0.021g [-0.044, 0.002]). Interactions

between origin and the two age terms (linear: 0.007 g/day [0.003, 0.012]; quadratic: -3.7e-4 g/day2 [-6.5e-4, -1.0e-4]) indicated that initial increases in mass were greater in wild-living individuals than laboratory-living individuals (Figure 4). Laboratory-born, wild-living beetles gained mass faster in the first 8 weeks or so, becoming heavier than lab-living counterparts at about four weeks old (Figure 4). The difference in mass was maximised at 10 weeks old, and then the masses began to converge.

4.4 Discussion

4.4.1 Overview

This study primarily aimed to investigate how shifts in the environment from the laboratory to natural settings influence actuarial aging rates in beetles. The secondary aims were to characterize age-specific patterns in body mass (an indicator of condition) and aspects of population dynamics that are often absent or inherently artificial in laboratory populations (population size and sex ratio). To this end, we conducted laboratory experiments tracking beetle cohorts from birth to death to observe aging patterns. Simultaneously, we collected longitudinal data from genetically similar beetle populations in the wild to estimate age-specific mortality rates and changes in body mass. Additionally, employing mark-recapture methods, we determined minimum and 'super' population sizes and examined population sex ratios across time and between field sites.

While baseline mortality was higher among wild-living individuals, age-specific increases in mortality were only apparent among individuals maintained in the laboratory. Moreover, age-specific patterns in body mass differed qualitatively between laboratory-maintained and wild-living individuals. Wild-living beetles exhibited a pattern of greater mass gain in early life followed by a deceleration or overall decline in mass in late life, whereas laboratory-maintained beetles displayed continuous mass gain into late life. Finally, we provided the first estimation of the population characteristics for a natural, urban population of *N. vespilloides*. We found that apparent population densities varied temporally and spatially, and that these natural populations were generally female-sex biased.

4.4.2 Population dynamics in the wild

Variations in population size, density, sex ratio, and inter- or intra-specific competition levels can influence the patterns of ageing and behaviour through individual plasticity (Mysterud *et al.*, 2001, 2005; Williams *et al.*, 2006; Rodríguez-Muñoz *et al.*, 2019a). Additionally, these population characteristics can modulate selection on trade-offs between reproduction and somatic maintenance, with consequences for ageing rates (Bonduriansky *et al.*, 2008; Lemaître *et al.*, 2015, 2020a). Considerable temporal and spatial variation in population density has been reported in natural *Nicrophorus* spp. populations (Springett, 1967; Easton, 1979; Smith *et al.*, 2000). In this case, estimated population size varied between years and

sites. The similarity in trapping area in Blackford between 2021 and 2022 suggest that the population density increased by over 40%. As we only analysed records of the Corstorphine population from 2022, it is unclear whether there was significant inter-annual variation in population size or density here, or whether any variation would have been occurred in concert with that observed in Blackford. However, within 2022, the apparent population density in Corstorphine was significantly lower than in Blackford. Such variation in population density may have consequences for patterns of ageing (Mysterud *et al.*, 2001; Nussey *et al.*, 2007). Individuals developing under high population densities generally have smaller body sizes, lower fecundity, and shorter lifespans (Nussey *et al.*, 2007; Descamps *et al.*, 2008; Fantinou *et al.*, 2008; Dey & Joshi, 2018; Than *et al.*, 2020). However, increased developmental densities can also trigger investment into dispersal-related traits (Applebaum & Heifetz, 1999), induce stress responses that increase resistance to thermal challenges (Sørensen & Loeschcke, 2001), or improve starvation resistance in adulthood (Baldal *et al.*, 2005). Under increased adult densities, body mass may be lower, and mean mortality rates higher due to increased competition for resources or increasing rates of costly antagonistic intra-specific interactions (Joshi & Mueller, 1997; Mysterud *et al.*, 2001; House *et al.*, 2019). If population density affects different age groups differently, the intensity of selection against late-life mortality may either increase or decrease, depending on which ages are most influenced by density-dependent survival (Abrams, 1993). If increased density affects survival early ages more negatively than later ages (or if increased density lowers reproductive rates across ages), selection at late ages will be strengthened, slowing ageing rates. However, increased sensitivity to density effects at later ages may weaken selection against mortality in late life, increasing ageing rates.

The cause of observed inter-annual variation in density is unclear. However, this may have reflected improved environmental conditions during the active season (e.g., less rainfall and higher mean temperatures) facilitating greater individual activity, and thus representation in traps, or increased population growth rate during the 2022 relative to the 2021 season (Merrick & Smith, 2004; Urbański & Baraniak, 2015; St. Aubin, 2017). Improved environmental conditions may also have improved the reproductive success of the wild-living population, facilitating more rapid population growth (Grew *et al.*, 2019). Alternatively, warmer winter conditions prior to the 2022 field season may have increased the population size in 2022 relative to 2021. *N. vespilloides* overwinter as adults, remaining buried in the soil to avoid inclement weather and cold temperatures (Easton, 1979). Lower average and minimum temperatures in the winter preceding the 2021 season, relative to the period before the 2022 season, may have negatively impacted overwinter survival, reducing the size of the initial breeding population and subsequent population size (Schnell *et al.*, 2008; Wettlaufer *et al.*, 2023). A similar temporal trend in population density across sites might have lent more support to environmental drivers of variation population size, whereas diverging patterns might have suggested that spatially distinct populations experience independent fluctuations in population size. Perhaps more simply, diverging patterns in the two sites might have suggested that our introduction of laboratory-born individuals to the Blackford site bolstered the breeding population and led to an increase in overall population numbers.

Previous work in wild populations of Nicrophorus spp. have observed sex ratios can vary between years and fluctuate greatly throughout the active season (Springett, 1967; Easton, 1979). Whether our observation of generally female-biased wild populations reflected true population trends or sex-specific differences in foraging behaviour, and whether these biases, if real, were pronounced enough to be biologically relevant to shaping ageing is unclear. For example, in laboratory populations of seed beetles, *Tenebrio mollitor*, biases in sex ratio can have sex-specific effects on ageing, perhaps because reproductive costs are shifted primarily on to one sex in response to rarity (Jehan *et al.*, 2020). Alternatively, in wild populations of field crickets, *Gryllus campestris*, female-biased sex-ratios may result in overall lower ageing rates, due to reduced levels of intra-sexual competition in males, and reduced costs associated with male interactions in females (Rodríguez-Muñoz *et al.*, 2019a). While males and females were equally likely to be recaptured after first encounters, we captured more females than males overall. Sex- or age-specific patterns in foraging or carcass-searching have not been extensively explored in this species in either laboratory or natural contexts (but see the following for considerations of general flight: Attisano & Kilner, 2015; Chapter 2). However, Hopwood et al. (2016b) found that fewer males arrived on carcasses with resident males than unattended carcasses, while females arrived on carcasses in equal numbers, regardless of male presence. In this case, our apparent female bias in captures may result from males avoiding traps that already contain male competitors. Further, even if these sex biases reflect true trends in the population, the relatively small deviation from an even sex ratio may have little effect on sexual competition or reproductive trends that may shape ageing rates. In *N. vespilloides*, carcass availability is the limiting factor on reproduction (Scott, 1998; Royle *et al.*, 2013); there are many more potential parents in a population than opportunities to obtain a carcass. Levels of intrasexual competition in this species may therefore be less sensitive to changes in sex ratio, and more sensitive to carrion abundance.

4.4.3 Age-specific mortality in wild- and lab-living populations

Overall apparent mortality rates are to be expected in higher wild-living populations than in laboratory-maintained groups. Wild-living individuals are exposed to heterogenous environments, foraging costs, inter- and intra-specific competition, predation, and inclement weather that are effectively eliminated under standard laboratory conditions (Zajitschek *et al.*, 2020). Nevertheless, our estimated age-independent weekly death rate in the wild population of approximately 25% is generally lower than that reported in other studies of wild insect populations. Springett (1967) characterised seasonal trends in mortality in wild populations of *N. investigator* and *N. humator*, finding weekly death rates greater than 40% during their active periods. Similarly, weekly death rates were high in wild populations of *P. litigata* (62%; Bonduriansky & Brassil, 2002), *T. angusticollis* (males: 59%; females: 84%; Kawasaki *et al.*, 2008), and honey bees, *Apis mellifera* (63%; Dukas, 2008).

Low recapture rates increase uncertainty around survival estimates (Villella *et al.*, 2004; Pike *et al.*, 2008; Papadatou *et al.*, 2012), exacerbating the issue of accurately

estimating mortality. At the peak of our field seasons, recapture rates were approximately 10% (2021: 6.9%; 2022: 12.5%). These levels fall at the low end of those reported in other insect mark-resight or mark-recapture studies (damselflies, *Coenagrion puella*, approximate range: 0 – 100%; butterfly, *Polyommatus daphnis*, approximate range: 10 – 75%; field crickets, *G. campestris*, range: 43 – 65%; Sherratt *et al.*, 2010; Rodríguez-Muñoz *et al.*, 2019b; Sielezniew *et al.*, 2020). The difficulty of recapturing individual insects, as opposed to vertebrates, in wild populations has been repeatedly noted as a major challenge to assessing ageing in such systems (Hagler & Jackson, 2001; Kawasaki *et al.*, 2008; Zajitschek *et al.*, 2020). For example, recapture rates of wild-living populations of vertebrates are often reported > 85% (Soay sheep, *Ovis aries*: Catchpole *et al.*, 2000; red deer, *Cervus elaphus*: Catchpole *et al.*, 2004; bighorn sheep, *Ovis canadensis*: Festa-Bianchet *et al.*, 2019) facilitating much more accurate estimation of age-specific mortality rates. It is unclear, given the ability of individuals to migrate in and out of the study area through time, whether transience in site occupancy or low efficacy of trapping individuals that were present were the major contributors to this low recapture rate.

Further, emigration from the field sites and low overall recapture rates almost certainly inflated our estimates of baseline mortality rates. High emigration/dispersal poses a challenge to the assumption that individuals disappearing from the recapture record have died, increasing the contribution of emigration relative to mortality in apparent mortality rates. For example, the mean distance travelled between captures was 180 metres in our study, with previous work suggesting that individuals can fly several kilometres per day (Chapter 2; Attisano & Kilner, 2015). Easton (1979) found that migration of *N. vespilloides* was higher between remote field sites (up to 6km apart) connected by similar habitats than between closer field sites (approximately 200m) separated by a body of water. Previous work in wild insect populations has generally considered more sedentary or philopatric species, or geographically isolated systems (Nussey *et al.*, 2013), in which emigration was rare, and conflating disappearance with death was perhaps more appropriate there (Sielezniew *et al.*, 2020). Some such studies have noted the rarity of individuals travelling between much more closely placed primary field sites, insect burrows, or recorded emigration from much smaller study areas (Kawasaki *et al.*, 2008; Zajitschek *et al.*, 2009b; Bretman *et al.*, 2011; Angell *et al.*, 2020). Here, we attempted to account for the increased dispersal ability, relative to other insect systems, of *N. vespilloides* by conducting trapping over large areas (over 30 ha per site), but these efforts were likely not sufficient to encompass individuals' capacity for foraging or dispersing over large distances (Easton, 1979).

Previous work comparing ageing rates in captive and wild-living populations of vertebrates or invertebrates has found empirical support for both faster and slower ageing in wild populations, relative to captive populations (Kawasaki *et al.*, 2008; Tidière *et al.*, 2016; Carroll & Sherratt, 2017; Mautz *et al.*, 2019). It is perhaps unsurprising that actuarial ageing was only detected in laboratory-maintained populations, and not among wild-living cohorts. Actuarial ageing may be expected to manifest more clearly under benign conditions (i.e., in the laboratory), where external sources of mortality are low and intrinsic declines in condition

are more pronounced (Flatt & Partridge, 2018). Our inability to detect senescence in wild populations may reflect important demographic and environmental influences that are absent in the laboratory.

First, the effects of individual heterogeneity and selective disappearance may be more pronounced in natural populations than laboratory populations (Brunet-Rossinni & Austad, 2005). Selective disappearance, the non-random removal of low-quality individuals over time, may result in lower apparent population-level ageing rates than that experienced by component groups of the population that may differ in quality (Vaupel & Yashin, 1985; Fay *et al.*, 2018). Such a pattern is consistent with late-life decelerations in the force of mortality (Vaupel & Yashin, 1985; Chen *et al.*, 2013), as observed in our laboratory-maintained populations. Cohorts reared in the laboratory are subject to relatively homogenous conditions, presumably minimising variation in quality (Baumans & Van Loo, 2013), whereas environmental stochasticity experienced by wild populations may exacerbate inter-individual differences in quality. This greater variation in quality in wild-living populations may serve to lower apparent ageing rates.

Second, we assumed that recapture probability was constant across ages in the wild, which may not be the case (Senar *et al.*, 1999). Little is known about age-specific patterns of dispersal in natural populations of *N. vespilloides*, though our results suggest that individuals are highly mobile between captures, and laboratory studies have indicated that individuals can fly great distances in a short period of time (Attisano and Kilner, 2015; Chapter 2). If young individuals are more dispersive than older individuals, this might result in in higher rates of emigration. In this case, recapture rates might increase with age and inflate apparent mortality estimates in younger age classes, reducing apparent ageing rates. Conversely, if older individuals are less capable or likely to engage in flight, as previous work has suggested (Chapter 2), this would lead to under-representation of old individuals recovered in traps, increasing apparent ageing rates. Considering our low overall recapture rates, and relatively low spatial resolution of movement between captures (discussed in greater detail below), it was difficult to properly explore these potential age-effects. However, preliminary analyses found no significant signal of age-dependent dispersal patterns. Easton (1979) suggested that propensity for migration was greatest in the first generation of individuals emerging in field season, and less so amongst individuals emerging late in the season. This seasonal effect may further complicate the detection of age-dependent patterns of dispersal, as such patterns change as a function of both time in the season and age. Further, as there may be age-dependent variation in carcass preferences (von Hoermann *et al.*, 2013), our sampling efforts may not have been equally effective for all age classes. Our traps contained small packages of carrion bait that may have been more attractive to older individuals ready to reproduce than younger individuals that prefer large carcasses on which antagonistic intra-specific competitive interactions are less likely (Wilson & Knollenberg, 1984). Future work may vary both the degree of decomposition and size of carrion bait, to perhaps sample all ages more effectively and evenly.

