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Individual strategies to cope with environmental change: A test of the pace-of-life syndrome hypothesis

By

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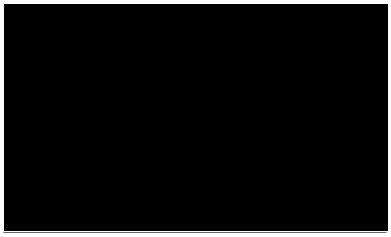
Declaration of Authenticity

The work presented in this thesis is, to the best of my knowledge and belief, original except as acknowledged in the text. I hereby declare that I have not submitted this material, either in full or in part, for a degree at this or any other institution.



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Abstract

Understanding the evolutionary causes and effects of diverse life-history strategies (i.e. how organisms allocate limited energy resources throughout their lifetime) is a principal aim of life-history theory. The pace-of-life syndrome (POLS) hypothesis expands the slow-fast continuum of life-histories to incorporate associations with physiological and behavioural traits to explain life-history variation at the individual or population level. An important prediction of the POLS hypothesis is that variation in single traits (e.g. metabolic rate) cannot be understood by measuring them in isolation, because specific combinations of traits have co-evolved as integrated syndromes with environment- and state-dependent consequences to fitness. The POLS hypothesis suggests individuals at the "slow"-end of the continuum will exhibit particular trait values, such as low metabolic rates, low activity levels, shy behavioural types, increased survival rates, and low rates of growth and reproductive output. In the same environment, other "fast" POLS individuals might exhibit the opposite set of trait values, with equal long-term fitness consequences.

Correlational selection of traits to form optimal syndromes could provide an explanation for the perplexingly high amount of variation in single behavioural and physiological traits that seem likely to be under strong directional selection. Metabolic rate, for example, is a trait that is likely to have important effects on fitness, yet this trait often varies several-fold even among individuals of the same population. The persistence of variation in metabolic rate could be explained if it represents one component of a correlated suite of traits that, acting as an integrated syndrome, provides an individual with increased fitness under specific environmental or intrinsic conditions. Hence, the POLS hypothesis, although not entirely a new idea, provides a unifying theory for predicting the importance of variation in key traits at the individual level.

Despite the attraction of the POLS hypothesis, empirical studies are needed to test assumptions regarding links between behaviour and metabolism, and their ecological consequences in different environments. The current research project addresses that need using wild caught house mice (*Mus musculus*) as a model species. The research conducted here provides a robust test of the POLS hypothesis in a wild animal population by determining whether individuals exhibit consistent and correlated differences in key behavioural and

physiological traits. Additionally, the research addresses a clear gap in our knowledge about the physiological ecology of wild-living house mice in Australia.

The first two chapters of this thesis review the literature on life-history theory and the developing POLS hypothesis, with a particular focus on the necessity of incorporating thermoregulatory effects on metabolic energy expenditure. These chapters highlight gaps critical to the progress of the POLS hypothesis and the importance of a better understanding of the mechanisms causing co-variation among physiological, behavioural and life history traits. Specifically, thermal physiology and thermoregulatory behaviour are important drivers of the energy budget of small mammals, and have widespread effects on physiological processes and their relations with various life history traits. Evidence also suggests that individuals vary consistently in thermoregulatory metabolic traits. Despite their significance to regulating individual energetic performance, thermal physiology and thermoregulatory behaviour (e.g. torpor use) have not been considered in the context of the POLS hypothesis. Indeed, integration of energetics with the POLS hypothesis has so far been limited to estimates of basal metabolic rate – a snap-shot index with questionable relevance at the individual scale. Clearly there is a need to progress past the use of basal metabolic rate as a single index of metabolism in the POLS hypothesis to derive meaningful predictions about the relevance of variation in metabolic energetics. To understand the ecological significance of individual variation in energy expenditure, researchers should test for consistent individual differences not only in constant state values but also in metabolic responses to key environmental conditions, such as temperature changes and food availability.

Empirical research described in chapters three to five address whether individuals exhibit consistent (repeatable) and correlated differences in key thermal and metabolic (ch.3) and behavioural (ch.4) traits, as required by the POLS hypothesis. These chapters address gaps in our understanding of the POLS hypothesis by incorporating measurements of behaviour and metabolism that are ecologically relevant and integrating changes in environmental conditions. The research describes essential aspects of individual behaviour and metabolism to provide a robust test of predictions from the POLS hypothesis.

Chapter three focuses on the thermal and metabolic physiology of *M. musculus.* Open-flow respirometry was used to determine the effects of changes in ambient temperature (15 °C, 20 °C and 31 °C), food availability and time on the metabolic response of *M. musculus.* The mice showed a decrease in all metabolic responses and a propensity to use torpor when faced with

low ambient temperatures and food restriction, indicating a physiological regulation of energy metabolism to adaptively cope with energetically stressful periods. Additionally, multiple components of the daily metabolic budget were found to be repeatable across the entire three-month measurement period. In particular, I found high individual consistency in daily energy expenditure, resting energy expenditure and metabolic responses at 15 °C, relative to the total population variation.

Chapter four addresses the behavioural responses of *M. musculus* to modified open-field tests (OFT) lasting 15 hours, to determine whether responses were consistent among individuals of the same population. Further aims were to explore the short-term temporal stability of the measured behavioural traits to see how behavioural responses, correlations between behavioural traits and repeatability estimates are affected by OFT duration (i.e. first hour versus full 15-hour experiment). Consistent individual differences in boldness and exploration were detected over both the first hour and entire 15-hour test duration. Some evidence for behavioural syndromes linking boldness and exploration were observed, whereby bolder individuals were more explorative, however this was only evident from behavioural variables measured over the first hour of the OFT.

Chapter five builds on the findings from chapters three and four to determine whether there are consistent and correlated individual differences in the measured physiological and behavioural traits. I found weak but significant associations ($r \leq \pm 0.30$) between many of the mass-specific metabolic measurements and our indices for boldness and exploration. In addition, I investigated how individuals' metabolic response to food restriction correlated with their behavioural measurements. Bolder individuals exhibited lower levels of energy expenditure when food was available and shyer individuals exhibited a stronger metabolic response to food restriction. Individuals that were more explorative had higher levels of energy expenditure and showed a more pronounced metabolic response to food restriction compared with less explorative individuals.

Our use of integrated measurements provides a unique insight into factors that determine rates of energy expenditure and assist in providing a more comprehensive understanding of the associations between various components of the daily energy budgets of small mammals. The correlations were found between an individual's behaviour and their metabolic responses to the environmental conditions (e.g. food restriction and changes in temperature) indicate that energy expenditure is part of a syndrome that involves behaviour and other life history

traits as is suggested by the POLS hypothesis. These results are significant in helping to understand the ecological importance of within-population variation in key behavioural, physiological and life-history traits. In particular, they explain how selection drives functional traits (e.g. metabolic activity and foraging rates) and the integrated mechanisms allowing small mammals to cope with changes in their environmental conditions. As a result, this will improve both our understanding of how adaptations arise and our ability to predict how populations cope with natural and human-induced environmental change.

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List of Abbreviations

BMR	Basal metabolic rate
DEE	Daily energy expenditure
REE	Resting energy expenditure
RMR	Resting metabolic rate
AEE	Average energy expenditure
RMR_15	RMR at 15 °C
RMR_20	RMR at 20 °C
RMR_31	RMR at 31 °C
AEE_15	AEE at 15 °C
AEE_20	AEE at 20 °C
AEE_31	AEE at 31 °C
AEE_Rest	AEE over rest period (0700 h – 1900 h)
AEE_Active	AEE over active period (1900 h – 0700 h)
PCA	Principal components analysis
T_a	Ambient temperature
T_b	Body temperature
OFT	Open-field test
POLS	Pace-of-life syndrome

Chapter 1

General introduction

1.1 Life-history theory

A principal aim of life-history theory is to understand the causes and effects of diverse life-history strategies (i.e how organisms allocate limited energy resources throughout their lifetime) by researching the evolution of life-history traits and how they interact. Whilst species differ widely in terms of their life-history strategies, there is a strong tendency for the life-history traits within groups of related organisms to co-vary systematically along a continuum (Pianka, 1970; Stearns, 1983; Dunham and Miles, 1984). This was recognised in one of the first predictive frameworks for life-history evolution; the theory of r/K selection (MacArthur and Wilson, 1967).

According to classical r/K selection theory, selective pressures associated with different levels of crowding are believed to drive evolution in one of two generalised directions: r- or K-selection (where r refers to the maximal intrinsic rate of growth and K to carrying capacity). Species referred to as r-selected are suited to unstable environments below their carrying capacity whilst K-selected organism are adapted to stable environments nearing their carrying capacity. Species that are characterised as r-selected tend to mature quickly, have small body sizes, be short-lived, have short gestation periods and produce numerous offspring, many of which die before they reach reproductive age. R-strategists thrive in resource rich, unstable habitats where they are capable of rapid growth when the environmental conditions are favourable. In contrast, K-selected species generally mature slower, live longer, are larger and have lower fecundity (MacArthur and Wilson, 1967). K-strategists are suited to highly competitive, resource limited environments where the optimum strategy is to invest in maintenance over productivity, where they can produce fewer offspring that have a high chance of survival. Although no organism is considered completely r- or K- selected, all are hypothesised to reach a compromise between the two extreme life-history strategies (Pianka, 1970).

While the classical theory of r/K selection was central in driving the research on comparative life-history strategies it is generally criticised as being an overly simplified view of the

evolution of life-histories (Wilbur *et al.,* 1974; Stearns, 1977; Reznick *et al.,* 2002). Within this framework life-histories are categorised based on cursory descriptions and many populations do not match the predictions of this theory (Reznick *et al.,* 2002). A focus on density dependent versus density independent selection neglects other important agents of selection that we now realise are crucial in selecting for life-history adaptations. In particular, mortality patterns and selective pressures among different life-history stages were not included in the r/K selection framework (Stearns, 1977). As a result, life-history theory has shifted from the narrow focus on r/K selection towards a more comprehensive approach to understand diverse life-history strategies.

1.2 Inter-individual variation within populations

In the past two decades there has been a surge of interest towards inter-individual variation within wild populations to help understand the evolution of life-history diversity (Wilson, 1998). Genetically based phenotypic variation has traditionally been considered as the raw material on which selection acts, however it has been suggested that it should be considered more as the end product of natural selection. Behavioural variation, in particular, has often been neglected and considered as noise surrounding an adaptive population-average optimum (Wilson, 1998; Dall *et al.,* 2004; Fisher *et al.,* 2015). For example, most of the studies on optimal foraging theory assume there is a single optimal way to forage and individual differences from the population's average foraging behaviour are non-adaptive (Stephens and Krebs, 1986). Yet, within a single population in a diverse environment, individuals will often inhabit different niches and be subjected to different ecological and evolutionary forces (Wilson, 1998). Within-population differences may be indicative of consistent and adaptive differences in the behavioural responses of individuals to their environment (Mather and Anderson, 1993).

Inter-individual differences in behavioural traits specifically have received considerable attention with numerous recent studies showing that inter-individual differences in behaviour are consistent over time and across situations, and are likely to be adaptive (Dall *et al.,* 2004; Sigh *et al.,* 2004; Bell *et al.,* 2009; Reale *et al.,* 2010). Consistency does not mean that measured traits values remain permanently fixed and cannot vary depending on the environmental conditions but that the differences among individuals remain stable (i.e. consistent individual rank order; Reale *et al.,* 2007). Repeatability is

often used to measure consistency and is estimated through repeated measurements of the same individuals in the same context across two or more time points. It can be described by the fraction of total phenotypic variation that is attributable to the among versus within individual level (Falconer and Mackay, 1996; Reale *et al.,* 2007; Griffen *et al.,* 2015). Traits that have comparatively low estimates of intra-individual variance compared to high inter-individual variance are more repeatable (Hayes and Jenkins, 1997; Bell *et al.,* 2009).

Behavioural consistency has been observed at separate levels. Animal personality is repeatable (i.e. consistent over time) individual differences in single behavioural traits (e.g. individuals that are bolder in a novel environment will tend to be bolder when measured at a later point in time). Behavioural syndromes are consistent individual differences in correlated suites of functionally different behavioural traits (e.g. individuals expressing high levels of territorial aggression also tend to be bolder; Huntingford, 1976; Dingemanse and Wolf, 2010; Garamszeigi and Herczeg, 2012). Consistent individual differences in single behavioural traits (i.e. personality) have been recorded in a wide range of taxa including mammals, reptiles, insects and birds (Brodie and Russell, 1999; Sih and Watters, 2005; Sinn *et al.,* 2006; Groothuis and Carerer, 2005; Sinn *et al.,* 2006; Fisher *et al.,* 2015). Similarly, correlations between behavioural traits (i.e. behavioural syndromes) are widespread across numerous species, with common correlations being observed between boldness, activity and dispersal (Fraser *et al.,* 2001; Dingemanse *et al.,* 2003), and between aggression and exploration (Sih *et al.,* 2004). Correlations between separate behavioural traits may indicate that the traits have co-evolved as a suite of traits rather than on independent evolutionary pathways.

Behavioural consistency can have important fitness consequences and in some situations appear maladaptive as it implies limited behavioural plasticity which can lead to non-optimal behaviour (Sih *et al.,* 2004; Smith and Blumstein, 2008). Several hypotheses have been suggested to try to explain these inter-individual behavioural differences. A popular explanation is that consistent individual differences in energy expenditure might promote consistent individual differences in behaviour (Careau *et al.,* 2008; Biro and Stamps, 2010). This idea lays the basis for a potential framework that the evolutionary significance of

behavioural consistency may only be understood when also considering other associated life-history traits.

1.3 Pace of life syndrome hypothesis – linking behaviour, metabolism and life-histories

The concept of the classic slow-fast (r/K) continuum of life-histories (MacArthur and Wilson, 1967) has been expanded to incorporate associations with behavioural and physiological traits in the holistic framework of the pace-of-life syndrome hypothesis (POLS) to explain life-history variation at the individual or population level (Sih *et al.,* 2004; Stamps, 2007; Reale *et al.,* 2010). A central prediction of the POLS hypothesis is that variation in single key traits can only be fully understood when considering their function as part of a suite of behavioural, physiological and life-history traits that have co-evolved as integrated syndromes. Accordingly, consistent individual differences in behaviour should covary with a wide variety of consistent individual differences in physiological and life-history traits forming predictable and stable associations (Reale *et al.,* 2010).

The POLS hypothesis predicts that, within a species or population, individuals can be ranked along a pace-of-life continuum of optimal trait value combinations, ranging from "slow" and reactive to "fast" and proactive life styles (Ricklefs and Wikelski, 2002; Juette *et al.,* 2014; Briffa, 2015). "Slow" POLS characterised individuals are more risk-averse and prioritise investment in survival. They are expected to be associated with particular trait values such as low levels of activity, thorough exploration, shyness (risk adverse), lower metabolic and reproductive rates, longer development times, and a longer lifespan (low mortality). On the opposite end of the continuum, "fast" POLS characterised individuals prioritise high reproductive success at a cost to their survival and are predicted to be associated with contrasting trait values (Réale *et al*., 2010; Galliard *et al.,* 2013; Hall *et al.,* 2014; Urszan *et al.,* 2015).

The conceptualisation that behavioural and physiological traits are linked to other life-history differences raises questions regarding how these trait combinations interact and why such co-variation might evolve. The maintenance of divergent life-history strategies may be associated with variation (spatial or temporal) in the surrounding environment causing unstable selective pressures that favour different life-history strategies depending

on the environmental conditions (Reale *et al.,* 2010). Poor, unstable habitats, such as those with low and intermittent energy availability and high predation risks, are expected to favour slow-characterised individuals. In contrast, benign environmental conditions with high and constant energy availability and low predation risks should favour individuals that are characteristic of the faster traits (Biro and Stamps, 2008; Reale *et al.,* 2010). This prediction is based on the idea that a fast personality (high activity levels and thorough explorer) is more likely to encounter higher levels of energy acquisition thus requiring a metabolic system capable of processing high energy intake, and hence driving higher growth rates and reproductive effort. Consequently, these fast-characterised traits are associated with a higher risk of mortality from predation, resulting in a similar lifetime reproductive success than low activity (Galliard *et al.,* 2013).

The suggestion that variation in physiological, behavioural and life-history strategies has coevolved to form optimal syndromes provides a comprehensive explanation for the perplexing within-population (i.e. inter-individual) variation seen in many key behavioural and physiological traits that seem likely to be under strong directional selection (Biro and Stamps, 2008; David *et al.,* 2015). Metabolic rate, for example, is a trait that is likely to have important effects on fitness, yet often varies several-fold even among individuals of the same population (Speakman *et al.,* 2004; Turbill *et al.,* 2013; Vignes *et al.,* 2012). The persistence of variation in metabolic rate could be explained if it represents one component of a correlated suite of traits that, acting as an integrated syndrome (i.e. pace-of-life syndrome), provides an individual with increased fitness under specific conditions or intrinsic states. Inter-individual variation in POLS are expected to be maintained as they yield equal life-time fitness depending on i) the frequency of individuals exhibiting other syndromes, and/or ii) variability in intrinsic capacities or environmental context (Stamps, 2007; Réale *et al*., 2010; Niemela *et al.,* 2012; Urszan *et al.,* 2015). For instance, bold individuals visiting a risky, high-return foraging area risk increased predation and mortality rates for higher foraging success whilst shy individuals gain less energetic intake but experience higher levels of survival. When combined with other life history and physiological traits, such as differences in energy expenditure, both strategies may result in equal fitness. According to this concept, the maintenance of individual behavioural, physiological and life history variation is ultimately underpinned by fundamental life

history trade-offs between reproduction and survival (Biro and Stamps, 2008; Hall *et al.,* 2014; Montiglio *et al.,* 2014).

1.4 Support for the POLS hypothesis

There has been a surge of interest in empirical research to test for correlations between behavioural, physiological and life-history variation and the predictions of the POLS hypothesis. Much of the research so far has lent support to this theory (David *et al.,* 2012; Yli-Renko *et al.,* 2014; Urzan *et al.,* 2015; Gangloff *et al.,* 2017; Monceau *et al.,* 2017). For instance, it has been shown that in some breeds of domestic dogs growth rate, mortality and energy expenditure were positively correlated with variation in activity, boldness and aggression (Careau *et al.,* 2010); longitudinal research on wild guppy populations with contrasting life-history strategies showed differences in boldness and learning ability (Gilliam and Fraser, 1987; Burns and Rodd, 2008); growth rate and boldness were shown to be positively correlated in the crayfish *Cherax destructor* (Biro *et al.,* 2014); in mealworm beetles, a relationship between the behavioural syndrome (comprising of four personality traits) and reproductive success was observed whereby high risk-taking females produced more offspring than low risk-taking females, as predicted by the POLS hypothesis (Monceau *et al.,* 2017); Shearer and Pruitt (2014) showed that in two species of orb-weaving spiders (*Larinioides cornutus* and *Larinioides patagiatus*) increased boldness was positively correlated with increased heart rate; and a review by Biro and Stamps (2010) concluded that resting metabolic rate is generally positively correlated with growth rate and activity.

Despite the support in the contemporary literature for the POLS hypothesis no study has found evidence to support all the predictions at the same time. Furthermore, some results have presented contrary evidence to the specific predictions of the POLS hypothesis model (i.e live fast, die young; Careau *et al.,* 2011; Thomas *et al.,* 2016). For instance, in brown trout (*Salmo trutta*) Zavorka *et al.,* (2016) found that fast-growing, highly active individuals had higher levels of survival than reactive conspecifics. Other studies have found partial support for a pace-of-life syndrome linking only some of the behavioural and metabolic traits measured. For example, in bluegill sunfish relationships between boldness and aerobic metabolism were found but not between boldness and anaerobic metabolism (Binder *et al.,* 2016). Such findings may suggest that different physiological capacities are

subject to different selection pressures and that some of the traits included in the POLS hypothesis may not be as tightly linked as has previously been suggested.

There have also been many studies that have detected no relationship between behaviour and life-history or physiological traits, opposing the predictions of the POLS hypothesis and cautioning against its excessive generalisation (Lantova *et al.,* 2011; Kluen *et al.,* 2014; Laskowski *et al.,* 2016). In house crickets (*Acheta domesticus*), for example, whilst substantive correlations between behavioural traits (e.g. activity and exploration) and both metabolic and life–history traits (e.g. routine metabolic rate and mass) were found separately, no evidence of an integrative syndrome involving behavioural and metabolic traits were observed (Royaute *et al.,* 2015); in wild meadow voles (*Microtus pennsylvanicus*) no correlation was observed between consistent individual differences in behaviour in a novel environment and mass-adjusted resting metabolic rate (Timonin *et al.,* 2011); and no evidence of correlations between metabolic rate and exploratory behaviour was found in salamanders (*Desmognathus brimleyorum*; Gifford *et al.,* 2014). Such conflicting results highlight the importance of further empirical research to clarify the interactions between behavioural, physiological and life-history traits.

1.5 Current limitations of POLS studies

To date, many studies testing the predictions of the POLS hypothesised have restricted themselves to analysing the relationship between single behavioural, life-history and or physiological traits (Krams *et al.,* 2013; Bijleveld *et al.,* 2014). As many behavioural and physiological traits are fundamentally multidimensional it seems imperative that further investigations progress past the use of single traits and employ multiple integrative measurements to examine the relationship between behavioural, physiological and life-history traits and avoid misleading results (Monceau *et al.,* 2017).

Some of the strongest support for the POLS hypothesis has been shown in domesticated species (Careau *et al.,* 2010) or lab maintained animals (Careau *et al.,* 2011). In contrast much of the research challenging the POLS hypothesis have involved field studies with wild individuals (Dingemanse *et al.,* 2004; Adriaenssens and Johnsson, 2010; Timonin *et al.,* 2011). This infers that the associations between the behavioural, physiological and life-history traits in a pace-of-life syndrome may be more variable in a natural environment

where the levels of predation, competition and resource abundance are frequently changing (Zavorka *et al.,* 2015). To provide more ecologically relevant results, future research should therefore measure behavioural and physiological traits in experimental conditions that more accurately mirror the conditions individuals would experience in the wild. For example, the traditional open-field test (OFT) is commonly used to measure behavioural traits by quantifying activity and emotional reactivity in a novel environment (Hall, 1934) and has dominated research looking at correlations between behavioural and life-history or physiological traits. A more complex test environment than the traditional OFT may be more appropriate to study traits such as boldness and exploration (Thomas *et al.,* 2016). Moreover, many studies focussing on the associations between metabolic and behavioural traits measure metabolism under conditions that the individuals are unlikely to experience in their natural environment, and consequently are unlikely to be representative of natural metabolic energy demands. Basal metabolic rate (BMR), for example, is one of the most frequently used proxies for minimum energy expenditure in studies investigating the ecological and evolutionary significance of individual differences in energy expenditure (McNab, 1997; Speakman *et al.,* 1999; Speakman *et al.,* 2004). Yet, there is doubt that BMR is an accurate index of minimum energy expenditure as it does not account for energy saving strategies, such as torpor by endotherms, that individuals may use in the wild (Mathot and Dingemanse, 2015). It has been argued that daily energy expenditure (DEE) is a more natural index of energy expenditure as it can be measured under field conditions and would therefore be more useful to use when looking at associations among individual differences in metabolism and behaviour (Careau *et al.,* 2008).

1.6 Conclusion – a multifactorial approach

The POLS hypothesis provides a mechanistic link between environmental conditions and life-history outcomes whereby ecological conditions favouring a specific life-history strategy will subsequently affect multiple associated traits. Environmental context is crucial for predicting fitness consequences of trait value combinations. For instance, it is predicted that environments with high, constant food availability would favour “fast”, proactive life-style over “slow” reactive life-styles.

Despite the considerable attention this research area has received our understanding of the evolutionary causes of inter-individual variation remains inconclusive, leaving a crucial gap in the study of evolution (Wilson, 1998; Reale *et al.,* 2010). Although not entirely a new idea, the POLS hypothesis provides a unifying theory to understand the causes and maintenance of individual variation in key traits that have traditionally been studied in isolation from each other. It provides a compelling argument to support shifting our research paradigm away from its traditional focus on mean values of individual traits for a population or species and towards an integrative study of individual variation in correlated suites of behavioural, physiological and life-history traits (Reale *et al.,* 2010; Careau and Garland, 2012). Moreover, the existence of individual diversity in the form of syndromes may be important for enhancing population stability during times of environmental variability (Moore *et al.,* 2014).

There has been substantial theoretical literature suggesting that integrative links between behavioural, physiological and life-history traits should be expected (Sih *et al.,* 2004; Reale *et al.,* 2010), yet support for the POLS hypothesis has been mixed and inconclusive. There is a need for further empirical research testing the assumptions that underlie the POLS hypothesis, that: i) individuals within a population exhibit consistent differences in trait values, ii) there exists correlations among behavioural, physiological and life-history traits across environmental contexts and iii) there are different fitness consequences of contrasting pace-of-life syndromes. Such research will be necessary to evaluate the relationship between behaviour, metabolism and life-history, and their ecological consequences in different environments, which is a fundamental question in physiological ecology.

1.7 Thesis Overview

This work uses wild derived house mice (*Mus musculus*) as a model species to test key predictions of the POLs hypothesis to increase our understanding of the generation and maintenance of within population variation in key traits. A major objective was to test for the presence of consistent individual differences in key behavioural traits (e.g. boldness, exploration) and physiological traits (e.g. daily energy expenditure) measured repeatedly over the expected life span of *M. musculus* in the wild. The next aim was to investigate whether individuals exhibited correlations between these behavioural and physiological

traits. In addition to helping uncover the integrated mechanisms that allow small mammals to cope with changes in environmental conditions, such as food availability and predation pressure, this research also has an important applied aspect in terms of understanding the causes of vulnerability to environmental degradation and potential for population resilience to future environmental variability.

1.8 Model species: *Mus musculus*

Mus musculus are small, nocturnal, rodents generally weighing under 20g. They are omnivorous having a varied and flexible diet that include invertebrates and plant material like grains and seeds (Sage, 1981; Phifer-Rixey and Nachman, 2015). The house mouse belongs to the Eurasian *M. musculus* and *Mus domesticus* species complex (Watts and Kemper, 1989; Tomlinson *et al.,* 2007). First introduced to Australia around 250 years ago with the arrival and settlement of the first European colonists (c. 1788) *M. musculus* has become a highly successful invasive species, currently widely distributed across mainland Australia (Dickman, 1992; Gabriel *et al.,* 2011). They inhabit wide-ranging environments and occur in two types of situation, commensal, living near artificial food sources and shelter (e.g. bird aviaries and grain silos), and feral, living in natural grasslands and forested environments (Latham and Mason, 2004; Singleton *et al.,* 2007). In Australia, and worldwide, *M. musculus* are significant environmental and agricultural pests, particularly of cereal crops and stored grain (Stenseth *et al.,* 2003; Singleton *et al.,* 2005).

Mus musculus have short generation times with a gestation period of around three weeks and reach sexual maturity at 6-8 weeks (Phifer-Rixey and Nachman, 2015). In Australia the breeding season generally starts in spring and declines in late autumn when food quality declines. During years with above-average rainfall in autumn, which prolongs the availability of high quality food, the breeding season may extend through autumn and winter (Bomford and Redhead, 1987; Singleton, 1989; Singleton and Readhead, 1990). In the wild *M. musculus* have a short life expectancy and seldom survive beyond three months (Berry and Jakobson, 1971; Rowe *et al.,* 1987; Pocock *et al.,* 2004).

Like many native Australian small mammals, *M. musculus* are capable of using daily torpor in response to poor environmental conditions such as low food availability (Hudson and Scott, 1979; Tomlinson *et al.,* 2007; Geiser and Kortner, 2010). Torpor is a decrease in

body temperature at low ambient temperature to conserve energy expenditure. Torpor use greatly increases survival rates and has been associated with a reduced risk of extinction among mammals (Geiser and Turbill, 2009). In laboratory mice torpor has been observed in numerous studies though we know relatively very little about the torpor characteristics and the extent to which torpor occurs in *M. musculus* in their natural environment.

Mus musculus provide an ideal model for testing co-variation among behavioural, physiological and life history traits as their physiology and behaviour are well understood and their thermoregulatory responses are similar to that of many native Australian small mammals. While *M. musculus* has been well studied under laboratory conditions, comparatively little is known about the physiological and behavioural ecology of wild house mice (Phifer-Rixey and Nachman, 2015). This research will fill a gap in our knowledge about the ecology of wild house mice. Additionally, understanding the amount of inter-individual variation in behavioural and physiological traits within a wild population will complement studies that have been carried out with laboratory mice and be essential to understand natural levels of variation in POLS traits.

1.9 Thesis Outline

The thesis is structured into several main objectives and chapters. This introductory chapter has reviewed the literature on life-history and the POLS hypothesis, highlighting the gaps critical to the progress of the POLS hypothesis and the need for rigorous empirical studies like the present project. The proposed mechanisms that underlie co-variation among traits within single populations have been described along with evidence supporting the proposed links between metabolic rate and behaviour.

Chapter 2 addresses the need to incorporate thermoregulatory effects on metabolic energy expenditure with the POLS hypothesis and reviews consistent individual differences in thermal physiology (e.g. body temperature) and thermoregulatory behaviour (e.g. torpor use) at different levels (i.e. population and individual). This chapter also demonstrates the limitations of using BMR as a single index for metabolic rate. So far the integration of metabolism with the POLS hypothesis has been limited to estimates of BMR. It is an untested assumption that BMR is a valid proxy for other components of the energy

budget, and this chapter explores how this deficit might lead to misinterpretation of the POLs hypothesis.

Chapter 3 focusses on the impact of ambient temperature, food restriction and time on the metabolic response of *M. musculus* at both the population and individual level, and investigates the repeatability (consistency) of various metabolic responses. Additionally, the study assesses the relationship between various standardised and integrative measures of the daily energy budget to determine the most useful predictor of daily energy expenditure.

Chapter 4 investigates the behavioural response of *M. musculus* to long term OFTs to assess the presence of consistent individual differences in behavioural traits and correlations between the measured behavioural traits (i.e. behavioural syndromes). Further aims were to explore the short-term temporal stability of the measured behavioural traits to determine whether behavioural responses, correlations between behavioural traits and repeatability estimates are affected by OFT duration.

Chapter 5 incorporates the findings from the chapters three and four to assess whether individuals exhibit correlated differences in key physiological and behavioural traits, as required by the POLS hypothesis.

Chapter 6 synthesises the key findings of this research to provide a cohesive discussion of all the hypotheses and findings, including future directions.

Chapter 2

Integrating thermal physiology with the pace-of-life syndrome hypothesis

2.1 Abstract

Currently, integration of energetics in the pace-of-life syndrome (POLS) hypothesis has relied exclusively on one selected index of variation in metabolic rate; a standardised measure of resting metabolic rate measured under a specific set of conditions termed standard (in ectotherms) or basal (in endotherms) metabolic rate (BMR). Relying on a single measurement of metabolism like BMR does not allow us to properly assimilate metabolic rate with the POLS hypothesis. Basal metabolic rate does not provide sufficient information regarding an individual's energetic response to environmental variation and for many species is not a good proxy for minimum resting energy expenditure (REE). I aim to demonstrate the need for stronger theoretical descriptions of the linkages between metabolic, behavioural and life-history traits. These relationships are underpinned by known effects of body temperature on metabolic rate and thermoregulatory responses to intrinsic and environmental conditions. To date thermoregulatory responses have been largely neglected from POLS studies despite being a key mechanism that affects the REE of endothermic animals. I am highlighting the need to re-evaluate how metabolic rate is conceptualised and move beyond the use of individual static traits like BMR which oversimplify metabolic rate. A metabolic "reaction norm" approach which characterises an individual's energetic response to variation in environmental conditions (e.g. food restriction and ambient temperature) will enable better defined and more realistic hypotheses about how consistent individual differences in energy expenditure relate to key POLS traits.