Third, we may not have the power to detect actuarial ageing in wild-living populations. Estimating ageing rates precisely requires large sample sizes (Ricklefs, 2000; Ricklefs & Scheuerlein, 2001), with the Weibull shape parameter (i.e., the rate of ageing) estimated from small initial sample sizes potentially biased downwards, towards one (ie., no ageing; Ricklefs, 2000). High levels of age-independent mortality pose a further challenge for detecting signals of ageing, eroding sample sizes of progressively later age classes. In our case, the weekly mortality rate of wild-living populations was approximately 25%, resulting in our initial sample size being more than halved by age four weeks, and halved again by age six weeks.

Last, as discussed above, our use of time from first capture as a proxy for age among wild-living populations may be expected to bias ageing estimates towards one (McArdle, 2003; Crespin *et al.*, 2006b). However, simulations suggest that the uncertainty introduced by using this proxy for age may have only a minor impact on power to detect ageing (Crespin *et al.*, 2006b).

In our interrogation of patterns of ageing in the lab, we did not observe the same striking qualitative differences between cohorts (in this case, between years) present in previous experiments (Chapter 3). It is unclear why this may be the case, but it may point to methodological or environmental consistency in the current work absent in the former. The previous study was carried out by several researchers over a period of two years, while the current work was conducted largely by a single researcher. Inter-individual variation in methods of care, handling, or feeding may have contributed to shifts in patterns of mortality in the prior study (Chapter 3).

Further, all cohorts in the present study were generated and lived mainly through the summer months, corresponding to the period of activity in natural populations (Easton, 1979; National Biodiversity Network, 2024). The previous study reared cohorts throughout summer and winter. While conditions in the laboratory are expected to be relatively stable with respect to temperature and light, changes in other environmental cues (e.g., humidity, barometric pressure, pollen or other natural compounds) may lead to changes in behaviour or activity levels (St. Aubin, 2017), contributing to variation in patterns of ageing.

Alternatively, the previous laboratory population was derived from an admixture of two genetic backgrounds – a population in the Netherland and populations local to Edinburgh. The current study considered a laboratory-maintained population entirely derived from the natural Blackford Hill population. The previous qualitative variation in patterns of ageing may have reflected genetic differences in the initial Netherlands-sourced population (Tully, 2023) that progressively hybridised with local populations over the course of the study, or greater plasticity in the hybridised cohorts.

Finally, we describe here patterns of ageing across summer cohorts as a whole, and not at as fine a temporal scale as previous work. We may not have sufficient power to interrogate within-season changes in patterns of ageing as monthly cohorts contained, on average, 66 individuals (Ricklefs & Scheuerlein, 2001). The previous study had much greater power (up to several hundred per cohort) to detect such variation in ageing.

Several of the concerns outlined above - low recapture rates, understanding of age-dependent dispersal patterns, and uncertainty around individual age - could potentially be addressed with methodological improvements to our field sampling efforts. A number of previous studies assessing ageing in wild insects have relied on the release and tracking only of laboratory-born individuals, eliminating uncertainty around age on recapture (Kawasaki *et al.*, 2008; Mautz *et al.*, 2019; Angell *et al.*, 2022). In our case, we released a total of 513 laboratory-born individuals over two years, of which only 62 were ever recovered. Given the issues outlined above, this was likely insufficient to adequately characterise ageing rates. Future work may involve the release of several hundreds of more laboratory-born individuals in the course of a single season (as in: Bonduriansky & Brassil, 2002; Mautz *et al.*, 2019; Angell *et al.*, 2020). As natural population sizes in our field sites were unknown at the onset of the study, our number of releases was intended to be conservative so as not to unduly distort the dynamics of resident populations. However, the size of the Blackford population appeared to fluctuate by 1000 individuals or more between 2021 and 2022, suggesting that the introduction of a similar number of individuals may be appropriate, and not outwith the bounds of natural annual variation.

Further, a more intensive trapping effort, with the use of a grid or radial design might improve overall recapture rates, improving estimation of ageing rates, and giving an insight into patterns of movement with respect to both age and time in the season (Easton, 1979; Kim *et al.*, 2020). In our study, trap density was higher in the Corstorphine site (2022: approximately 0.49 traps/ha), relative to the Blackford site (2021: 0.34 traps/ha; 2022: 0.41 traps/ha). The proportion of individuals recaptured appeared to increase with increasing density across years and sites, suggesting more intense sampling of the field sites would yield better results (Easton, 1979). However, this association may be erroneous, and may in fact reflect population dynamics, response to weather conditions, or differences in site composition or habitat fluctuating over time, independent of our efforts. Further, our trap placement was determined in large part due to human activity and land management practices (e.g., tree felling and clearing of undergrowth), resulting in non-uniform transects. Previous studies in *N. vespilloides* and closely related species have employed much more structured trapping designs (Springett, 1967; Easton, 1979; Kim *et al.*, 2020) to estimate population dynamics, population density, habitat choice, or movement between sites. While these studies reported low recapture rates overall, Springett (1967) estimated that they had sampled 40% of all individuals present during a season in an isolated island population (Inner Farne, Northumberland, UK). A regular grid with closely spaced traps would likely increase recapture rates (Easton, 1979) while allowing for greater resolution of individual movement within the study area, whereas a trapping web (wherein individuals are released at the centre of multiple expanding rings of traps) might similarly improve recapture rates and shed light on whether individuals in a population differ in habitat exploration or use from a common release point. In this instance, released individuals may disperse randomly, or engage in non-random dispersal in response to habitat quality or cues. Finally, our field sampling was conducted

relatively small areas of woodland (approximately 70 ha in 2022) contiguous with larger woodlands, meadows, and grasslands that may house burying beetles, or carrion. Either increasing the coverage of traps over the entirety of these field sites, or focussing on more remote habitats (i.e., islands: Springett, 1967; Easton, 1979; discrete woodlands: Sun *et al.*, 2020) might serve to effectively reduce emigration rates, increasing overall captures, and strengthening the interpretive association between individual disappearance and mortality.

4.4.4 Age-specific trends in body mass in wild- and lab-living populations

Among insects, increased body mass may be associated with improved cold tolerance, drought resistance, immune function, fecundity, and survival (Rantala & Roff, 2005; Stoehr, 2007; Yoder *et al.*, 2010; Sturm, 2016; Badwan & Harper, 2021b). General trends in insect body mass with age, which may underpin patterns in mortality or lifespan, are unclear, with empirical evidence for late-life declines (De Luca & Cocroft, 2011; Halle *et al.*, 2015; Pásztor *et al.*, 2022) or mild increases or plateaus in mass (Hilligsøe & Holmstrup, 2003; Tabata & Teshiba, 2018). Previous work in captive populations of *N. vespilloides* has described rapid increases in body mass following emergence as an adult and plateauing in early adulthood at a mass assumed to be adaptive for searching for reproductive resources (Hopwood *et al.*, 2013; Trumbo & Xhihani, 2015). In holometabolous insects, such as *Nicrophorus* spp., variations in body mass following the development of flight muscles may be expected to be minor (Bowler & Terblanche, 2008).

In our case, we observed monotonic increases in mass in laboratory-maintained individuals and late-life plateaus or possibly declines in mass in wild-living individuals. These patterns may have reflected very different processes in the two environments. Laboratory-maintained individuals were largely sedentary, restricted to movement within a small plastic container. This precluded most flight activity, and only allowed for locomotion or shallow burial or hiding behaviours. In concert with *ad libitum* feeding, it is perhaps not surprising that individuals in this environment exhibited a linear increase in mass with age. Further, aside from elevated mortality of the smallest emerging adults at early ages, there was no clear linear signal of selective disappearance which might suggest that larger individuals had longer or shorter lifespans than intermediate-sized counterparts.

Wild-living individuals, however, were presumably highly active, with some degree of flight activity necessary for entry to traps. The greater rate of initial mass increase in wild-living individuals may have reflected a more suitable diet than that consumed by laboratory-maintained individuals (organic beef). Alternatively, this trend in body mass may have reflected the presence of bonanza resources (i.e., carrion) in the wild, or our provision of carrion bait as a means of attracting wild-living individuals. Captured individuals had access to a high-quality food source (i.e., carrion bait) for up to seven days prior to each assessment of mass in the laboratory, which may result in rapid increases in mass and stabilizing at weight determined by body size. Elevated rates of mortality in the wild translated to a greater potential for selective disappearance to shape apparent age-specific trajectories in body mass

(Hämäläinen *et al.*, 2014). As ages at death were unknown for the wild-living, laboratory-born cohort, we could not account for selective disappearance in this cohort. If body mass confers a survival benefit, as in other insects (Badwan & Harper, 2021b), the apparent population-level trend in mean mass may be strongly positive with respect to age, even where individual mass is constant, or only weakly positive with age (Fay *et al.*, 2018). Future, more intensive, schedules of release of known-age individuals and their recapture in the wild may provide greater insights into within-individuals trends in body mass.

4.4.5 Conclusion

Our study presents the first attempt to characterise ageing in *N. vespilloides* across the divide of benign laboratory and the complex and challenging natural conditions experienced in the wild. Previous studies that have described ageing in wild insect populations have focussed on sedentary, philopatric, or spatially isolated populations (Nussey *et al.*, 2013; Zajitschek *et al.*, 2020). Here, we attempted to develop *N. vespilloides*, a highly mobile, dispersive species, as a model for study under natural conditions. While our work highlights the challenges typical of working in natural insect populations, our results suggest that this is a promising system in which to pursue future characterisation of ageing in the wild.

Future work may implement more spatially complex trapping regimes, expanded release of known-age individuals, consider populations occupying discrete, isolated habitats, or more intense trapping within a given site to overcome low recapture rates or uncertainty introduced by age estimation. Analytical approaches that relax the assumption that time since first capture is equal to age (i.e., BaSTA; Colchero *et al.*, 2012) or account for individual heterogeneity in survival (i.e., random effect or mixture models (Gimenez *et al.*, 2018) performed poorly in the current study. Greater coverage of individuals in future field studies (i.e., more recaptures per individual or a greater proportion of right-censored individuals to interval-censored individuals) may also facilitate the use of these more complex statistical tools. Future versions of BaSTA purport to incorporate flexible modelling of recapture probabilities (https://github.com/fercol/basta2.0), providing a means to account for uncertainty in ages with no sacrifice of flexibility in account for seasonal effects in recapture rates that appear so striking in natural populations of *N. vespilloides*.

Our analyses also highlight outstanding questions of phenotypic ageing or behavioural variation in natural populations that remain unexplored. For example, teasing apart age-specific or seasonal changes in flight or dispersal behaviours from estimates of apparent survival would not only shed light on sources of phenotypic variation in natural populations, but also improve estimates of ageing rates. Previous work has shown that several aspects of flight behaviour can be measured easily and non-destructively in this species (Attisano & Kilner, 2015; Chapter 2).

Finally, more work is needed to understand the nature and scale of variation in population dynamics in *N. vespilloides*. Such work may aim to the rate, if any, of gene flow between similar, spatially isolated, populations for which evidence is sparse (Easton, 1979; but

see: Sun *et al.*, 2020). Common garden experiments (relying on natural variation between discrete populations) or manipulative experiments could be conducted to explore if and how these population characteristics may shape ageing. These studies may explore whether population characteristics of discrete populations (that are nevertheless proximate to one another) vary with respect to shared environmental cues (e.g., temperature or rainfall) or in response to population-specific trends (e.g., density-regulation).

5 Computer-vision methods to identify individuals of *Nicrophorus vespilloides* on the basis of elytral patterns

5.1 Introduction

In wild populations, the task of assessing population abundance, dynamics, or demography often relies on some means of tracking and identifying individuals across time and space (Walker & Wineriter, 1981; Bolger *et al.*, 2012). In large or easily-handled species such as deer, sheep, or birds, tags, collars, rings, or brands can be applied to facilitate individual identification on future encounters or sightings (Sherwin *et al.*, 2002; Tavecchia *et al.*, 2005; Moyes *et al.*, 2006; Pruett-Jones *et al.*, 2010). However, for very large, very small, or elusive species, adhering tags, applying brands, or other markings may be impractical, dangerous to researchers, harmful to the organism in question, or ineffective over longer timescales (Zúñiga *et al.*, 2002; Gompper *et al.*, 2006; Butler *et al.*, 2012). For example, toe clipping for the purpose of individual identification in reptiles or amphibians may be harmful to individual survival, and ineffective given the prevalence of natural toe loss in this taxon (McCarthy & Parris, 2004; Schmidt & Schwarzkopf, 2010; Gould *et al.*, 2023). Capturing or handling large carnivores in the course of mark-recapture studies may be dangerous to animals and researchers alike, and it can be challenging given that these species often occur at low densities or are elusive (Akenson *et al.*, 2001; Gompper *et al.*, 2006; Moriarty *et al.*, 2012).

Marking and subsequently identifying small-bodied insects poses a unique challenge (Walker & Wineriter, 1981; Piper, 2003). Adhesive tags and paints rely on a large enough surface area on which to attach or apply a unique marking (Walker & Wineriter, 1981). These applications may be harmful to survival if they attract predators or collect debris over time (Butler *et al.*, 2012). Further, these adhered tags may be lost due to natural behaviours such as moving through soil or other substrates (Zúñiga *et al.*, 2002; Butler *et al.*, 2012). The clipping or branding of insect elytra, though permanent, has similar disadvantages (Walker & Wineriter, 1981). Removing or weakening sections of the elytra through branding may expose the wing to injury and affect flight, thereby harming individuals' ability to forage or avoid predation (De Souza & Alexander, 1997; Jenkins *et al.*, 2016). Clipping of the elytra may also affect sexual signalling (e.g. stridulation) that negatively affects reproductive success (Hall *et al.*, 2015; Schrader & Galanek, 2022). However, many species of vertebrates and invertebrates have information that derives from naturally occurring characteristic marks – wing markings, scars, spots or scales - that can be leveraged using manual or sophisticated computer-vision methods as the basis of individual identification (Van Tienhoven *et al.*, 2007; Bolger *et al.*, 2012; Díaz-Calafat *et al.*, 2018; Quinby *et al.*, 2021; Langley *et al.*, 2022). In these cases, researchers have increasingly turned to photographic-based identification rather than more traditional, invasive methods.

Several custom tools and pipelines have been developed with specific use-cases, species, and degrees of automation in mind (MYDAS for green turtles; Carter *et al.*, 2014), AmphIdent for amphibians and reptiles (http://www.amphident.de/en; Matthé *et al.*, 2008), I3S Classic and I3S Spot for sharks and rays (https://reijns.com/i3s/; Van Tienhoven *et al.*, 2007), Wild-ID for giraffes (Bolger *et al.*, 2012), and Hotspotter for specific taxa (Crall *et al.*, 2013). While several of these tools are freely available (e.g., various I3S applications; ExtractCompare: Hiby & Lovell, 1990; Wild-ID; Hotspotter; APHIS: Moya *et al.*, 2015), others are proprietary and require a one-off purchase (e.g., AmphIdent) or operate under a subscription model (e.g., Wild Me; https://www.wildme.org/). These different platforms address specific challenges in pattern recognition and matching: changes in subject posture or observer viewing angles that affect identifying patterns; localisation of individuals against complex backgrounds; and varying levels of automation.

Original use-cases aside, most of these applications can perform well in processing patterns and identifying individuals in a wide range of taxa (Matthé *et al.*, 2017; Langley *et al.*, 2022; de Lorm *et al.*, 2023; Givord-Coupeau & Rey, 2023). Several recent studies have affirmed the viability of this approach using discrete elytral patterns in the cerambycid beetle (*Rosalia alpina*; Caci *et al.*, 2013; Rossi de Gasperis *et al.*, 2017) and in burying beetle species (*Nicrophorus americanus* and *N. orbicollis*; Quinby *et al.*, 2021) and mandible shapes in stag beetles (*Lucanus cervus*; Romiti *et al.*, 2017).

Despite the increasing complexity and efficacy of computer-vision methods, many of these tools developed to aid photographic record-based identification revolve around the same process (illustrated in Figure 1). However, a negative consequence of the growth of specialised use-cases in this space is the lack of flexibility of these packages to process simplified datasets. This results in additional manual processing of images, thereby reducing the efficiency and scalability of the overall process in large datasets. The complexity of an image – the presence of a complex background that may obscure the relevant individual pattern, poor quality or inconsistent lighting effects (e.g., shadows or over-exposure), or flexible body positioning – may increase the amount of preprocessing required or reduce the efficacy of 'fingerprint' extraction. For example, when images of subjects are captured at multiple angles, manual annotation of reference points is often required (e.g., I3S Classic, I3S Pattern, I3S Spot, I3S Contour, APHIS, AmphIdent, ExtractCompare). These reference points facilitate transformations of the original images into a standard plane to better align 'fingerprints' for extraction and comparison (Van Tienhoven *et al.*, 2007; Moya *et al.*, 2015). However, this manual annotation stage can generally not be avoided even in cases where all images are standardised with respect to scale and angle. With small-bodied insects, standardising these aspects of image capture is straightforward. Insects can generally be captured with greater ease than many large vertebrates and immobilised for image capture, if necessary, using CO2 or brief chilling (Gasperin & Kilner, 2016; MacMillan *et al.*, 2017; Groening *et al.*, 2018). Further, rigid exoskeletons or large, flat wings that exhibit characteristic markings minimise the challenge of flexible body positioning or observer

viewing angle present in vertebrate systems that might increase the apparent within-individual variation in extracted 'fingerprints'.

Regions of interest in a photograph may also need to be manually annotated (I3S Classic, I3S Pattern, I3S Spot, I3S Contour, Wild-ID, Aphis, ExtractCompare, MYDAS) or a machine-learning model trained on a particular taxonomic group or species to locate said regions (Wild Me, and that described in: Ferreira *et al.*, 2020). Either annotation process may be time-consuming or computationally demanding, and in the latter case, may be highly specific to study species, image layout, or background complexity. For example, Wild Me offers pre-trained models for annotating images of 18 different taxonomic groups. For species with discrete colour banding (i.e., identifying regions are discrete, binary patches of colour) and in images with simplified backgrounds, simpler methods may be more effective. Many insect species exhibit body markings characterised by discrete bands or patches of colour. These patterns are present on rigid wing cases (elytra), carapaces, or on wings themselves, and are thus reasonably constant with respect to motion or body position. The ease of handling of many small-bodied insects facilitates the capture of images against neutral backgrounds under constant lighting conditions and devoid of environmental complexity. In this case, segmenting images into 'relevant pattern' versus 'non-relevant background' by colour range may be far more efficient (see: Weller *et al.*, 2024, for an overview on segmentation of animal colour patterns). Further, individual colouration may be indicative of condition, diet, or age (Wormington & Luttbeg, 2018; Badejo *et al.*, 2020; McMurry *et al.*, 2023). Therefore, standardising image capture may serve the dual purposes of improving the efficacy of image segmentation and improve the fidelity of colouration data contained within images that is necessarily extracted during the segmentation process.

Finally, most current packages implement only one, or at most two, 'fingerprinting' and matching algorithms (described in Methods). Among the 10 packages described above, nine distinct 'fingerprinting' algorithms are used, and only a single package (APHIS) implements two 'fingerprinting' methods simultaneously. Different algorithms may be sensitive to different types of noise (image rotation, relative angle or distance of the subject to the camera, lighting, etc.; Tareen & Saleem, 2018; Bansal *et al.*, 2021). This means that a fingerprinting and matching method that performs well in one context, or with respect to a certain biological system, may not be as effective in a different context (de Lorm *et al.*, 2023). A more robust approach would be to employ several algorithms concurrently and compare matches across these different selection criteria.

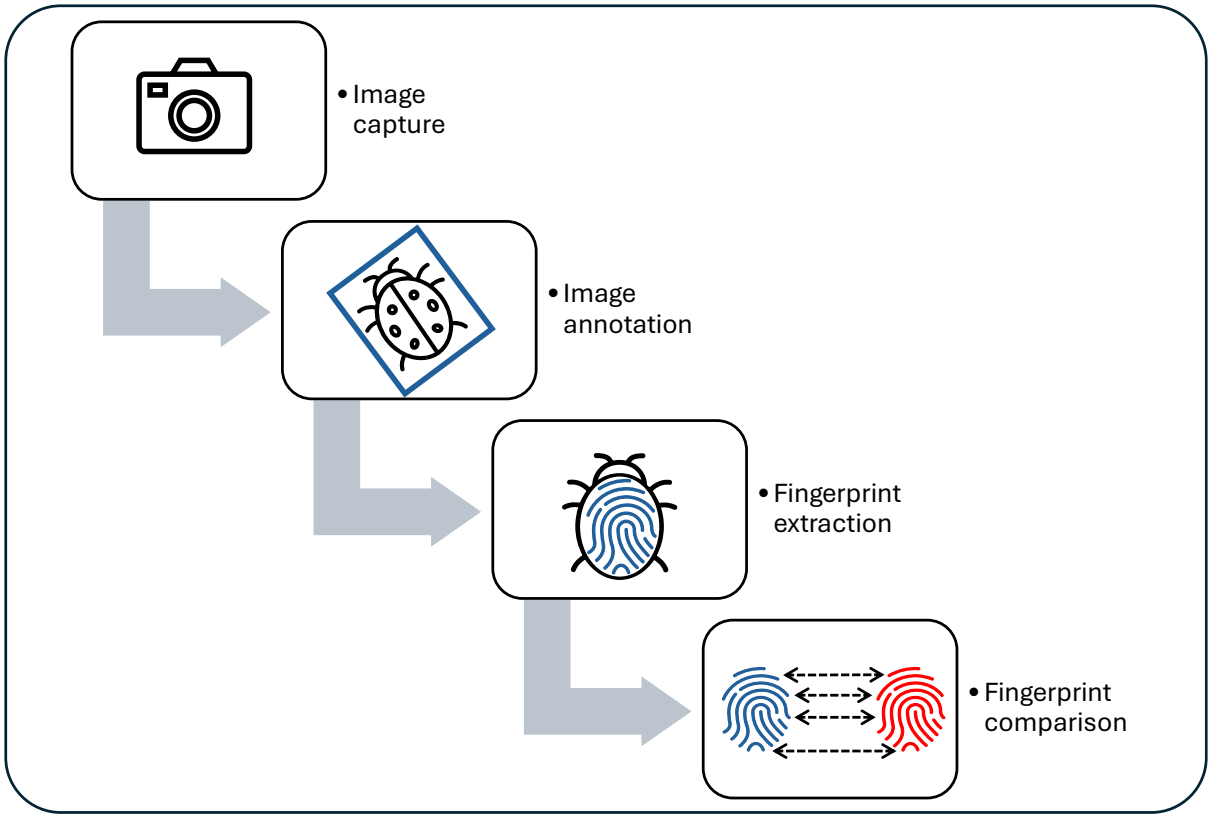

Figure 5-1: The general pipeline of photographic record-based identification packages. First, an image is captured containing one or more individuals one wishes to identify. Second, this image is annotated to localise individuals or identifying regions to be analysed. Third, a 'fingerprint' is generated from the features highlighted in this image using a pre-determined algorithm (discussed below). Fourth, this 'fingerprint' is compared against a database of similarly-generated 'fingerprints', and some selection of potential matches is offered for the researcher to confirm. Depending on the complexity of the 'fingerprinting' methods, this may involve scaling two candidate images to the same size and noting matching pixels (eg. APHIS, AmphIdent, or calculating some 'distance' between multi-dimensional descriptors of similar key-points in either image (APHIS, Wild-ID, I3S Pattern, Hotspotter).

Here we present a method of image capture and individual identification that reduces pre-processing demands, employs several algorithms for 'fingerprint' extraction, and is broadly applicable to invertebrates with discrete colour patterns. This workflow aims to reduce the complexity of images at the data-collection stage and lessen the need for complex image pre-processing or manual image manipulation. Our approach differs from most other packages described above in employing several different 'fingerprinting' algorithms simultaneously: two 'fingerprinting' algorithms common to several of the existing packages described (I3S Pattern, Wild-ID, Hotspotter) and an additional computer vision algorithm not currently included in animal recognition packages (described below). This approach is designed to allow for rapid individual identification while maximising accuracy and scalability to large datasets. We describe the general features of this workflow and pipeline and illustrate its efficacy using a photographic record collected from a laboratory population of burying beetles, *Nicrophorus vespilloides*, a small-bodied insect with discrete colour markings.

5.2 Methods

5.2.1 Image capture

Beetles can be challenging to photograph to a high standard due to small body sizes, reflective exoskeletons, and convex body shapes (Riedel *et al.*, 2005). Further, we eschewed anaesthetising individuals due to potential harmful effects (Bartholomew *et al.*, 2015; Overgaard & MacMillan, 2017; Groening *et al.*, 2018), increasing the challenge of capturing detailed, focussed photographs of elytral patterns. Therefore, images were captured in a standardised manner balancing the competing needs of adequate illumination, detail, speed, glare reduction, and depth of field (i.e., keeping the subject in focus across a wide range of depths). The exact arrangement of physical orientation, lighting, and camera settings that follow are not intended to be prescriptive guide to optimal photography methods but to indicate aspects of data-collection that can, and should, be standardised.

Images were captured using a tripod-mounted digital single lens reflex camera (DSLR Canon EOS 500D) with a macrophotography lens (Canon EF 100mm f/2.8 USM) in a fixed orientation approximately perpendicular to the subject. Individuals were mounted on a neutral blue background (*N. vespilloides* body colours are black and orange) a set distance from the camera sensor. This achieved the primary aims of standardising viewing angle and reducing image complexity for fingerprint extraction.

Variation in focal length can result in the expansion or compression of depth in an image (Kingslake, 1992; Banks *et al.*, 2014; Třebický *et al.*, 2016), potentially distorting individual identifying patterns. In general, shorter focal lengths can enhance the appearance of depth in an image, exacerbating the apparent convexity of insect body shapes. Longer focal lengths appear to compress depth in images (i.e., subjects appear flatter than they truly are). Therefore, all images were taken at a fixed focal length of 60mm. Further, while the depth-of-field in images increases with decreasing shutter aperture, this comes at an increased cost of artefacts caused by diffraction, particularly at high magnification (Riedel, 2005), as in our case. Therefore, we chose an aperture value of 6.3 as a compromise between these two concerns.

Shutter speed affects the amount of light that falls on a camera sensor. For a given aperture setting, slower shutter speed increases the brightness at a potential cost of over-exposing an image (i.e., capturing too much glare) or causing motion blur. Further, iso settings affect the sensitivity of the camera sensor to light, affecting exposure and colour accuracy. Therefore, shutter speed and iso were set at 1/30 and 400, respectively, to avoid overexposure and reduce image noise and blurring.

The light-stage in which images were captured was also standardised with the use of two tri-pod mounted LED panels (5500 Kelvin, 1000 lumen). These panels were angled approximately 90^{O} from each other, oriented toward the subject, and placed approximately 15 cm from the subject. This arrangement effectively flooded the viewing area of the camera

with a consistent level and temperature (i.e., colour) of light, without producing excessive glare from beetle elytra.