2.2 Introduction

Metabolism integrates all physiological processes and is fundamental to animal ecology. The acquisition and processing of energy is essential for growth, reproduction and survival in all organisms (Schmidt-Nielsen, 1991). Most, if not all behaviours (e.g. activity, aggressiveness) have consequences for the energy budget (Careau *et al.,* 2008). It is a logical postulation that metabolic energy expenditure is informative to understanding the

proposed associations between behaviour and life history, and metabolic rate is a key trait that must be integrated with the POLS hypothesis. Despite this, few experimental studies have integrated thermal physiology (e.g. body temperature) and thermoregulatory behaviour (e.g. torpor use) with other key traits as proposed by the POLS hypothesis.

The POLS hypothesis suggests that consistent individual differences in behaviour covary with physiological and life-history traits. Different trait combinations (syndromes) fall along a pace-of-life continuum ranging from "slow", reactive, to "fast", proactive, life styles or syndromes (Stearns, 1983; Gaillard *et al.,* 1989; Roff, 2002; Juette *et al.,* 2014). "Slow" characterised individuals are more risk-averse and prioritise investment in survival. They are predicted to be associated with particular traits including low metabolic rates, low activity levels, shy behavioural types, and low rates of growth and reproductive output. On the other hand, "fast" characterised individuals prioritise high reproductive success at a cost to their survival. These individuals are expected to be associated with a contrasting set of co-varying traits. (Williams 1966; Reale *et al.,* 2010; Le Galliard *et al.,* 2013; Hall *et al.,* 2014).

The experimental studies that have attempted to integrate thermal physiology and thermoregulatory behaviour with the POLS hypothesis have limited themselves to the use of BMR as a measure of metabolic state (Tieleman *et al.,* 2005; Reale *et al.,* 2010). Basal metabolic rate is a useful measure for metabolic rate and can be used to indicate the demands for resources an individual will place on its environment. At a gross level (i.e. showing some relationship among many data at the species level) BMR has been valuable in predicting field metabolic rates and daily energy expenditure (DEE). However, to more fully understand the relationship between REE and intra-specific variation in behavioural and life history traits, it is crucial that future studies move beyond the sole use of BMR and adopt additional measures of metabolic rate that incorporate the effects of thermoregulation and its adjustments on metabolism. Single measures, like BMR, are not informative about an organism's energetic response to environmental variables (e.g. temperature and food availability). Measuring multiple aspects of REE as an integrated response to different environmental contexts (i.e. a metabolic reaction norm; Terblanche *et al,* 2008; Careau *et al.,* 2014) will incorporate the effects of variation in thermoregulatory physiology and behaviour that are neglected with BMR.

This perspective aims to demonstrate that to properly assimilate metabolic rate with the POLS hypothesis at the individual level and understand how REE is linked with behavioural syndromes and life history variation it is necessary for future research to i) move beyond the sole use of BMR and incorporate more relevant, informative and useful proxies of among-individual variation in energy requirements, ii) incorporate thermoregulatory effects as a key mechanism affecting the REE of endothermic animals. An integrative focus on individual variation in multiple correlated traits will increase our understanding of the physiological mechanisms influencing ethology and affecting how individuals cope with natural and anthropogenic variations in their surrounding environment.

2.3 How does resting energy expenditure link with the energy budget?

The current hypotheses proposing how REE relates to the non-resting components of the energy budget are underpinned by how limited resources may be allocated to competing physiological processes. Predicting the direction of the relationship between REE and fitness is difficult as sound theories have been proposed for both positive and negative relationships. The increased intake hypothesis assumes that REE is proportional (i.e. positively correlated) to an organism's maximum capacity for production and represents the cost of maintaining the "metabolic machinery" (Drent and Daan, 1980; Boratynski and Koteja, 2010; Biro and Stamps, 2010). A high REE is therefore an inevitable cost of a high rate of total energy intake and this hypothesis predicts that REE will be positively correlated with fitness.

On the other hand, the "compensation hypothesis" proposes that a lower REE has a general fitness advantage as it allows a higher proportion of limited resources to be allocated to other functions such as growth and reproduction. This hypothesis predicts that REE will be negatively correlated with fitness (Metcalfe *et al.,* 1995; Burton *et al.,* 2011). It has also been suggested that the relationship between REE and fitness is dependent upon the quality of environmental conditions (e.g. food availability). In the "context dependent" hypothesis high REE is expected to be associated with increased fitness in favourable environments and low fitness in poor quality environments. Individuals with low REE are better protected against the environment due to their lower

costs of maintenance. Therefore, low REE is associated with relatively high fitness in poor environments but lower fitness than higher REE individuals in favourable environments (Biro and Stamps, 2010; Burton *et al.,* 2011). To date, a significant limitation on the theory of how REE relates to fitness is how REE is defined and a reliance on BMR as a single index of metabolism.

2.4 Limitations of BMR as an indicator of individual energetic phenotype

Basal metabolic rate is defined as the minimum rate of REE expressed by a non-reproducing mature endotherm measured during the normal inactive phase of its daily cycle, when that individual is post-absorptive and resting in its thermoneutral zone (Kleiber, 1961; McNab, 1997; Careau *et al.,* 2009). Early in the study of animal metabolism it became necessary to outline a set of specific conditions that would be equivalent across all animals to serve as a standardised index for measuring metabolic rate and enable meaningful comparison among studies. Basal metabolic rate has been widely used as a single index of energy expenditure over the last 50 years in a variety of species creating a comprehensive dataset for comparative physiologists. Basal metabolic rate has been instrumental in comparative studies at the inter-specific level where differences in size, insulation and thermal response make finding an appropriate temperature for comparison difficult. Its usefulness at the individual (intra-specific) level, as a substitute for other metabolic states, is debatable (McNab, 1997; Frankenfield, *et al.,* 1998; Speakman *et al.,* 1999; Hulbert and Else, 2003). It has been suggested that the relevance of BMR as a valid proxy for DEE is an insufficiently tested assumption (Mathot and Dingemanse, 2015). If so, this would indicate that observed patterns between metabolic rate and behaviour for a wide range of animals may be misinterpreted. Despite this, the use of BMR has dominated studies investigating the ecological and evolutionary significance of individual differences in energy expenditure.

For many species the use of BMR is not a good representation for minimum REE. Basal metabolic rate is most suitable for homeotherms that do not experience wide variations in their body temperature, though even these species may adjust body temperature to affect REE (e.g. Bush rats, *Rattus fuscipes*; Seebacher and Glanville, 2010). It is well established that body temperature influences an individual's metabolic rate and several species vary their body temperature over comparatively large ranges. Yet, it is not a requirement for

measuring BMR that the individual keep its body temperature at euthermic or a standard level (Speakman *et al.,* 1999; Gillooly *et al.,* 2001). Whilst several studies analysing among-species variation and scaling of BMR have adjusted for body temperature effects, this does not take into consideration that the among species or among individual variation in body temperature (and hence its effect on metabolism) could be adaptive as opposed to representing only a phylogenetic historical effect (White and Seymour, 2004*;* Clarke *et al.,* 2010).

Another limitation of BMR is that there are no conditions regarding the season that the measurements be taken, despite both being factors that heavily influence body temperature and metabolic rate (and hence BMR; Weathers, 1979; Speakman *et al.,* 1999). There is numerous evidence showing that daily circadian rhythms and seasonal rhythms affect REE in a wide range of taxa (Aschoff and Pohl, 1970; Rutter *et al.,* 2002; Eckel-Mahan and Sassone-Corsi, 2013). In many small mammals, particularly rodents, it has generally been noted that BMR is significantly higher in winter than during summer (Degen, 1997; Wan-long & Zheng-kun, 2012). Even in several large ruminants (e.g. white-tailed deer (*Odocoileus virginianus*) and red deer (*Cervus elaphus*) REE has been shown to vary seasonally with the lowest metabolic rates recorded in the winter, and highest in the summer (Silver *et al.,* 1969; Moen, 1978; Turbill *et al.,* 2011). There is a lack in appreciation that resting metabolic rate varies periodically (i.e. daily and seasonally) and the extent of such variation might be repeatable (at the appropriate level e.g. individual, population or species) and hence represent an among individual (or population or species) difference in energy requirements equally as large as the difference in BMR at a fixed point in time.

Basal metabolic rate is frequently used to represent a measure of minimum resting energy requirements, yet it purposefully excludes adjustment in regulated body temperature, which is the most common mechanism used to adjust minimum energy requirement. This is a key oversight because the aspects of resting metabolism not included in BMR are likely to be important mechanisms used by animals to adjust their energy expenditure. This unmeasured variation could be important in defining how resting metabolic rate should relate to other components of the energy budget and other fitness-related traits. One such factor is the capacity of an individual to reduce its resting energy costs during periods of reduced energy availability. For most mammals this involves a decrease in body

temperature from normothermic values as maintaining high metabolic heat production requires a high energy intake (Geiser, 2004). This ability is an important strategy in terms of survival and is presumed to be the primary fitness cost of a high BMR. The relationship between BMR and the capacity to minimise REE is not accounted for when just measuring BMR. Though there has been some research at the species level to see how BMR can be used to predict an organism's capacity to minimise their REE, this link is still not well understood (Cooper and Geiser, 2008). It is feasible that an individual could have a high BMR under normal conditions with abundant food but also exhibits very low REE by employing thermoregulatory behaviours like torpor during periods of low energy availability. Current hypotheses linking resting metabolic rate and fitness do not consider these possibilities. Hence, these hypotheses are greatly hampered by their reliance on what is arguably a severely limited proxy for resting metabolic rate.

Single measurements like BMR inform little about an individuals' energetic response to changing conditions (e.g. food withdrawal or temperature variation), which are challenges individuals face in their natural habitat. It is a standardised measurements quite removed from ecological reality. Clearly, REE is not a static characteristic but varies periodically (daily and seasonally) and can be adjusted in response to changes in intrinsic and environmental conditions. Such unaccounted-for factors are likely to be causing variation in recorded measurements of BMR (Speakman *et al.,* 1993). The extent of this variation may be repeatable and hence represent an among individual (or among population) difference in energy requirements equally as large as the difference in BMR at a fixed point in time. Often metabolic rate is oversimplified and approached as if it were a static value. Instead individual energetic phenotypes should be considered in regard to their metabolic response to environmental cues (i.e. metabolic reaction norm). As seen with individual traits, it appears that populations are in general comprised of individuals that exhibit consistent differences in their metabolic response to environmental change. For example, the reduction in REE in response to food shortage or increase in REE in response to colder temperatures can range from small to large among individuals whereas within-individual variation is small.

2.5 Influence of body temperature and thermoregulatory behaviour on metabolism

Body temperature and thermoregulatory behaviour are central mechanisms regulating individual energetic strategies that have widespread effects on metabolism and energy expenditure. It has been suggested that physiological constraints related to energy expenditure may underlie behavioural syndromes and limit phenotypic plasticity in behaviour (Gillooly *et al.,* 2001; Stamps, 2007; Careau *et al.,* 2008). Yet so far, there is a lack of theoretical or experiment studies that have integrated thermal physiology (i.e. body temperature) and thermoregulatory behaviour (i.e. torpor use) with other key traits as proposed by the POLS hypothesis (Reale *et al.,* 2010). The few studies that have addressed this subject have produced contradictory results (Careau *et al.,* 2011; Le Galliard *et al.,* 2013). This is an important topic as body temperature and thermoregulatory behaviour are strongly linked with metabolism and REE, and intraspecific variation in metabolic physiology may be a crucial evolutionary mechanism for the persistence of populations at times of environmental change (Speakman *et al.,* 1999; Morrison *et al.,* 2008; Seebacher 2009). Consistent individual differences in thermoregulation could have a functional influence on behaviour, physiology and life-history traits and so a clearer understanding of whether differences in thermal physiology can be explained by integration with other pace-of-life traits is essential.

In both ectothermic and endothermic animals body temperature has a large influence on rates of metabolism owing to underlying effects of temperature on all biochemical reactions. Hence, animals with lower body temperature generally exhibit lower metabolic rates than those with higher body temperatures (Speakman *et al.,* 1993; Clarke and Johnston, 1999; Ooijen *et al.,* 2001). Such reductions in metabolic rate at lower temperatures can be explained by the effect of temperature on biochemical reactions. An increase in body temperature accelerates biochemical reactions, this effect is often exponential and frequently expressed as the rate of change over a 10 °C increase in temperature (Q_{10}; Heldmaier and Ruf, 1992; Geiser, 2004). Thus, an organism's body temperature regulates the rate of cellular biochemical reactions that are involved in their metabolism. Although the influence that body temperature has on metabolism is well known the mechanisms that link body temperature and metabolic rate in different groups of animals remain relatively obscure. Better understandings of this relationship would help

explain links between physiology and ecology, furthering the field of evolutionary physiology (Feder *et al.,* 2000; Clarke and Fraser, 2004).

To maintain normothermic body temperature individuals will employ a variety of thermoregulatory mechanisms, which can be energetically costly. For example, a significant proportion of the daily energy budget in endotherms is used to generate endogenous metabolic heat to maintain a relatively constant body temperature over a wide range of T_a. As the surface area to volume ratio increases with decreasing size, thermoregulatory costs are higher in smaller sized endotherms, which lose their internal body heat at proportionally greater rate than larger animals (Kleiber, 1975). Many endothermic animals do not maintain a constant normothermic body temperature; instead they often experience controlled bouts of hypometabolism. Heterothermic responses, comprising of voluntary and temporary decreases in body temperature and metabolism in response to cues in the external environment (i.e low ambient temperature, decreased energy acquisition) are very common among endotherms (Grigg *et al.,* 2004; McKechnie and Mzilikazi, 2011). Through reducing an individual's resting metabolic rate these thermoregulatory behaviours significantly decrease an individual's energy expenditure resulting in an important adaptation for coping with variable environmental conditions. In some large mammals (i.e. ungulates) resting metabolic rates are linked with variations in core and peripheral body temperatures and change according to seasonal endogenous signals synchronised to photoperiod and short torpor like bouts of hypometabolism during winter (Arnold *et al.,* 2004; Turbill *et al.,* 2011).

Daily torpor is an energy conservation strategy that has evolved in many heterothermic mammals and birds to significantly reduce energetic requirements through a gradual and controlled reduction in body temperature, metabolic rate and other physiological functions. Torpor is an effective thermoregulatory process that reduces an individual's minimum metabolic rate by 5-30% of the basal normothermic levels. It is frequently employed in high cost environments, such as low and variable food availability and low temperatures (Geiser, 2004; Gilbert *et al*., 2009). Torpor expression is not a fixed response; instead it is determined by both intrinsic factors and extrinsic environmental context. Under benign environmental conditions (i.e. constant food availability, low predation risk) individuals may not enter torpor, avoiding the energetic cost of arousal

from torpor (Lovegrove *et al.,* 1999). The ability to undergo torpor bouts has been associated with reduced extinction risk, particularly in smaller mammal species which experience higher energetic advantages than larger species (Geiser and Turbill, 2009; Hanna and Marcel, 2014). Although torpor is most commonly observed in response to adverse environmental conditions as a way to balance the daily energy budget, torpor is often used in situations where individuals have access to food and are experiencing no immediate energetic stress (Geiser and Brigham, 2012). For example, hummingbirds utilise torpor to enhance fat storage at night during their migratory period and prevent a future energy shortage (Carpenter and Hixon, 1988).

2.6 Conclusions and future perspectives

I am recommending the need to re-evaluate how metabolic rate is conceptualised and how it should be linked with behaviour and life history. A large assumption of research exploring links between metabolism and behaviour has been that the metabolic measurement used, usually BMR, is a reliable index for REE and predictor of active energy expenditure (Mathot and Dingemanse, 2015). Daily energy expenditure is a more all-inclusive measure for looking at consistent individual differences in metabolism and behaviour yet evidence supporting a link between BMR and free-ranging DEE among small mammals is very weak (Speakman *et al.,* 1999). It is clear that REE is not a fixed characteristic but one that is heavily influenced by variations in intrinsic properties and the surrounding environment. Therefore, to get a clearer understanding of how individual variations in metabolism are linked to the POLS hypothesis, it is necessary to adopt a holistic approach when measuring REE. This should involve moving beyond the use of BMR as the sole index of an individual's REE to employ multiple measures of metabolic rate in order to create a complete metabolic profile over a long period of time (Bouwhuis *et al.,* 2014). A metabolic "reaction norm" approach characterises how individuals respond energetically to variation in environmental conditions (e.g. food restriction and ambient temperature). This approach will enable better defined and more realistic hypotheses about how consistent individual differences in energy expenditure relate to key POLS traits. Thus, leading us to better understand the links between different components of the daily energy budget and how these components respond to energy supply or use.

Future approaches must recognise the effects of variation in thermoregulatory physiology and behaviour on metabolism. A focus on individual variation, rather than the current emphasis on mean values of single traits, could integrate all relevant factors, such as BMR and individual variation in energy saving mechanisms (i.e. torpor). Thorough research on factors influencing individual variation will assist in explaining the evolutionary mechanisms driving the presence of consistent individual differences and determine whether these individual differences represent adaptive plasticity in response to local conditions (Thornton and Lukas, 2012).

A multidisciplinary approach would allow the assessment of the relative influence that each factor's variation has on REE and help understand how individual differences in metabolic rate are linked to key POLS traits. The POLS hypothesis could explain the individual differences that are seen in regard to how REE is affected by environmental and intrinsic conditions. This would aid our understanding of the relationship that REE has with lifetime fitness in a natural environment.

Chapter 3

Thermal and metabolic physiology of wild-caught House Mice (*Mus muculus*)

3.1 Abstract

Research is still needed to understand the ecological significance of metabolic rate and its relation to other key traits that determine animal performance and evolutionary fitness. Metabolism has widespread impacts on an individual's energetic demand on their environment and individual differences in metabolism can influence fitness**.** Environmental conditions such as food availability and temperature affect metabolism but it is unclear how these effects vary among individuals. Past efforts to determine the ecological and evolutionary significance of intraspecific variation have relied on basal metabolic rate (BMR) as a single index of individual differences in metabolism. Yet, for small endotherms, metabolic rate is strongly affected by thermoregulatory behaviour and food availability, and individual differences in metabolic strategies to environmental conditions (e.g. food restriction) could be important repeatable traits.

This study used long-term respirometry to determine how changes in ambient temperature and food restriction affected the metabolic rate of wild-caught house mice (*Mus musculus*). In particular, the repeatability of individuals' metabolic responses were calculated. The relationship between standardised and integrative measures of metabolism was also calculated to determine which would be most useful predictors of DEE. Overall, the standard physiological responses to temperature and food withdrawal were typical of a murine rodent. Mice decreased their energy expenditure and exhibited a propensity to use torpor when faced with low temperature and food withdrawal. Strong evidence of repeatability for multiple components of metabolic energy expenditure was observed. In particular, there was high individual consistency in daily energy expenditure (DEE), REE and average energy expenditure (AEE) at 15 °C, relative to the total population variation. Resting metabolic rate and AEE at 15°C were more accurate as relative predictors of DEE than measurements at 31°C, which lacked a thermoregulatory component (similar to BMR). These results provide valuable information on the lifetime changes of physiological traits in wild caught mice. Future studies should aim to use

measurements that include the significant variation in resting energy expenditure (REE) that is not incorporated in standard measures of BMR.

3.2 Introduction

Metabolism is the biological processing of energy and has widespread impacts on the dynamics of ecological systems by determining organisms' energy budgets and consequently their demands on their environment for resources (Brown *et al.,* 2004). Despite the significant ecological consequences of metabolism there remain many gaps in our understanding of the metabolic physiological adaptations employed by animals that enable them to survive and reproduce in the face of seasonal and day-to-day variation in their environmental conditions. Metabolic energy expenditure and thermoregulatory mechanisms that save energy (e.g. torpor by endotherms) are fundamental for coping with environmental change as they determine the minimum resources required for an individual to survive. In addition, energy expenditure regulates the rate at which an individual can convert energy into somatic growth, maintenance and reproductive output making them intrinsically related to fitness (Ricklefs and Wikelski, 2002; Brown *et al.,* 2004; Geiser and Turbill, 2009; Careau *et al.,* 2014b). Subsequently, predicting changes in energy expenditure would enable further insight into numerous aspects of animal ecology, physiology and behaviour.

Studies commonly find substantial individual variation in various aspects of the metabolic budget such as mass-specific resting metabolic rates, total energy expenditure and propensity to use torpor within populations (Speakman *et al.,* 2003; Nespolo and Franco, 2007; Versteegh *et al.,* 2008, Biro and Stamps, 2010). In the last few decades, researchers have increasingly realised the possible ecological and evolutionary significance of inter-individual variation in wild populations (Bennett, 1987). Consequently, there has been a significant shift in our general approach towards focussing on variation among individuals, rather than on the mean population responses, to gain a better understanding of variation in key biological traits (Hayes and Jenkins, 1997; Careau *et al.,* 2008). It remains uncertain how much of the observed variation reflects random variation of little interest and how much is consistent individual differences that might be considered a trait affecting survival, growth or reproductive output.

For natural selection on a biological trait (e.g. metabolic rate) to be effective, that trait in question must be consistent for a significant period of an individual's lifetime. Repeatability is often used to estimate trait consistency and can be estimated as the proportion of total variance in a measured trait that occurs among rather than within individuals (Falconer, 1960; Lessells and Boag, 1987). High consistency does not necessarily imply that the measured trait is permanently fixed. For instance, an individual's metabolic rate may gradually vary over repeated measurements, whilst that individual's relative placement (i.e. ranking) within a population is largely maintained and considered repeatable (Reale *et al.,* 2007). Demonstrating repeatability is important because it implies that an individual's trait value (or rank) measured at one particular time point will be a reasonable predictor of its trait value at another time point. Many, but not all, ecological studies measure an index of metabolism (typically basal metabolic rate) only once for each animal and assume that measurement is representative of longer term among individual differences (Speakman *et al.,* 1994).

Past efforts to determine the evolutionary significance of intraspecific variation in metabolism have frequently relied on BMR as a single index of energy expenditure (Bech *et al.,* 1999; Labocha *et al.,* 2004). Basal metabolic rate represents the minimum rate of energy required for maintenance in an adult endotherm when it is post-absorptive, at rest, non-reproductive and within its thermo-neutral zone (TNZ). Other levels of metabolism (e.g. daily energy expenditure (DEE), field metabolic rate (FMR), average and maximum metabolic rates) are often assumed to be relatively constant multiples of BMR (McNab, 1980, Koteja, 1991). Basal metabolic rate is one of the most frequent metabolic measurements used in captivity and is a valuable standardised index for measuring metabolic rate which has enabled meaningful comparisons across studies (McNab, 1997; Speakman *et al.,* 2004). Whilst BMR is useful as an indicator of a low precision index of metabolic capacity for broad scale among taxa comparisons, at the individual scale it is an inadequate indicator of energetic capacity. For instance, the metabolism of small endotherms like *M. musculus* is strongly affected by environmental thermal conditions (because of their high thermal conductance), and changes in thermoregulatory behaviour (e.g. torpor use), and consequently is strongly influenced by intrinsic and environmental changes. Basal metabolic rate does not account for energy saving strategies (e.g. torpor) that significantly reduce energy requirements whilst at rest and is widespread among small

mammals (Ruf *et al.,* 1991; Stawski and Geiser, 2010). Additionally,as it is unlikely that individuals experience the strict conditions required for measuring BMR when in their natural habitat (Turbill *et al.* 2011) BMR may be of limited use for predicting energy expenditure of animals under natural conditions. Past studies have corroborated this by showing weak evidence for a link in intra-specific studies of small mammals (< 4 kg) between BMR and daily energy expenditure in the field (Speakman, 2000). Despite this, BMR is commonly cited to represent maintenance energy costs in the wild (Koteja, 2991; Hulbert and Else, 2003; Mathot and Dingemanse, 2015).

In addition to inter-individual variation in single metabolic traits (e.g. BMR), individuals may differ in their metabolic responses to significant biological conditions (e.g. variation in food availability and ambient temperature). Reaction norms can be used to demonstrate an animal's capacity to adjust their metabolism to environmental change. To date, few studies have incorporated metabolic reaction norms to see whether individuals vary in how their energy expenditure responds to changes in ambient temperature or food availability (Careau *et al.,* 2014).

The large effects of thermoregulation have thus far been neglected in the current literature. Thermoregulation is energetically costly, particularly in small mammals which lose body heat rapidly, and has substantial implications on physiological processes and how they are associated with key life history traits. Thermoregulatory costs could be an important aspect of energy expenditure that significantly differs among individuals. This may be the case, for instance, if individuals differ in thermal conductance (e.g. as a function of body size, or difference in pelt quality). Moreover, body temperature (T_b) and hence metabolic requirements for thermoregulation is not fixed over time or among individuals. Body temperature set-point and resting metabolic rate vary daily and seasonally (Aschoff and Pohl, 1970; Rutter *et al.,* 2002; Eckel-Mahan and Sasson-Corsi, 2013). Over time, even in large mammals, reductions in peripheral temperature are a common adaption for energy savings (Turbill *et al.,* 2011; Arnold *et al.,* 2004). In smaller mammals, controlled temporary reductions in T_b set-point and resting metabolic rate (i.e. torpor) are a common mechanism to reduce energy requirements in response to harsh conditions such as low ambient temperatures and food restriction (Barnes, 1989; Geiser and Ruf, 1995). The functional effects of torpor on physiology, behaviour and life history

reach beyond the immediate energy budget. In addition to increasing survival by reducing daily energy requirements during environmentally stressful times, daily torpor may also reduce the risks of predation through reducing minimum required foraging activity (Schubert *et al.,* 2010).

The two most important environmental variables influencing metabolic rate are thermal conditions and food availability (Kleiber, 1932; Gillooly *et al.,* 2001; Liu and Fu, 2007). Here, I investigated the impact of ambient temperature, dietary energy availability and time on the energetic response of wild caught *M. musculus.* Temperature generally varies widely over the course of each day and adverse weather or other environmental factors can prevent foraging for 24 h or more. Hence, in this chapter the metabolic energy expenditure of wild-caught *M. musculus* exposed to a daily temperature cycle (15 °C, 20 °C and 31 °C) and alternate-day food withdrawal was measured over six days and repeated three times at one-month intervals. Under this regime, I gained repeated estimates not only of resting metabolic rate without a thermoregulatory component (similar to BMR) but also the response in metabolic rate to cooler temperature, reflecting thermal conductance.

This chapter provides an initial overview of the thermal and metabolic physiology of *M. musculus* at the population level before focussing on individual level responses. Specifically, the detailed longitudinal measurements were used to examine: (i) the mean effects of temperature and food availability, (ii) the relationship between standardised measurements similar to BMR and integrative measures of metabolic energy expenditure to assess the most useful predictor of daily energy expenditure, (iii) repeatability of various metabolic responses both within a series of measurements (i.e. over six day respirometry run) and over longer periods (i.e. over three months), and (iv) how *M. musculus* uses daily torpor in response to period of food withdrawal. It was hypothesised that; (i) typical responses to food restriction and lowered temperatures would be observed, specifically a decrease in metabolic responses and propensity to use torpor, (ii) resting metabolic rates at temperatures below the thermoneutral zone which took into account a thermoregulatory component (e.g. torpor) would be more accurate as relative predictors of DEE than measurements that lack a thermoregulatory component, (iii) multiple components of the daily metabolic budget would be repeatable across the entire three

month measurement period, and (iv) the mice would show a strong propensity to use torpor in response to periods of food withdrawal.

3.3 Materials and methods

Approval to conduct this study was granted by the University of Western Sydney's Animal Care and Ethics Committee (Animal research authority #A10445) and all procedures met federal standards for animal care and welfare (National Health and Medical Research Council, 2013).

3.3.1 Study animals and colony maintenance

This study was conducted on 69 wild caught house mice (*M. musculus*) captured in Elliott aluminium live traps on private agricultural land in Wilberforce, NSW, Australia (GPS 33°33'40.8 S, 150°50'0.8 E). Traps were baited with balls of rolled oats, honey and peanut butter, left open overnight and checked at dawn the following morning. Upon capture, mice were checked for breeding condition, weighed and measured. Palpably pregnant females or females exhibiting signs of lactation (exposed nipples) were released at capture. Length of the animal taken from the base of the tail to the nose tip (HB) was used to determine whether the individuals were juveniles (0.5 weeks old; HB < 64 mm), sub-adults (5-8 weeks old; 64 mm ≤ HB ≤ 71 mm) or adults over 8 weeks old (HB > 71 mm; Newsome, 1969 and Singleton, 1983). Only sub-adults were included in this study to compare individuals of similar age and avoid the possibility of using senescing individuals. During each trapping session, I aimed to catch 16 adult non-reproductive mice, with trapping sessions taking place over one night at approximately three-month intervals between July 2015 and July 2016.

Once captured the mice were brought to a rodent holding facility on the Western Sydney University's Hawkesbury Campus where they were weighed, sexed and treated topically with one drop (c. 10µl) of antiparasitic agent (Ivermectin, 0.83 mg/mL) on the back of their neck. When mice were not being used for experimental measurements they were housed individually in standard mouse cages (1248L Eurostandard Type II polysulfone cages with filter top lids; Techniplast, Italy) with *ad libitum* quantities of maintenance rodent pellets (Gordon's Specialty Stockfeeds P/L, Australia) and water. All mice were housed in a single room where the T_a was maintained at 23 ± 2°C and the mice experienced natural

photoperiods. Cages contained 500 ml of Pura cob bedding substrate (Able Scientific, Australia), a handful of shredded paper and a cardboard tube for nesting material and environmental enrichment. The mice remained in captivity for the duration of the measurement period.

3.3.2 Measurement of metabolic traits

Following capture a number of physiological and behavioural traits were measured using a computer controlled high-resolution open-flow respirometry system (Promethion Metabolic and Behavioural Data Acquisition System, Sable Systems, Las Vegas, U.S.A.). Each individual underwent three respirometry runs; the first respirometry run started one day after capture and the following two were carried out at approximately one-month intervals. The mice were placed individually in unsealed "live-in" respirometry chambers (21 x 37 x 14 cm) with the same size dimensions as the mice's normal cages. These chambers were housed inside a temperature-controlled cabinet (Panosonic MIR-554) and their metabolic response to daily variation in temperature and food availability was recorded continuously over six days (144 hours; hereafter termed a respirometry run).

3.3.2.1 Respirometry System

The indirect calorimetry system measured up to 16 individuals at a time. The 16 respirometry chambers were separated into two groups of eight chambers, located within each of two temperature-controlled cabinets. The system analysed the gas exchange (O_2, CO_2 and water vapour) for each group of eight chambers in two parallel lines of gas flow, each measured by a mass flow meter, O_2, CO_2 and water vapour pressure analysers. The airflow for each group of eight chambers and an additional baseline cage was measured and regulated by one of two nine-channel mass flow generators (FR-8, Sable Systems, Las Vegas, U.S.A). These flow generators pulled a constant flow of air from all chambers simultaneously at a rate of 2,000 mL min^{-1} (Sable Systems, 2013). Each of the two parallel lines was connected to a separate gas analyser (GA-3, Sable Systems, Las Vegas, U.S.A) contained within a custom electronic enclosure. This setup simplifies system layout and reduces plumbing length between the analysers, thereby minimising lag effects that complicate data analysis. The air flow from one chamber of each eight-chamber group was selected by each FR-8 to be directed through the two gas analysis blades. In the GA-3 gas analyser, a subsample of each of the two selected air streams was pulled out of the main

flow (250 ml min^{-1}) and pushed in series through the spectrophotometric CO_2 analyser, integrated fuel cell O_2 analyser and capacitive water vapour partial pressure analyser (Sable Systems, 2013).