To improve the efficacy of colour-segmentation, image-processing (described below), and the fidelity of colour in captured images, we periodically photographed a grey card (Häuser, 2005; Troscianko & Stevens, 2015). Grey cards, objects with neutral, 18% reflectance across the visible spectrum, are tools for correcting image exposure and colour accuracy under known lighting conditions (Troscianko & Stevens, 2015). Calibrating a series of images of subjects against images of this known reflectance value corrects for variations in the temperature of ambient lighting that may change between photography sessions.

5.2.2 'Fingerprinting' algorithms

We employed several 'fingerprinting' algorithms to generate three independent sets of descriptors describing each elytral pattern. Each of these methods (described below, in brief) has different strengths and weaknesses with respect to complexity, processing time, and robustness to different sources of noise (Tareen & Saleem, 2018; Bansal *et al.*, 2021). In combination, this suite of 'fingerprinting' techniques was intended to account for image scale, rotation, blurring, changes in illumination, or change in viewing angle to a far greater extent than any single approach could offer. Regardless of 'fingerprinting' method, determining the similarity between two patterns (and thus potential individual matches) requires calculating the Euclidean or Hamming distances between sets of 'fingerprints', with low values indicating similar patterns, and high values indicating dissimilar patterns (Noble, 2016; OpenCV: Feature Matching, 2024).

5.2.2.1 SIFT

Scale-invariant fast transformation (SIFT; Lowe, 2004) is a widely used 'blob' detector in computer-vision applications and is employed by both Wild-ID and Hotspotter. Broadly speaking, SIFT works by progressively blurring an image, and comparing the location of influential pixels (extrema of pixel values) between these degrees of blurring (OpenCV: Introduction to SIFT (Scale-Invariant Feature Transform), 2024). The exact locations of these key-points are then determined using a thresholding value. The orientation of these key-points are assessed based on local image gradient (the relative angle of light and dark clusters of pixels), accounting for the scale of the key-point and magnitude of the gradient. Key-points are then defined by size, orientation of the major axes of pixel values, and local gradients of pixel values (i.e., 'descriptors').

This approach is broadly robust to image scale and rotation and to changes in 3D viewpoint and changes in lighting (Lowe, 2004). However, it is mathematically complex relative to other algorithms, is slower at generating 'fingerprints', and performs poorly with blurred or distorted patterns (OpenCV: Introduction to SIFT (Scale-Invariant Feature Transform), 2024; Tareen & Saleem, 2018; Bansal *et al.*, 2021).

5.2.2.2 SURF

Speeded-up Robust Features (SURF; Bay *et al.*, 2008) is a faster version of the SIFT technique and is employed by the I3S Pattern application. The steps of key-point (i.e., 'blob') detection are broadly similar, with simplified methods of blurring and a sliding-window wavelet calculation to determine key-point orientation (OpenCV: Introduction to SURF (Speeded-Up Robust Features), 2024). SURF also uses the sign of the Laplacian to distinguish bright regions on dark backgrounds from dark regions on light backgrounds. In the 'fingerprint' comparison stage, this means only descriptors of similar contrast are compared, allowing for faster processing. This approach is robust to image blurring and rotation but may not perform as well as SIFT in accounting for changes in illumination or scale (OpenCV: Introduction to SURF (Speeded-Up Robust Features), 2024; Tareen & Saleem, 2018; Bansal *et al.*, 2021).

5.2.2.3 ORB

Oriented FAST and Rotated BRIEF (ORB; Rublee *et al.*, 2011) was designed as an efficient alternative to the previous two techniques. ORB uses Features from Accelerated Segment Test (FAST) and Harris corner measures to find corners (rather than blobs) based on pixel values (light or dark) and the profile of pixel gradients (OpenCV: ORB (Oriented FAST and Rotated BRIEF), 2024). Key-point orientation is determined by calculating the vector linking the intensity-weighted centroid of a patch, and its corner. Key-points are then described using Binary Robust Independent Elementary Features (BRIEF) which selects a series of location pairs in each key-point and compares pixel intensities within these pairs. ORB can be faster than both SURF and SIFT and extracts fewer but more meaningful features from images (OpenCV: ORB (Oriented FAST and Rotated BRIEF), 2024). This technique is robust to scale and rotation but may not perform well with blurry or distorted images (Tareen & Saleem, 2018; Bansal *et al.*, 2021).

5.2.3 Image processing, segmentation, and matching

Images should first be colour-standardised against a grey card (SpyderCheckr 24, Datacolor UK Ltd) in the open-source image-processing program *darktable* (v4.6.1). This involves setting the white balance of images containing the grey card against the card itself and applying those values to any images captured immediately following the calibration image. While this is somewhat a manual process, colour-correction can be applied to dozens or hundreds of images simultaneously, depending on the schedule of photography.

While not strictly necessary, images can be named informatively, if desired, and exported in large numbers from the *darktable* program. Tags, manually applied to individual images or batches of images simultaneously, along with metadata (e.g., date of capture, GPS location, etc.) contained within the images themselves can be included in naming templates.

All further image processing, fingerprinting, and individual matching was conducted in Python 3.10 using the package opencv2 (v4.7.0). A minimal graphical user interface was built using the package PyQt6 (v6.6.1) for the latter stages of image comparison. The python

pipeline described below includes a number of convenience functions that are not strictly required for proper function but allow for error-investigation and correction and streamlined project formats. Such additional functions will be noted throughout. All user-specified settings, and their functions, are defined in Table 1. The relevant scripts are available at https://github.com/KynanDelaney/Beetle_ID.

Optional Step 1. Project folder setup – this convenience function creates a folder in a user-specified directory in which to deposit the photographic record. This set-up script establishes the main structure of the project – separate sub-folders for unprocessed photos, data files, scripts, processing errors, fingerprints, and a general 'temporary' folder. Additionally, this script creates log files for pre-processing errors, fingerprinting errors, pair-wise comparison errors, and processing times.

Step 2. Image segmentation and annotation - once deposited into the 'unprocessed photos' folder, the main cropping and rotating function can be applied. Colour corrected images are first converted to a binary mask. In this step, a user-defined colour range (in our case, dark red through to light yellow) is used to discretise the original image into foreground (i.e., the colour pattern of interest) and background (i.e., everything else). A minimum bounding box is drawn around the foreground region and the original image and mask rotated and cropped to that area (Figure 1). The cropped image and mask are then written to the 'fingerprint' folder. Users may optionally set a scaling factor that expands the minimum bounding box area, in the event that this area is too restrictive (outlined in step 6).

Each pair (e.g., "img0001_mask.png" and "img0001_cropped-image.png") are written to a folder named after the original photo ("img0001"). Informative file names in the format "[Date]_[ID]_[Example]" can make this folder more human-readable. However, informative names aren't needed as long as there's a way to connect photo names to user data.

Errors detected at this stage are written to a log file, and the offending images transferred to a 'crop and rotate' error folder. Errors currently are defined as images that exceed a user-defined size threshold or a user-defined aspect-ratio or are missing a foreground region. In the case of *N. vespilloides*, correctly processed images (fewer than six million pixels) are appreciably smaller than input images (twelve million pixels), with an approximately even ratio of image length to width. Additionally, images not containing a subject cannot be masked, generating an error detected by the opencv2 package.

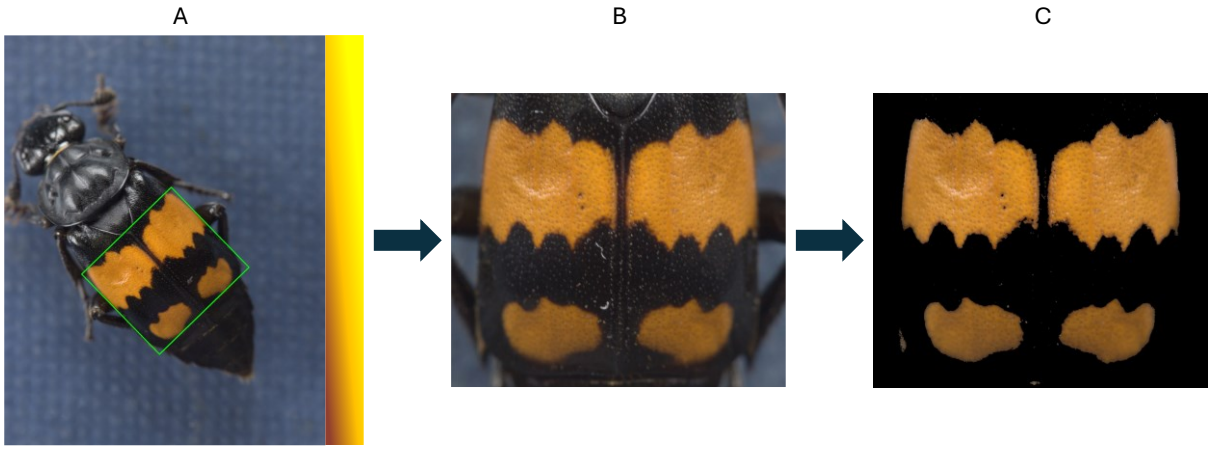

Figure 5-2: An example image crop, rotation, and annotation process. Automatic pattern localisation and segmentation is based on colour values (in the red – yellow colour-space A – a colour-corrected image with an automatically annotated bounding box highlighting the relevant pattern. Regions of identifying patterns are defined by pixels that fall into the colour range displayed alongside image. B – an automatically cropped view of the relevant pattern. C – an automatically-generated mask of the pattern from which a 'fingerprint' can be extracted.

Step 3a. Fingerprint extraction - Once the fingerprint folder is populated with cropped and rotated masks, fingerprints can be extracted. Fingerprints are generated from masks from all example images (Figure 2). User-specified settings are limited to a 'hessian threshold' determining how parsimonious the SURF algorithm defines key-points (higher values are increasingly restrictive) and the number of key-points to be defined by the ORB and SIFT algorithms (Table 1). The resulting fingerprints are stored as text files for later use.

Errors detected at this stage are written to a log file, and the folder involved (including cropped mask, image, and any fingerprints already extracted) are moved to a fingerprinting error folder. Errors of this nature are defined as failed extractions detected by the opencv2 package.

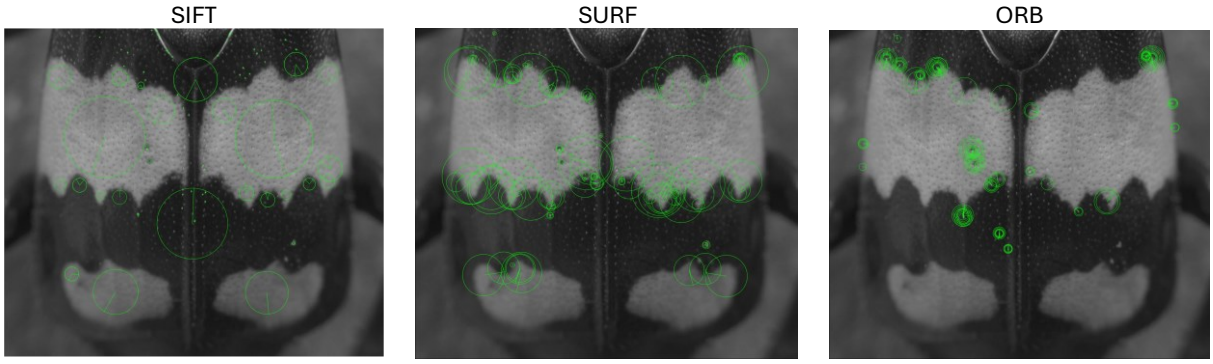

Figure 5-3: Example 'fingerprints' extracted from a single reference image, according to three algorithms (SURF, SIFT, and ORB). Images are in grey-scale to highlight 'fingerprint' features, key-points are in green.

Optional Step 3b. Within-individual assessment - Following fingerprint extraction, a user may opt to conduct an error-checking exercise by running a series of within-individual fingerprint comparison. All known examples of a given individual, either within or between photography session or sighting dates, can be compared in a pairwise manner. If fingerprinting has progressed satisfactorily, all within-individual comparison values (described in step 5) should fall within a similar range. Outlier values may then indicate poorly processed or mis-labelled images that require the attention of the user.

Step 4. Generating pairwise comparisons - Pairwise comparisons between focal and test individuals are drawn from text files defining a single focal individual or a group of individuals ('focal set'), and the images or individuals against which comparisons should be conducted ('test set'). Text files should include the name of an individual or filenames associated with said individual and the date the relevant images were taken. Additional data, such as sex, body size, or location of capture may also be included.

This stage iterates through the 'focal set', finding potential matches in the 'test set' based on matching meta-data values. Currently, this process can look backwards in time, forwards in time, or within the same interval, but not within a specific date range. Numeric meta-data such as body size can be restricted to exact matches, or within a user-defined range (perhaps accounting for measurement error). Meta-data such as sex or location are currently restricted to exact matches. However, missing values in either the focal or test set are considered suitable matches.

The 'focal set' and 'test set' may both include all entries in the database, in which case all valid comparisons will be evaluated, or be restricted to mutually exclusive or overlapping subsets. The output of this step is a text file containing a suitable subset of pairwise comparisons for fingerprint matching.

Step 5. Pairwise fingerprint comparisons – Once a list of valid comparisons is generated, fingerprint matching can be conducted according to one, or all, of three matching algorithms. This process loads fingerprints in pairs using a brute-force matcher to find similar key-points in both patterns and calculates the 'distances' between them. The sum of these dimensionless 'distances' is divided by the number of key-points compared, and this 'distance' is saved. Across all algorithms, smaller values indicate highly similar fingerprints and larger values indicate more pronounced discrepancies. These values are grouped by individuals in the 'focal set' and reduced to a user-specified number of the best-matching 'test set' images for confirmation or rejection by the user.

Errors detected at this stage are written only to a log file. Images involved are not removed to an error-catching folder, as this can cause cascading errors due to missing files in subsequent comparisons.

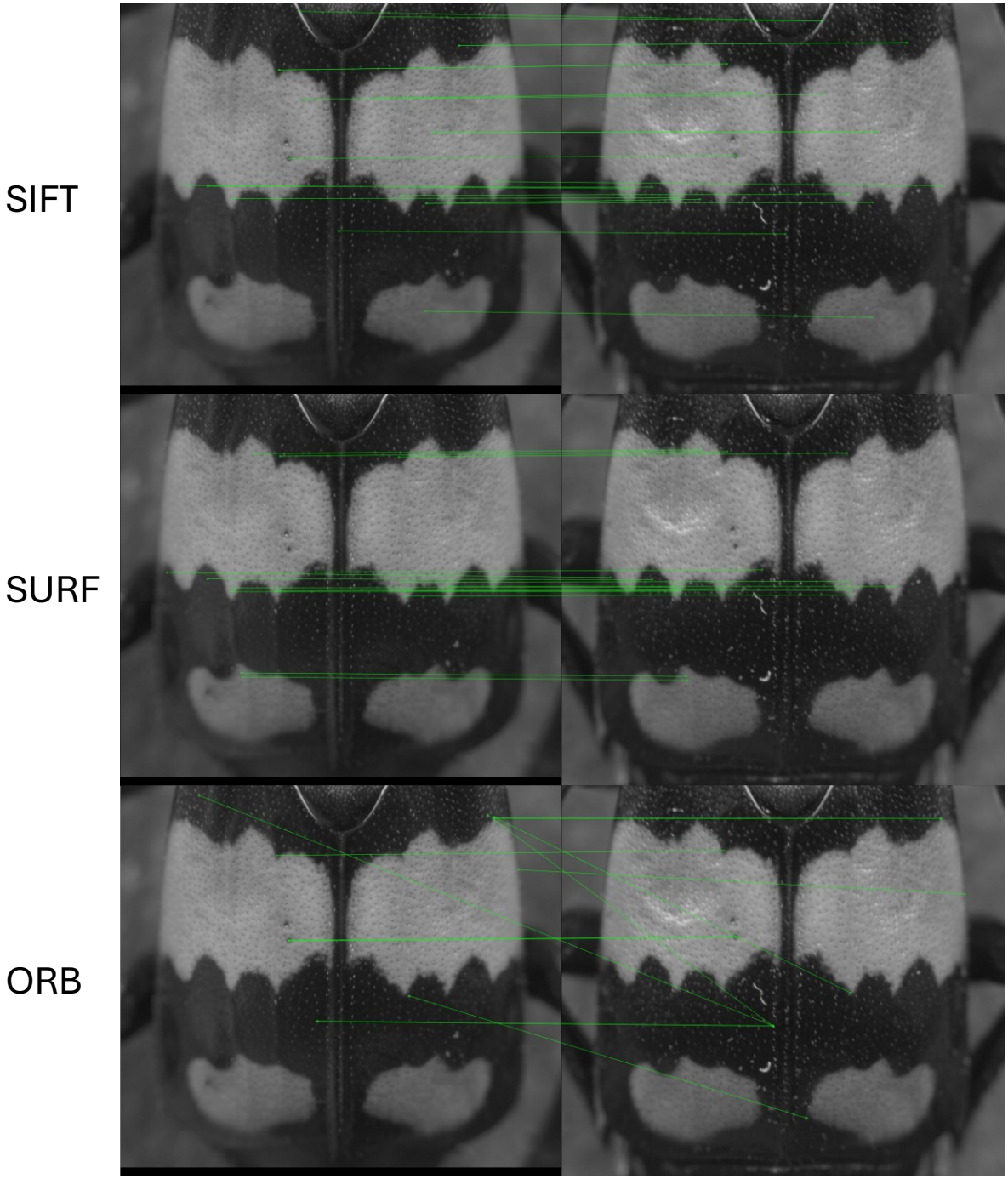

Figure 5-4: Example 'fingerprint' matching between two images of the same reference individual according to three algorithms. Lines connecting images indicate matching key-points according to the relevant algorithm. Images of the reference individual were taken three weeks apart and are in grey-scale to highlight matching features.

Step 6. Confirmation – This stage inherits the sets of best-supported matches according to fingerprint comparison (above) and displays an example of an individual from the 'focal set' and a user-specific number of potential matches from the 'test set'. If non-pattern identifying features (e.g., notches, scars, discolouration) are sometimes present, the view outwith the primary identifying region can be increased (scaling factor set in step 2).

The graphical user interface (GUI) is minimal but presents meta-data about the focal and test individuals (e.g., size and ‘name’) and calculated distance values. Matching pairs are confirmed by clicking the button “Individual is a match”, and the absence of suitable matches is indicated by selecting the button “No matches here". The GUI can optionally allow for single or multiple matches between focal and test individuals, depending on preference. Difficult matches can be skipped or returned to by clicking “Next” or “Previous”.

Focal individuals that have received a confirmation (“ID1 = ID2”) or rejection (“ID3 = unknown”) from the user are saved to a text file and are flagged by the system as completed. Exiting and restarting the GUI will return the user to the first unprocessed focal individual, skipping any previously processed individuals. Finally, the resulting chains of “ID1 = ID2 = ID*n*…..” are aggregated, and the resighting history of individuals resolved.

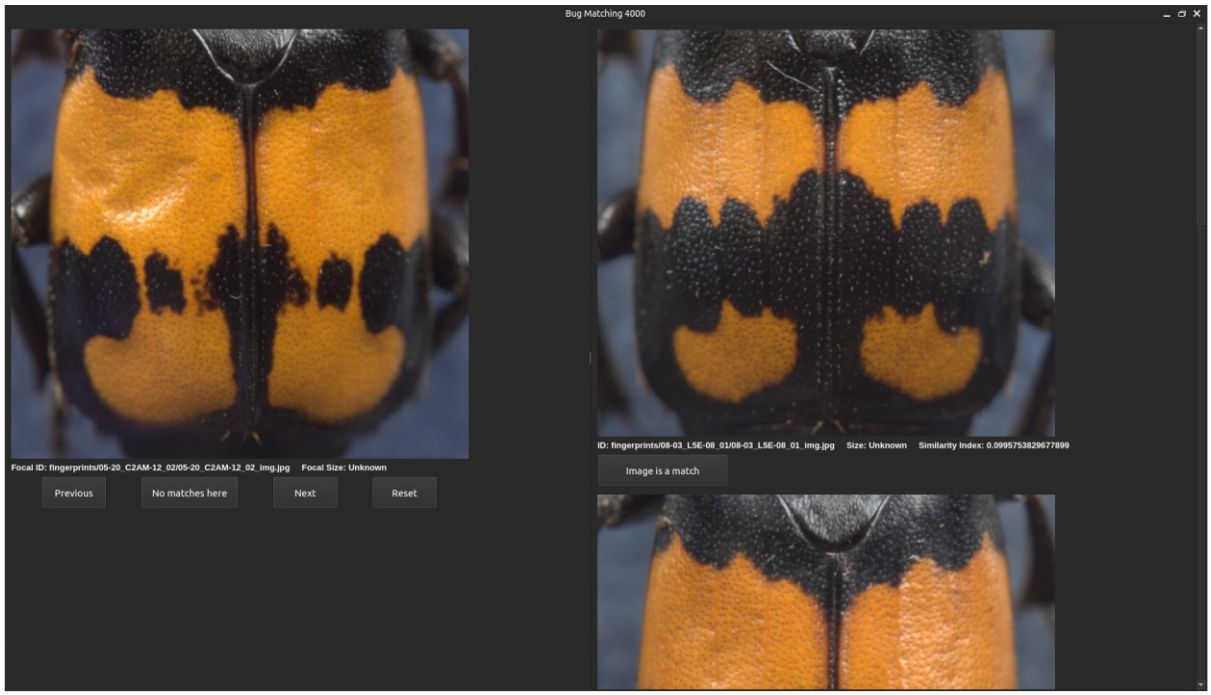

Figure 5-5: An example view of the final manual confirmation or rejection step. Focal individuals are displayed one at a time in the left pane, and n *potential matches displayed in a scrolling window on the right. Focal individuals can be viewed without processing by selecting “Previous” or “Next”. “Reset” resets the view for the current focal individual. Selecting “No matches here” automatically progresses to the next focal individual. Exiting and restarting the GUI will set the user back to the last un-processed (i.e., no decision recorded) focal individual.*

Table 5-1: The complete set of user-specified values and their effect on the performance of the pipeline.

Stage	User input	Outcome
Project setup	*Project name*	A convenience setting to name a project in the event several separate photographic records are being analysed.
Image segmentation and annotation	*Colour thresholds*	The lower and upper bound of colours by which images are segmented (Figure 1). Exact values will depend on study species and variability in colour patterns. Default colour space is Hue-Saturation-Value (HSV) in the format H{0:180}, S(0:255}, V{0:255}. In broad terms, Hue refers to colour (red, green, etc.), Saturation refers to colour-depth, and Value refers to brightness.
	Grey-scale threshold	A simplified thresholding value that segments a grey-scale version of the focal image (0 = black, 255 = white).
	Colour-patch size threshold	An optional setting that ignores small colour patches or noise during image segmentation. Value is in pixels and varies depending on focal species size, degree of zoom in captured images, and typical colour-patch sizes.
	Number of colour-patches	An optional setting that ignores excessive numbers of colour-patches associated with image noise or other artefacts.
	Scale view	A scaling factor that expands the annotated area from the minimum view based on colour-segmentation.
	Cropping threshold	An optional setting that determines atypically large images to be poorly cropped. Value is in pixels and varies depending on focal species size and degree of zoom in captured images.

Fingerprint extraction	*Hessian threshold*	A value used by the SURF algorithm to filter out key-points that are considered non-influential. Larger values are more selective and eliminate a greater number of candidate 'fingerprint' features.
	Number of features	The maximum number of features to be extracted from images according to the SIFT and ORB algorithms.
Generating pairwise comparisons	*Size offset*	Discrepancies in body size accepted during the filtering of potential pairwise comparisons. Units are on the same scale as the provided body size measures (e.g., millimetres, inches, etc.).
Pairwise comparisons	*Number of features*	A limit applied to 'distance' calculation for SURF fingerprints. Sets the maximum number of best-matching 'fingerprint' features to be evaluated. Can be inherited from the variable of the same name (above) for consistency in methods across 'fingerprinting' algorithms.

5.2.4 Case study

We evaluated the efficacy and efficiency of the (above) workflow and pipeline using a laboratory population of *N. vespilloides*. This and closely related Nicrophorus spp. are characterised by black bodies with discrete red and orange markings on their elytra. These markings are persistent and individually distinct, facilitating individual recognition without the need for adhesive or destructive markings (Quinby et al., 2020; J. Moorad, pers. comm.). We imaged the elytral patterns of 708 individuals over a period of six months. Individuals were sexed and pronotum width (a proxy for body size in this species; Müller *et al.*, 1990; Creighton, 2005) was recorded using digital callipers (CD-6" CSX, Mitutoyo Corp.; accuracy: +/-0.02mm). Individuals were imaged between one and eight occasions over this period (312 individuals imaged on multiple occasions, 396 individuals imaged on a single occasion). This yielded 5,385 images for colour-correction, segmentation, annotation (Step 2, above), and 'fingerprint' extraction (Step 3a, above). This entire image database was treated as both 'focal set' and 'test set', that is, we performed exhaustive searches of all individuals against all other images in the photographic record. A pairwise list of comparisons to conduct (Step 4) was generated, filtering suitable examples from the 'test set' by sex and body size. Comparisons were only conducted between sampling occasions (i.e., across days, rather than within days) to avoid images of an individual taken in series (i.e., within seconds of each other) inflating our success

rates. All identities were known to the researchers, therefore the success of the 'fingerprint' matching and confirmation process (steps 5 and 6, above) was measured against this certainty.

We first evaluated the efficacy of the three core 'fingerprinting' algorithms by visually comparing the distributions of 'self' vs 'non-self' comparisons. We considered an algorithm to be an effective agent of identifying individuals if these distributions were largely non-overlapping. For example, if all 'distance' values associated with 'self' comparisons were less than one and all 'non-self' comparison 'distances' were greater than one, then the system would perfectly discriminate between all 'self' and 'non-self' examples. As the overlapping region of 'distance' values associated with each class of comparison increases, the system becomes less effective at segregating 'self' from 'non-self' examples.

We then considered whether low 'distance' values could be leveraged to automatically assign positive identifications in the photographic record. Therefore, we estimated the false-positive rate that would occur if all matches were accepted based on 'distance' values, according to each algorithm (SIFT, SURF, and ORB). The incidence of naïve false-positives ('self' and 'non-self' comparison; 0 and 1, respectively) was analysed with a binomial model using a complementary log-log link function. This link function is suited to Bernoulli data with many zeros or many ones (Zuur *et al.*, 2009), as in our case. We analysed the false positive rate of each algorithm separately, with each model including only a smooth of 'distance' value. This analysis was conducted using the package using mgcv v1.8-41 (Wood, 2011, 2017) in R v4.3.1 (R Core Team, 2023).

We then narrowed our focus to individuals with a known match somewhere in the photographic record (312 individuals). As these individuals were each imaged on up to eight occasions, this resulted in 733 distinct searches for a match. For this subset, we determined the number of positive identifications within the *n* best matches (determined by lowest 'distance' values). Previous case studies of other pattern matching software in vertebrate and invertebrate taxa have considered a positive identification within the 20 closest matches to be a success (Halloran *et al.*, 2015; Morrison *et al.*, 2016; Quinby *et al.*, 2021; Langley *et al.*, 2022). Therefore, we evaluated up to 20 matches per search.

Finally, as efficiency was a core focus of developing this pipeline, we report the processing times of each stage from image segmentation through to pairwise fingerprint comparisons. Though brief, the prior steps (imaging and colour-correction) and subsequent step (confirmation of matches) are manual and vary in duration depending on the investigator's ability.