For each parallel group of eight chambers and analyser blade, the measured airstream was switched between a baseline chamber (identical to each animal chamber) and each animal chamber every fifteen seconds. Thus, O_2 consumption and CO_2 production were measured for each individual for 15 seconds at four-minute intervals (Sable Systems, 2013). Water vapour in the sample airstream was also measured and used to continuously correct the VO_2 and VCO_2. According to the set flow rate (2000 mL min^{-1}) and the cage volume, time until 99% volumetric washout would be around five and a half minutes (Lighton, 2008). The raw data were adjusted using the *Z*- transformation to correct the data for dampening in measured response caused by the slow washout time relative to sampling rate (also termed: instantaneous correction). The Z value was calculated to optimise the data during prior calibration using the exact set-up of the respirometry system. The Z transformation of the raw data extrapolates the instantaneous changes in metabolic rate at each sampling point (Bartholomew *et al.,* 1981), which radically improves the time resolution and detailed structure of the metabolic data.

Fractional concentrations of O_2 depletion and CO_2 enrichment were determined from the raw O_2 and CO_2 traces by subtracting out traces with baseline values (with drift-correction as necessary). Oxygen consumption (VO_2) and CO_2 production (VCO_2) were calculated using following equations for VO_2 (mL O2) and VCO_2 (mL CO_2) were used (Lighton, 2008):

$$VO_2 = FR_e\,[(F_iO_2 - F'_eO_2) - F_iO_2(F'_eCO_2 - F_iCO_2)]/(1-F_1O_2)$$

and

$$VCO_2 = FR_e[(F'_eCO_2 - F_iCO_2) + F_iCO_2\,(F_iO_2 - F'_eO_2)]/(1 + F_iC0_2)$$

Where FR_e is the flow rate of the excurrent air, F_i is the fractional concentration of O_2 or CO_2 in the incurrent airstream and F'_e is the fractional concentration of O_2 or CO_2 in the excurrent airstream. The ratio of CO_2 production to O_2 consumption was used to calculate respiratory quotient; an indicator of nutrient utilization.

Energy expenditure was determined by converting rates of CO_2 production and O_2 consumption using the abbreviated Weir equation and expressed in units of kilocalories per hour (Weir, 1949):

$$Kcal\ h^{-1} = 60*(0.003941*V_{O2} + 0.001106*V_{CO2})$$

Energy expenditure was then converted to Watts:

$$W = kcal\ h^{-1} \times 1.163$$

Rates of metabolism were converted to Joules:

$$J = W \times seconds$$

Data collection and equipment control were regulated by the data acquisition software Metascreen v.1.9.2 (Sable Systems) and raw data were transformed using ExpeData analysis software v. 1.9.2 (Sable Systems) involving the use of a customised automated analysis script detailing each step of the data transformation.

3.3.2.2 Chamber Structure

The unique "live-in" designed respirometry chambers (21 x 37 x 14 cm) used normal mouse cages (i.e. Eurostandard Type II) with a modified lid, and hence remained very similar to the mice's normal cages. This setup helped to minimise stress responses caused by novel or much smaller respirometry chambers. The larger chambers, which included food, water and shelter, also enabled the respirometry runs to last for several days, whereas almost all previous studies measured for periods lasting < 24h. The chambers contained standard bedding material and were equipped with a water bottle, a food hopper connected to a mass load cell to monitor real time food intake and a suspended enrichment tube also connected to a mass load cell for continuous body mass monitoring. Each chamber also contained a metal running wheel connected to a magnetic reed switch, providing continuous measurement of wheel revolutions. A continuous flow of air was pulled into the chambers via holes in the lid and then through micro-perforations in a

stainless steel respirometry manifold located along three sides at the inner bottom rim of the chamber.

3.3.2.3 System Calibration

Gas analysers were hand calibrated every two months with pure analytical grade nitrogen (zero CO_2) and a CO_2 span gas containing a certified known concentration of CO_2, O_2 with balance N_2. The GA-3 unit was also programmed to perform calibration measurements at the beginning of each run. During the pre-run calibration ambient air, containing water vapour, was switched to flow through the GA-3 unit before the airstream was temporarily directed through a chemical scrubber column containing magnesium perchlorate, a very effective drying agent, sandwiched between layers of Ascarite, to remove CO_2 from the airstream. This operation allowed the water vapour analyser to be calibrated using the technique of O_2 dilution (Lighton, 2008). The worldwide fractional concentration in dry air is extremely close to 0.2094. The incurrent dry air, in combination with barometric pressure correction to the standard pressure of 101.325 kPa, was used to span the GA-3 O_2 analyser at the fractional concentration of 0.2094 and zero the water vapour pressure analyser. The chemical scrubber column was then switched out of the circuit allowing the O_2 concentrations to become diluted by the incoming "wet" ambient air. The water vapour pressure in the airstream was then calculated from the degree that the O2 concentration falls and used to automatically span the water vapour pressure analyser (Lighton, 2008). During this automated water vapour analyser calibration, the CO_2 is chemically scrubbed from the airstream enabling the CO_2 analyser to be zeroed. This was the only time that the air stream was dried during each respirometry run. For the remainder of the run, water vapour was continuously measured and its dilution effect on O_2 and CO_2 was compensated for mathematically, using data from the GA-3's water vapour and barometric pressure analysers (Lighton, 2008; Sable Systems, 2013).

3.3.3 Data Collection

Before the start of the respirometry run, mice were weighed between 1400 h and 1500 h and placed individually into the respirometry chambers. At 1600 h the respirometry measurements started and on the last day of the run the respirometry measurements were stopped at 1600 h. Mice were then removed from their chambers, weighed and returned to their home cages.

3.3.3.1 Food Availability and wheel access

Access to the food hoppers was controlled by remotely controlled doors that were programmed to open and close at set times and days. On alternating days, food access was denied by closing the door at 1700 h for 24 hours. Wheel access was restricted between 1200 h and 1700 h on non-food days by inserting a metal rod to block wheel rotations. Data collected between 1700 h and 1800 h were not used as the incubators were opened over this period to confirm the status of the food access doors, remove the wheel block (if necessary) and check on the mice's welfare.

3.3.3.2 Temperature Profile and Photoperiod

Mice were exposed to a 24-hour temperature cycle with three differing temperature regimes of; 1200h to 2000h: 31 °C, 2000h to 0400h: 20 °C and 0400h to 1200h: 15 °C. Ambient temperature within the incubators was recorded every five minutes using temperature-logging iButton data loggers (resolution: 0.065°C; Maxim Integrated, U.S.A). One iButton was positioned on the top shelf and another on the bottom shelf in both incubators to record any temperature variation within the incubators. Temperatures within the incubators took up to 50 minutes to stabilise during a temperature regime change. As a result, the hour immediately following each temperature change was excluded from analysis. The mice were subjected to a 12 h light- 12 h dark cycle where the lights were turned off at 1900 h and back on at 0700 h for all respirometry runs.

3.3.4 Data analysis

3.3.4.1 Metabolic measurements

For all analyses of metabolic data, an experimental day was designated as starting at 1600 h and ending the following day at 1559 h. Each day was separated by their photo phase into an active (lights off to lights on; 1900 h -0700 h) and rest (lights on to lights off; 0700 h -1900 h) phase. The rest phase of day one therefore began on the same experimental day as the active phase of the previous night. Daily energy expenditure (DEE) was calculated by averaging an individuals' energy expenditure over each 24-hour period (experimental day) the mouse was in the respirometry run. Resting energy expenditure (REE) was calculated by combining average energy expenditure over the late active (0000 h-0700 h) and early resting phases (0700 h-1200 h). Resting metabolic rates (RMR) were calculated daily for

each temperature by averaging the lowest consecutive 12- minute period within each temperature period (excluding the first hour). Average energy expenditures (AEEs) for each temperature were also calculated daily by averaging the energy expenditure at all three temperatures. Metabolic characteristics that are not referred to as whole animal measurements refer to mass adjusted values for brevity.

3.3.4.2 Statistical analysis

All data provided in the text are reported as means of individual means ± SD (*n* = the number of individuals, *N* = the number of observations). Correlation tests were used to determine whether body mass interacted with whole animal DEE and REE on food days. Data were pooled from replicated samples per individual per run to account for issues associated with pseudo replication. The effects of food availability and temperature on mass-specific metabolic responses were estimated using linear mixed effect models (R package "lme4", "lmertest") within the R statistical interface v3.3.3 and RStudio 1.0.136 (R Core Team 2015; R Studio Team 2016; Bates *et al.,* 2015; Kuznetsova, Brockhoff and Christensen 2016). Fixed effects included "temperature", "food", "day" and "sex" and their associated interactions. "ID" and "run" (i.e. respirometry run) were included as random effects to account for repeated measures within individuals and among runs. Terms that were not significant were not included in the final model. The fixed effect of "body mass" and "sex:bodymass" were included in models incorporating whole animal metabolic characteristics. Mixed models were also used to compare mean differences in RMR and AEE between respirometry days and runs, and to determine the effect of food availability on AEE over the different photo phases (rest and active). Pair-wise differences in estimated mean effects were compared using a Tukey-Kramer *post hoc* test (using the *glht* function in the R package *multcomp*). Where interaction effects were significant with more than two levels separate LME models were used in place of *post hoc* tests.

The distribution of RMR at 15 °C (RMR_15) on days without food compared with those days where food was present was visually analysed and used to determine an arbitrary threshold of 0.14 W to designate periods of torpor use (Fig. 3.2A). Very little data for RMR_15_F fell below 0.2 W, 0.14 W was chosen as the torpor threshold as it represented the upper limit of the lower mode in the RMR_15_NF data. Use of torpor was analysed between 0000 h and 1200 h only after visual inspection of the metabolic traces of all

individuals indicated that this period was when an individual was likely to use torpor if they had any propensity to do so. All torpor bouts, save one, occurred on a non-food day. To define a torpor bout an individual's metabolic rate had to remain below the metabolic torpor threshold (0.14W) for a minimum of 30 minutes. The torpor bout ended when the individual's metabolic rate rose above this threshold for more than 12 consecutive minutes. Where an individual did not arouse from torpor by 1200 h the characteristics of the torpor bout were analysed until the individual had aroused and not artificially cut off at 1200 h. Likewise, torpor bouts that started prior to 0000 h were fully included.

Permutational analysis of variance (PERMANOVA), combining all torpor characteristics in an individual model, determined no significant difference between males and females in the observed torpor characteristics. Consequently, sexes were combined for all further analysis. Linear mixed models (as above excluding fixed effects of "food" and "temperature") were used to observe the effects of day and run on the measured torpor characteristics. The behavioural variable "percentage of time in torpor" was log transformed to normalise residuals. An mixed model was also used to determine the effects of body mass and food intake (i.e. mass of food eaten) on torpor duration and the dependent variable "torpor duration" was log-transformed to normalise residuals.. Correlations were used to observe the individual relationship between each of the RMRs and AEEs with DEE. Significance for all tests was set at $P = 0.05$.

Repeatability (R), the proportion of total variance that could be attributed to among individual differences over the three runs, was estimated following Araya-Ajoy *et al.* (2015) and a semi-parametric bootstrap method ("lme4" package in R) was used to calculate the 95% confidence intervals (CI) for R from 100 simulations. When the CIs did not overlap with zero, the R estimate was considered significant.

3.4 Results

Seventy-two mice underwent three runs of respirometry measurements, however, due to equipment error, the second run of metabolic measurements for nine individuals were not included in analyses on individual repeatability across runs.

The mean body mass of the individuals, taken immediately prior to the commencement of respirometry measurements, ranged from 8.69 - 19.5 g, with an average (mean ± SD reported here and elsewhere in the text) initial body mass of 14.50 ± 2.25 g (*n* = 72; Table 3.1). Male mice (*n*= 32) started the experiment with a higher mean initial body mass of 14.94 ± 2.28 g compared to female mice, which had an average body mass of 14.14 ± 2.18 g (*n*=40). Food was available only on three of the six days (day two, four and six) of each respirometry run and total food consumption over the entire run averaged over the three days when food was available was 8.58 ± 2.50 g day^{-1} (*N* = 207). Female mice (*n*= 113) consumed an average of 8.07 ± 2.06 g day^{-1}, compared to 9.19 ± 3.01 g day^{-} for male mice (*n*=94). There was a weak positive relationship between total food consumed and body mass, with larger mice eating more food (*r* = 0.19, t = 2.71, *P* = 0.005, N = 207 ; Fig. 3.1).

Mean T_a within the temperature cabinets at the three temperature levels were 15.35 ± 0.31 (range: 14.32 to 15.94) °C, 20.25 ± 0.27 (range: 19.20 to 20.82) °C and 31.21 ± 0.39 (range: 29.52 to 32.26) °C. Overall, the T_a differed by 0.43 ± 0.38 °C between the two cabinets, and within the incubators the bottom shelves were on average 1.11 ± 0.55 °C lower than the top shelves. To minimise temperature differences within the incubators individuals were rotated randomly between and within the incubators over their three runs.

On food days whole animal DEE averaged 38,490 (± 6764) J day^{-1} (range: 10,708 to 55,131 J day^{-1}), with more than half of that energy expenditure on average occurring during the late active and early rest phase (when T_a was also coolest): whole animal REE (0000 h - 1200 h) was 22,212 (±4313) J /12 h (range: 1,481 to 32,881 J/12 h). As expected, there was a significant positive relationship between whole animal DEE on food days (DEE_F) and body mass *(r* = 0.34, t = 4.95, *P* < 0.001, *N* = 192) and also whole animal REE on food days (REE_F) and body mass *(r* = 0.26, t = 3.67,, *P* < 0.001, *N* = 192; Fig. 3.2). When food was available, whole animal AEE over the rest phase (0700 h -1900 h; 12 h) averaged 19,104 ± 4,320 J/12 h (range: 2,902 to 30,754 J/12 h) and 19,264 ± 3,681 J/12 h (range: 3,922 to 30,546 J/12 h) over the active phase (1900 h – 0700 h; 12 h).

The whole animal metabolic response of *M. musculus* to food availability and T_a varied substantially among individuals within the respirometry runs (Fig. 3.3). Regular daily torpor

patterns at 15°C on non-food days were observed in some individuals (Fig. 3.3A), whereas others displayed a much lower propensity to use torpor (Fig. 3.3B) and some individuals showed little variation in their daily metabolic patterns on food and non-food days (Fig. 3.3C).

3.4.1 Metabolic response to variation in food availability and ambient temperature

As described in section 3.3.4. where metabolic responses are not referred to as whole animal they are mass adjusted. There was a significant negative effect of food withdrawal on both DEE and REE, whereby, on average, DEE decreased by 20% and REE decreased by 26% on days without food (Table 3.2). Food withdrawal also had a significant effect on the AEE over both the active (2000 h -0700 h) ($F_{2, 392}$ = 27.38, P < 0.001) and rest phase (0700 h -2000 h) ($F_{1,392}$ = 214.40, P < 0.001). Specifically, AEE decreased when food was unavailable in both the resting and active phases but had a more pronounced effect when mice were in their resting phase (Fig. 3.4).

Increasing the T_a had a significant negative effect on both RMR ($F_{2, 584}$ = 568.16, P < 0.001) and AEE ($F_{2, 584}$ = 534.73, P < 0.001) on days where food was available. A linear relationship between T_a and RMR was expected, however RMR_20 (resting metabolic rate at 20 °C) appeared slightly elevated (Fig. 3.5). This is most likely a result of measuring RMR_20 during the mice's active phase, consequently RMR_20 did not represent a true resting measurement.

Food withdrawal significantly affected the negative relationship between temperature and RMR (Fig. 3.5A). Resting metabolic rate was significantly lower when food was not available at all temperatures, though the degree that food withdrawal affected RMR varied depending on the T_a. Specifically, the interaction between food availability and temperature was strongest at 15 °C where RMR decreased by two-thirds (67%) on food withdrawal days, compared to an 18% decrease at 20 °C. Even at 31 °C, however, RMR decreased by 30% on no-food days. The frequency distrubition of RMR at 15 and 20 on non-food days showed a distinct bimodal distibution, which presumedly represented the RMR of normothermic versus torpid indivdiuals (Fig. 3.6A and B). Food withdrawal had a similar effect on AEE at all T_as (Fig. 3.5B); at all temperatures, AEE decreased when food

was unavailable, with the strongest effect occuring at 15 °C where AEE decreased by 34%, compared to a 7% decrease at 20 °C and 31% decrease at 31 °C.

There was a positive correlation between RMR at all three T_a and body mass on both food and non-food days. On days with food this was strongest for RMR_20 whereas on food withdrawal days this was strongest for RMR_31 (Fig. 3.7). On food days there was a significant effect of an interaction between temperature and body mass ($F_{2, 584}$ = 3.98, P = 0.019). The interaction between temperature and body mass was stronger on food-withdrawal days ($F_{2, 584}$ = 4.92, P = 0.008). Food availability had a significant effect on the intercept of the relationship between body mass and RMR.

3.4.2 Temporal effects on metabolic response among runs

Run in interaction with food had a significant effect on DEE ($F_{2, 972}$ = 3.22, P = 0.040) and REE ($F_{2,972}$ = 3.50, P = 0.031). Therefore, separate LME models including the fixed effects of run, age, sex and food availability were used to determine the effect of respirometry run on these mass-adjusted metabolic characteristics. Respirometry run had a significant effect on DEE and REE on both food and non-food days (Fig. 3.8). Specifically, DEE and REE on non food days were significantly lower in the first run, wheras DEE_F and REE_F were highest in run two. There was a three-way interaction effect of run, food and temperature on RMR ($F_{4,3318}$ = 3.56, P = 0.007) and AEE ($F_{4,3318}$ = 2.97, P = 0.018). Individual mixed models showed that on both food and non-food days run had an effect on RMR at all temperatures (Fig. 3.9). In particular, RMR_15_F was lowest in run three, whereas RMR_15_NF was lowest in the first run, both RMR_20_F and RMR_20_NF were lowest in the first run and RMR_31_F and RMR_31_NF were lowest in run three. Overall, run had a significant effect on average energy expenditure on food days (AEE_F) and average energy expenditure on non-food days (AEE_NF) at all temperatures, although how run affected AEE at each temperature varied (Fig. 3.10). At 15 °C AEE_15_NF was lowest in the first run, whereas AEE_15_F was lowest on the final run. On both food and non-food days AEE _20 was lowest in the first run and AEE_31 was lowest in the final run.

3.4.3 Temporal effects on metabolic response within runs

Separate LME models, including the effects of day, age, sex and food availability were used to examine the effect of respirometry day on the the mass-adjusted metabolic response of

M. musculus. Respirometry day had a significant effect on DEE on both food and non-food days, and on REE but only on non-food days (Fig. 3.11). Within the runs DEE_NF (daily energy expenditure on non-food days) and REE_NF (resting energy expenditure on non-food days) were both lowest on the first non-food day (respirometry day one) and DEE_F increased over the three food days (respirometry days two, four and six).

On food days, day of the respirometry run did not have an effect on RMR at any temperature, whereas there was a significant effect of day on RMR_15_NF and RMR_31_NF where the RMR for both temperatures were lowest on the first non-food day (respirometry day one) (Fig. 3.12). There was no effect of day on AEE_15_F, whereas, AEE_20_F increased over the three food days within the run, AEE_31_F was highest on the first food day (respirometry day two) and at all temperatures, AEE_NF was lowest on the first non-food day (respirometry day one) (Fig. 3.13).

3.4.4 Daily torpor

Many individuals exhibited frequent, pronounced reductions in their energy expenditure on non-food days (Fig. 3.1A). When these periods of reduced metabolic rates crossed an arbitrary threshold (0.14 W) set at the upper boundary of a lower mode in the data, they were analysed as torpor bouts (Fig. 3.6A & B). Fifty-three of the 74 individuals (71%) were observed entering daily torpor at one point over the three respirometry runs. A PERMANOVA (used to test treatment effects on all measured torpor characteristics) showed that the torpid metabolic characteristics (torpid RMR, torpor duration, torpid EE and times of arousal and entry) of males and females were not significantly different, therefore the results for males and females were combined ($F_{1,272}$ = 0.39, P= 0.674).

Torpor bout durations ranged from 30 to 980 minutes with a median duration of 108 minutes (mean: 159.43 ± 136.07 minutes (n= 53, N=274; Fig. 3.14). The majority (96%) of the torpor bouts occurred during the period of 15 °C, which also coincided with the early rest phase (0400 h -1200 h). Mean AEE over the length of all torpor bouts was 22.26 ± 5.55 J h^{-1} g^{-1} at 15 °C (N=263) and 28.10 ± 4.92 J h^{-1} g^{-1} at 20 °C (N=11). Mean torpid RMR was 0.004 ± 0.002 W g^{-1} at 15 °C and 0.006 ± 0.001 W g^{-1} at 20 °C. Including all torpor bouts, there was an effect of torpor duration on torpid RMR ($F_{1,242}$= 109.37, P < 0.001) with the two significantly negatively correlated at 15 °C (r = -0.61, t = -7.43, P < 0.001, N = 93),

though this was not significant using the small data set for the torpor bouts that occurred at 20 °C (r = -0.43, t = -1.43, P = 0.186, N = 11; Fig. 3.15). The AEE over torpor bouts was also significantly correlated with the logarithm of torpor duration at 15 °C (r = -0.45, t = -4.86, P < 0.001, N = 93) but not for torpor bouts occurring at 20 °C (Fig. 3.16). Hence, in general, individuals were in torpor for longer when their rate of energy expenditure was lower. Neither body mass ($F_{1,68}$= 1.67, P = 0.200) nor food intake ($F_{1,68}$= 1.09, P = 0.300) had a significant effect on torpor duration. Both DEE and REE were negatively correlated with the percentage of the day spent in torpor (Fig. 3.17A and B, respectively). For example, the DEE of mice, on average, was reduced by 9% for every 10% increase in proportion of the day spent in torpor.

The time of entry into torpor ranged from 1825 h to 1145 h with two main peaks, the first occurring between 0500 h and 0600 h shortly after the time when the temperature profile dropped from 20°C to 15°C (0400 h), and the second between 0900 h and 1000 h (Fig. 3.18A). The time-period when torpor arousal occurred was narrower than the range over which torpor entry was recorded. Torpor arousal ranged from 0432 h to 1530 h, with a main peak between 1100 h and 1200 h, just prior to the temperature profile warming up at 1200 h (Fig. 3.18B).

The only torpor characteristic that varied between days within a run was the overall number of torpor bouts that occurred on each day, with more individuals entering torpor on the first day of the respirometry run (Table 3.3). The day of the run did not have a significant effect on torpor duration, torpid RMR at 15 °C, percentage of the day spent in torpor, AEE over the torpor bout nor the entry and arousal times (Table 3.3).

Respirometry run had a significant effect on many of the measured metabolic characteristics of torpor bouts (Table 3.4). In particular, torpor bout duration was significantly shorter in the final run whereas torpid RMR at 15 °C was significantly higher in run three and the AEE over the torpor bouts increased over the course of the three runs (Fig. 3.19). In the final run there was also a decrease in the total time (percentage of each day) spent in torpor ($F_{1,253}$ = 4.43, P= 0.013). Neither the time of entry nor time of arousal varied between different runs.

3.4.5 Relationship between metabolic measurements and daily energy expenditure

On food days, the best RMR predictor of DEE was RMR_15_F (r =0.84), followed by RMR_20_F (r =0.73) and lastly RMR_31_F (r=0.57; Fig. 3.20). On food days AEE_15_F was the best AEE predictor of DEE (r =0.93) and AEE_31_F (r=0.58) the weakest (Fig. 3.21). On food days AEE over the rest phase (0700 h – 1900 h) was a slightly better predictor of DEE (r =0.90) than AEE over the active phase (1900 h – 0700 h) (r =0.88; Table 3.5). On non-food days, the best RMR predictor of DEE was RMR_15_NF and RMR_20_NF (r =0.77; Fig. 3.20). For AEE, AEE_15_NF remained the best predictor of DEE (r =0.91) and AEE_31_NF the worst predictor (r =0.69; Fig. 3.21). On non-food days AEE over the active phase and rest phase remained the same (r =0.91). Torpid AEE was a better predictor of DEE (r =0.60) than torpid RMR (r =0.47). Overall, as expected REE was the best predictor of DEE (r =0.97).

3.4.6 Repeatability of metabolic responses

Separate LME models were used to explain DEE, REE and both RMR and AEE at all temperatures (Table 3.6).

3.4.6.1 Average individual reactions norms

Most of the estimates of the average individual responses to food availability (i.e. reaction norm intercepts and slopes) over all three runs were repeatable (Table 3.7). The estimated average individual reaction norm intercept ($R_{intercept}$) for RMR_20, and the average individual reaction norm slope (R_{slope}) for RMR_20 and AEE_20 were not repeatable as the 95% CI's overlapped with zero. For all metabolic measurements, the R estimates were higher for the average individual reaction norm intercept (i.e. values when food was available; range: 0.29 to 0.66) than the reaction norm slope (i.e. response to no food; range: 0.15 to 0.45). The R estimates of individual $R_{intercept}$ between runs one and two were lower for most of the metabolic measurements, compared to the individual $R_{intercept}$ estimates across runs two and three, or all runs, with the exception of the metabolic measurements at 30 °C (RMR_30 and AEE_30) which were higher. Between runs one and two the individual R_{slope} for REE, DEE, RMR_20 and RMR_31 were not repeatable. The individual R_{slope} for RMR_15 and AEE_15 had lower repeatability estimates between runs one and two, compared to estimates over all three runs, whereas AEE_31 had a slightly higher repeatability estimate across runs one and two compared to over all three runs.

With the exception of the $R_{intercept}$ for AEE_31, the repeatability estimates for all metabolic measurements were highest between runs two and three and only the R_{slope} for AEE_20 was not repeatable.

3.4.6.2 Long-term repeatability ($R_{long\text{-}term}$) of individual reaction norms

Estimates of the long-term repeatability ($R_{long\text{-}term}$) of individual reaction norms also had 95% CI above zero for all metabolic measurements apart from $R_{intercept}$ for RMR_20 and R_{slope} for RMR_20 and AEE_20 (Table 3.8). The $R_{long\text{-}term}$ estimates for all metabolic measurements over the three runs were higher for all $R_{intercept}$ measurements (range: 0.14 to 0.44) than R_{slope} measurements (range: 0.08 to 0.29). The $R_{long\text{-}term}$ estimates between runs one and two, compared to all runs, were less repeatable for all measurements save $R_{intercept}$ and R_{slope} for AEE_31. More of the $R_{long\text{-}term}$ estimates were not repeatable between runs one and two, particularly for R_{slope} where only the RMR_15 and AEE_31 were found to be repeatable. With the exception of $R_{intercept}$ of RMR_30 and AEE_30 the $R_{long\text{-}term}$ estimates between runs two and three were more repeatable than those across all runs and just between runs one and two. Overall, estimates of $R_{long\text{-}term}$ were lower than R for average individual reaction-norm responses.

3.4.6.3 Short-term repeatability ($R_{short\text{-}term}$) of individual reaction norms

Estimates of the short-term repeatability (i.e. within each run; $R_{short\text{-}term}$) of individual reaction norms were 95% above zero (Table 3.9) and there was less variation between $R_{short\text{-}term}$ estimates for $R_{intercept}$ (range: 0.21 to 0.70) and R_{slope} (0.23 to 0.75) than observed for the average individual R and $R_{long\text{-}term}$. All $R_{short\text{-}term}$ estimates including all runs were very similar to the estimates including runs one and two, and two and three. As expected the estimates for $R_{short\text{-}term}$ were higher than the average individual R and $R_{long\text{-}term}$ for most metabolic measurements.

3.5 Discussion

This chapter provides an in-depth look at the thermal physiology of wild caught *M. musculus* and how they respond to the thermoregulatory demands of a daily cycle in T_a (15 °C, 20 °C and 31 °C) combined with intermittent periods of fasting. Incorporating measurements using the “Promethion” metabolic phenotyping system provided a continuous trace of metabolic rate over six days for animals living under variable

conditions of air temperature and food availability. This enabled me to collect a more detailed dataset than traditional respirometry, which often provide just a single value of metabolic rate for each trial. Whilst laboratory strains of *M. musculus* are frequently used as model systems in evolutionary physiology and physiological ecology, wild derived mice are used far less regularly. From an ecological perspective wild derived mice are far more suitable for making inferences about adaptation and other evolutionary processes in the wild as it is difficult to determine whether the metabolic responses of laboratory bred animals reflect general mechanisms applicable in the wild or are a result of the genetic consequences and conditioning to captivity (Richardson *et al.,* 1994; Swindell, 2012).

3.5.1 Metabolic response to variation in food availability and T_a

I examined the effect of food withdrawal and T_a on multiple components of the individual's energy expenditure. In general, the metabolic responses to food withdrawal and variation in T_a were consistent with those expected for an average endotherm and reported in other rodents (Chappell, 1985; Barker *et al.,* 2012; Zhu *et al.,*2013; Kaseloo *et al.,* 2014). I found that food withdrawal had a negative effect on all metabolic responses, for example, DEE declined by 20% on non-food days. Similar decreases in energy expenditure in small mammals challenged with poor quality food or restricted food availability is commonly recorded (Rothwell and Stock, 1982; Ma and Foster, 1986). When energy acquisition is limited animals employ adaptive strategies to compensate for the reduced energy intake, often resulting in decreases in energy expenditure. A stronger negative effect of food withdrawal was observed over the rest phase (0700 h - 2000 h) compared to the active phase (2000 h - 0700h) due to the energy conserving thermoregulatory responses (e.g. torpor) used by the mice in the first half of their rest phase (early-late morning).

As was expected temperature negatively impacted metabolism whereby AEEs and RMRs were highest at 15 °C and lowest at 31 °C. It is well established that T_a has a considerable effect on animal physiology. When at rest within their TNZ (lower critical temperature of the TNZ for *M. musculus* is around 30 °C) endotherms produce a minimum rate of energy expenditure that is sufficient for maintaining normothermic T_bs, but when they are exposed to lower temperatures additional sources of heat production are necessary (e.g. increased activity, shivering or non-shivering thermogenesis) to sustain their T_b. These

internal thermoregulatory processes cause an increase in energy expenditure and explain the higher energy expenditure recorded at the lower temperatures. Slightly elevated levels of metabolism were observed at 20 °C than expected, likely because this temperature period was always measured during the mice's active phase; hence metabolic measurements taken at this temperature may have been elevated relative to measurements taken during the rest phase.

The interactive effects of food availability and temperature on metabolism were strongest at 15 °C on non-food days; RMR decreased by 67% and AEE decreased by 34%. This period of food withdrawal at 15 °C delivered the most physiologically stressful conditions for the mice. To cope with these high energetic demands, it is presumed that mice allowed a controlled reduction in T_b (i.e. torpor), which explains the larger decrease in energy expenditure at 15 °C (further described in section 3.5.4).

3.5.2 Temporal effects on metabolic response among runs

In general, the measurements of metabolic energy expenditure were lowest in the first run compared to runs two and three. The increase in energy expenditure between the first and subsequent runs may be an effect of age and changing body mass, which increased significantly between runs one and two as mice matured from sub-adults to mature adults. In the first run the mice were sub-adults and recently captured from the wild. Considering the prolonged period of restricted activity and *ad libitum* access to higher quality food (i.e. higher in fats and carbohydrates) that mice experienced in long-term captivity, they were likely in differently physical condition (e.g. increased body fat stores) in the subsequent two respirometry runs, then when recently captured mice at the start of the experiment (Larcombe and Withers, 2007). The lower energy expenditures of mice on non-food days in the first run could be associated with lower body fat stores which can affect thermoregulatory behaviours like torpor. Differences in thermoregulation were observed in the first run (discussed in detail in section 3.5.4) and would have had a significant effect on energy expenditure among runs. For example, torpor bouts recorded in the final run were shorter and shallower than in previous runs, leading to reduced energy savings.

Alternatively, the increase in energy expenditure between the first and subsequent runs could be a result of the mice acclimating to captivity. Long-term captivity can have an

effect on metabolic variables (Skadhauge and Bradshaw 1974; Geiser *et al.,* 1990; Warkentin and West, 1990), although how captivity effects metabolism has produced contrasting results. In some studies, no differences are described between freshly caught individuals and individuals that have been in captivity long-term (Weathers *et al.,* 1983). In other studies, captive individuals have higher metabolic rates (Selman, 1998; Larcombe and Withers, 2007) whilst in others metabolism is lower in captive individuals (Piersma *et al.,* 1996). However, many studies assessing the effects of captivity on metabolism do so by comparing wild and captive individuals (of similar mass) of the same species rather than investigating how metabolism varies within an individual over long-term captivity (Mansour, 2005).