5.3 Results

Our exhaustive search of potential matches based on individual sex, body size, and date of image capture yielded 5,472,469 pairwise 'fingerprint' comparisons that were passed to the 'fingerprint' comparison step. Of these pairwise comparisons, 25,605 pairs were within-individual (or 'self') comparisons, and 5,444,416 were inter-individual ('non-self') comparisons. 2448 instances found no suitable matches (an outcome for all images associated

with the first sampling occasion wherein no previous cohort is available to compare against or cases in which no suitable matches within the body-size threshold were found).

Visually comparing the performance of the three' fingerprinting' algorithms suggested that SURF and SIFT were more effective at discriminating between 'self' and 'non-self' image pairs than ORB (Figure 4A). However, there was a significant degree of overlap in 'self' and 'non-self' 'distance' values in all cases (Figure 4A). Further, false positive rates were high at almost all 'distance' values, suggesting that an arbitrary (i.e., very low or very high) value of 'distance' may not, in itself, be a good threshold for accepting or rejecting a match in the photographic record (Figure 4B).

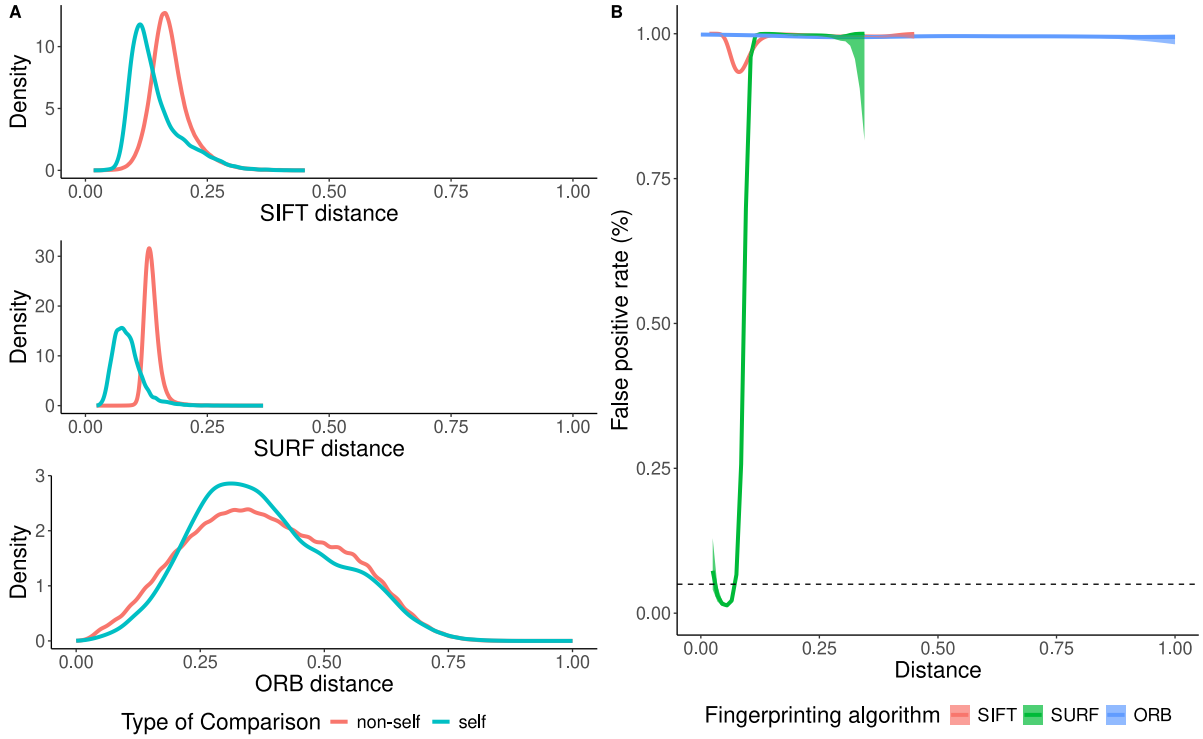

Figure 5-6: A - the density distributions of 'distance' values arising from 'self' and 'non-self' fingerprint comparisons. 'Distance' values were transformed to be presented on a similar scale for visual comparison. SIFT and ORB distance values (Euclidean and Hamming distance, respectively) were reduced by a factor of 1000 and 100, respectively. SURF distances (Euclidean distance) are on the latent scale. B – the apparent false positive rate according to each algorithm if 'distance' values are used as a naïve indicator of a positive match. Ribbons denote 95% confidence intervals; dashed line indicates an arbitrary threshold of 5% false positive rate.

The fingerprinting and matching methods were much more effective as a means of sorting potential matches for focal individuals for whom a match was known to exist in the photographic record (Figure 5). With SURF, the closest matching fingerprint (sorted by lowest 'distance') provided a positive identification in 98.8% of cases (up to 99.6% within the 20 lowest 'distances'). SIFT and ORB were much less effective as a method of sorting potential matches (SIFT: 70.8% effective within 20 lowest 'distances'; ORB: 14.1%). Therefore, choosing

the most effective 'fingerprinting' algorithm for a given species or set of patterns (in our case, SURF) effectively reduced thousands of unnecessary manual comparisons.

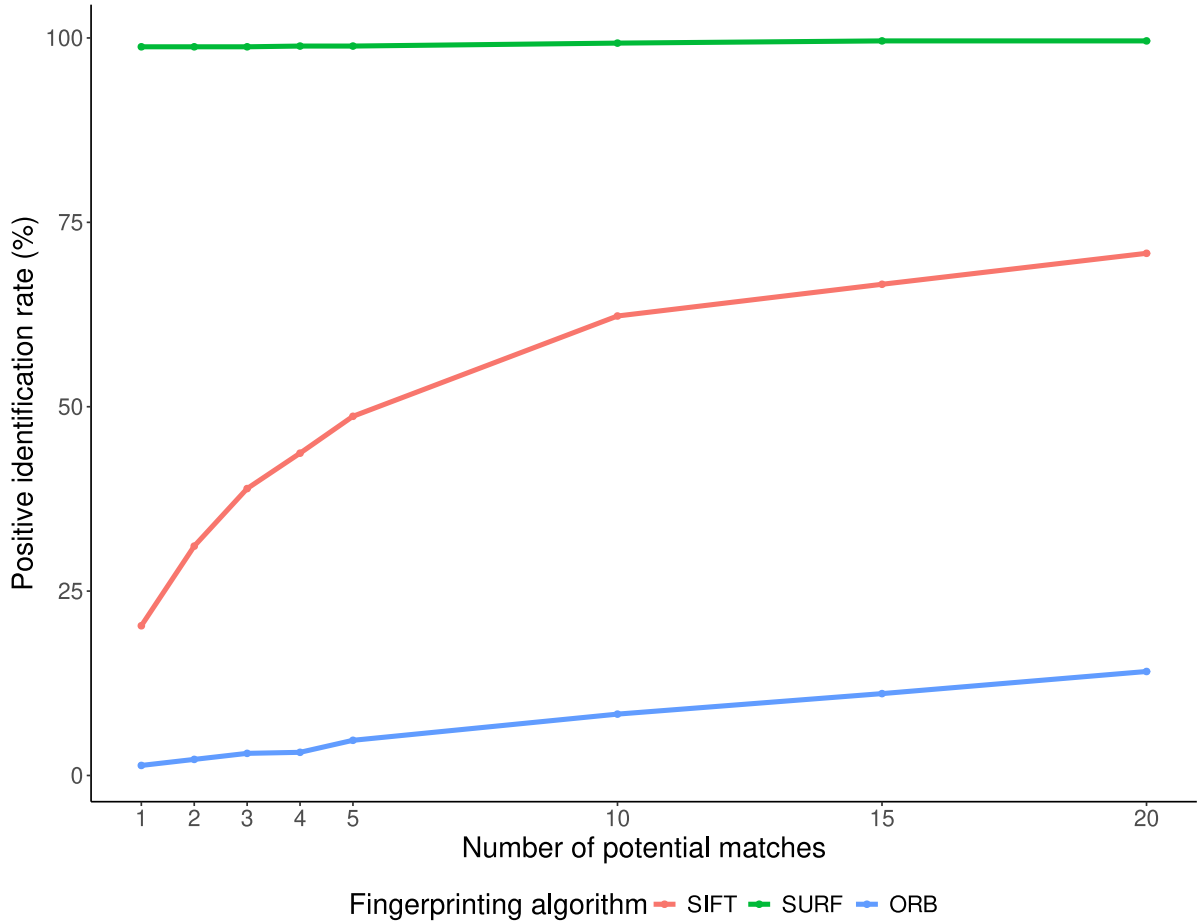

Figure 5-7: Efficacy of SIFT, SURF, and ORB at finding positive matches in the photographic record, given that a match was present. Positive identification rate was calculated as the number of searches (number of individuals x the number of occasions they were imaged; N = 733) in which a positive match was found over the total number of searches.

Our 5835 starting images were batch segmented and annotated in 7 minutes and 46 seconds (approximated 12.5 images second^{-1}). 126 images were identified as being processed incorrectly (poorly cropped or no subject in the image) and transferred to an error folder for manual inspection. Following error-inspection, 'fingerprints' (SIFT, SURF, and ORB) were extracted from 5782 images in 12 minutes and 44 seconds (approximately 7.6 images second^{-1}). A suitable pairwise comparison list (5,472,469 pairs) was generated in 2 minutes and 34 seconds. Pairwise fingerprint comparison was then processed separately according to each algorithm (SIFT – 3:15:10; SURF – 3:28:51; ORB – 2:07:42). This resulted in overall high per-second pairwise comparison performance (SIFT – 467.3 pairs second^{-1}; SURF – 436.7; ORB – 714.2).

5.4 Discussion

Here we described a method of data collection and Python pipeline for efficient, high-volume processing of large numbers of unique and discrete colour patterns in small-bodied insects for the purpose of assigning identities. This method combines approaches employed by several existing image and pattern recognition packages to achieve high levels of performance while eliminating a large degree of unnecessary image pre-processing and annotation.

The case study presented here represents a specific use-case of this system: processing an entire season's worth of data in a single batch. However, our approach may be of greater utility in the rapid processing of continuously collected data. Processing and comparing even hundreds of newly collected images against a large existing photographic record is orders of magnitude faster than that outlined here. As a tool for continuous processing of smaller sets of images, our approach may allow for close to real-time (i.e., same day) identification of captured individuals from natural or artificial populations.

Here SURF outperformed both SIFT and ORB in correctly sorting and identifying individuals in our photographic record, but this may not be the case for other species with different natures of colour patterns. Assessing the performance of these three algorithms on a case-by-case basis is highly recommended to maximise the effectiveness and efficiency of our pipeline. Further, users should be aware that while one or more algorithms might be an effective method of sorting potential matches, low 'distance' values alone may not be a robust selection criterion for matching images. Assessing false positive rates in a known-identity sample, as described here, is advised prior to considering any threshold 'distance' value for automatic acceptance.

Finally, this approach is currently designed for small-bodied insects with discrete, binary colour patterns. However, this could easily be extended to ternary or quaternary colour patterns if each colour layer is extracted sequentially. Relevant pairs of colour-specific 'fingerprints' could then be compared in series. Future versions of this method may aim to incorporate more sophisticated image annotation methods (i.e., a machine-learning model for insect localisation), 'fingerprinting' algorithms, or methods of comparing fingerprints (Local Naïve Bayes Nearest Neighbour approach – Crall *et al.*, 2013; Artificial or Convoluted Neural Networks - Carter *et al.*, 2014; Ferreira *et al.*, 2020). More complex image annotation methods would lessen the requirement for either neutral backgrounds in the image-capture stage or strictly colour-based image segmentation in the early processing stage (Lin *et al.*, 2018; Ferreira *et al.*, 2020). This would effectively eliminate the only image pre-processing required by our workflow. The current image segmentation approach could be used as a base from which to produce large training datasets of annotated images on which to train machine-learning models to localise colour-patterns in an image (Ferreira *et al.*, 2020). Similarly, more effective methods of generating or matching 'fingerprint' features between images may reduce the overlap in 'distance' values between 'self' and 'non-self' comparisons, allowing for the use of an automatic acceptance threshold. This would further reduce the already limited number of manual comparisons required from the investigator, improving overall efficiency.

6 General discussion

6.1 Overview

Variation in juvenile and adult environments may be expected to contribute to age-independent variation in adult phenotypes (e.g., mean body size, frailty, reproductive success; Grafen, 1988; Monaghan, 2008). Further, environmental effects may manifest on rates of ageing (either actuarial, reproductive, or functional; Nussey *et al.*, 2007; Cooper & Kruuk, 2018; Spagopoulou *et al.*, 2020). My thesis explores this broad theme of plasticity, determining the consequences of environmental variation for age-specific declines in survival, reproduction, flight, and body mass.

In this chapter I will outline the main findings of this thesis and contextualise these outcomes against the broader concern of the generalisability of laboratory-observed trends to natural settings.

According to contrasting life-history theory predictions (namely the silver spoon theory or the 'live fast, die young' theory), benign early-life conditions may result in reduced or accelerated rates of ageing, respectively (Preston *et al.*, 2011; Lemaître *et al.*, 2015; Cooper & Kruuk, 2018). In Chapter 2, I found that benign early-life conditions (i.e., food abundance) gave rise to larger, faster-flying individuals, consistent with silver-spoon effects. However, early-life background did not appear to affect the rate of ageing in any flight trait considered (propensity to fly, time to initiate flight, distance flown, or peak flight speed). A net, albeit weak, speed advantage conferred by a benign developmental background, with no apparent cost of accelerated ageing is certainly more consistent with 'silver spoon ageing' than a 'live-fast, die young' outcome. The overall similarity in performance in several components of flight (propensity to fly, time to initiate flight, and distance flown) between individuals from both developmental backgrounds was also consistent with plasticity in allocation of limited resources into competing traits (Nettle & Bateson, 2015; Pigeon *et al.*, 2019). Individuals exposed to poor early-life conditions may invest a greater proportion of developmental resources into flight traits to compensate for poor body condition.

We did not consider the broader implications of early-life food abundance on other aspects of ageing, however, nor how costs associated with flight activity may manifest on other traits. Measuring individual lifespans and providing opportunities for reproduction in addition to assessing flight performance would have offered a more holistic view of the consequences of early-life conditions. Particularly, a positive covariance between flight and fitness-related trait (lifespan and fecundity) would indicate that food abundance in development has general benefits for individual quality and lend greater support to the observed "silver spoon ageing" (*sensu*: Cooper & Kruuk, 2018). Alternatively, negative covariances between flight, lifespan or reproduction would indicate that unaccounted-for

trade-offs between these key traits might have acted to minimise otherwise apparent differences in individuals from both backgrounds. Further, trade-offs between flight and lifespan or reproduction may be exposed by experimentally manipulating costs associated with flight (by imposing greater levels of flight activity on individuals) and measuring subsequent survival and fecundity.

In Chapter 3, I found that the presence or absence of maternal care during development played a role in shaping patterns of ageing and lifespan. Adults that had not received maternal care when young were smaller and exhibited lower mean mortality and longer lifespans than those that had received care. In the previous chapter, I identified positive associations between body size and some aspects of flight performance; here I found that increased body size may incur a cost to lifespan. It is possible that greater levels of (unobserved) selective disappearance among larvae that did not receive care can shape apparent mortality rates among adult populations or that large body sizes incur some cost that is reflected in reduced lifespans. Adjusting experimental methods to control initial brood sizes would allow for the potential for selective disappearance to be interpreted as the known proportion of individuals surviving to emergence as adults.

In Chapter 4, I considered how shifts in the environment, from the laboratory to natural settings, influenced ageing rates in *Nicrophorus vespilloides*. As expected, mean mortality rates were higher among wild-living cohorts than laboratory-maintained cohorts. Actuarial ageing was only observed in the laboratory-maintained group, which may have reflected the challenges (e.g., low recapture rates, emigration, uncertainty in the ages of wild-born individuals) of measuring ageing in wild populations of small-bodied insects. Nevertheless, I did find that age-specific trends in body mass differed between laboratory-maintained and wild-living groups. Further, we found that population densities varied between our field sites, and between years; these natural populations were generally female-sex biased.

Chapter 5 outlined the methods that facilitated individual recognition based on elytral patterns. High quality photos of elytral patterns proved the basis of a robust, efficient method of individual recognition. These approaches were applied to laboratory and wild populations to identify individuals through time and build recapture histories from the mark-recapture study described in Chapter 4.

6.2 Variation in ageing rates in the laboratory

It can often be difficult to measure ageing (Vaupel & Yashin, 1985; Partridge & Barton, 1997; Monaghan *et al.*, 2008). A large part of this is due to the effects individual heterogeneity (i.e., selective disappearance) distorting or masking patterns of ageing (Monaghan *et al.*, 2008; Nussey *et al.*, 2008). Population-level (i.e., between-individual) ageing rates may differ from within-individual or 'true' ageing rates due to selective disappearance (Vaupel & Yashin, 1985;

Fay *et al.*, 2018). With phenotypic traits, we can relate individual declines in performance to some measure of age at death to separate within- (i.e., 'true') and between-individual age effects in the trait in question (van de Pol & Verhulst, 2006). However, since death is only observed once per individual, it isn't possible to directly account for these effects of individual heterogeneity in actuarial ageing (Nussey *et al.*, 2008).

A recurring theme of the survival analyses applied to laboratory-maintained cohorts of *N. vespilloides* in this thesis was the qualitative and quantitative variation in ageing rates between experimental blocks and groups despite attempts to minimise environmental variation. In our case, in Chapters 4 and 5, failing to account for block-level or temporal variation may have acted to reduce laboratory ageing estimates, minimising apparent differences in mortality between laboratory-maintained and wild-living cohorts. Here, fluctuations in patterns of ageing were highlighted by our use of non-standard survival analyses (i.e., generalised additive mixed models). It may be that such temporal or cohort-level variation in ageing trajectories is a common occurrence in laboratory-based studies of ageing (as in: Fukui *et al.*, 1996; Barks *et al.*, 2018). It would seem to be the case that the manifestation of cryptic heterogeneity on ageing rates is unavoidable, even in environments designed to minimise individual differences in quality.

This makes comparing ageing rates between the laboratory and the wild particularly difficult. Population-level actuarial ageing rates, as we measure them, are a function of both true ageing rates and the magnitude of individual heterogeneity (Vaupel & Yashin, 1985; Brunet-Rossinni & Austad, 2005). Both of these components likely differ between artificial and natural environments. It is not clear how these two components might be teased apart. Heterogeneity in mortality risk can be inferred, based on grouping individuals by some measured trait (sex, body size, etc.; Vaupel & Yashin, 1985; Nussey *et al.*, 2008) but cryptic heterogeneity will likely always manifest on measured ageing rates. It may be the case that future considerations of ageing across environments must simply be aware of this concern and temper their interpretations or expectations of 'faster' or 'slower' ageing in one context over another.

6.3 *N. vespilloides* as an insect model for ageing in the wild

Assessing ageing in populations of wild insects is challenging (Zajitschek *et al.*, 2020). This is the case for sedentary or spatially restricted species and populations considered in previous studies but particularly so in small-bodied, highly dispersive species such as *N. vespilloides*. Nevertheless, I consider that the suite of life-history traits (e.g., parental care, reliance on bonanza resources to reproduces, flight behaviours) and identifying elytral patterns characteristic of *N. vespilloides* make this system highly amenable and valuable to future investigations of ageing in natural settings. This portion of my work may be considered preliminary development of a largely novel system for long-term mark-recapture in the wild.

Future work may consider a wider set of questions to address in such contexts. For example, in Chapters 2 and 3, I identified key early-life cues (food availability and maternal

loss) in the laboratory that, under benign, homogenous conditions, can have lifelong effects on survival and phenotypic traits. However, a major focus of my work was to consider how trends observed in the laboratory may not be representative of those in natural settings (Briga & Verhulst, 2015; Reichard, 2016; Zajitschek *et al.*, 2020). It is not clear whether the observed outcomes of these early-life manipulations would be qualitatively similar across environments. For example, the negative association between the receipt of maternal care and lifespan, mediated via body size, may be reversed or exaggerated further in wild conditions. Intrasexual competition and predation are likely much more relevant to survival and reproduction in wild populations than in the laboratory. *N. vespilloides*, being one of the smaller burying beetle species in the UK, face challenges in securing carcasses for reproduction due to their size disadvantage (Easton, 1979; Otronen, 1988). Smaller individuals may face greater danger from larger conspecifics and be exposed to predation from larger congeneric challengers. This may act to increase mortality rates in smaller individuals and reverse the lifespan benefit of small body size apparent in the laboratory. Alternatively, small *N. vespilloides* may avoid direct competition, as observed in wild males (Hopwood *et al.*, 2016b), to secure reproductive success, perhaps preserving the observed lifespan benefit apparent in the laboratory. Alternatively, body size effects on lifespan may be mediated by increased growth or metabolic costs characteristic of different body sizes (Hooper *et al.*, 2017). Across species, larger individuals may store greater quantities of resources and be more resistant to starvation than small individuals (Gergs & Jager, 2014). Smaller individuals may also require fewer absolute resources to survive (but more, relative to body size) than large individuals, improving survival where food is scarce. Either case may yield lifespan benefits under different environmental circumstances. Future work should therefore consider whether the effects of maternal care, or the more obvious effects of body size, on lifespan are consistent across environments.

Similarly, I considered the role of quantity of developmental resources and age in flight performance in the laboratory. Little is known about patterns of dispersal or movement in natural populations. Movement through natural environments has consequences for fitness in gaining access to breeding resources and escaping predators. Future work might consider how flight performance in wild-living individuals differs from those in the laboratory. For example, in Chapter 2, I suggested that resources acquired during adulthood may be sufficient to mask the effects of food restriction during development (which limited the resources available for body formation) on flight behaviours. Under natural condition, with increased foraging costs or less reliable food sources, variation in individual quality in flight performance may be more pronounced. Given the rapid individual identification methods developed in Chapters 4 and 5 and the flight mill methods of Chapter 2, assessing the flight performance of wild-living individuals of known age is now increasingly feasible. Additionally, it would be interesting to consider the consequences of reduced movement ability in more direct terms by considering the succession of arrival of individuals on carcasses in the wild. For example, a field study in which traps are monitored more frequently (i.e., daily or more) than in Chapter

4 might indicate the individual characteristics (e.g., body size, mass, sex, or age) associated with more rapid arrival on a breeding resource.

6.4 Conclusion

The work here represents an exploration of how the environment may shape actuarial, reproductive, or functional ageing in *N. vespilloides*. In this thesis, I have explored the potential for juvenile nutritional and maternal care environments to shape ageing (Chapter 2, 3). However, I tried to remain conscious of (and contextualise my findings with respect to) the gulf that exists between the complexity of the environment in which ageing evolved (the wild) and the environment in which ageing is so often measured (the laboratory). To this end, I attempted to develop *N. vespilloides* as a novel system in which to assess ageing in natural conditions. This species' unique life-history traits set it apart from existing insect systems for the study of ageing in the wild. There exists a breadth of questions about the relationships between parental care, individual condition, and ageing that can and should be asked in the future.

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Supplementary Information for Chapter 2

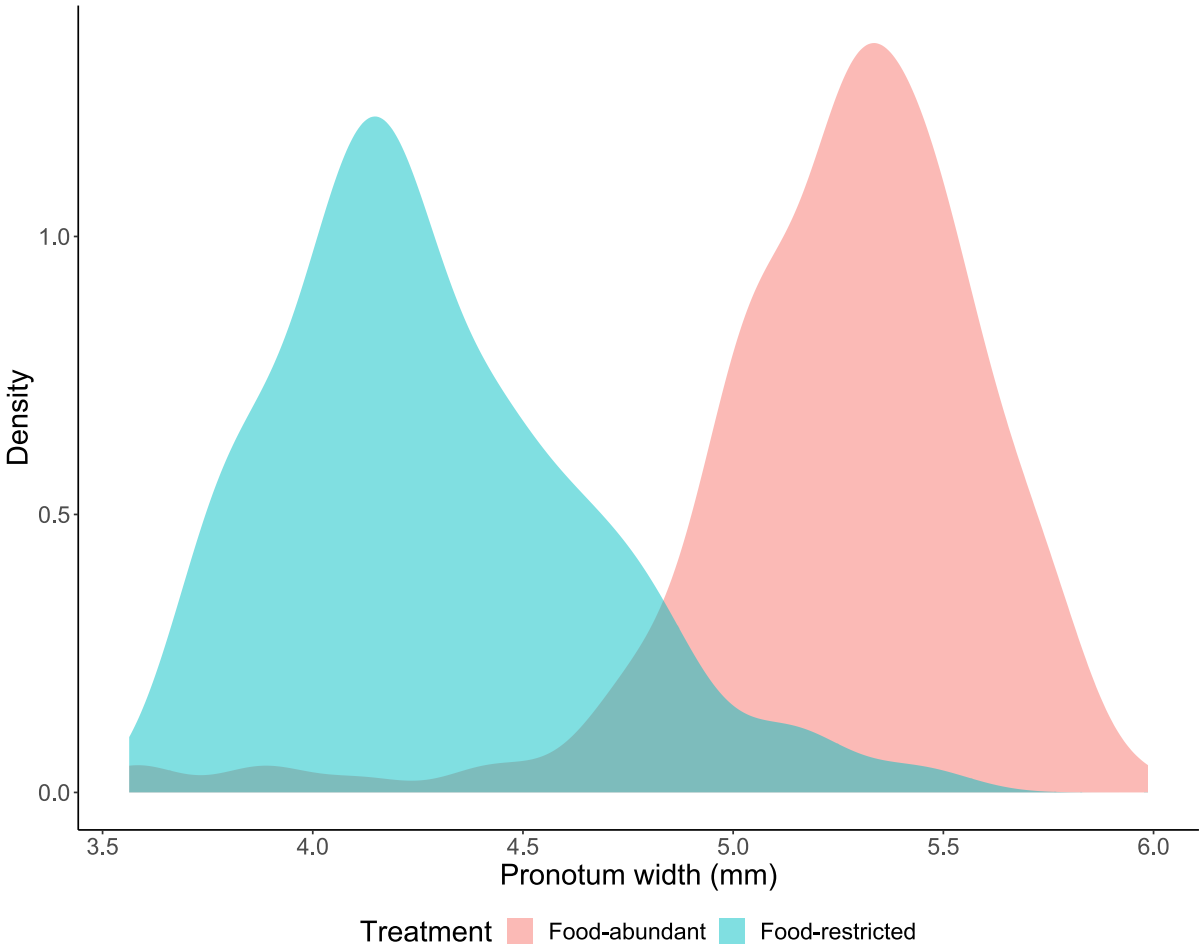

Figure 0-1: Distribution of body sizes (as measured by pronotum width) according to experimental early-life food abundance. Sample sizes: Food abundant = 166 individuals; Food-restricted = 148)

Table 0-1: Effects of early-life food availability and age on propensity to fly and time to initiate flight, accounting for body-size effects.