Metabolic measurements for days when food was available tended to decline over time (i.e. over the three runs). In a range of species, age related declines in energy expenditure, particularly at the TNZ, in senescing adults have been associated with a decline in metabolically active tissue (Roberts and Rosenberg, 2006; Broggi *et al.,* 2009; Moe *et al.,* 2009). Although this experiment was carried out over the natural expected lifespan of an individual in the wild (due to high rates of mortality from environmental causes, e.g. predation) the estimated ages of the mice in the third run (c. 5-6 months) were too young for senescent changes to have begun (10-15 months; Dutta and Sengupta, 2016).

3.5.3 Temporal effects on metabolic responses within runs

An effect of respirometry day was observed for many of the metabolic responses, particularly on non-food days, whereby metabolism was generally lowest on the first food available and non-food day. The lower metabolic responses for some of the measurements (e.g. DEE_NF and REE_NF) on the first non-food day (respirometry day one) can be partially explained by the mice having a higher propensity to use torpor on the first non-food day. Since torpor reduces energy expenditure, an increase in torpor expression on the first day of food withdrawal would lead to lower rates of DEE and REE (discussed further in section 3.5.4). Predictability of food has been shown to influence torpor use in small mammals and it is possible that the mice learnt that food would follow a period of food restriction. This may have led to the reduced propensity to enter torpor that was observed during the second and third period of food restriction (Munn, 2010).

The lower observed metabolism for some of the measurements could also be an artefact of stress. The chamber setup was very similar to the home cage and was a more enriched environment than traditional respirometry chambers to minimise any effects of stress and their associated metabolic artefacts (Lighton, 2017). Compared to traditional respirometry studies this methodology made substantial progress in reducing the amount of stress the animals experienced, however, handling and transfer into a novel area (the respirometry chamber) could not be avoided and is likely to have affected the animals (Hayes *et al.,* 1992a). An anxious or stressed mouse will often display elevated physical activity, which may have been the case when the mice were first in the respirometry chambers (Speakman, 2013). The first few hours that the mice were in respirometry were not included in these analyses as this period included the system calibration and a temperature change, and to allow the animals to acclimatise after transfer. During this period the mice likely displayed higher than normal activity and stress levels. Following this, the mice may have had to compensate for an energy deficit caused by the period of heightened activity by lowering the energy expenditure until food was next made available.

These results highlight how even in the short term an individual's metabolic responses can change dramatically. Metabolic measurements taken from short-term respirometry experiments may not give an accurate indication of an individual's normal state as, specifically at the start of a respirometry experiment, individuals may be affected by behavioural characteristics (e.g. heightened stress) that have significant metabolic repercussions. The highly significant effect of measurement duration on metabolic responses have been highlighted in an array of studies (Hayes *et al.,* 1992a; Connolly and Cooper, 2014). For examples, Winwood-Smith and White (2018) showed that short-duration measurements lead to underestimates of metabolic rate in amphibians and Cooper and Withers (2009) demonstrated in small mammals that short-term measurements lead to over inflation of BMR. Hayes *et al.,* (1992a) observed that the measurement duration influenced the values of metabolism in wood mice (*Apodemus sylvaticus*) and short tailed field voles (*Microtus agrestis*), attributing elevated values in short-term experiments to be a result of stress, which can last for several hours (Hayes *et al.,* 1992a; Steffenson, 2002). My results have further provided evidence demonstrating

that appropriate measurement duration is a crucial consideration for metabolic measurements.

3.5.4 Shallow daily torpor

In heterothermic endotherms the T_b during a torpor bout will undergo a profound decrease from high normothermic values (c. 32 to 42 °C) to values from -3 °C to 30 °C allowing core T_b to approach T_a. On average, torpid RMR is around 5-30% of BMR (including hibernators; Geiser, 2004). The energy savings from the torpor bouts observed in this study were not as high as commonly seen in other rodents. For instance, the golden spiny mouse reduces metabolic rates to around 17% of normothermic rates (Ehrhardt *et al.*, 2005). The energy savings exhibited by *M. musculus* in the present study are closer to those observed in the native Australian ash grey mouse which reduce metabolism to 44% of normothermic values (Barker *et al.,* 2012). Although most of the torpid RMRs in this study were not as low as 30% of BMR, the duration and metabolic characteristics of these relatively moderate decreases in metabolism were consistent with incidences of daily shallow torpor observed in other wild muroid rodents and marsupials in response to poor environmental conditions (Schubert *et al.,* 2010; Barker et al., 2012). These results show that *M. musculus* readily use shallow daily torpor when faced with short-term changes in T_a along with food withdrawal. This was expected as a reduction in T_a and food restrictions are the most common stimuli for torpor in small mammals (Hudson, 1973; Morton, 1978). In other studies *M. musculus* has similarly been shown to use shallow bouts, sometimes with torpid metabolic rates as high as 70 % of BMR (Hudson and Scott, 1979). Whilst the torpor bout duration observed in this study were lower than has been recorded for *M. musculus* in other studies (Schubert *et al.,* 2009), the torpid resting metabolic rates observed in the present study are similar to those reported in other studies of *M. musculus* (Geiser, 2004; Ruf and Geiser, 2015). The relatively small metabolic reductions of individuals undergoing torpor suggest that torpor is not the primary thermoregulatory strategy for this species.

Although no sex-specific differences were found in the metabolic characteristics of the observed torpor bouts, a disparity in willingness to enter torpor, with female mice being more likely to use daily torpor, was detected between sexes. This concurs with studies involving other rodent species demonstrating that males can be reluctant to enter torpor

(Lovegrove and Raman, 1998; Mzilikazi and Lovegrove, 2002). High levels of testosterone may inhibit the incidence of torpor in male rodents and the limited heterothermic capacity of males in my study to use torpor may also be a consequence of their reproductive status (Lee *et al.,* 1990; Ouarour *et al.,* 1991). In the South African pouched mouse (*Saccostomus campestris*) torpor was only observed in testosterone inhibited males with low concentrations of gonadal hormones (Ruby *et al.,* 1993; Mzilikazi and Lovegrove, 2002). Like *S. campestris,* male *M. musculus* can be opportunistic, rather than strongly seasonal, breeders, and often have a relatively high level of testosterone throughout the year which may moderate the ability of males to use torpor (Bomford and Redhead, 1987; Singleton, 1989; Singleton, 1990). Other studies have observed that torpor expressed in male small mammals is often shallower and shorter than in females, however no such effect was observed in this study (Munro *et al.,* 2005; Rojas *et al.,* 2014).

In the wild, animals will frequently experience a daily T_a cycle. An interaction between T_a and time of day has been shown to be a significant cue for the timing of torpor entry and arousal (Turbill *et al.,* 2003). Generally, small nocturnal mammals are most likely to employ torpor in the early morning when daily T_a is lowest, and arouse around midday or in the early afternoon, presumably as a result of increasing T_a and hence opportunities for passive rewarming (Geiser, 2004; Geiser and Kortner, 2010). In the present study the majority of torpor bouts occurred in the early morning on non-food days with only one torpor bout detected on a day where food was available. I observed that entry into daily torpor was strongly influenced by temperature, with the majority of recorded torpor bouts occurring during the minimum daily T_a (15 °C : 0400 h -1200 h). There were two distinct peaks for torpor entry; the first coincided with when the temperature profile dropped to its minimum level (15 °C), indicating this decline in T_a was a strong cue for entry into torpor. The second peak in torpor entry occurred between 0900 h and 1000 h, a few hours after lights on. Many of the mice remained in a single relatively deep torpor bout for most of the morning. Other individuals underwent rhythmic short-term decreases and increases in energy expenditure after entering torpor shortly after T_a decreased in the early morning. These torpor bouts would last for one to three hours and were interrupted by spontaneous arousals with short normothermic (<1-2 hours) periods before re-entering torpor.

These results show that *M. musculus* can spontaneously arouse (undisturbed) from daily torpor as demonstrated in other studies (Hudson and Scott, 1978; Tomlinson *et al.,* 2007). The timings of torpor arousals were closely synchronised; a distinct peak was observed between 1100h and 1200h, before food was available and prior to the T_a increasing from 15 °C to 31 °C. While times of arousal in wild animals often coincide with an increase in T_a or exposure to solar radiation in laboratory studies under stable T_a (Geiser *et al.,* 2004), animals frequently arouse from torpor several hours prior to their nocturnal active phase as seen in the present study (Kortner and Geiser, 2000). This tendency to arouse from torpor in the late morning may indicate that torpor arousal is partially regulated by an endogenous circadian cue and individuals have an innate propensity for torpor arousal to overlap with rising T_a and passive rewarming in the wild (Turbill, *et al.,* 2008). It should be noted that despite efforts to keep disturbance to a minimum it is possible that some arousals occurred because of noise and vibrations in the laboratory.

Torpor duration was negatively correlated with both torpid RMR and torpid AEE, whereby longer torpor bouts included lower metabolism. Previously, torpor duration has been shown to be a negative function of body mass, with smaller individuals employing longer torpor bouts. Longer torpor bouts increase the extent of energetics savings, which are likely more important in smaller individuals to counteract their high relative heat loss during cold exposure and food shortages (Geiser, 1988). Despite this, no effect of body mass on torpor duration was detected.

The only torpor characteristic that was affected by respirometry day was the mice's propensity to use torpor. The mice showed a higher propensity to use torpor on the first day of the respirometry run. Torpor is often characterised as a response to environmental stressors. It is possible that initial stress associated with a novel environment (i.e. respirometry chamber) may have contributed to the increase in torpor frequency during the first day of the respirometry run (Hudson, 1973). Respirometry run had a significant effect on torpor bout duration, whereby torpor duration was shorter in the final run. Additionally, torpor bouts were shallower in the final run resulting in reduced energetic savings. Although many studies have considered the effects of age on torpor expression, the majority of these studies have compared juveniles and adults and concluded that juveniles have a higher propensity to use torpor and their torpor bouts tend to be longer

and deeper than in adults (Giroud, *et al.,* 2012; Healy *et al.,* 2012). Fewer studies have looked at how the age of mature individuals affect torpor use (Rojas *et al.,* 2014). In this study it appears that there could be an effect of age on torpor, with older individuals using shorter and shallower daily torpor. Alternatively, these shorter and shallower torpor bouts in the later runs may be a result of habituation by the mice to repeated exposure to the respirometry chambers. In the earlier runs mice may have been more exhibited higher stress responses such as higher activity levels and higher associated energy expenditure, which would have been energetically costly. To compensate for stress associated energetic costs in earlier runs individuals may have employed longer and deeper torpor bouts. It would be useful for future studies to test for such effects of stress by measuring stress hormones. Conversely, the shorter and shallower torpor bouts in the later runs could be a result of time in captivity. After three months in captivity with food available *ad libitum* the mice may have built up substantial fat reserves (Larcombe and Withers, 2007). This could have been providing the mice with surplus energy compared to the first two runs where mice may have exhibited longer torpor bouts to conserve their smaller fat reserves.

This chapter provides a comprehensive and unique look at use of torpor in wild *M. musculus.* Frequently, studies on torpor in mice involve laboratory mice that will not have experienced the normal daily T_a cycles in their early development, as did the mice in this study, and in general are often not an accurate reflection of the natural state of mice. Although wild house mice use underground burrows that provide a relatively stable microclimate and relief from temperature extremes, they will still experience daily T_a cycles that affect their T_b and energy expenditure and are likely important cues for torpor entry and arousal (Turbill *et al.,* 2003). Despite this, many studies use stable temperatures to assess torpor use. My use of a T_a that are similar to the natural conditions mice would experience in the wild and continuing the measurements over the expected lifespan of an individual in the wild enables a more ecologically relevant result (Pocock *et al.,* 2004).

In conclusion, like many other small mammals the house mouse is capable of undergoing shallow daily torpor in response to poor environmental conditions. There is evidence that torpor in mice can occur in response to high foraging costs in poor environmental conditions (Schubert, *et al.,* 2010; Turbill and Stojanovski, 2018). Torpor use has also been associated with a reduced risk of extinction among mammal species (Geiser and Turbill,

2009). This highlights the ecological relevance of torpor and the importance of improving our understanding of hypometabolic states in mice to get an accurate idea of how this physiological adaptation are associated with limited energy budgets over the course of an individual's lifetime.

3.5.5 Relationship between metabolic measurements and daily energy expenditure

Basal metabolic rate is frequently used as a single proxy for an energy expenditure of free-living animals. As BMR is a major component of the total energy budget, it is assumed that other levels of metabolism (e.g. daily energy expenditure) are correlated with BMR. Yet, few studies have attempted to test if such correlation exists and whether other integrative measures of metabolism are more appropriate if trying to predict DEE (Koteja, 1991).

My results demonstrated that average energy expenditure at 15 °C on days where food was available and on non-food days were both among the strongest predictors of DEE. These measurements were calculated by averaging the energy expenditure over the entire temperature period (8 hours), as opposed to the RMR measurements which were calculated by averaging the lowest consecutive 12-minute period within each temperature phase. 15 °C was the minimum T_a the mice experienced and was the period when the strongest thermoregulatory changes were most likely to occur. These results highlight how even small thermoregulatory changes can have a significant effect on DEE. At both 15 °C and 20 °C the average energy expenditures over these temperatures were correlated more strongly with DEE than the resting metabolic rates (a short-term metabolic measure), showing how "snap-shot" (i.e. short term) measurements might not be representative of true maintenance energy costs and that integrative measurements taken over a longer temporal period are more appropriate for predicting DEE. For example, the metabolic measurements during torpor bouts were some of the least correlated measurements with DEE so there would be no benefit in using them to predict DEE over the other measurements.

In this study, RMR_31 on non-food days was measured under similar conditions to that required of BMR (i.e. measurements were taken in non-reproducing, post absorptive individuals and measured whilst the individuals were resting in their thermoneutral zone during their inactive phase; Kleiber, 1961; McNab, 1997). However, it should be noted that

the mice's thermal insulation in the live-in respirometry cages with bedding would be different from traditional measures of BMR. Therefore, my measurements of RMR_31 on non-food days are not a true measurement of BMR and was viewed as being a representation of BMR. Whilst my measurements of RMR_31 on non-food days were lower than BMR values reported for *M. musculus* in many studies (Geiser, 2004; Mathias *et al.,* 2004) they were within the range of BMR values cited in other studies for *M. musculus* (Degen *et al.,* 1998; Johnston *et al.,* 2007). Though mass specific RMR_31 on non-food days and DEE were strongly positively correlated this metabolic measure was one of the weakest predictors of DEE compared to the other measurements taken. This was because RMR_31 did not encompass physiological mechanisms, like torpor, that were used to adjust the rates of energy expenditure to unfavourable environmental conditions (i.e. low temperature). On both food and non-food days RMR_15, which does encompass individual thermoregulatory metabolic responses was a more accurate predictor of DEE than RMR_31. In conclusion, although RMR_31 on non-food days (representing BMR) was strongly correlated with DEE it is not the most appropriate representative of true maintenance energy costs. The relative predictive value of metabolic traits measured at 15 °C were more useful for predicting DEE and responses in DEE to food variation.

3.5.6 Repeatability of metabolic responses

As biologically meaningful variation in metabolism is underpinned by consistent individual differences I was interested in showing that the measured physiological traits and metabolic responses were significantly repeatable (Bell *et al.,* 2009). I found that the average individual responses to food availability of most of the metabolic measurements were significantly repeatable over the expected lifespan of a wild mouse (Pocock *et al.,* 2004). For all metabolic measurements the *R* estimates for the average individual metabolic responses when food was available (reaction norm intercept) were more repeatable than the average individual response to food withdrawal (reaction norm slope). The slope of the reaction norm defines an individual's response to the environment (food withdrawal), showing the level of phenotypic plasticity. This shows that the individual's metabolic response to their normal state, when food is available, is more consistent than how they respond to food withdrawal. Energy expenditure on non-food days is intrinsically more variable than on food days because of the "sliding scale" of the reduction in T_b set-point. Consequently, this causes more variation in the slope of the reaction norm

(metabolic response) than the intercept. The reaction norm slopes were repeatable at all among-individuals, showing that some individuals generally show a stronger response to food withdrawal than others.

Except for measurements taken at 31 °C, all repeatability estimates for average individual responses were lower and often insignificant between runs one and two compared to between two and three and across all runs. This is largely due to a lower degree of among individual variance between the first two runs whereas individual differences in metabolism were largely maintained between the second and third runs. A possibility for the lower among individual differences in their various metabolic responses between the first two runs could associated with the life stages of the mice during measurements. In run one the mice were estimated to be sub-adults, and therefore had yet to complete a significant developmental phase (sexual maturation) that would have associated physiological changes, whereas between runs two and three all individuals would have been in the same life history period (mature adults) for both runs. Possibly, some metabolic responses are more important in terms of survival and therefore consistent across individuals, in the sub-adult phase.

These results highlight the possibility that the metabolic responses of sub adults might not be a good indicator for how they respond in their later life. Alternatively, the higher *R* estimates between the latter two runs could be a result of habituation to the respirometry chambers and acclimatisation to captivity. Estimates of *R* are frequently considerably lower in free-living animals than those from animals living under more homogenous laboratory conditions (Auer *et al.,* 2016). In the first run the mice had recently been captured from the wild compared to the second and third run where they had been in captivity for a significant period.

In many species the degree of repeatability of metabolic characteristics has been shown to decrease with an increase in the interval between measurements (Chappell *et al.,* 1996; Bell *et al.,* 2009; Auer *et al.,* 2016). This was corroborated in these results where the short-term *R* estimates among individuals within each run were higher than the long-term *R* estimates across all runs. For the short-term *R* estimates the animals were of very similar body and physiological state (e.g. size and age) for all the measurements as they were

taken in only days apart, whereas for the long-term *R* estimates each individual is likely to have changed developmentally and physiologically (e.g. sexual maturity) over the three months, significantly increasing the likelihood of variance between measurements (Bell *et al.,* 2009). Alternatively, the decrease in repeatability over the three runs may be associated with time in captivity. In the wild, the metabolism of mice would be affected by habitat, daily temperature cycles, food acquisition and dominance hierarchies amongst numerous other ecological processes. The absence of such effects in a captive environment may reduce long-term repeatability, which could have been preserved under more natural conditions. Additional research would be beneficial to understand the long-term repeatability of metabolic traits in a semi-natural or natural environment.

3.5.7 Conclusion

These integrated measurements provide a unique insight into factors that determine rates of energy expenditure in wild caught *M. musculus* and assist in providing a more comprehensive understanding of the associations between various components of their energy budget. This approach expands upon the traditional "snap-shot" view into an individual's metabolic energy expenditure during a very specific period that does not necessarily reflect the natural state of the mice. My approach to characterising the energetic phenotype of wild-caught mice differed from past attempts in several key ways. First, by manipulating important environmental variables (i.e. variation in food availability and daily T_a cycle) my methodology reflected the conditions mice would experience in their natural habitat and allowed me to investigate metabolic responses to key environmental conditions. Secondly, the use of live-in respirometry cags substantially reduced the stress-related artefacts to metabolism and allowed for long-term measurements (6 days). Finally, these detailed measurements were repeated three times over the mice's natural expected lifespan. This produced results that are more representative of the "real world" metabolic energetics for *M. musculus* and more ecologically relevant than previous studies.

Through providing a complete metabolic profile over a long period of time these results offer valuable information on the lifetime changes in physiological traits of wild caught *M. musculus.* In summary, mice decreased their energy expenditure and displayed a propensity to use torpor when faced with low temperatures and food withdrawal,

indicating a physiological regulation of energy metabolism to cope with energetically stressful periods. This methodology allowed me to determine the most useful aspects of the daily energy budget to measure to obtain estimates of DEE. Results showed that RMR and AEE at 15 °C were more accurate predictors of DEE than measurements at 31 °C. This highlights that studies using a single index of energy expenditure to represent metabolism should use measurements that incorporate the significant variation in REE that is not incorporated in the standard measure of BMR. Additionally, this chapter provides evidence of significant repeatability of multiple components of metabolic energy expenditure in *M. musculus*. In particular, high individual consistency in DEE, REE and energy expenditure at 15, relative to the total population variation was observed. It is plausible that this repeatable metabolic variation has a significant impact on fitness, though this would depend on whether the individual variances detected here are heritable.

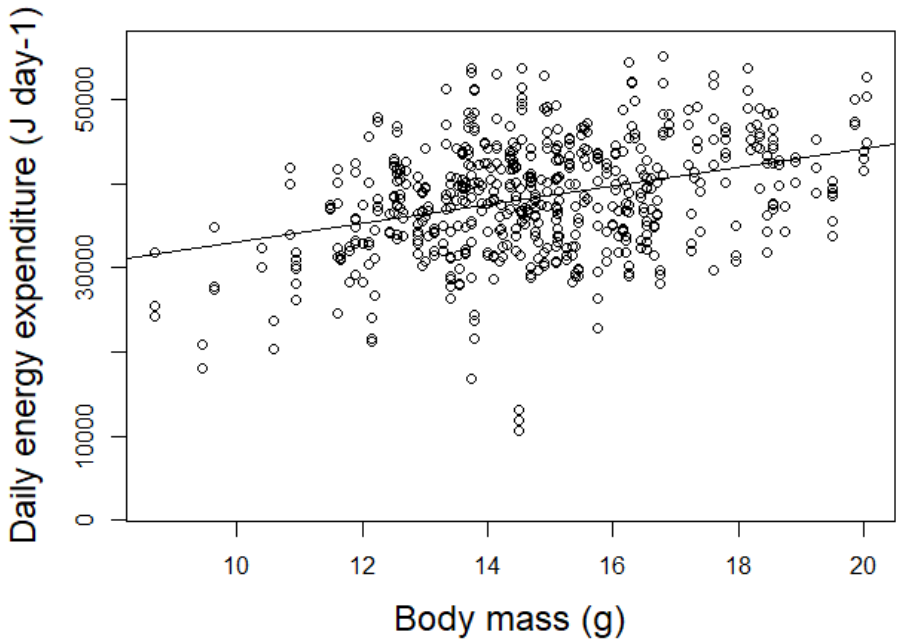
Table 3.1. Pre-experimental measurements (time in captivity and initial mass) and the effects of food availability on metabolic characteristics of male and female wild caught house mice over three respirometry runs. Means (±SD) shown.

Variable		Food Days						Non-Food Days					
		Females			Males			Females			Males		
		n	Mean	SD	*N*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD
Time in captivity (days)	Run 1	40	2.00	0	32	2.00	0	40	2.00	0	32	2.00	0
	Run 2	34	46.09	10.92	23	46.88	8.84	34	46.09	10.92	23	46.88	8.84
	Run 3	38	87.83	17.72	31	85.32	16.28	38	87.83	17.72	31	85.32	16.28
Initial Mass (g)	Run 1	40	13.94	2.19	32	14.48	2.05	40	13.94	2.19	32	14.48	2.05
	Run 2	34	13.96	1.73	23	16.23	2.46	34	13.95	1.73	23	16.23	2.46
	Run 3	38	14.16	1.78	31	15.98	2.03	38	14.16	1.78	31	15.98	2.03
	Overall	110	14.02	1.93	87	15.50	2.30	110	14.02	1.92	87	15.50	2.30
Daily energy expenditure (J day^{-1})	Run 1	40	37,546	7,517	31	37,820	6,170	40	28,778	9,466	32	29,391	8,978
	Run 2	34	37,944	6,043	24	41,049	6,838	34	30,612	7,482	24	33,664	7,509
	Run 3	36	38,159	7,129	31	39,364	6,013	36	31,031	7,316	31	32,160	6,441
	Overall	110	37,871	6,956	87	39,278	6,430	110	30,082	8,263	87	31,557	7,932
Resting energy expenditure (J/12 h)	Run 1	40	21,598	5,041	32	21,876	3,708	40	14,837	6,537	32	15,781	6,127
	Run 2	34	21,969	3,878	23	23,976	4,099	36	16,044	5,466	24	18,632	4,764
	Run 3	38	21,971	4,536	31	22,522	3,718	38	16,495	4,974	31	17,398	3,857
	Overall	110	21,833	4,570	87	22,695	3,912	110	15,752	5,772	87	17,143	5,160
Rest phase average energy expenditure (J/12 h)	Run 1	40	19,447	4,541	31	19,571	3,831	40	11,745	5,946	32	12,977	5,268
	Run 2	34	18,580	3,934	24	20,859	4,703	34	12,532	4,841	24	14,901	4,451
	Run 3	36	18,186	4,506	31	18,484	3,870	36	12,484	4,004	31	13,437	3,257
	Overall	110	18,762	4378	87	19,539	4,208	110	12,230	5,043	87	13,672	4,474
Active phase average energy expenditure (J/ 12 h)	Run 1	40	18,073	3,946	32	18,101	3,457	40	16,575	4,282	32	16,039	4,252
	Run 2	34	19,604	3,166	23	20,219	3,210	36	17,627	3,461	24	18,456	3,704
	Run 3	38	19,751	3,680	31	20,651	3,616	38	18,045	4,104	31	18,298	3,810
	Overall	110	19,002	3,700	87	19,594	3,632	110	17,381	4,031	87	17,511	4,103

Variable		Food Days						Non-Food Days					
		Females			Males			Females			Males		
		n	Mean	SD	*N*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD
Resting metabolic rate at 15°C (W)	Run 1	40	0.3740	0.0984	32	0.3782	0.0908	40	0.1700	0.1542	32	0.2031	0.1362
	Run 2	34	0.3692	0.0781	23	0.4200	0.0965	34	0.1966	0.1296	23	0.2765	0.1173
	Run 3	38	0.3529	0.0867	31	0.3716	0.0605	38	0.2038	0.1193	31	0.2584	0.0945
	Overall	110	0.3656	0.0888	87	0.3875	0.0852	110	0.1893	0.1364	87	0.2431	0.1211
Resting metabolic rate at 20 °C (W)	Run 1	40	0.2986	0.0787	32	0.3071	0.0559	40	0.2340	0.1144	32	0.2471	0.1059
	Run 2	34	0.3136	0.0618	23	0.3290	0.0581	34	0.2499	0.0978	23	0.2908	0.0800
	Run 3	38	0.3221	0.0773	31	0.3131	0.0609	38	0.2659	0.1157	31	0.2703	0.0702
	Overall	110	0.3110	0.0738	87	0.3154	0.0588	110	0.2493	0.1104	87	0.2374	0.0888
Resting metabolic rate at 31 °C (W)	Run 1	40	0.1921	0.0534	31	0.1894	0.0516	40	0.1207	0.0503	32	0.1320	0.0485
	Run 2	34	0.1715	0.0376	24	0.1873	0.0486	34	0.1241	0.0358	24	0.1431	0.0403
	Run 3	36	0.1655	0.0590	31	0.1624	0.0526	36	0.1140	0.0332	31	0.1209	0.0327
	Overall	110	0.1770	0.0523	87	0.1791	0.0525	110	0.1196	0.0411	87	0.1311	0.0420
Average energy expenditure at 15 °C (J/8 h)	Run 1	40	15,404	3,914	32	15,697	3,132	40	9,103	5,126	32	10,358	4,719
	Run 2	34	15,409	3,296	23	17,230	3,403	36	9,937	4,602	24	12,198	3,689
	Run 3	38	15,045	3,902	31	15,404	3,121	38	10,236	3,903	31	10,947	2,831
	Overall	110	12,288	3,721	87	16,019	3,284	110	9,732	4,605	87	11,076	3,903
Average energy expenditure at 20 °C (J/ 8 h)	Run 1	40	12,342	2,781	31	12,493	2,114	40	11,810	3,115	32	11,228	3,065
	Run 2	34	13,0.23	2,418	24	13,788	2,469	34	12,471	2,307	24	13,052	2,711
	Run 3	36	13,655	2,581	31	14,095	2,617	36	12,660	3,048	31	12,913	2,774
	Overall	110	12,986	2,656	87	13,432	2,499	110	12,293	2,882	87	12,332	2,979
Average energy expenditure at 31 °C (J/ 8h)	Run 1	40	8,082	2,113	32	7,865	2,027	40	5,179	1,904	32	5,388	1,772
	Run 2	34	6,832	1,913	23	7,901	2,514	36	5,052	1,490	24	5,620	1,944
	Run 3	38	7,020	2,449	31	7,075	2,221	38	4,769	1,102	31	5,113	1,204
	Overall	110	7,343	2,237	87	7,590	2,266	110	5,006	1,555	87	5,354	1,653

Figure 3.1. The relationship between food consumption (g day^{-1}) over respirometry runs, which included three food days, and average body mass (g) (r = 0.19, P = 0.007, N = 207). Body mass was measured immediately prior to and at the end of each respirometry run, an average of these two measurements was used to calculate body mass.

A.



B.

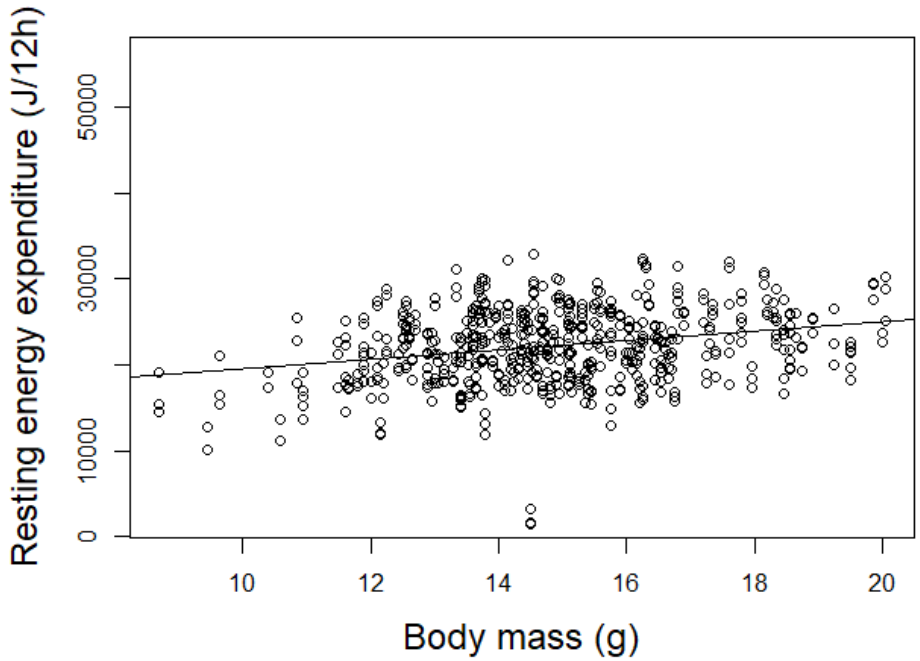


Figure 3.2. The relationship of daily energy expenditure (DEE) with body mass (A) and resting energy expenditure (REE) with body mass (B) in *M. musculus* on days where food was available. Body mass was measured immediately prior to and after each respirometry run, an average of these two measurements was used to estimate body mass over run.