	Propensity to fly				**Time to initiate flight (s)**			
Predictors	*Odds Ratios*	*CI*	*Statistic*	*p*	*Estimates*	*CI*	*Statistic*	*p*
(Intercept)	0.044	8.2e-4 – 2.362	-1.537	0.124	656.692	23.585 – 182 84.737	3.822	**1.32e-4**
Age [days]	0.963	0.949 – 0.977	-5.115	**3.1e-7**	1.026	1.016 – 1.036	5.237	**1.63e-7**
Early-life conditions [food-restricted]	1.901	0.740– 4.888	1.334	0.182	0.743	0.347 – 1.590	-0.766	0.444
Size [mm]	2.586	1.216 – 5.499	2.469	**0.014**	0.833	0.448 – 1.549	-0.578	0.563
Selective disappearance [death between ages 60 – 90 days]	1.146	0.384 – 3.419	0.245	0.806	1.693	0.778 – 3.686	1.327	0.185
Selective disappearance [death post 90 days]	1.948	0.603 – 6.292	1.115	0.265	2.703	1.201 – 6.083	2.403	**0.016**
Age [days] * Treatment [food-restricted]	0.991	0.974 – 1.009	-0.977	0.329	0.998	0.985 – 1.012	-0.246	0.806
Random Effects								
Variance	0.892 $_{ID}$				1.776 $_{ID}$			
	0.106 $_{Family}$				3e-6 $_{Family}$			
	1e-6 $_{Block}$				1.8e-5 $_{Block}$			
N	324 $_{ID}$				314 $_{ID}$			
	42 $_{Family}$				42 $_{Family}$			
	5 $_{Block}$				5 $_{Block}$			
Observations	650				525			

Table 0-2: Effects of early-life food availability and age on distance flown and flight speed, accounting for body-size effects.

	Distance flown (rotations)				Peak flight speed (m/s)			
Predictors	*Log-mean*	*CI*	*Statistic*	*p*	Estimates	*CI*	*Statistic*	*p*
(Intercept)	2.092	0.504 – 3.680	2.582	**0.010**	-0.037	-0.543 – 0.468	-0.145	0.885
Age [days]	-0.002	-0.008 – 0.005	-0.476	0.634	-0.004	-0.006 – -0.002	-3.929	**8.5e-5**
Early-life conditions [food-restricted]	-0.012	-0.364 – 0.341	-0.066	0.947	0.065	-0.053 – 0.183	1.074	0.283
Size [mm]	0.179	-0.116 – 0.473	1.190	0.234	0.326	0.235 – 0.418	6.977	**3.02e-12**
Selective disappearance [death between ages 60 – 90 days]	-0.203	-0.557 – 0.152	-1.120	0.263	-0.041	-0.146 – 0.064	-0.769	0.442
Selective disappearance [death post 90 days]	-0.178	-0.550 – 0.194	-0.938	0.348	-0.068	-0.184– 0.049	-1.143	0.253
Age [days] * Treatment [food-restricted]	-0.003	-0.011 – 0.006	-0.605	0.545	0.002	-0.001 – 0.004	1.100	0.271
Dispersion Parameter								
(Intercept)	-0.445	-0.551 – -0.339	-8.204	**2.33e-16**	-1.052	-1.165 – -0.938	-18.148	**<2.22e-16**
Age [days]	-0.008	-0.011 – -0.004	-4.363	**1.28e-5**	0.008	0.005 – 0.011	5.594	**2.22e-8**
Random effects								
Variance	1.5e-4 $_{ID}$			$\tau_{00\ ID}$	0.000000 $_{ID}$			
	0.085 $_{Family}$			$\tau_{00\ Family}$	0.002 $_{Family}$			
	2.3e-5 $_{Block}$			$\tau_{00\ Block}$	0.007 $_{Block}$			
N	314 $_{ID}$				314 $_{ID}$			
	42 $_{Family}$				42 $_{Family}$			
	5 $_{Block}$				5 $_{Block}$			
Observations	525				525			

Supplementary Information for Chapter 4

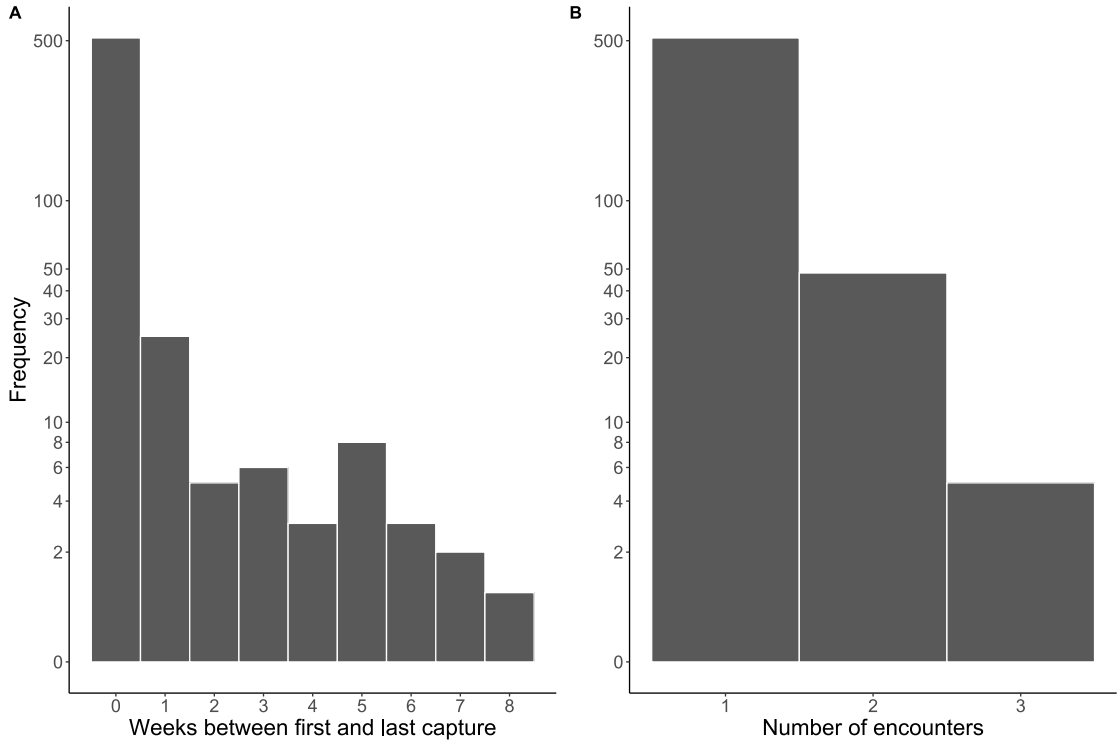

Figure 0-1: Summary of distribution of captures and recaptures in 2021. A) the distribution of the interval between first encounter (a release from the lab or capture of a novel individual from the wild) and last capture. B) the number of individuals caught only once, or multiple times.

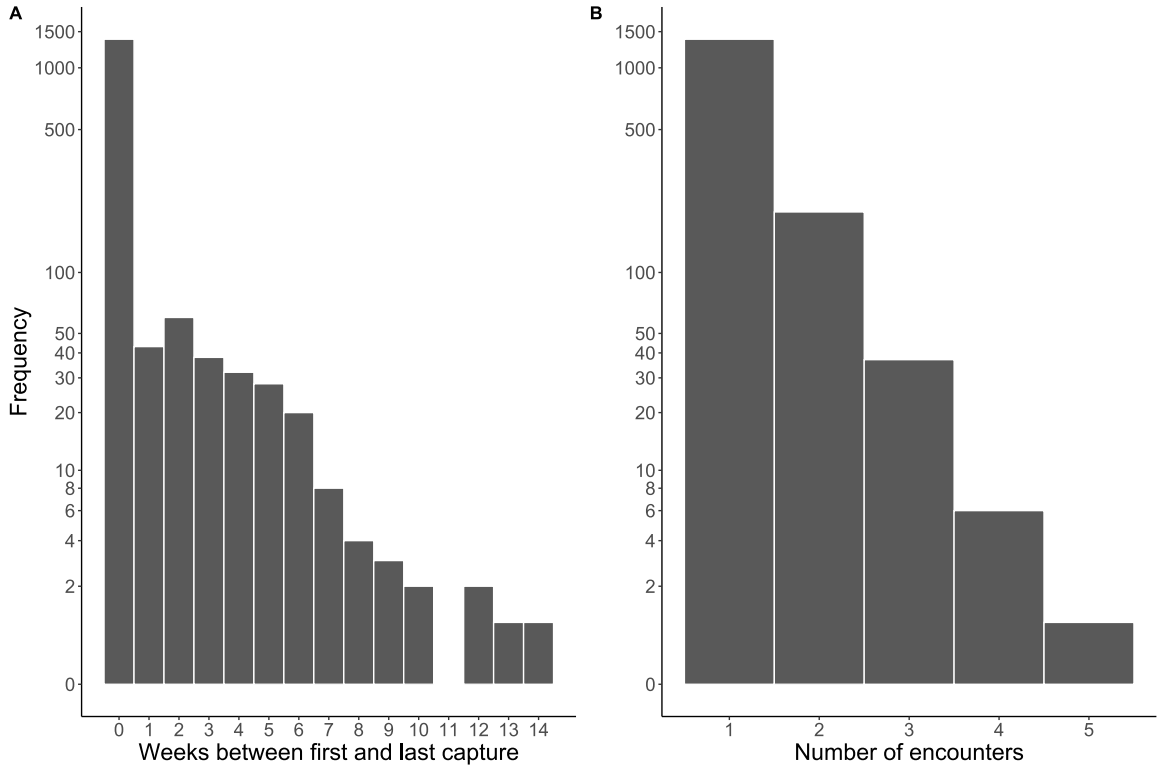

Figure 0-2: Summary of distribution of captures and recaptures in 2022. A) the distribution of the interval between first encounter (a release from the lab or capture of a novel individual from the wild) and last capture. B) the number of individuals caught only once, or multiple times.

Table 0-1: Results of AICc model selection for ageing functions in wild-living populations in 2021 using approximations of survival functions in MARK.

Apparent survival model (φ)	***Recapture probability model (ρ)***	***Approximate survival function***	***df***	***AICc***
~1	~poly(Time, 2)	Exponential	4	557.271
~Ln(Age)	~poly(Time, 2)	Weibull	5	558.946
~Age	~poly(Time, 2)	Gompertz	5	559.166
~1	~Time	Exponential	3	570.412
~Age	~Time	Gompertz	4	572.134
~Ln(Age)	~Time	Weibull	4	572.437
~Age	~1	Gompertz	2	583.856
~1	~1	Exponential	2	584.100
~Ln(Age)	~1	Weibull	3	586.119

Table 0-2: Results of AICc model selection for ageing functions in wild-living populations in 2022 using approximations of survival functions in MARK.

Apparent survival model (φ)	***Recapture probability model (ρ)***	***Approximate survival function***	***df***	***AICc***
~Ln(Age)	~poly(Time, 2) + site	Weibull	6	2562.081
~Age	~poly(Time, 2) + site	Gompertz	6	2562.125
~1	~poly(Time, 2) + site	Exponential	5	2562.833
~Ln(Age)	~poly(Time, 2) * site	Weibull	8	2564.847
~Age	~poly(Time, 2) * site	Gompertz	8	2564.847
~1	~poly(Time, 2) * site	Exponential	7	2565.557
~1	~poly(Time, 2)	Exponential	4	2582.768
~Age	~poly(Time, 2)	Gompertz	5	2583.073
~Ln(Age)	~poly(Time, 2)	Weibull	5	2583.278
~Age	~Time + site	Gompertz	5	2634.573
~Ln(Age)	~Time + site	Weibull	5	2635.324
~Age	~Time * site	Gompertz	6	2635.475
~Ln(Age)	~Time * site	Weibull	6	2636.208
~1	~Time + site	Exponential	4	2641.594
~1	~Time * site	Exponential	5	2642.625
~Age	~Time	Gompertz	4	2660.610
~Ln(Age)	~Time	Weibull	4	2661.818
~1	~Time	Exponential	3	2665.931
~Age	~site	Gompertz	4	2666.457
~Ln(Age)	~site	Weibull	4	2667.544
~1	~site	Exponential	3	2672.722
~Age	~1	Gompertz	3	2685.346
~Ln(Age)	~1	Weibull	3	2686.694
~1	~1	Exponential	2	2690.148

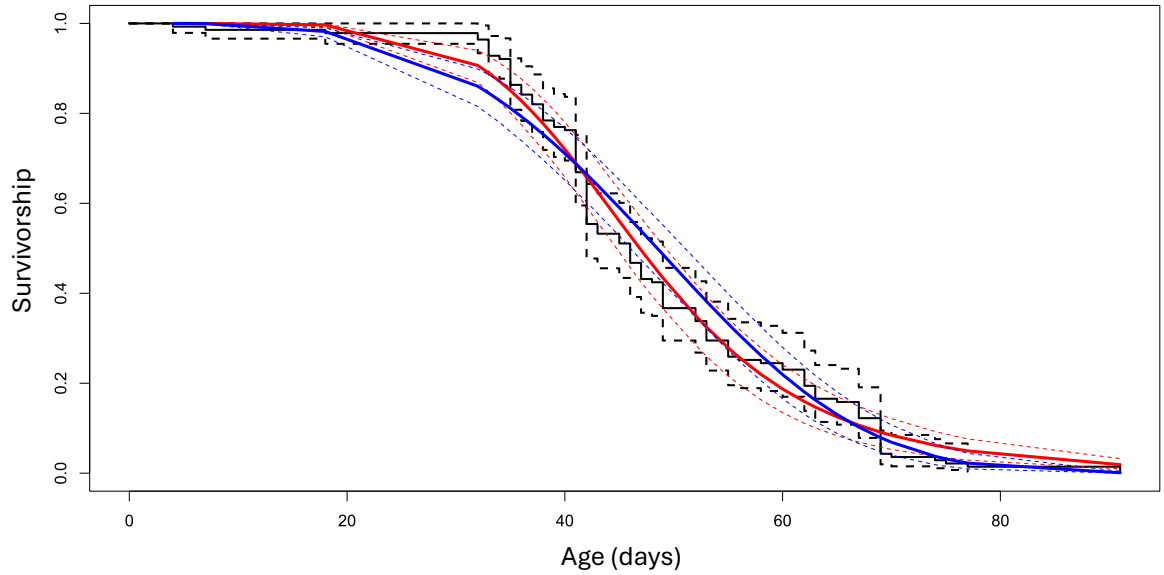

Figure 0-3: Comparison of model fit of Log-logistic (red) and Weibull (blue) functions to lab survival data in 2021.Dashed lines denote 95% confidence interval. Non-parametric survival curve is in black.