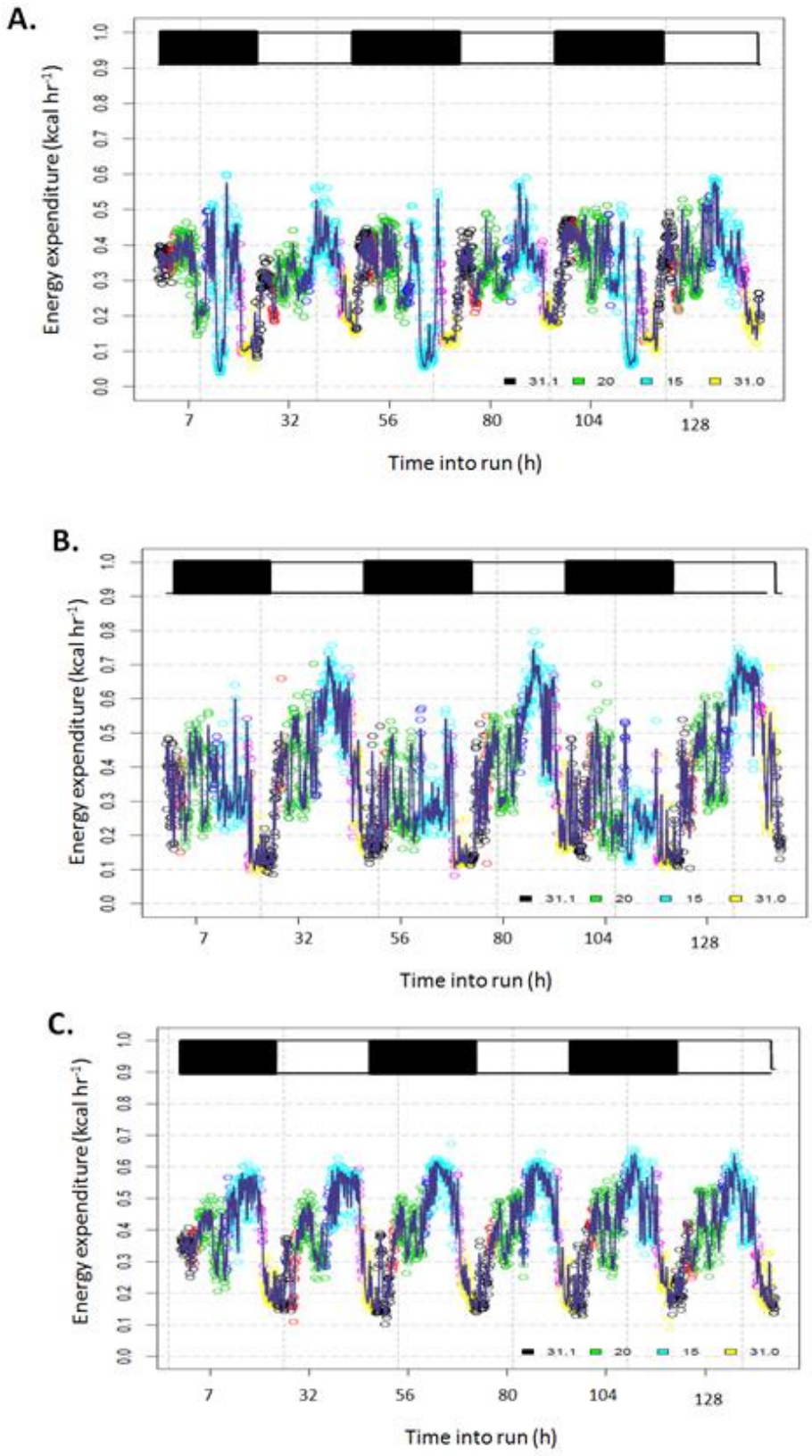


Figure 3.3. Metabolic profiles of three contrasting individuals (all female *Mus musculus*) over their first six-day respirometry run. On day one, three and five of the respirometry run food was restricted (represented by black bars). The colours represent 15 °C (blue), 20 °C (green) and 31 °C (yellow). Red, black and purple colours denote sections that were not included in analyses due to interim temperature fluctuations.

Table 3.2. Effect of food availability on whole animal daily energy expenditure (DEE), resting energy expenditure (REE) and average energy expenditures (AEE) of *Mus musculus* at all temperatures and resting metabolic rate (RMR) at all temperatures averaged over all respirometry runs. Overall means (± SD) shown. * Significance indicated by separate mixed-effect linear-models for each response variable that tested for an effect of food, in addition to including effects of "day" "body mass", "sex" and a "sex by body mass" interaction term. Run and individuals were included in the model as random effects. Terms that were not significant (cage and age) were not included in the final model.

Treatment	**DEE** ($J\ day^{-1}$)	**REE** (J/12h	**AEE_15** (J/8h)	**AEE_20** (J/8h)	**AEE_31** (J/8h)	**RMR_15** (W)	**RMR_20** (W)	**RMR_31** (W)
Food	38488 ± 6814	22212 ± 4315	15610 ± 3551	13182 ± 2595	7452 ± 2251	0.375 ± 0.088	0.313 ± 0.068	0.178 ± 0.052
Non-food	30734 ± 8154	16367 ± 5555	10325 ± 4357	12310 ± 2923	5160 ± 1607	0.125 ± 0.042	0.257 ± 0.102	0.125 ± 0.042
Test results:	$F_{1,388}$ = 142.43; $P <$ 0.001*	$F_{1,388}$ = 180.80; $P <$ 0.001*	$F_{1,387}$ = 223.07; $P <$ 0.001*	$F_{1,387}$ = 16.18; $P <$ 0.001*	$F_{1,387}$= 194.59; $P <$ 0.001*	$F_{1,388}$ = 292.31; $P <$ 0.001*	$F_{1,388}$ = 69.18; $P <$ 0.001*	$F_{1,388}$ = 199.88; $P <$ 0.001*

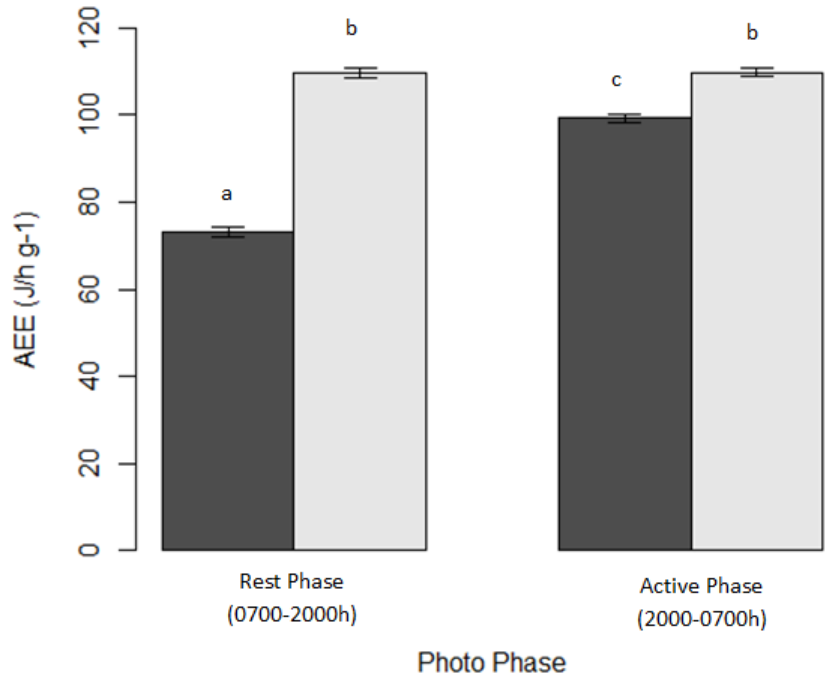
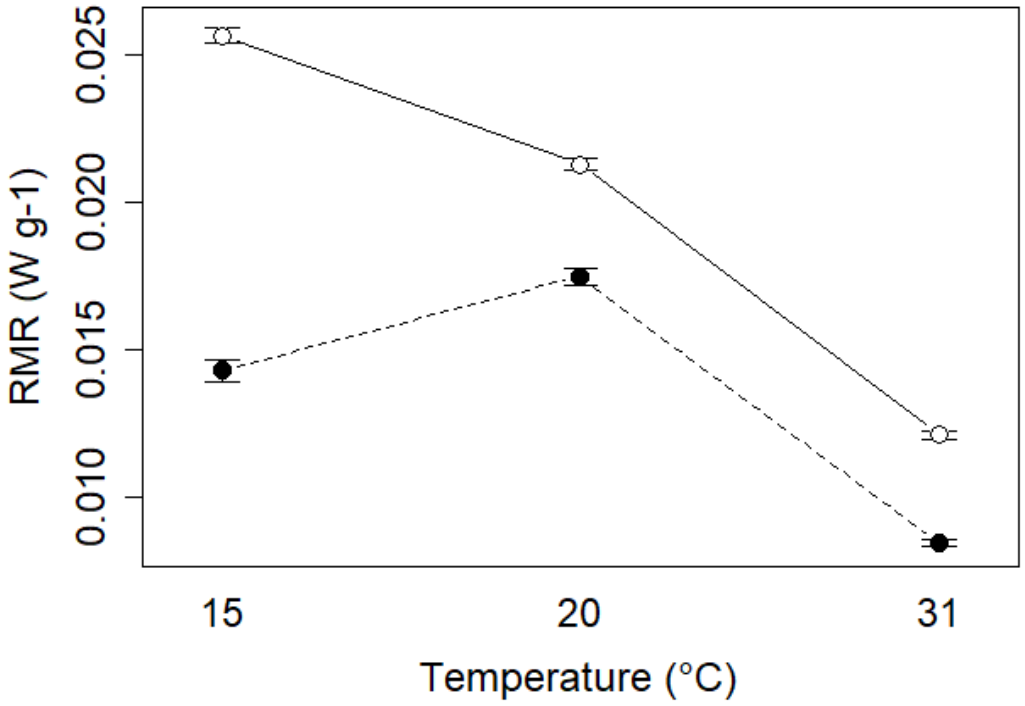


Figure 3.4. Effects of photo phase (rest and active phase) on average energy expenditure (AEE) of *Mus musculus* on food days (light bars) and non-food days (dark bars) over all respirometry runs. Mean population values (± SE) shown. Bars with the same letters were not significantly different ($P < 0.05$).

A.



B.

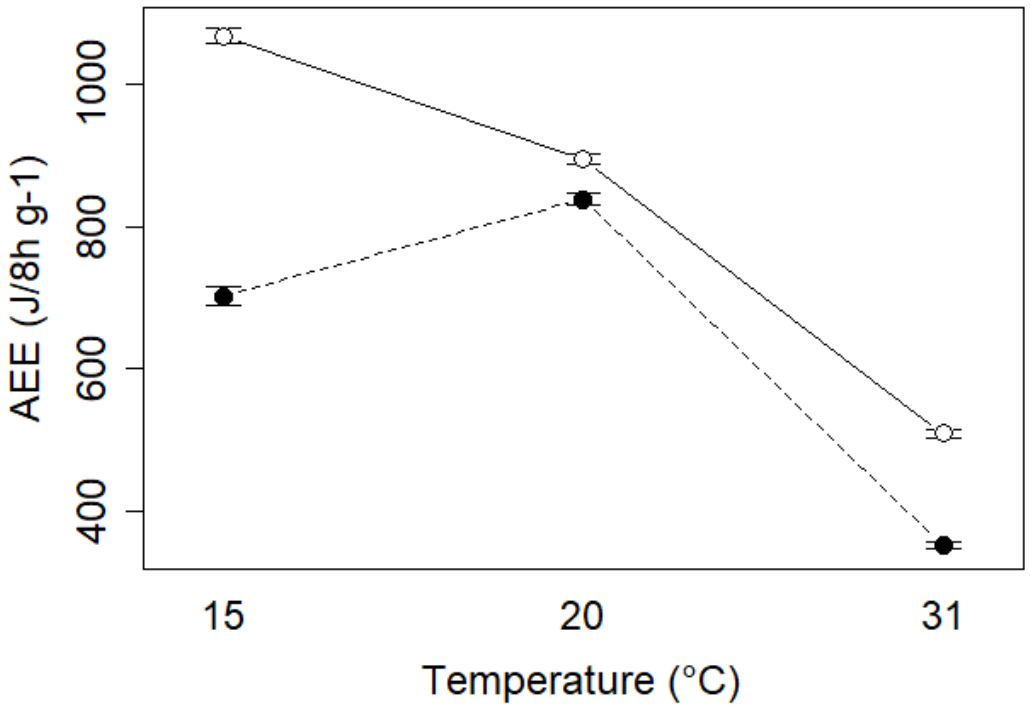


Figure 3.5. Effects of temperature on the resting metabolic rate (A) and average energy expenditure (B) of *Mus musculus* with (white circles) and without (black circles) food across three respirometry runs. Mean values (± SE) shown.

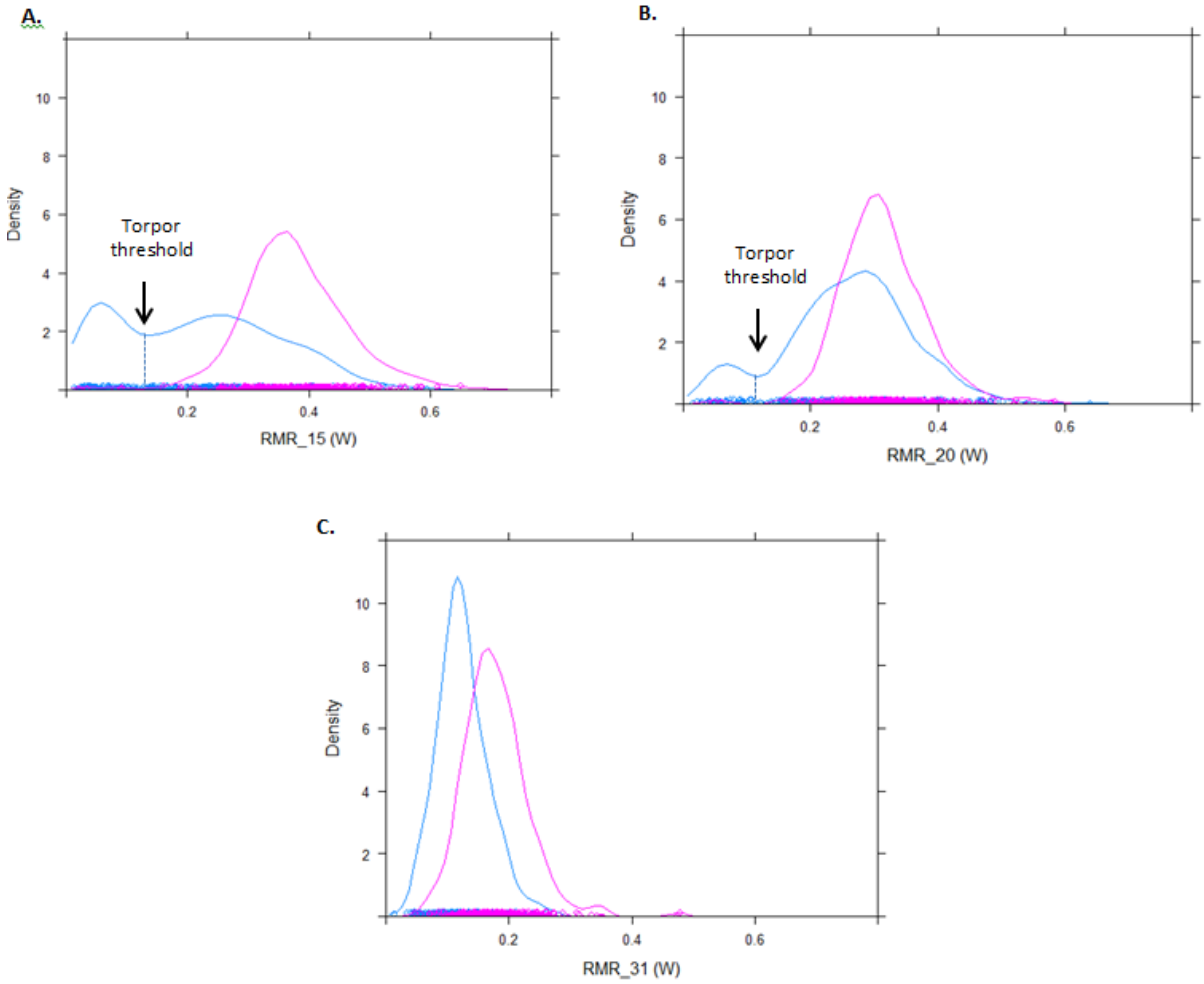


Figure 3.6. Frequency density distribution of resting metabolic rates (RMRs) (W g-1) of *Mus musculus* at 15 °C (**A**), 20 °C (**B**) and 31 °C (**C**) on food (pink lines) and non-food (blue lines) days (including data point distribution). A threshold of 0.14 W was selected to define a period of torpor as resting metabolic rates at 15 °C and 20 °C on days when food was available seldom dropped below this threshold (**A**).

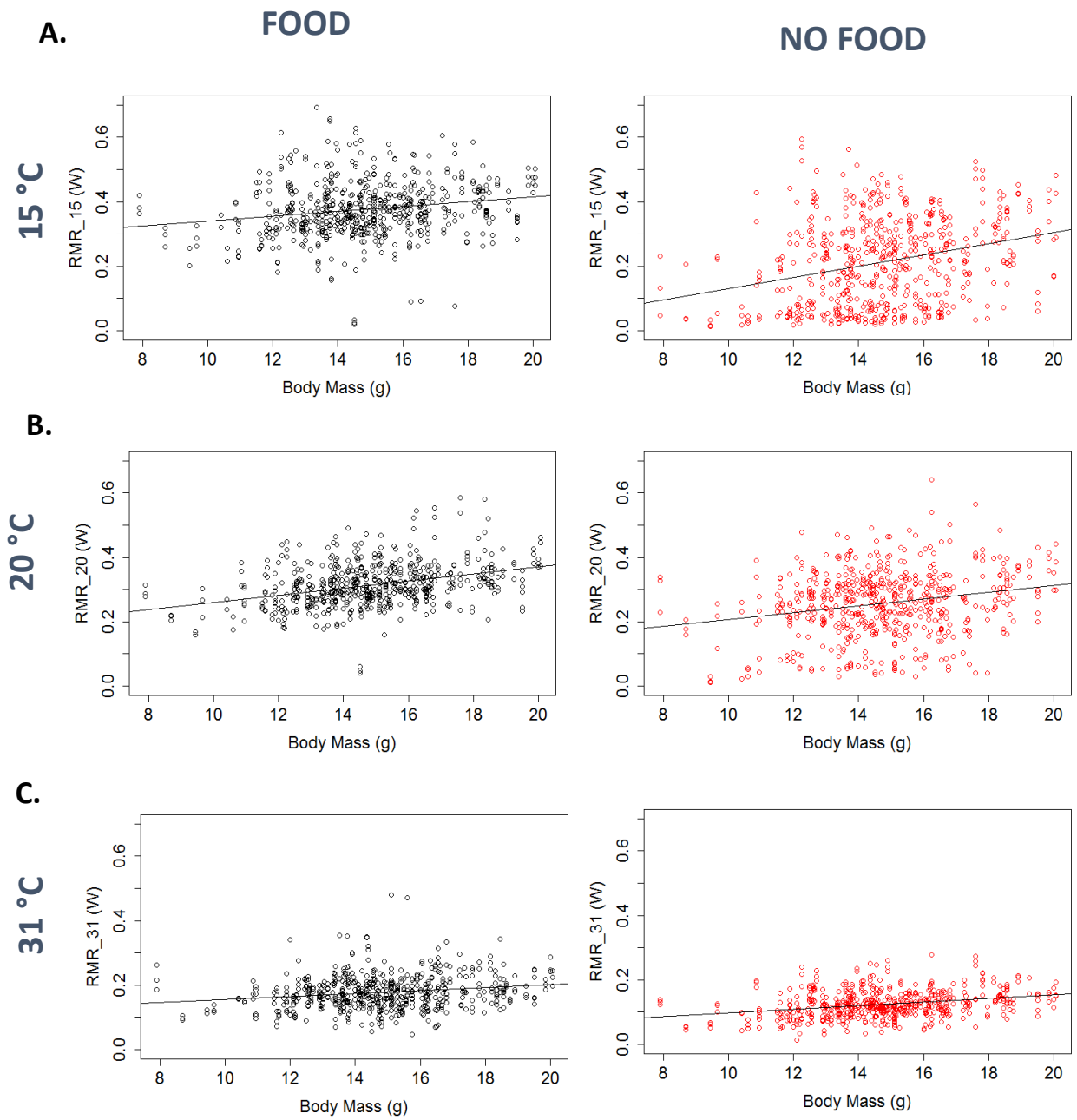


Figure 3.7. The relationships between resting metabolic rate (RMR) and body mass on food (black symbols) days at 15 °C (r = 0.188, P < 0.001), 20 °C (r = 0.355, P < 0.001) and 31 °C (r = 0.192, P < 0.001) and non-food (red symbols) days at 15 °C (r = 0.288, P < 0.001), 20 °C (r = 0.228, P < 0.001)and 31 °C (r = 0.299, P < 0.001). N = 586 for food days and 591 for non-food days.

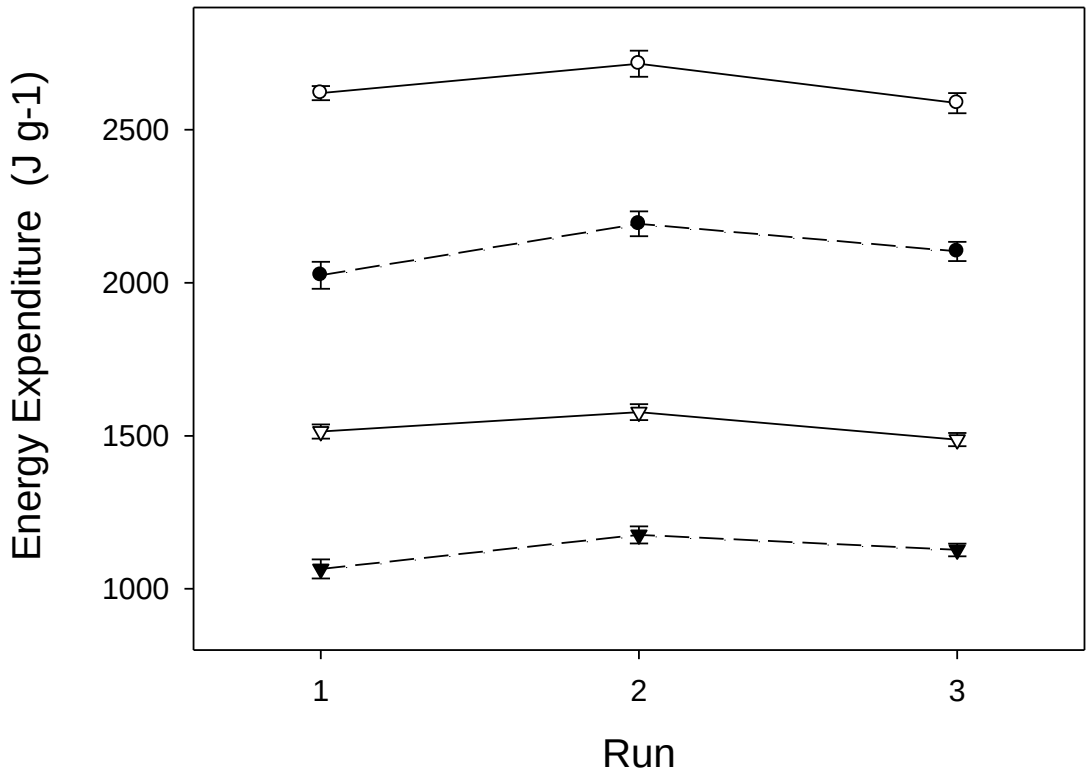


Figure 3.8. The effect of run on daily energy expenditure (DEE J $day^{-1}g^{-1}$) (circles) and resting energy expenditure (REE J/12 h g^{-1}) (triangles) of *Mus musculus* on food (white) (DEE : $F_{2,126}$ = 4.445, P = 0.014; REE : $F_{2,126}$ = 4.90, P = 0.009) and non-food days (black) (DEE: $F_{2,126}$ = 5.72, P = 0.004; REE : $F_{2,126}$ = 5.35, P = 0.006;) across three respirometry runs. Mean values (± SE) shown.

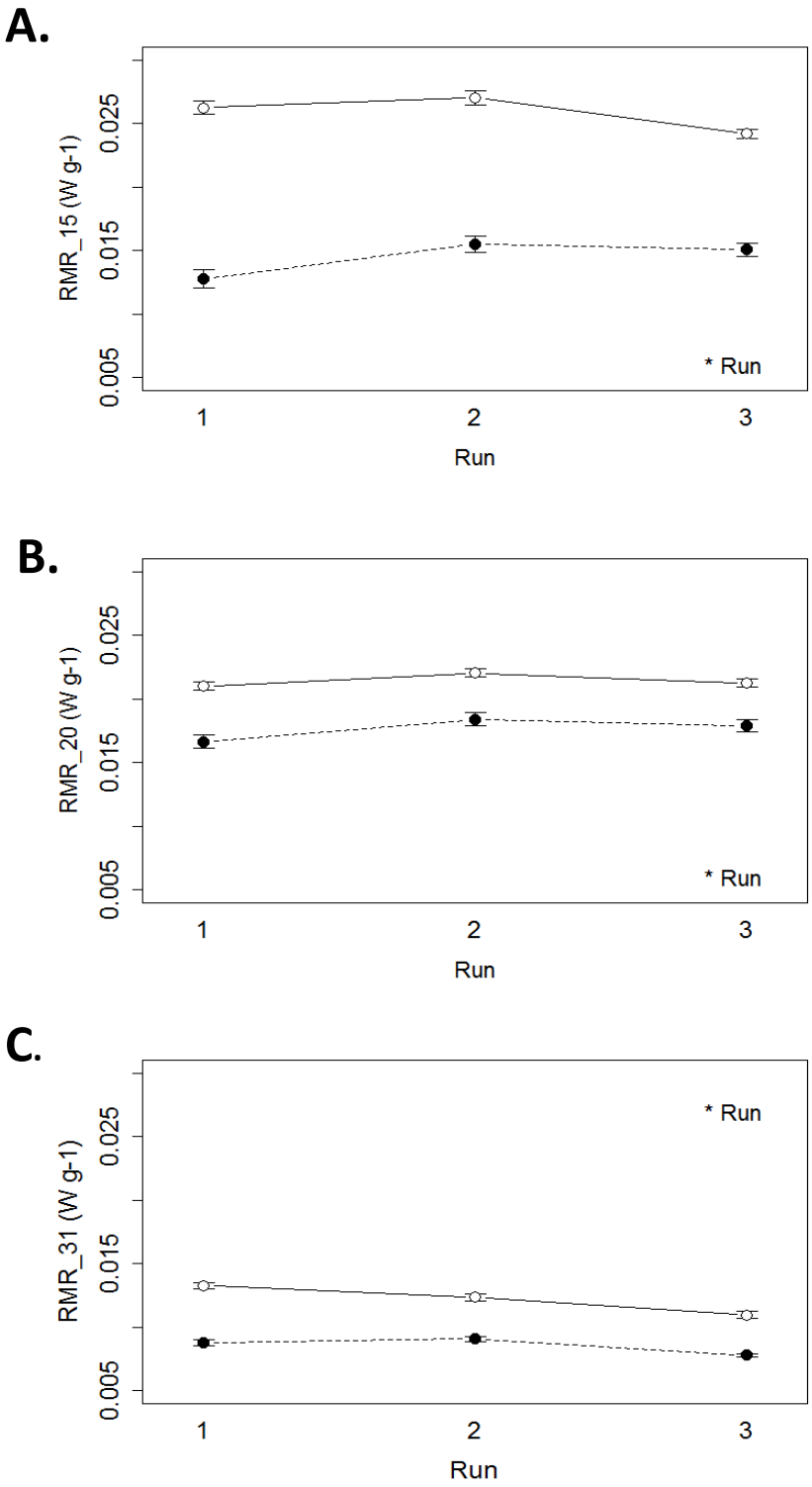


Figure 3.9. The effect of run on resting metabolic rate (RMR) of *Mus musculus* at 15 °C (**A**), 20 °C (**B**) and 31 °C (**C**) on food (open circles) (**A** : $F_{2,119}$ = 5.58, P = 0.005; **B** : $F_{2,117}$ = 3.09, P = 0.049; **C** :- $F_{2,130}$ = 15.86, P < 0.001) and non-food days (black circles) (**A** : $F_{2,130}$ = 4.68 , P = 0.011; **B** : $F_{2,131}$ = 3.38, P = 0.037; **C** : $F_{2,131}$ = 5.00, P = 0.008) across three respirometry runs. Mean values (± SE) shown. Significant variables from mixed models displayed by * (P < 0.05).

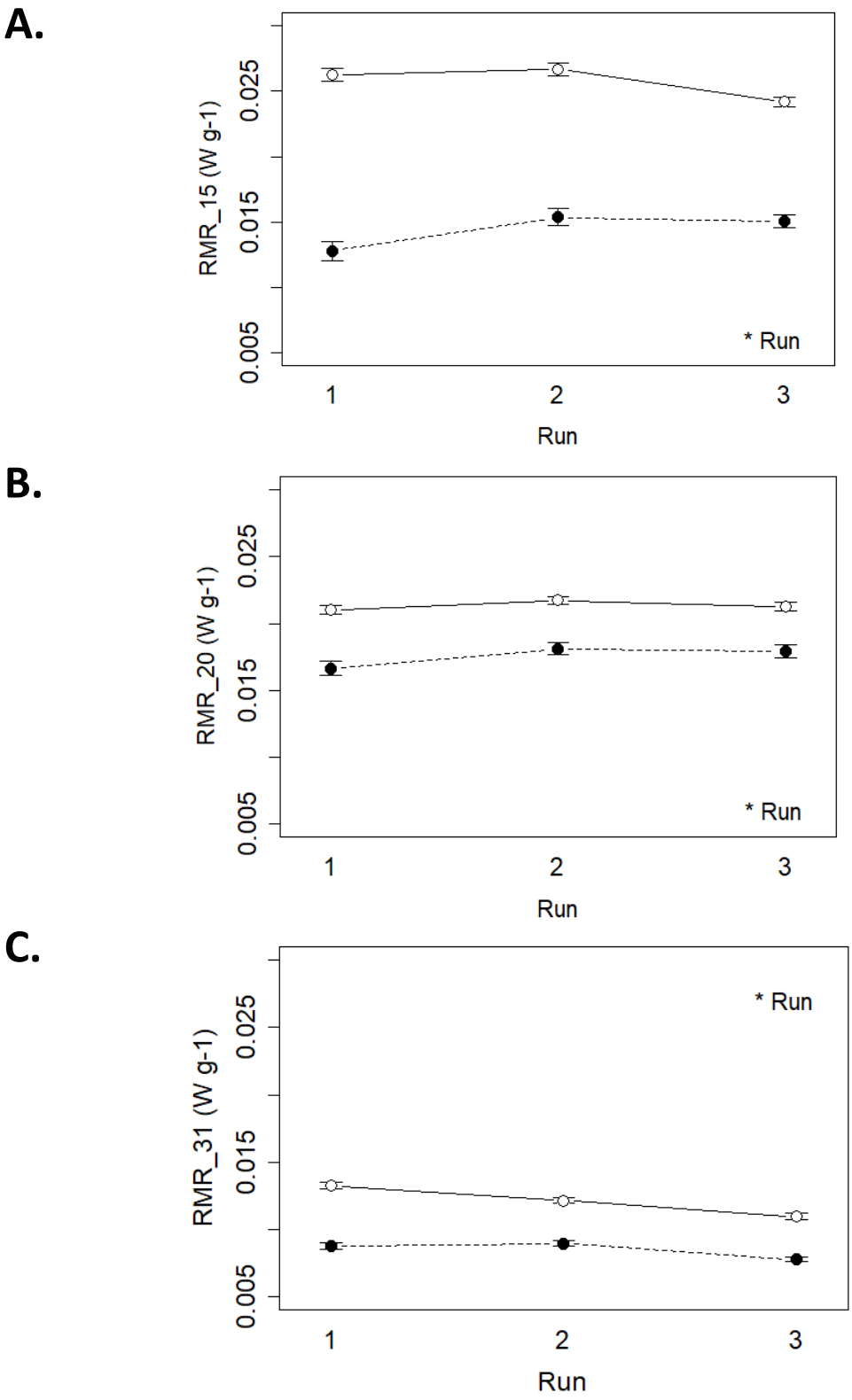


Figure 3.10. The effect of run on average energy expenditure of *Mus musculus* at 15 °C (**A**), 20 °C (**B**) and 31 °C (**C**) on food (open circles) (**A** : $F_{2,119} = 4.39$, $P = 0.014$; **B** : $F_{2,117} = 7.11$, $P = 0.001$; **C** :- $F_{2,134} = 8.76$, $P < 0.001$) and non-food days (black circles) (**A** : $F_{2,128} = 3.18$, $P = 0.045$; **B** : $F_{2,128} = 4.42$, $P < 0.014$; **C** : $F_{2,133} = 4.07$, $P < 0.019$) across three respirometry runs. Mean values (± SE) shown. Significant variables from mixed models displayed by * ($P < 0.05$).

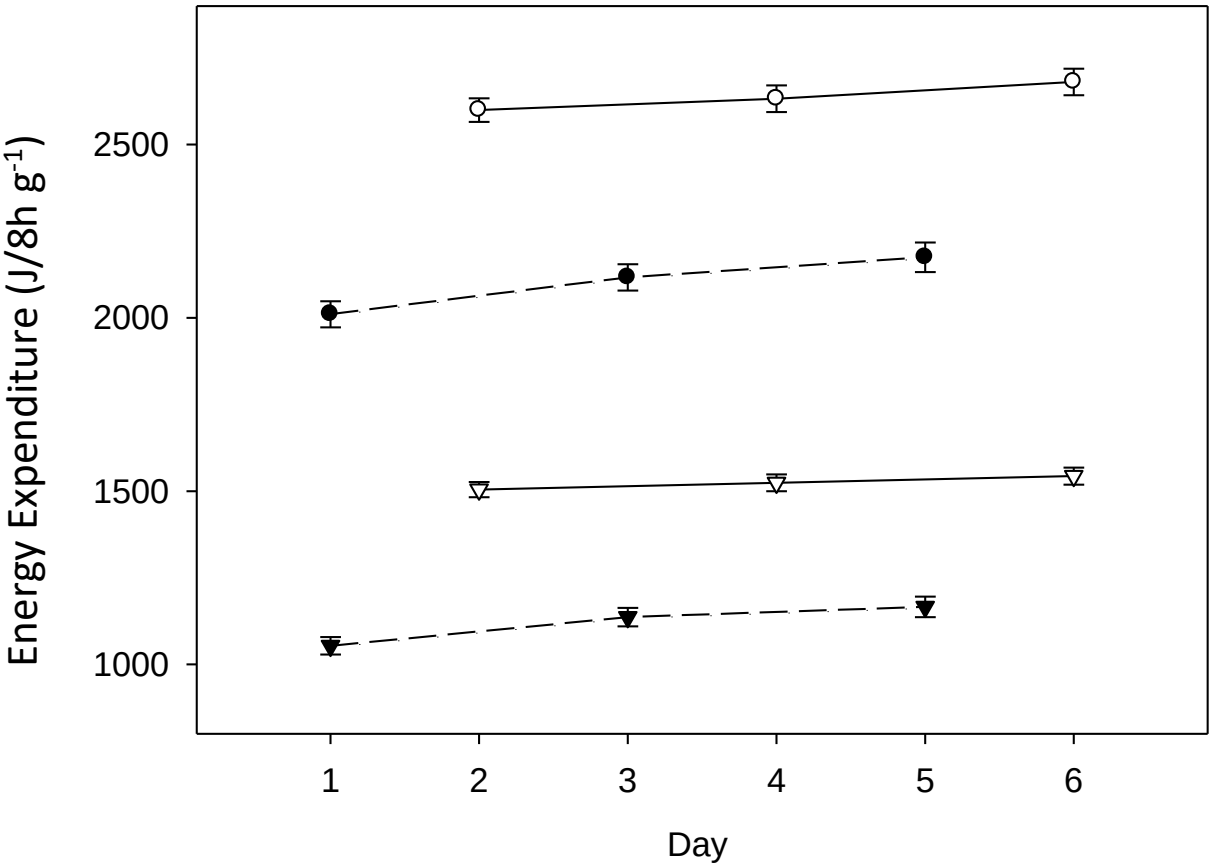


Figure 3.11. The effect of respirometry day on daily energy expenditure (DEE) (circles) and resting energy expenditure (REE) (triangles) of *Mus musculus* on food (white) (DEE : $F_{2,387}$ = 10.65, P < 0.001; REE : $F_{2,387}$ = 5.13, P = 0.006) and non-food days (black) (DEE : $F_{2,392}$ = 15.54, P < 0.001 0; REE : $F_{2,392}$ = 13.98, P < 0.001) across three respirometry runs. Mean values (± SE) shown.

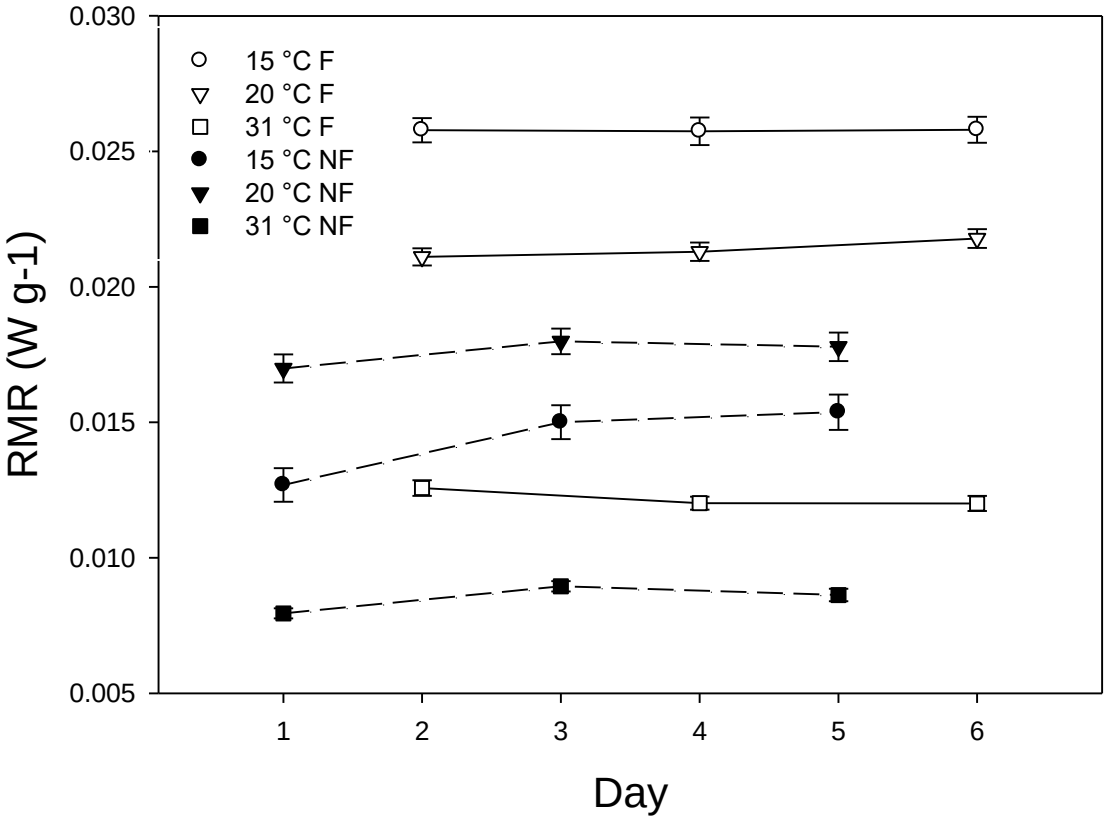


Figure 3.12. The effect of day on resting metabolic rate (RMR) of *Mus musculus* at 15 °C (circle), 20 °C (triangle) and 31 °C (square) on food (white) (15 °C: $F_{2,387} = 0.05$, $P = 0.956$; 20 °C : $F_{2,388} = 2.64$, $P = 0.072$; 31 °C :- $F_{2,389} = 3.17$, $P = 0.043$) and non-food days (black) (15 °C: $F_{2,392} = 12.3018$, $P < 0.001$; 20 °C : $F_{2,392} = 2.398$, $P = 0.092$; 31 °C : $F_{2,392} = 16.87$, $P < 0.001$) across three respirometry runs. Mean values (± SE) shown.

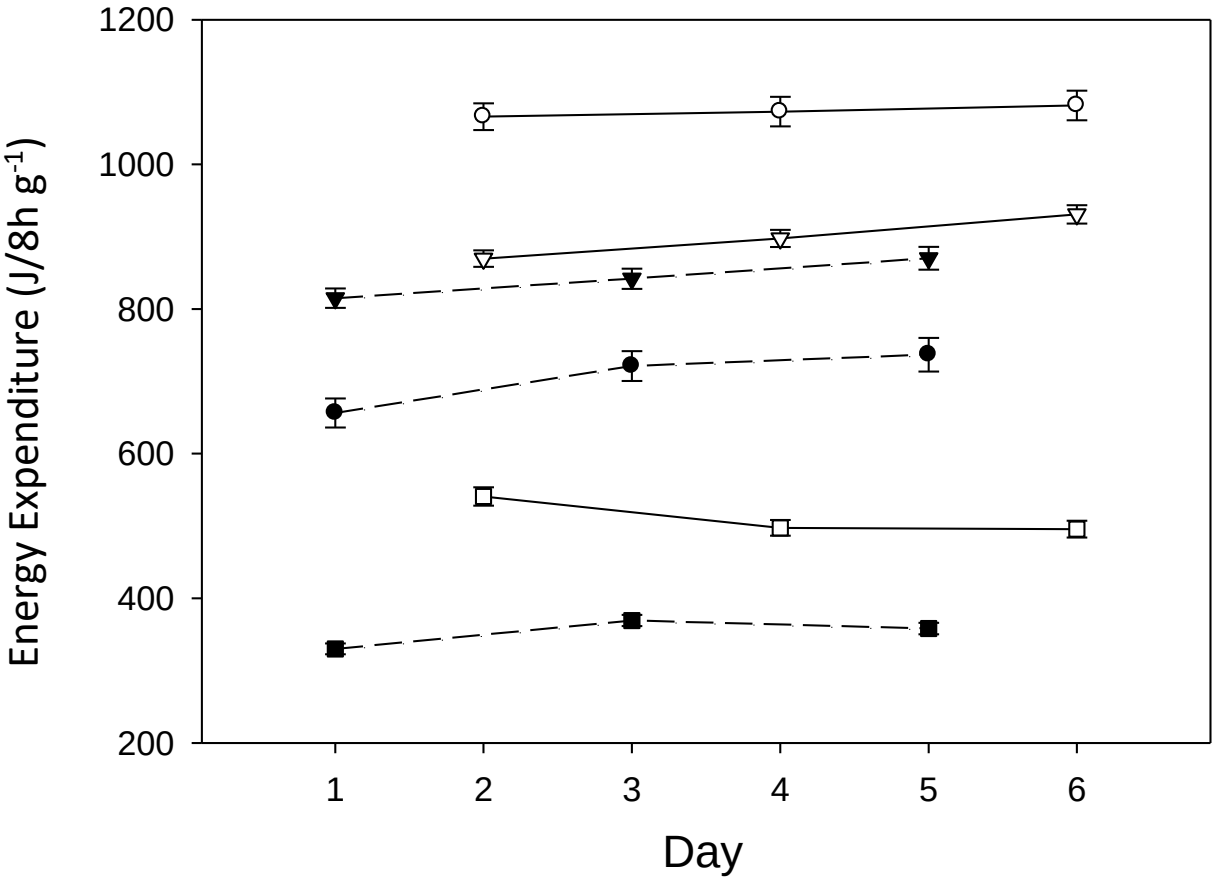

1200
1000
800
600
400
200
Energy Expenditure (J/8h g-1)
1
2
3
4
5
6
Day

Figure 3.13. The effect of day on average energy expenditure (AEE) of *Mus musculus* at 15°C (circle), 20°C (triangle) and 31°C (square) on food (white) (15 °C : $F_{2,387}$ = 1.254, P = 0.287; 20 °C: $F_{2,387}$ = 21.271 , P < 0.001; 31 °C : $F_{2,388}$ = 14.51, P < 0.001) and non-food days (black) (15 °C : $F_{2,392}$ = 11.36 , P < 0.001; 20 °C : $F_{2,392}$ = 9.60, P < 0.001; 31 °C : $F_{2,392}$ = 20.390, P < 0.001) across three respirometry runs. Mean values (± SE) shown.

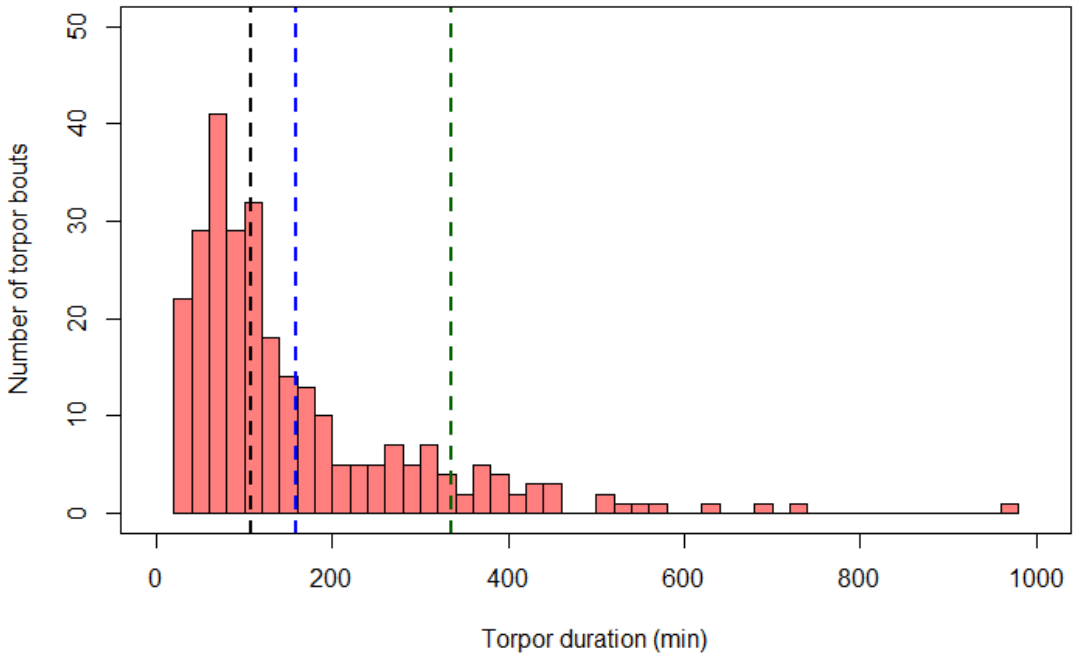


Figure 3.14. Frequency distribution for torpor bout duration (min) in *Mus musculus* (*n*= 275*)* across three respirometry runs. Dashed lines represent the population median (black), mean (blue) and 90th percentile (green).

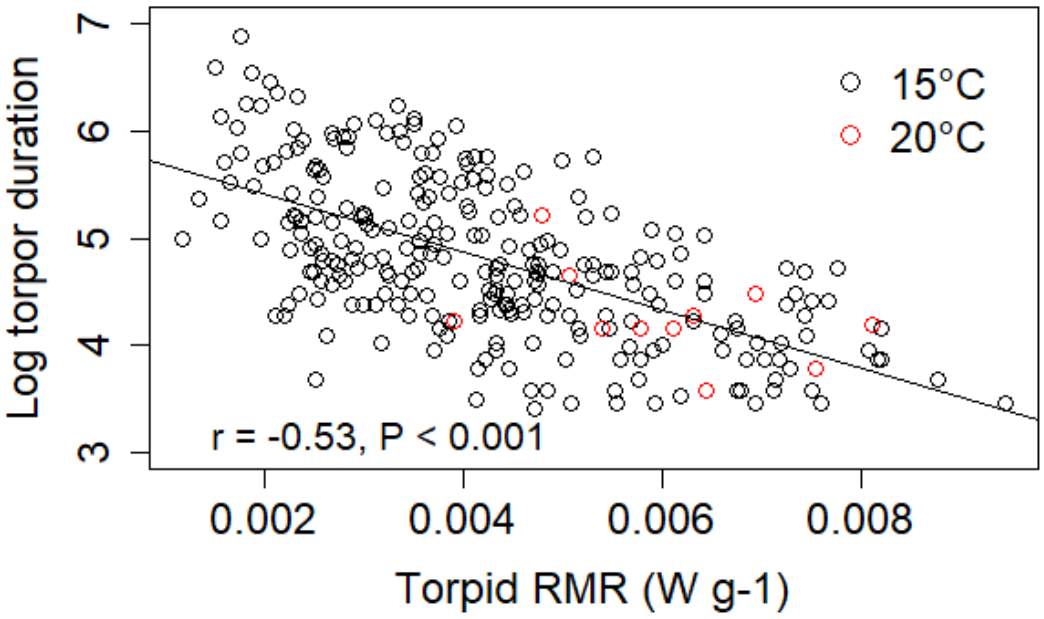


Figure 3.15. Effect of torpor bout duration on mass specific torpid resting metabolic rate (RMR) at 15 °C (black) and 20 °C (red) in *Mus musculus* across three respirometry runs. *N* = 274.

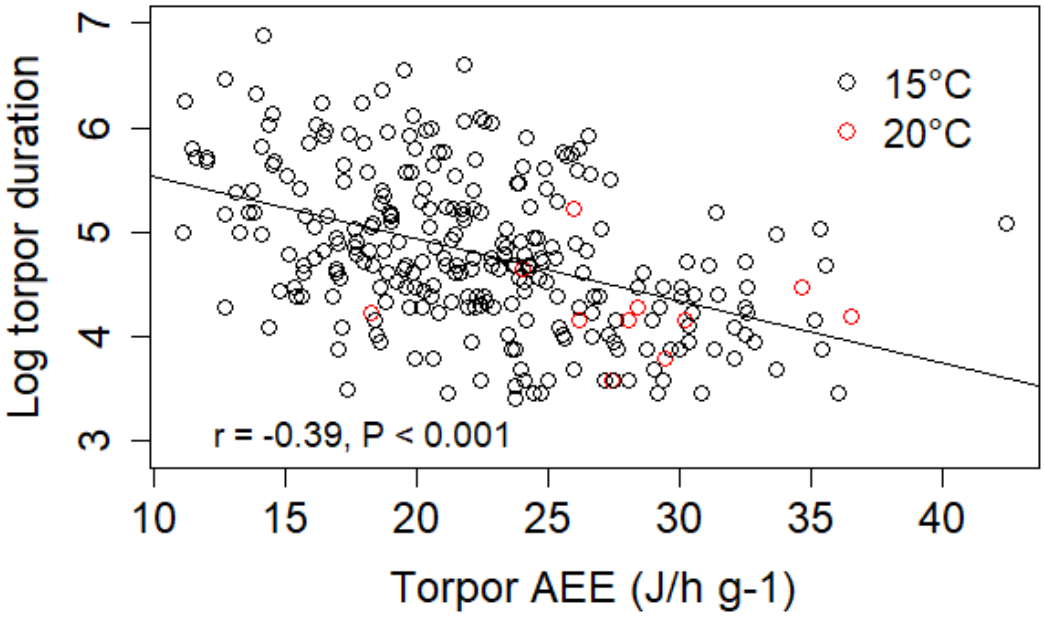


Figure 3.16. Effect of torpor duration on average energy expenditure (AEE) during torpor at 15 °C (black) and 20 °C (red) in *Mus musculus* across three respirometry runs. *N* = 274.

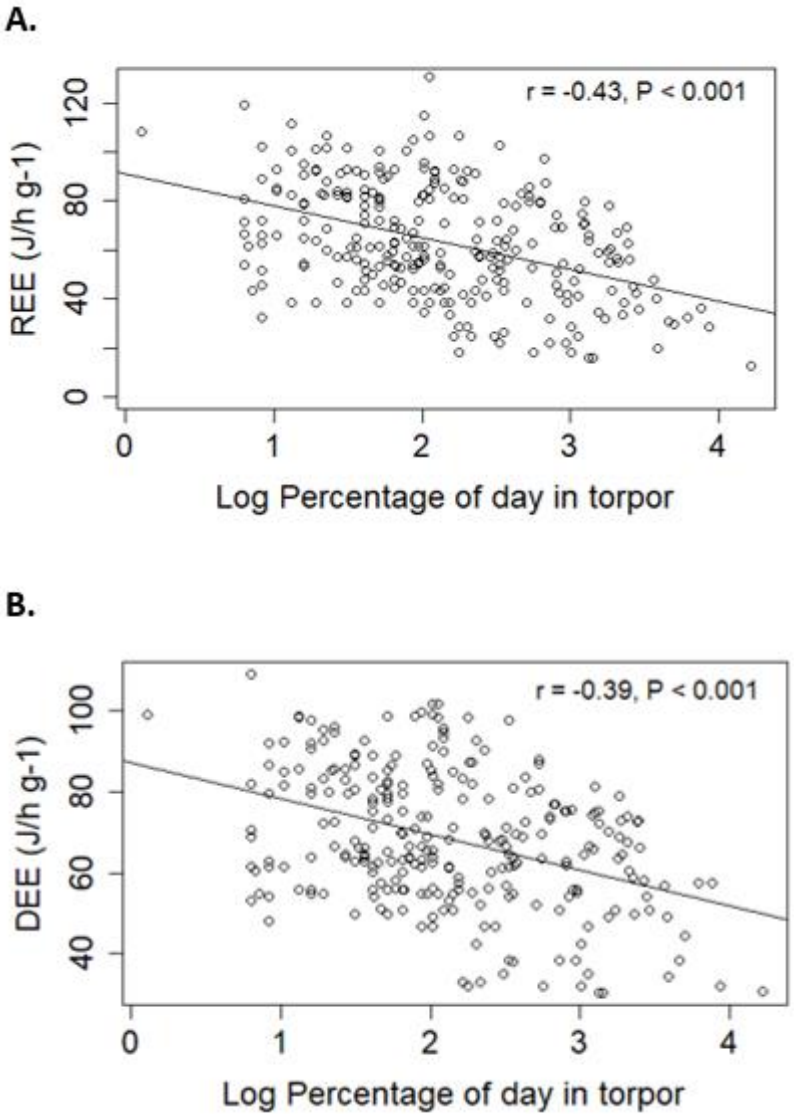


Figure 3.17. Mass specific daily energy expenditure (DEE) (**A**) and resting energy expenditure (REE) (**B**) in *Mus musculus* as a function of the logarithm of percentage of the day spent in torpor across three respirometry runs. *N* = 274.

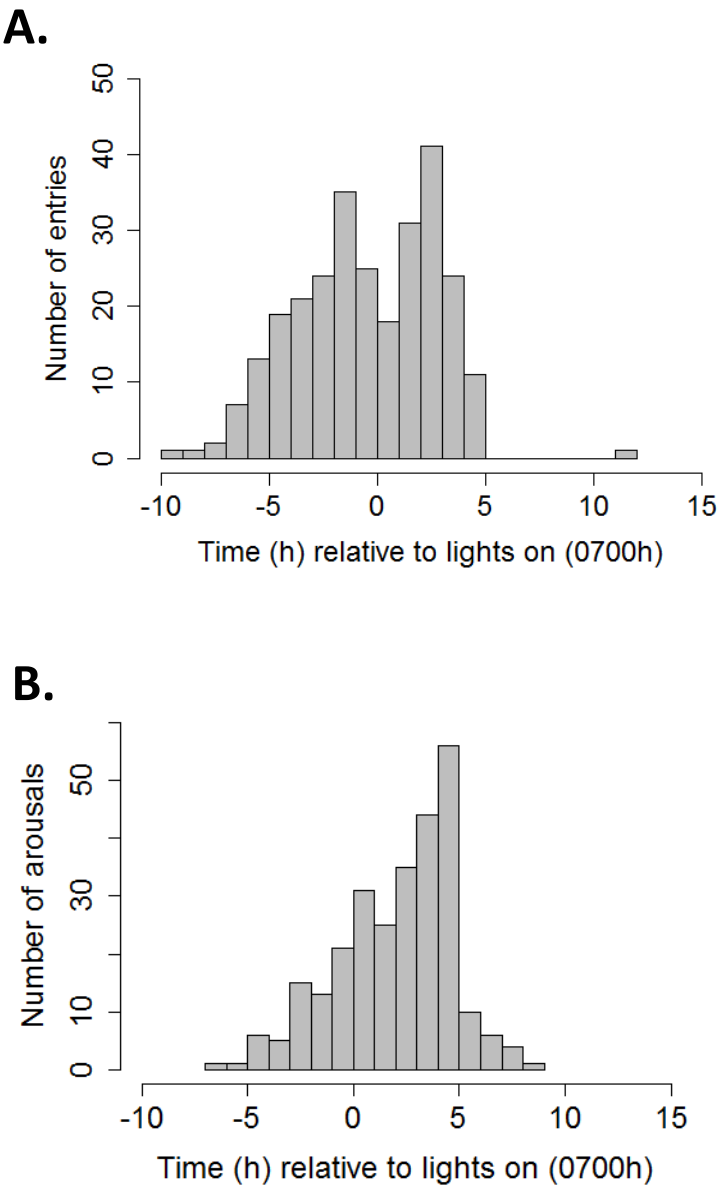


Figure 3.18. Times of entry into (A) and arousal from (B) torpor bouts in *Mus musculus* across three respirometry runs.

Table 3.3. Effect of day on the metabolic characteristics of daily torpor (number of torpor bouts, torpor duration, torpid resting metabolic rate (RMR), average energy expenditure (AEE) over torpor bouts, time of entry of torpor bout and time of arousal from torpor bout) for *Mus. musculus* across three respirometry runs. Overall mean ± SD and linear mixed effect model statistics shown.

Day	No. of torpor bouts	Torpor duration (min)	Torpid RMR at 15 °C (W)	Torpor AEE (J h^{-1})	Time of entry relative to lights on (h)	Time of arousal relative to lights on (h)
1	115	164.36 ± 137.50	0.057 ± 0.023	304.15 ± 73.45	-0.743 ± 2.966	1.995 ± 3.012
3	79	142.45 ± 127.40	0.059 ± 0.024	302.54 ± 68.09	0.079 ± 3.195	2.453 ± 2.608
5	81	169.77 ± 145.34	0.063 ± 0.024	320.94 ± 66.91	-0.702 ± 3.373	1.836 ± 2.390
Test results:		$F_{2, 254}$ = 1.34; P = 0.260	$F_{2, 250}$ = 1.62; *P* = 0.203	$F_{2, 7}$ = 1.28; *P* =0.282	$F_{2, 268}$ = 1.69; *P* = 0.190	$F_{2, 268}$ = 0.65; *P* = 0.524

Table 3.4. Effect of respirometry run on the metabolic characteristics of daily torpor (number of torpor bouts, torpor duration, torpid resting metabolic rate (RMR), average energy expenditure (AEE) over torpor bouts, time of entry of torpor bout and time of arousal from torpor bout) for *Mus musculus* across three respirometry runs. Overall mean ± SD and linear mixed effect model statistics shown. P-values highlighted in bold indicate significance (*P* < 0.05).

Run	No. of torpor bouts.	Torpor duration (min)	Torpid RMR at 15 °C (W)	Torpor AEE (J h^{-1})	Time of entry relative to lights on (h)	Time of arousal relative to lights on (h)
1	154	170.87 ± 148.42	0.058 ± 0.024	299.73 ± 70.49	-0.283 ± 3.447	2.410 ± 2.792
2	59	172.97 ± 141.08	0.056 ± 0.023	307.61 ± 67.13	-1.252 ± 2.559	1.631 ± 2.545
3	63	119.63 ± 90.09	0.066 ± 0.023	331.58 ± 68.44	-0.322 ± 2.872	1.672 ± 2.652
Test results:		$F_{2, 253}$ = 4.44; ***P* = 0.013**	$F_{2, 253}$ = 4.57; ***P* = 0.011**	$F_{2, 244}$ = 1.68; ***P* = 0.003**	$F_{2, 266}$ = 1.68; *P* = 0.188	$F_{2, 261}$ = 0.63; *P* = 0.536

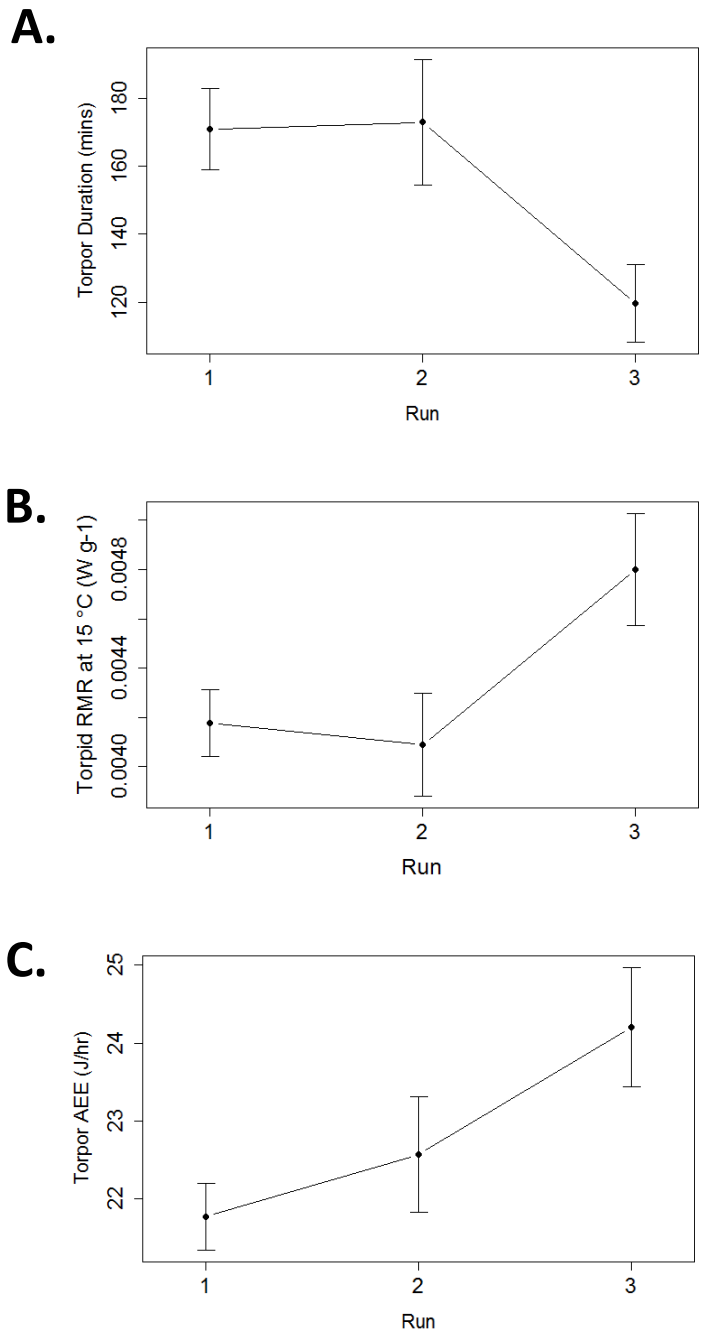


Figure 3.19. Effects of run on torpor bout duration (minutes) (A), mass specific torpid resting metabolic rate (RMR) at 15 °C (B) and average energy expenditure (AEE) over torpor bouts (C) in *Mus musculus* across three respirometry runs. Mean values (±SE) shown.

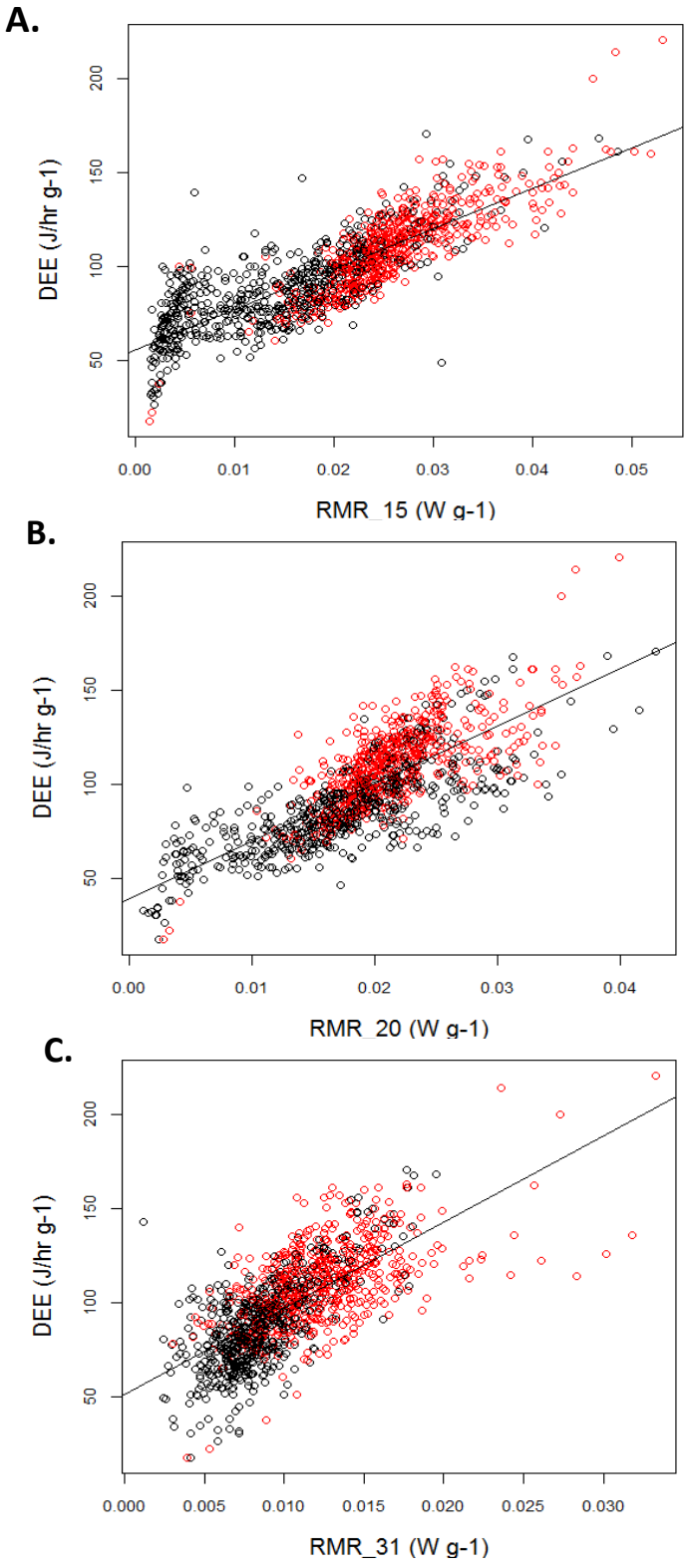


Figure 3.20. Showing relationship of resting metabolic rate (RMR) of *Mus musculus* at 15 °C (**A**), 20 °C (**B**) and 31 °C (**C**) and daily energy expenditure (DEE) on food (red; **A**: r = 0.84. $P < 0.001$; **B**: r = 0.73. $P < 0.001$; **C**: r = 0.57. $P < 0.001$; N = 586) and non-food (black; **A**: r = 0.77. $P < 0.001$; **B**: r = 0.77. $P < 0.001$; **C**: r = 0.570. $P < 0.001$; N = 591) days across three respirometry runs.

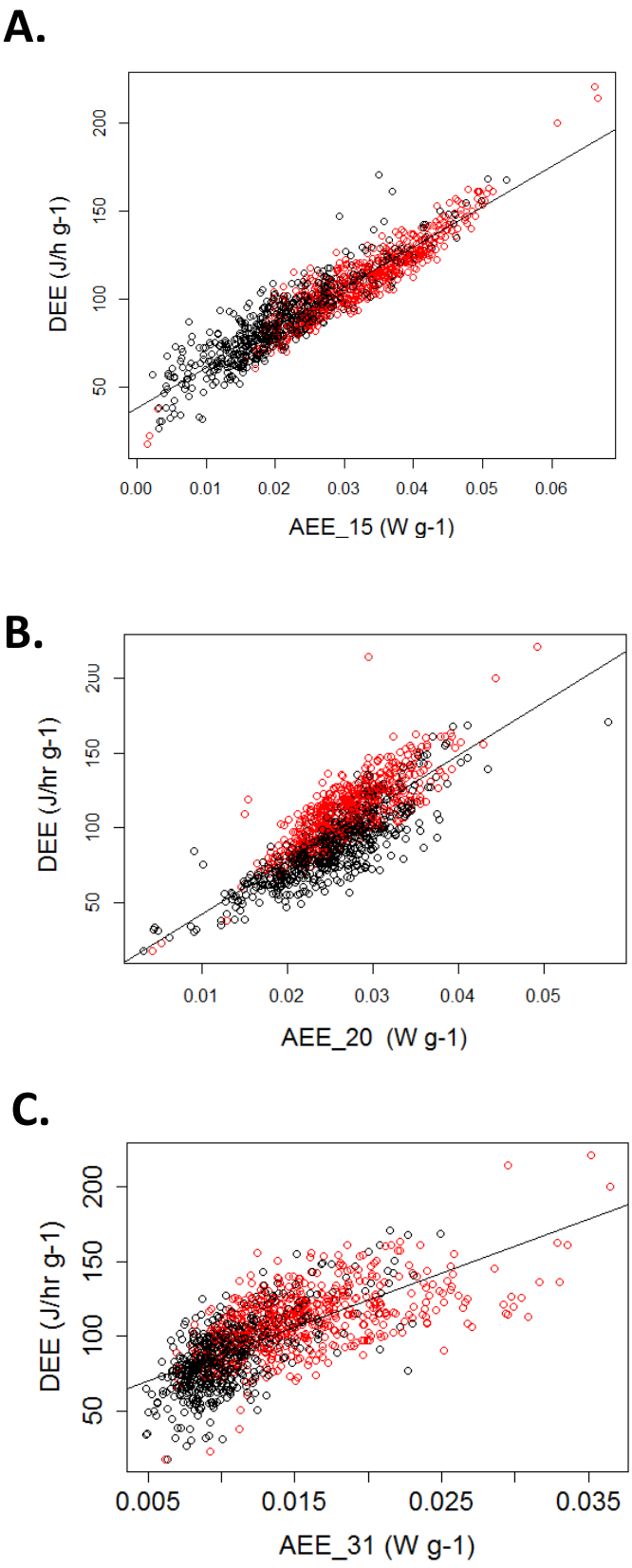


Figure 3.21. Showing relationship of average energy expenditure at 15 °C (**A**), 20 °C (**B**) and 31 °C (**C**) and daily energy expenditure (DEE) on food (red; **A**: r = 0.93, P < 0.001, df = 584; **B**: r = 0.81, P < 0.001, df = 584; **C**: r = 0.58, P < 0.001, df = 584) and non-food (black; **A**: r = 0.91, P < 0.001, df = 589; **B**: r = 0.84, P < 0.001, df = 589; **C**: r= 0.69, P < 0.001, df = 589) days in *Mus musculus* across three respirometry runs.

Table 3.5. Regressions of various metabolic characteristics with DEE of *Mus musculus* across three respirometry runs. All regressions significant at *P*=<0.001

Metabolic measure	Period	*r*	*df*	95% Confidence Interval	
				Lower bound	Upper bound
RMR 15°C	Food	0.84	584	0.811	0.860
	No food	0.77	589	0.740	0.805
	All days	0.84	1175	0.820	0.854
RMR 20°C	Food	0.73	584	0.686	0.762
	No food	0.77	589	0.739	0.804
	All days	0.77	1175	0.746	0.792
RMR 31°c	Food	0.57	584	0.514	0.624
	No food	0.70	589	0.651	0.735
	All days	0.70	1175	0.699	0.728
AEE 15°C	Food	0.93	584	0.918	0.940
	No food	0.91	589	0.896	0.924
	All days	0.93	1175	0.926	0.941
AEE 20°C	Food	0.81	584	0.785	0.840
	No food	0.84	589	0.811	0.860
	All days	0.8	1175	0.776	0.818
AEE 31°C	Food	0.58	584	0.520	0.629
	No food	0.69	589	0.643	0.728
	All days	0.69	1175	0.660	0.720
Torpid RMR	NA	0.47	272	0.432	0.605
Torpid AEE	NA	0.60	272	0.523	0.675
AEE rest phase (0700 - 1900h)	Food	0.90	584	0.887	0.917
	No food	0.91	589	0.894	0.922
	All days	0.92	1175	0.913	0.930
AEE active phase (1900 - 0700h)	Food	0.88	584	0.860	0.897
	No food	0.91	589	0.901	0.927
	All days	0.89	1175	0.874	0.898
REE (0000-1200h)	Food	0.97	584	0.968	0.977
	No food	0.97	584	0.968	0.977
	All days	0.98	1175	0.977	0.980

Table 3.6. Summary model statistical analyses of individual metabolic measures of *Mus musculus* across three respirometry runs.

Metabolic measure					
DEE					
	Variance component	**Variance**	**SD**		
Random effects					
Food|Series	*Vind0*	12191349	3492		
	Vind1	13716983	3704		
Food|ID	*Vind0*	17275320	4156		
	Vind1	2584303	1608		
Residual		12534880	3540		
	Coefficient	**SE**	***t*-value**	**df**	***P*-value**
Fixed effects					
Body mass	1488	210	7.08	132	< 0.001
Day	1346	232	5.79	1949	< 0.001
Run	-262	634	-0.41	120	0.681
Food	-7319	423	-17.29	85	< 0.001
Trial x food	937	720	1.30	157	0.195
REE					
	Variance component	**Variance**	**SD**		
Random effects					
Food|Series	*Vind0*	4349641	2086		
	Vind1	7174698	2679		
Food|ID	*Vind0*	7208195	2658		
	Vind1	1310435	1145		
Residual		6594620	2568		
	Coefficient	**SE**	***t*-value**	**df**	***P*-value**
Fixed effects					
Body mass	833	136	6.143	133	< 0.001
Day	786	169	4.667	866	< 0.001
Run	-316	401	-0.787	118	0.433
Food	-5588	305	-18.310	85	< F.001
Trial x food	773	521	1482	155	0.140
RMR_15					
	Variance component	**Variance**	**SD**		
Random effects					
Food|Series	*Vind0*	0.0015	0.0389		
	Vind1	0.0043	0.0658		
Food|ID	*Vind0*	0.0025	0.0497		
	Vind1	0.0037	0.0605		
Residual		0.0044	0.0664		
	Coefficient	**SE**	***t*-value**	**df**	***P*-value**
Fixed effects					
Body mass	0.014	0.003	5.324	118	< 0.001
Day	0.011	0.004	2.498	681	< 0.001
Run	-0.022	0.009	-2.522	118	0.013
Food	-0.160	0.010	-15.369	58	< 0.001
Trial x food	0.040	0.013	3.031	113	0.003

Metabolic measure					
RMR_20					
	Variance component	**Variance**	**SD**		
Random effects					
Food|Series	*Vind0*	0.0007	0.0274		
	Vind1	0.0015	0.0383		
Food|ID	*Vind0*	0.0003	0.0176		
	Vind1	0.0003	0.0164		
Residual		0.0038	0.0618		
	Coefficient	**SE**	***t*-value**	**df**	***P*-value**
Fixed effects					
Body mass	0.013	0.002	7.455	88	< 0.001
Day	0.009	0.004	2.114	683	0.035
Run	0.005	0.007	0.738	117	0.462
Food	-0.051	0.005	-9.380	61	< 0.001
Trial x food	0.006	0.010	0.600	113	0.549
RMR_31					
	Variance component	**Variance**	**SD**		
Random effects					
Food|Series	*Vind0*	0.0007	0.0261		
	Vind1	0.0012	0.0344		
Food|ID	*Vind0*	0.0007	0.0257		
	Vind1	0.0002	0.0156		
Residual		0.0010	0.0309		
	Coefficient	**SE**	***t*-value**	**df**	***P*-value**
Fixed effects					
Body mass	0.006	0.001	5.707	113	< 0.001
Day	0.002	0.002	1.201	680	0.230
Run	-0.0232	0.005	-4.744	118	< 0.001
Food	-0.0536	0.004	-13.729	59	< 0.001
Trial x food	0.0144	0.007	2.201	114	
AEE_15					
	Variance component	**Variance**	**SD**		
Random effects					
Food|Series	*Vind0*	2948970	1717		
	Vind1	5742195	2396		
Food|ID	*Vind0*	6075782	2465		
	Vind1	2623559	1620		
Residual		4306398	2075		
	Coefficient	**SE**	***t*-value**	**df**	***P*-value**
Fixed effects					
Body mass	546.4	111.1	4.919	131	< 0.001
Day	444.1	136.2	3.261	1233	0.001
Run	-725.4	328.6	-2.208	118	0.029
Food	-5157.4	313.1	-16.474	58	< 0.001
Trial x food	848.4	449.6	1.887	112	0.062

Metabolic measure					
AEE_20					
	Variance component	**Variance**	**SD**		
Random effects					
Food|Series	*Vind0*	1645578	1282		
	Vind1	533375	730		
Food|ID	*Vind0*	797763	893		
	Vind1	215668	646		
Residual		2475332	1573		
	Coefficient	**SE**	***t*-value**	**df**	***P*-value**
Fixed effects					
Body mass	595.11	68.29	8.714	98	< 0.001
Day	730.60	103.19	7.080	566	< 0.001
Run	704.13	244.74	2.877	117	0.005
Food	-624.77	132.21	-4.725	62	< 0.001
Trial x food	-158.18	226.76	-0.698	114	0.487
AEE_31					
	Variance component	**Variance**	**SD**		
Random effects					
Food|Series	*Vind0*	1717217	1310		
	Vind1	2603365	1614		
Food|ID	*Vind0*	1537405	1240		
	Vind1	788729	888		
Residual		1419847	1192		
	Coefficient	**SE**	***t*-value**	**df**	***P*-value**
Fixed effects					
Body mass	258.33	43.47	5.942	121	< 0.001
Day	-107.76	78.25	-1.377	698	0.169
Run	-777.88	228.47	-3.405	118	< 0.001
Food	-2316.30	187.38	-12.361	67	< 0.001
Trial x food	378.80	288.33	1.314	140	0.191

Table 3.7. Estimates of the repeatability (*R*) of average individual variation in reaction norms of measured metabolic rates of *Mus musculus* in response to differences in food availability measured among three respirometry runs over three months.

	Runs 1-3		**Runs 1-2**		**Runs 2-3**	
	R	95% CI	*R*	95% CI	*R*	95% CI
Intercept						
REE	**0.64**	0.44 - 0.76	**0.49**	0.26 – 0.74	**0.68**	0.51-0.83
DEE	**0.59**	0.44 – 0.71	**0.53**	0.32 – 0.71	**0.62**	0.39-0.76
RMR 15°C	**0.64**	0.43 – 0.79	**0.51**	0.19 – 0.76	**0.72**	0.41-0.91
RMR 20°C	**0.31**	0.02 - 0.56	**0.01**	0.00 - 0.52	**0.43**	0.04-0.78
RMR 31°C	**0.49**	0.26 - 0.66	**0.50**	0.26 – 0.69	**0.46**	0.13-0.65
AEE 15°C	**0.66**	0.50 – 0.77	**0.59**	0.36 – 0.80	**0.68**	0.47-0.83
AEE 20°C	**0.29**	0.05 – 0.51	**0.15**	0.00 – 0.44	**0.46**	0.08-0.71
AEE 31°C	**0.45**	0.27 – 0.59	**0.64**	0.37 – 0.77	**0.45**	0.20-0.67
Slope						
REE	**0.15**	0.04 - 0.38	**0.07**	0.00 – 0.53	**0.48**	0.16-0.81
DEE	**0.15**	0.05 – 0.37	**0.09**	0.00 – 0.36	**0.44**	0.18-0.65
RMR 15°C	**0.45**	0.22 – 0.63	**0.30**	0.08 – 0.63	**0.72**	0.44-0.94
RMR 20°C	**0.15**	0.00 – 0.46	**0.26**	0.00 – 0.55	**0.48**	0.06-0.83
RMR 31°C	**0.18**	0.04 – 0.41	**0.12**	0.00 – 0.51	**0.35**	0.06-0.75
AEE 15°C	**0.31**	0.09 – 0.51	**0.24**	0.05 – 0.52	**0.61**	0.33-0.81
AEE 20°C	**0.29**	0.01 – 0.82	**0.56**	0.09 – 0.98	**0.49**	0.00-0.99
AEE 31°C	**0.23**	0.10 – 0.41	**0.30**	0.12 – 0.55	**0.42**	0.11-0.66

Table 3.8. Estimates of the long-term repeatability (*R*) among individuals in average reaction norm intercept and slope to differences in food availability from three respirometry runs carried out monthly in *Mus musculus*.

	Runs 1-3		**Runs 1-2**		**Runs 2-3**	
	R	95% CI	*R*	95% CI	*R*	95% CI
Intercept						
REE	**0.40**	0.29 - 0.50	**0.30**	0.14 – 0.46	**0.46**	0.28 – 0.59
DEE	**0.42**	0.25 - 0.53	**0.37**	0.23 - 0.05	**0.45**	0.25 – 0.59
RMR 15°C	**0.30**	0.21 - 0.41	**0.25**	0.10 – 0.39	**0.34**	0.16 – 0.47
RMR 20°C	**0.07**	0.00 - 0.15	**0.07**	0.00 – 0.15	**0.09**	0.00 – 0.18
RMR 31°C	**0.29**	0.18 - 0.41	**0.32**	0.12 - 0.45	**0.29**	0.15 – 0.43
AEE 15°C	**0.44**	0.32 - 0.54	**0.39**	0.19 - 0.52	**0.48**	0.29 – 0.61
AEE 20°C	**0.14**	0.04 - 0.26	**0.08**	0.00 – 0.23	**0.23**	0.04 – 0.36
AEE 31°C	**0.32**	0.17 - 0.43	**0.48**	0.31 – 0.60	**0.32**	0.16 – 0.52
Slope						
REE	**0.08**	0.03 – 0.20	**0.04**	0.00 – 0.23	**0.28**	0.11 – 0.49
DEE	**0.09**	0.03 – 0.22	**0.05**	0.00 – 0.23	**0.27**	0.07 – 0.47
RMR 15°C	**0.29**	0.16 -0.44	**0.20**	0.03 - 0.39	**0.45**	0.27 – 0.58
RMR 20°C	**0.05**	0.00 – 0.15	**0.07**	0.00 – 0.19	**0.18**	0.04 – 0.35
RMR 31°C	**0.11**	0.03 – 0.25	**0.07**	0.00 – 0.27	**0.19**	0.06 – 0.44
AEE 15°C	**0.21**	0.08 – 0.34	**0.15**	0.01 – 0.31	**0.41**	0.24 – 0.53
AEE 20°C	**0.07**	0.00 – 0.18	**0.16**	0.01 – 0.33	**0.12**	0.00 – 0.28
AEE 31°C	**0.16**	0.05 -0.30	**0.23**	0.08 – 0.44	**0.29**	0.04 – 0.46

Table 3.9. Estimates of the short-term repeatability (*R*) among individuals in average reaction norm intercept and slope to differences in food availability within each respirometry trial in *Mus musculus* .

	Runs 1-3		**Runs 1-2**		**Runs 2-3**	
	R	95% CI	*R*	95% CI	*R*	95% CI
Intercept						
REE	**0.63**	0.55 – 0.71	**0.62**	0.53 - 0.70	**0.67**	0.57 – 0.76
DEE	**0.70**	0.63 – 0.77	**0.70**	0.62 – 0.76	**0.73**	0.65 – 0.79
RMR 15°C	**0.47**	0.37 – 0.57	**0.49**	0.39 - 0.62	**0.47**	0.35 – 0.56
RMR 20°C	**0.21**	0.15 – 0.32	**0.24**	0.15 – 0.34	**0.21**	0.14 - 0.30
RMR 31°C	**0.59**	0.49 – 0.66	**0.63**	0.55 – 0.70	**0.71**	0.63 – 0.76
AEE 15°C	**0.67**	0.59 – 0.73	**0.65**	0.57 – 0.72	**0.71**	0.63 – 0.79
AEE 20°C	**0.50**	0.43 – 0.57	**0.52**	0.43 – 0.61	**0.51**	0.40 – 0.60
AEE 31°C	**0.70**	0.63 – 0.75	**0.74**	0.65 – 0.80	**0.71**	0.62 – 0.78
Slope						
REE	**0.56**	0.47 – 0.67	**0.56**	0.45 – 0.64	**0.58**	0.48 – 0.67
DEE	**0.57**	0.47 – 0.64	**0.58**	0.47 – 0.68	**0.73**	0.47 – 0.69
RMR 15°C	**0.65**	0.56 – 0.72	**0.67**	0.57 – 0.74	**0.62**	0.52 – 0.71
RMR 20°C	**0.31**	0.22 – 0.44	**0.28**	0.20 – 0.43	**0.37**	0.23 – 0.51
RMR 31°C	**0.59**	0.52 – 0.66	**0.68**	0.59 – 0.76	**0.56**	0.41 – 0.65
AEE 15°C	**0.67**	0.61 – 0.73	**0.66**	0.55 – 0.74	**0.67**	0.58 – 0.76
AEE 20°C	**0.23**	0.10 – 0.36	**0.28**	0.15 – 0.42	**0.28**	0.09 – 0.36
AEE 31°C	**0.72**	0.64 – 0.77	**0.77**	0.70 – 0.83	**0.71**	0.59 – 0.76

Chapter 4

Behavioural responses of House Mice (*Mus musculus*) to long-term modified open-field test

4.1 Abstract

Animal behaviour is often regarded as being flexible with individuals able to demonstrate a wide variety of responses. Yet in a wide range of taxa individuals appear to show limitations in their behaviour, consistently exhibiting finite responses. These consistent differences in behavioural traits (e.g. boldness) are stable within individuals but vary among individuals. Correlations between different behavioural traits have also been observed, forming behavioural syndromes. Individual differences in behaviour are believed to have some significant ecological consequences, including affecting how populations respond to environmental change. This chapter tested for the presence of consistent individual differences in behavioural traits and behavioural syndromes in wild derived house mice (*Mus musculus*). Multiple behavioural traits were measured using a modified open-field test (OFT) which was repeated three times at one-month intervals. This allowed the estimation of repeatability and analysis of syndrome structure. Here I focused on indexes of boldness and exploration, as these are significant behavioural traits that can be readily measured and have a significant impact on individual survival in the wild. Results showed that wild derived mice exhibit large and consistent difference in boldness and exploration. These individual differences were substantially repeatable over the three months of measurements, which comprises the average expected lifespan of feral house mice. In conclusion, I found that a large proportion of variation (~47%) in key behaviour traits occurs among individuals, that these among individual differences are highly repeatable over a mouse's natural lifetime and that some behavioural responses are correlated, suggesting the presence of consistent behavioural syndromes.

4.2 Introduction

4.2.1 Consistent individual differences in behavioural traits

Natural selection should favour lower phenotypic variation if an optimal behavioural phenotype existed (Fischer, 1930). However, individual animals often appear to consistently differ in their average behavioural responses over time and across

environments. Within a population, some individuals appear to be more aggressive, more explorative or bolder than others (Reale *et al*., 2007; Biro and Stamps, 2010; Carere *et al.,* 2013). Moreover, individuals tend to vary consistently in suites of correlated and functionally-distinct behavioural traits (Koolhaas *et al.,* 2001; Sih *et al.,* 2004). In rodents, for example, more aggressive individuals have also been shown to be bolder in a novel environment (Hurtado and Mabry, 2017). Structured behavioural differences are frequently observed within populations from a diverse range of species (Gosling, 2001; Sih *et al.,* 2004). Differences in behaviour among individuals might be indicative of consistent and adaptive differences in the behavioural responses of individuals to their environment rather than random noise, caused by measurement error or fluctuating behavioural responses within individuals, as has traditionally been believed (Mather and Anderson, 1993; Dall *et al.,* 2004).

Individual differences in behaviour can have significant ecological consequences (Wolf and Weissing, 2012). For instance, individual variation in behavioural responses can affect how populations respond to environmental change. Anthropogenic impact on the environment is a crucial issue in conservation biology, so understanding how populations respond to environmental change is a key issue (Dall *et al.,* 2004). Populations with diverse behavioural types are expected to be less vulnerable to environmental change as different individuals employ contrasting strategies in the face of the same ecological challenge (Benus *et al.,* 1987; Bergmuller, 2010; Reale *et al.,* 2007; Le Galliard *et al.,* 2013). Behaviourally diverse populations are more likely to have individuals that can survive the novel conditions, thus enhancing population stability and persistence (Hughes *et al.,* 2008; Wolf and Weissing, 2012). Consistent individual differences in behaviour have also been shown to have significant influences on population dynamics, ecological invasions, species invasions, reproductive success, intra- and interspecific competition and survival (Dingemanse *et al.,* 2004; Hughes *et al.,* 2008; Sih *et al.,* 2012, Yli-Renko *et al.,* 2014). The evidence of consistent individual differences in behavioural traits from a wide range of taxa and their ecological consequences encourages further research to better understand the development and fitness consequences of such variation (Sih and Watters, 2005; Sinn *et al.,* 2006; Groothuis and Carerer, 2005).

The number of studies aiming to quantify consistent individual differences in behaviour and explore their potential adaptive significance has increased considerably in the last decade (Bell, 2007; Dingemanse and Wolfe, 2010, Sih *et al.,* 2012). Despite this surge in interest in studies on animal behaviour and personality significant confusion concerning definitions and terminology exists within the field (Biro and Stamps, 2008). I use the term personality as referring to the existence of substantial variation among individual's (within a species and population) average behavioural response, with at least some individuals showing behaviour that is repeatable across time and/or situations (Reale *et al*., 2007; Griffin *et al.,* 2015). Behavioural syndromes are considered as correlations between two or more functionally different behavioural traits (Sih *et al.,* 2004; Garamszegi and Herczeg, 2012a). For instance, correlations have been observed between activity and aggressiveness in *M. musculus*, whereby more active mice are also more aggressive than less active and less aggressive individuals (Koolhaas *et al.* 1999).

Consistent behaviour (i.e. repeatable over time) does not mean that measured behavioural trait values remain permanently fixed and cannot vary depending on the environmental conditions, but that the rank of individual mean values remains consistent over some time-period. Repeatability is estimated through replicated measurements of the same individuals in the same context across two or more time points. It can be described by the fraction of total phenotypic variation that is attributable to the among versus within individual level (Falconer and Mackay, 1996; Reale *et al.,* 2007; Griffen *et al.,* 2015). Behaviours are more repeatable when relatively low amounts of the total variance in repeated measures over time occurs within individuals compared to the amount of variance among individuals (i.e. large relative differences among individual means; Hayes and Jenkins, 1997; Bell *et al.,* 2009).

4.2.2 Causes and persistence of consistent individual differences in behaviour

An important aim in this field of research is to better understand how consistent individual behavioural differences are generated and maintained in natural populations and whether such variation is adaptive (Reale *et al.,* 2010). Numerous hypotheses have been proposed to explain the causes and persistence of consistent individual differences in behaviour (Sih *et al.,* 2004; Stamps, 2007; Biro and Stamps, 2010). For example, variation in individuals' expression of behavioural traits may be constrained by an individuals' ability to capture

(i.e. sensory capabilities) and process information (Hazlett, 1995; Briffa *et al.,* 2015) or could be underpinned by physiological (e.g. metabolic rate; Biro and Stamps, 2010; Reale *et al.,* 2010) or genetic (van Oers and Mueller, 2010) individual differences. Existing differences in the state of an individual such as age or body condition are also believed to have an important role in individual behavioural differences (Luttbeg and Sih, 2010; Dosmann *et al.,* 2015). One of the most popular explanations for the occurrence of consistent behavioural differences in behavioural traits incorporates life-history trade-offs, where a trade-off between growth and survival maintains differences in behavioural traits (Stamps 2007; Reale *et al.,* 2010).

The repeatability of behavioural traits over the length of an individual's lifetime is thought to be affected by numerous state dependent variables. The effect of age on behavioural consistency has produced conflicting results with some studies showing no effect of age class on the consistency of behavioural traits (Bell *et al.,* 2009) whereas others report evidence for increased behavioural repeatability in adults compared to juveniles (Brommer and Class, 2015). During vertebrate maturation individuals experience considerable hormonal changes which are likely to affect individual behavioural consistency (Stamps and Groothuis 2010). Despite the various proffered hypotheses and frameworks to explain consistent individuals in behaviour there remains a lack of empirical research that has tested these theories.

4.2.3 Behavioural Syndromes

In numerous species it has been observed that distinct behavioural traits are frequently co-correlated and that species or populations can exhibit behavioural syndromes (i.e. suites of correlated behavioural traits). For example, individuals with higher activity levels may also exhibit greater boldness. Correlations between behavioural traits that lead to life-history trade-offs could assist in the maintenance of consistent individual differences in individual behavioural traits (Sih *et al.,* 2004; Boon *et al.,* 2008; Garamszegi *et al.,* 2012b). Specifically, a trade-off between survival and future reproductive success may preserve differences in multiple behavioural traits. An individual's investment in activity may be both beneficial (increased food intake and mating success) and hazardous (increased risk of predation). Consequently, such trade-offs may lead to variation in behavioural traits and enable both proactive and reactive individuals within a population

to have similar evolutionary fitness (Sih *et al.,* 2004, Stamps 2007). Care must be taken to avoid simplifying a complicated reality. Behavioural syndromes are often simplified and consequently misinterpreted as bimodal variables, when really it is believed that individuals within a population or species can be ranked along a continuum between two extremes (e.g. shy-bold; Reale *et al.,* 2007).

The theory of behavioural syndromes implies limited behavioural plasticity. Individuals are unable to express the full range of values of different behavioural traits present in the population and are therefore unable to exhibit the optimal behaviour in all contexts (Sih *et al.,* 2004; Reale and Dingemanse, 2010). Therefore, the ecological significance of a behavioural trait may not be apparent when that trait is studied in isolation or only in one context (e.g. high food availability or low predation risk) and can only be fully understood when viewed as one component of a correlated suite of behavioural traits that have evolved in tandem and will maximise fitness under specific conditions. Long-term repeatability has been observed in some behavioural traits and not others, yet most studies focus on short term behavioural measures within the same life-history phase (Schuster *et al.,* 2017). Furthermore, behavioural syndromes can be less stable as the individual ages (Wuerz and Kruger, 2015; Fischer *et al.,* 2016. Longitudinal studies that collect measurements over an individual's entire lifespan will be crucial in increasing our understanding of the stability of any interactions between individual traits in behavioural syndromes.

4.2.4 Open-field tests

The widespread use of a general protocol, such as the OFT, can provide a useful context for exploring the potential evolutionary causes and ecological consequences of consistent individual differences in animal behaviour (Dall and Griffith, 2014). The OFT is a commonly used experiment to measure behavioural traits by quantifying activity and emotional reactivity in a novel environment (Hall, 1934; Belzung, 1999; Boon *et al.,* 2007; Gould *et al.,* 2009). The OFT is used to measure boldness in rodents by comparing the amount of time an individual spends in the perceived "safe" edge zones compared to the more exposed and "unsafe" centre. Additionally, distance moved is frequently interpreted as an index of exploration, as the individual must move around the arena as they explore the novel environment (Russell, 1983).

Traditionally the duration of OFTs have ranged between two and ten minutes, though some studies have extended the OFT up to 60 minutes (Gross *et al.,* 2002; Gould *et al.,* 2009). A limitation of the OFT is that these relatively short tests can only produce measurements of immediate behavioural reactions to novelty, rather than the behaviour of interest, as upon entry to a novel environment a mouse's priority will always be to explore (Spruijt *et al.,* 2014), most likely in an attempt to hide. Limiting measurements to a period where the individual is habituating to novelty results in behavioural states being measured, rather than behavioural traits (Fonio *et al.,* 2012). Studies have indicated that individuals may differ in how their behavioural traits (e.g. activity level) vary over time within a single OFT (Wilson, *et al.,* 1976; Carere *et al.,* 2005). With some individuals exhibiting extreme behavioural responses at the start of the experiment which then change significantly over the course of the experiment (e.g. decreasing movement over time) and other individuals exhibiting more consistent responses throughout the entire experiment (Montiglio *et al.,* 2010). Despite indications that behaviour during the time intervals of traditional OFTs can vary significantly to behaviour observed later, few studies have adopted a methodology that acknowledges the difference between transitory behavioural responses brought on by a temporary cue (i.e. novel environment) and stable behavioural responses (Fonio *et al.,* 2012).

A further limitation of the traditional OFT is that behavioural responses are preceded by response selection, requiring the individual to "choose" from several options. Yet, the traditional OFT does not require the individual to choose a "positive" or "negative" outcome as multiple options are not available. This can lead to misinterpretation of behaviour as an individual in a traditional open field arena moving around the arena in search of refuge may be interpreted as expressing high exploration (Spruijt *et al.,* 2014). To counteract some of these limitations modifications to the traditional OFT are sometimes employed, such as the incorporation of novel objects in the centre of the arena to provide more drive for exploration (Rex *et al.,* 1996; Ishibashi *et al.,* 2006).

4.2.5 Experimental Objectives

This study aimed to test for the presence of consistent individual differences in behavioural traits and behavioural syndromes in wild-derived house mice (*Mus musculus*).

To achieve this, I measured a suite of behaviour traits using a modified OFT on three occasions at one-month intervals. Although in captivity mice can live over a year, in the wild *M. musculus* have a more shorter life expectancy of 100-150 days (Pennycuik *et al.,* 1986; Berry and Jakobson, 1971). Thus, these measurements, which took place over three months, cover a substantial period of the expected lifespan of *M. musculus* the wild (Berry and Jackobson, 1973, Pocock *et al.,* 2004). This is a rigorous test on how wild derived *M. musculus* respond to modified OFTs and whether there is any effect of sex on behavioural responses. This methodological approach allowed the measurement of behaviour in different contexts and was used to determine how much individuals from a single wild population differ in their behaviour and whether correlations between behavioural traits differed temporally (i.e. both short term and long term). I report estimates of repeatability and syndrome structure, focussing on indexes of boldness and exploration as these are significant personality traits that can be readily measured and are likely to affect individual survival in the wild. In this experiment I have interpreted boldness as being the propensity to take risks to use resources such as food and habitat, particularly in a novel and potentially risky situation (Coleman and Wilson, 1998). Exploration was represented by behavioural traits that were concerned with gathering information about the environment (e.g. percentage of experiment spent outside of the dark chamber (active) and distance moved).

In the present study the following specific questions were to address: (i) whether the individual behavioural traits (boldness and exploration) are correlated, indicating a behavioural syndrome as has been found in other species (Koolhaus *et al.,* 1999; Sih *et al.,* 2010; Dammhahn, 2012) and (ii) to what extent are the measured behavioural traits consistent (repeatable) over a substantial period of the expectant lifespan of wild mice (three months). Further aims were to explore the short-term temporal stability of the measured behavioural traits to determine whether behavioural responses, correlations between behavioural traits and repeatability estimates were affected by OFT duration.

4.3 Materials and methods

Approval for all procedures in this experiment was granted by the University of Western Sydney's Animal Care and Ethics Committee and met federal standards for animal care and welfare (A10445).

An open-field behavioural experiment was used to measure individual behavioural traits by quantifying locomotor activity (i.e. movement between and time in zones), exploration and stress responses in a novel environment (Boon *et al.,* 2007). Mice were exposed to a modified version of the standard OFT (Hall and Ballachey, 1932). The protocol used here differed from the standard test as it was conducted using a relatively large area that included a tray of food in the centre of the arena, a dark chamber and the test duration was 15 hours (i.e. a long-term alternative to the short-term tests that studies tend to use, covering the entire active phase of the individuals daily rest-activity cycle). Behavioural testing for this study starting at 1700 h and ran overnight until 0800 h. Individuals began the experiment in a dark chamber, which had free access to an illuminated enclosed arena (120 x 88 x 60 cm; Fig. 4.1). The animal's latency to emerge from the dark chamber and activity within the main arena were continuously recorded via an overhead digital USB-connected camera to a computer and later analysed using multi-zone motion tracking software (Ethovision XT, Noldus Information Technology, Utrecht, The Netherlands).

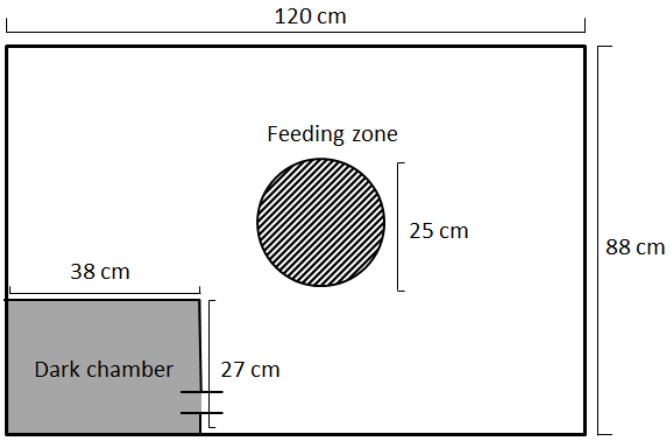


Figure 4.1. Schematic diagram representing the configuration in the open-field test

To determine the consistency of each individual's behavioural characteristics, three runs of behavioural experiments, using the OFT, were carried out. The first open field experiment took place within two weeks of capture and the subsequent two runs occurred at one-month intervals. The order that individuals were tested within each of the three experimental runs was randomly assigned. Prior to each of these behavioural experiments each individuals' metabolic characteristics were measured using open-flow respirometry (as detailed in Chapter 3). The OFT always took place at least seven days after the

respirometry measurements during which time the mice were monitored with minimum disturbance in their home cages.

4.3.1 Study animals and colony maintenance

Elliott traps were used to live trap 69 wild house mice (*Mus musculus*) in and around agricultural buildings located on private land in Wilberforce, NSW, Australia (GPS 33°33'40.779 S, 150°50'0.781 E). The traps were baited with a standard bait mixture (a teaspoonful of peanut butter, oats and honey), set between 1700 and 1800h and checked between 0700 h and 0800 h the following morning. Sampling took place over one-night trapping sessions which were carried out at approximately three month intervals between July 2015 and July 2016. Upon capture, mice were checked for breeding condition and their body length measured. Females showing signs of lactation or pregnancy were released at the capture site. Length of the animal taken from the base of the tail to the nose tip (HB) was used to determine whether the individuals were juveniles 0-5 weeks old (HB < 64 mm), sub-adults 5-8 weeks old (64 ≤ HB ≤ 71 mm) or adults over 8 weeks old (HB > 71mm; Newsome, 1969 and Singleton, 1983). Only individuals classified as sub-adults were included in this study in order to compare individuals of similar age and avoid the possibility of using senescing individuals.

Following capture, one drop (c. 10µl) of an antiparasitic agent (Ivermectin, 0.83mg/mL) was administered topically between the scapula and the mice were brought to a rodent holding facility on the University of Western Sydney, Hawkesbury Campus. The antiparastic was repeated weekly for three weeks. For the duration of the experiment, mice were housed individually in clear polysulfone mouse cages (425 x 266 x 155 mm, 1248 L Eurostandard Type 11 L, Techniplast, Italy) containing cotton nesting material, a cardboard tube and corncob bedding substrate (Able Scientific, Australia). Rodent pellets, containing 16 % protein (Gordans Specialty Stockfeeds P/L, Australia), and tap water were available *ad libitum* throughout the study period. Home cages were cleaned every three weeks and no behavioural experiments took place within three days of an individual's cage being cleaned. The colony was maintained in an air-conditioned room (23±2 °C) and exposed to natural photoperiods via ambient light entering through the windows.

4.3.2 Behavioural testing

The open-field consisted of a rectangular arena (120 x 88 cm) with a wooden floor painted white and enclosed by 60 cm high wooden walls. The mice were weighed and placed individually into an enclosed dark chamber (38 x 27 cm) within the main arena for 30 minutes prior to the start of each run. This 30 minute desensitisation period was used to reduce the effect of external stimuli on the individual's initial response. The dark chamber contained a toilet roll, a handful of shredded paper for bedding and access to water *ad libitum*. In the centre of the arena was a foraging tray (25 cm diameter) containing 6 g of seed ("Canary Mix") mixed in 1 L of sand. Dim illumination in the main arena was provided by a frosted incandescent light bulb mounted circa 120 cm above the floor of the centre arena (light level 35-55 lux as measured at the floor of the arena). All other lights in the test room were turned off for the duration of these behavioural tests. Immediately prior to the start of the experiment (1700 h) the doorway barrier between the dark chamber and main arena was removed allowing free access between the two areas. For the duration of the 15-hour experimental period, which covered the entire daily active phase of *Mus musculus*, the animals were left undisturbed in the testing room (Shuboni *et al.,* 2012).

After the end of each run (0800 h) the mice were reweighed to determine any change in body mass and returned to their home cage. Defecation was quantified by counting and weighing all faecal boles deposited during the test. The remaining seed was sieved from the seed and sand matrix to record the food consumption over the course of the experiment. Test room temperature, lighting and noise levels were consistent for all subjects. Ambient temperature over the course of each run was recorded using two temperature-logging iButton devices (resolution: 0.0625 °C; Maxim Integrated, U.S.A) placed at floor level outside the open-field arena. At the end of each run the arena was cleaned with warm soapy water and a 75% ethanol solution in order to eliminate any residual odours.

Recording and analysis of behavioural data

A digital camera (c525 High Definition 720p webcam; Logitech) was positioned circa 120 cm above the centre of the open-field arena to record the arena throughout the experiment. The computer monitor was situated adjacent to the arena with the screen covered to block all screen illumination and the video recordings were saved to an external hard drive. Recorded videos were collected and analysed with auto-tracking multi-zone

motion monitoring software (Ethovision XT and Media Recorder, Noldus Information Technology, Utrecht, The Netherlands). This software automatically detected the location of the mouse when it was in view (i.e. outside of dark chamber) and tracked its activity within the main arena over the course of each video.

The open field arena in the videos was subdivided into six zones: corners, edges, dark chamber, top of dark chamber, central arena and foraging zone. An individual was determined to have entered a zone when the centre of its body had passed the zone border. Using the Ethovision software, I obtained the following variables: the latency to first emerge from the dark chamber (s), latency to enter each zone (s), time spent in each zone (% of total recording time), time the individual was mobile (% of total recording time) and total distance travelled (cm) over the course of the experiment for each individual. In addition, the corners, edge of dark chamber, top of dark chamber and edge zones were grouped together and defined as the peripheral area ('edges') in order to gain a total score of thigmotaxis – the tendency to remain close to vertical surfaces (Walsh and Cummins, 1976).

Data Analysis

Sixty nine mice underwent three runs of behavioural analysis using an overnight (15 hour) OFT to examine differences in the behavioural response of the individuals. In addition to the variables scored using the Ethovision software (described above) the weight of faecal boli (g), amount of food consumed (g), initial mass (g) and mass change over the experiment were used to evaluate individual response (Table 4.1). The time spent in each zone (% of total recorded time) was converted to percentage of total time active (i.e outside of dark chamber) in each of the zones (i.e. percentage of active time spent in edges, centre and foraging area).

Data were analysed separately for the first hour of data and for the entire 15 hour experiment to see whether the behaviour over longer time periods were significantly different from behaviour observed over a shorter period. All data in the text were reported as means ± SD (*n* = number of individuals, *N* = number of observations). Permutational multivariate analysis of variance (PERMANOVA), combining all the main measured behavioural variables (Table 4.1), was used to test for an overall effect of group

(i.e. cohort individuals were measured in), run and sex. The effects of run and sex (factors that were significant in PERMANOVA analysis) on the measured response variables (Table 4.1) were determined using linear mixed-effects models (R package "lmer4", "lmertest") within the R statistical interface v3.3.2 and RStudio 1.0.136 (R Core Team 2015; R Studio Team 2016; Bates *et al.,* 2015; Kuznetsova, Brockhoff and Christensen *et al.,* 2014). The response variable "Perc.Food" (percentage of active time in foraging area) was log10 transformed, whilst response variables "Emergence" (time taken to emerge from dark chamber), "Food" (food consumed), "Distance" (distance moved), "Faeces" (faces produced) and "Lat.Food" (latency to enter foraging area) were square-root transformed to normalise their distributions. Fixed effect included "Initial body mass", "Run" and "Sex", and an interaction term between "Run" and "Sex". "ID" was included as a random effect on the intercept to account for repeated measures within individuals and differences in mean responses among individuals. Similar models were used to look at the effect of OFT duration on behavioural variables, with both "Run" and "ID" included as random effects to account for repeated measures. Models were simplified by removing non-significant fixed terms in a stepwise manner (in order of least significance) using associated *P* values taken from ANOVA model comparison. Terms that were not significant were not included in the final model.

Correlation matrices were used to examine bi-variate relationships between the behavioural traits by calculating Pearson correlation coefficients and associated *P* significance. Highly correlated variables ($r > \pm 0.6$) were closely inspected to evaluate their likelihood of being robust since multiple correlations were used (see results for further details). A principal component analysis (PCA) was performed on the correlation matrix of behavioural variables to summarise the relationships between the multiple behavioural variables measured during the OFT (Table 4.1) separately within the first hour and over the entire 15 hours experiment. The principal components explaining the highest contribution of individual behavioural traits were used as a composite behavioural measure, with each axis potentially representing a behavioural trait (Budaev, 2010). For each principal component, the measured variables with the largest loadings were used to interpret the behavioural trait that principal component represented. The PCA scores were used to rank the individuals for each principal component within the first hour and over the entire 15 hour experiment.

The multiple measurements of each individual's assigned ranks in the first hour and entire experiment, and the most informative of the individual behavioural variables, were used to estimate repeatability (*R*) over the three runs following Araya-Ajoy *et al.,* 2015. Repeatability is the proportion of total variance that could be attributed to among individual differences over the three runs (Falconer and Mackay, 1996). Linear mixed effects models described the effect of "body mass", "individual" and "run" on individual's ranks or individual behavioural variables from the OFTs. All models included "body mass" as a fixed term and a random effect of "individual" and random slope for "run". A semi-paramatric bootstrap method ("lme4" package in R) was used to calculate the 95% confidence intervals (CI) for *R* from 100 simulations. Confidence intervals that did not overlap with zero indicated a sufficiently high level of confidence that the estimated *R* was different from zero. Repeatability of ranks and behavioural variables were analysed for each sex separately and for the entire population as a whole.

4.4 Results

4.4.1 Performance characteristics

The mean ambient temperature in the test room where all the open field experiments were undertaken was 22.26 ± 1.56 °C (mean ± SD reported here and elsewhere in the text). The 69 individuals were captured in five groups of up to 16 individuals, which were staggered by three months. A PERMANOVA confirmed no significant effect ($F_{1, 209}$ = 1.85, P = 0.062) of group on the individual's main measured variables (Table 4.1).

At the start of the experiment mice weighed, on average, 16.05 ± 2.35 g for males (n = 31; range: 11.33 to 20.03 g) and 14.36 ± 1.95 g for females (n = 36; range: 10.02 to 20.02 g). The initial body mass of males was significantly higher than that of females but sex, nor its interaction with body mass, did not significantly affect the amount of food eaten, the amount of faeces produced or the change in body mass (Table 4.2). For the population as a whole the initial body mass, change in body mass and amount of food consumed were significantly lower in run one compared to runs two and three (Fig. 4.2 A-C). Male mice increased the amount of food eaten over the three runs ($F_{2,61}$= 14.34, P < 0.001), consuming the least in run one (0.89 ± 0.49g) and the most in run three (1.62 ± 0.88 g) (Table 4.2). Females also consumed the least food in the first run (1.04 ± 0.69g) ($F_{2,71}$=

11.10, $P < 0.001$) though there was no significant difference in the amount consumed between runs two and three (Fig. 4.2A). The amount of faeces produced did not vary between runs (Table 4.2).

4.4.2 First hour of open-field test

Behavioural traits

In seven of the 204 open field experiments the individual did not emerge from the dark chamber within the first hour of the experiment. These OFTs were excluded from the first hour analyses. These seven experiments with a non-emerging mouse involved four female individuals, two of which did not emerge in the first hour in one run, one that did not emerge in the first hour in two runs and one that did not emerge within the first hour in any of their runs. To emerge from the dark chamber mice took 709 ± 688 s for males (*n* = 93; range: 30 to 3420 s) and 464 ± 493 s for females (*n* = 108; range: 5 to 3300 s) including all runs (Table 4.2). The average amount of the first hour spent within the dark chamber was 72.75% (*N* =194; range: 13.63% to 99.8%) over all the runs. In 83% of all behavioural experiments the individuals spent at least 50% of the first hour within the dark chamber.

For the 197 runs in which the individual emerged within the first hour, mice did not venture into the central zone within the first hour in 11.94% of the experiments, and did not reach the foraging area in the very centre of the arena in 13.20% of all experiments. Individuals varied considerably in the amount of time they spent in the different zones whilst outside the dark chamber (active) within the first hour. In 97% (*n*=95) of the open field experiments the mice spent greater than 50% of their time active in the corners and edges.

During the first hour of the experiment, run had a significant effect on all behavioural characteristics except the mean distance moved (Table 4.3). In particular, the latency to emerge (Fig. 4.3A), the latency to forage (Fig. 4.3B), the time spent in the dark chamber (Fig. 4.3C) and time spent in the corners and edges (Fig. 4.3D) all generally decreased with time over the experimental runs. These characteristics were all significantly higher in run one compared with runs two and three. In contrast, time spent foraging (whilst active) was significantly lower in run one compared with runs two and three and generally increased over the three experimental runs (Fig. 4.3F). There was a weak interaction effect of run by

sex on the time spent in the dark chamber (Table 4.3), whereby males spent significantly longer in dark chamber than females in runs one and two, but not in run three (Fig. 4.3C). In general, males took longer to emerge from the dark chamber (Fig. 4.3A) and enter the foraging area (Fig. 4.3B), spent longer in the dark chamber (Fig. 4.3C) and in the corners and edges (Fig. 4.3D) and travelled less distance (Fig. 4.3F) than females. The percentage of time spent in the centre of the arena did not vary between sexes ($F_{1, 122} = 2.51$, $P = 0.085$) or runs ($F_{2, 130} = 2.51$, $P = 0.085$).

Relationships between behavioural variables in first hour

The relationships between the measured variables from the first hour of the experiment were examined using a correlation matrix for each run. However, correlation tests should be interpreted with caution and considered as indicative only as multiple tests can lead to false positive results (e.g. Type I errors). These issues were somewhat avoided by only regarding *r* values above ±0.6 as robust. Values where $r < \pm 0.6$ were not considered robust correlations regardless of whether $P < 0.05$. With the exception of amount of faeces produced, which was only significantly correlated with initial body mass and body mass change, most of the variables were significantly correlated with each other (Table 4.4). In general, the relationships observed between variables were similar between the three runs, though the strength of these relationships was variable. In run one (Fig. 4.4) several of the correlations were stronger and the number of significant correlations among variables was also greater than in subsequent runs (Fig. 4.5 & 4.6).

Seven correlations were consistently strong ($r \geq 0.6$) across all three runs:

i) **Latency to emerge from dark chamber** by **latency to enter centre** (positive).
ii) **Distance travelled** by **percentage of overall time in dark chamber** (negative).
iii) **Percentage of time spent in the corners and edges** by **percentage of time spent in the foraging area** (negative).
iv) **Latency to enter foraging area** by **latency to enter centre** (positive).
v) **Latency to emerge from dark chamber** by **latency to enter foraging area** (positive).
vi) **Distance travelled** by **latency to enter foraging area** (negative).
vii) Amount of **food eaten** by **change in body mass** (positive).

Multivariate analysis of behavioural responses

Principal components analysis was used to summarise the variation in the behavioural variables. With the exception of faeces and initial mass, most of the measured variables measured over the first hour aligned closely with the first principal component (PC1_1h) in all three runs. Within the first hour the amount of variance explained by PC1_1h decreased over the three runs (Table 4.5). In run one, PC1_1h explained 47.8% of the variance, and the second principal component (PC2_1h) explained a further 12.4%, for a combined total explanation of 60.2% of the variance in the measured traits (Fig. 4.17A). In the second run, PC1_1h accounted for 42.7% of the variance and PC2_1h explained a further 15.6% for a combined total explanation of 58.3% of the variance (Fig. 4.17B). In run three, the first two principal components explained the least amount of variance, with PC1_1h explaining 39.4% and PC2_1h explaining 13.9% for a combined total of 53.3% (Fig. 4.17C).

Individuals with a lower PC1_1h score emerged from the dark chamber and reached the centre and foraging areas earlier, spent less overall time in the dark chamber, consumed more food, travelled further and spent a greater proportion of their time, while outside of the dark chamber, in the centre and foraging zones as opposed to the edges or corners. As a result of how closely PC1_1h was associated with the behavioural variables important for identifying an individual's level of boldness (e.g. shorter latencies to explore novel areas and negative thigmotaxis), PC1_1h was interpreted as an index of how bold each individual was within the first hour of the experiment. Individuals were considered to be relatively bold if they had a lower PC1_1h score, whereas higher PC1_1h scores indicated shyer or more risk averse individuals. Principal component two was largely associated with the amount of faeces produced, initial body mass of the individual and body mass change over the experiment. Individuals with a lower PC2_1h value tended to have a higher initial body mass, produce more faeces over the course of the experiment and experience a higher change in body mass.

In each run, individuals were ranked according to their PC1_1h score, with the individual that had the lowest PC1_1h score (boldest) ranked one and the individual with the highest PC1_1h score (least bold) ranked 69 (Fig. 4.8). PC2_1h was not used to rank individuals over the first hour because it explained less than 15% of the variation in the dataset. For

50% of the population (*n* = 34), the change in PC1_1h ranks of the individuals over the three runs did not vary by more than 12 places (i.e. ranked units) (Fig. 4.9). Individuals in the top 10% (*n* = 7) of the population (ranked highest) in run one changed in rank, on average, 9 places over all runs. Whereas, individuals in the lowest 10% of the population (ranked lowest) in run one changed in rank, on average, 10 places over all runs (Fig. 4.9). An individual's PC1_1h rank in the first run was not correlated with its mean change in PC1_1h rank between the three runs (R=0.08, *df*= 65, *P*=0.55). The amount of variation in rank change between the second and third runs was slightly less than between the first and second runs (Fig. 4.8 and 9). For 50% of the population the change in PC1_1h rank, between runs one and two, was less than 12 places, whilst between runs two and three this decreased to 10 places. Specifically, the lowest 10% of the population (ranked lowest) in run one moved on average 12 places between runs one and two, and 9 places between runs two and three. The highest 10% (ranked highest) in run one moved 13 places between runs one and two, and 6 places between runs two and three.

A permutational analysis of variance (used to test the treatment effects on all measured behavioural traits (Table 4.1) in a single multivariate model) revealed that sex had a significant effect on the measured behaviour traits in runs one (PERMANOVA $F_{1, 63} = 4.62$, $P = 0.021$) and two (PERMANOVA $F_{1, 61} = 11.54$, $P = 0.001$), but not run three (PERMANOVA $F_{1, 62} = 2.29$, $P = 0.076$). In general, there was a slight tendency for females to have a lower PC1_1h score.

4.4.3 Fifteen hour open-field test

Behavioural variables

The seven mice that did not emerge from the dark chamber within the first hour were included within the analysis of the complete 15h OFT. Of these late emerging individuals, the longest latency to emerge from the dark chamber was 333 minutes (i.e. 5.6 hours). Compared to the first hour there was less variation among individuals in the amount of time spent within the dark chamber, with mice spending an average of 83.21% (*N* = 201; range: 40.91 to 99.05%) of the 15 hour experiment in the dark chamber (Table 4.2). In only 2% (*N*=4) of all experiments did any individual spend less than 50% of the 15 hour experiment outside of the dark chamber. During the 15 hour test mice spent a higher percentage of total time in the dark chamber ($F_{1,216} = 66.40$, $P < 0.001$), a lower percentage

of time active in the corners and edge zones ($F_{1,216}$ = 267.65, P < 0.001), a higher percentage of time active spent in the foraging area ($F_{1,216}$ = 463.82, P < 0.001) and covered more distance ($F_{1,216}$ = 368.29, P < 0.001) compared with the first hour. There was no significant difference in the percentage of time spent in the centre whilst active between the first hour and the entire experiment ($F_{1,216}$ = 12.62, P < 0.001).

Over the 15 hour experiment, run had a smaller effect on all behavioural measurements compared with the first hour (Table 4.3). Run had a significant effect on the percentage of active time spent in the corners and edges, as well as on the percentage of active time spent in the foraging area (Fig. 4.10). Mice on average spent more time in the corners and edges and less time in the foraging area in the first run. Sex had a significant effect on the distance travelled by the mice, whereby males travelled less distance (Fig. 4.10F) than females. Sex had no significant effect on the time spend in the dark chamber, corners and edges or the time spent foraging (Table 4.3). Similar to the first hour, the percentage of active time spent in the centre of the arena did not vary either between sexes ($F_{1,130}$ = 0.30, P = 0.586) or between runs ($F_{2,136}$ = 0.22, P = 0.801).

Relationships between behavioural measurements over 15 hour experiment

Correlation matrices were used to observe the relationships between the measured variables in all three runs for the data from the 15 hour experiment (Fig. 4.11, 4.12 and 4.13). There were fewer correlations, particularly strong correlations with r ≥ 0.6, in the entire experiment for all three runs compared with the first hour (Table 4.6). While the direction of the effects was consistent between the first hour and 15 hour experiment, the strength of the correlations were stronger within the first hour.

Of the seven correlations that were highly correlated (r > ±0.60) across all runs in the first hour five remained consistently highly correlated across all runs over the entire experiment:

i) **Latency to emerge from dark chamber** by **latency to enter centre** (positive).
ii) **Distance travelled** by **percentage of overall time in dark chamber** (negative).
iii) **Percentage of time active spent in the edges** by **percentage of time active spent in the foraging area** (negative).
iv) **Latency to enter foraging area** by **latency to enter centre** (positive).

v) **Amount of food eaten** by **change in body mass** (positive).

Distance and latency to reach foraging area were highly correlated in the first hour for all runs but the correlation between these two variables was much weaker over the 15 hour experiment in runs one and two, and in run two they were not significantly correlated.

Multivariate analysis of behavioural responses over 15 hour experiment

In general, the first two principle components explained the majority of data variance, with boldness characteristics lining up with PC1 and exploration characteristic lining up with PC2. The first principal component scores from the PCA for each run over the full experiment (PC1_15h) explained less of the variation compared to the PC1_1h whilst the second principal component scores (PC2_15h) accounted for more variation than in the first hour (Table 4.5). In run one, PC1_15h explained 30% of the variance and PC2_15h accounted for an additional 18.6% for a combined total explanation of 48.6% of the variance in the measured traits (Fig. 4.14A). In run two PC1_15h accounted for 29.9% of the variance and PC2_15h explained a further 20.6% for a total explanation of 50.5% of the variance (Fig. 4.14B). For the final run slightly more variance was explained with PC1_15h accounting for 32.8% and PC2_15h for 20.5% which when combined accounted for 53.3% of the variance (Fig. 4.14C).

Over the 15 hour experiment, PC1_15h was interpreted as an index for boldness as it was largely represented by latencies to enter novel areas (e.g. latency to emerge from dark chamber, and latency to enter the central and foraging areas) and amount of food consumed (see Table 4.5 for contributing PCA values of individual variables for each run). Individuals with a lower PC1_15h score tended to emerge from the dark chamber and first enter the central and foraging zones earlier, consume more food, produce more faeces and have a more positive change in body mass. The lower the PC1_15h score the bolder that individual was ranked. PC2_15h was largely represented by distance, percentage of active time spent in the centre and foraging areas, initial mass and percentage of overall time spent in the dark chamber (inactive) (Table 4.5). From these variables PC2_15h was interpreted as an index of exploration. Individuals with a lower PC2_15h score travelled further, spent more overall time outside of the dark chamber (active), and spent more of the time they were active in the corner and edge areas compared to the foraging area.

These individuals were determined to be more active than individuals with higher PC2_15h scores.

For each run all individuals were ranked according to their PC1_15h and PC2_15h scores, with a separate rank for each principal component. The individual with the lowest principal component scores were ranked one (Fig. 4.15 and 4.16). For 50% (n = 34) of the individuals, over all three runs the change in PC1_15h ranks did not vary by more than 13 places and the change in PC2_15h ranks did not vary by more than 11 places (Fig. 4.17). Rank order stability across all runs showed that the boldest and least bold individuals (i.e. those ranked in the top and bottom 10% for PC1_15h run one) changed in rank, on average, 16 and 9 places, respectively, over the three runs. The most and least explorative individuals (i.e. those ranked in the top and bottom 10% for PC2_15h run one) changed rank, on average, 19 and 6 places, respectively, over the three runs.

No correlation was observed between PC1_15h rank in the first run and mean change in PC1_15h rank over the three runs (R=0.013, df=66, P=0.916) or PC2_15h rank in the first run and mean change in PC2_15h rank over the three runs (R=0.129, df=66, P=0.296). Overall, the average rank change for PC1_15h and PC2_15h between runs one and two and two and three were very similar. Between runs one and two, 50% of the individual's PC1_15h rank did not change by more than 10 places, and 11 places between runs two and three (Fig. 4.17 A and B).

Rank order stability between individual runs showed that bolder individuals changed on average, 17 (between run one and two) and 13 (between runs two and three) places. The least bold individuals showed a smaller difference in average rank change between individual runs, moving on average 10 (between runs one and two) and 9 (between runs two and three) places. Whilst 50% of the individuals showed no rank change in exploration (PC2_15) between any of the runs the most and least explorative individuals had a more pronounced difference. The most explorative individuals changed in rank, on average, 21 (between runs one and two) and 16 (between runs two and three) places, and the least explorative individuals changed in rank, on average, five (between runs one and two) and eight (between runs two and three) places (Fig. 4.17 C and D).

A multivariate permutational analysis was used to test the effects of sex on all the measured behavioural traits at once. Sex did not have a significant effect on the measured behaviour traits (Table 4.1) in general in runs one (PERMANOVA, $F_{1, 66} = 2.40$, $P = 0.069$) and three (PERMANOVA, $F_{1, 66} = 1.56$, $P = 0.188$), but there was an effect of sex on the behavioural traits in general in run two (PERMANOVA, $F_{1, 66} = 3.60$, $P = 0.016$).

4.4.4 Repeatability of behaviour

Repeatability estimates were calculated from linear mixed models (LMM) that accounted for body mass and run. The individual rankings of PC1_1h, PC1_15h and PC2_15h scores were all repeatable over the three runs for both males and females (95% CI's non-overlapping with zero). The repeatability estimates did not differ significantly between sexes or between the three PCA rankings (overlapping 95% CI's) (Table 4.7). Including all individuals, the repeatability estimates of individual's PC1_1h ranks was 0.48 and the repeatability estimates of the individual's PC1_15h and PC2_15h ranks were both 0.46, showing that between 46 % and 48% of the total variance was associated with among individual differences in the intercept for the PCA rankings.

Additionally, for the whole population, all of the main measured behavioural variables from the first hour and the entire experiment were also repeatable over the three runs (Table 4.7). In general, the repeatability of these main behavioural variables were higher (by an average of 18%) over the 15 hour experiment than the first hour. For most of the behavioural variables the repeatability estimates did not differ significantly between sexes, though the repeatability estimates for latency to emerge from the dark chamber and latency to enter foraging area (over the entire run) were both significantly higher for females than males. The percentage of active time that males spent in the foraging area was the only behavioural variable that was not repeatable (95% CI overlapped with zero).

4.5 Discussion

This study found that wild derived house mice exhibit large and consistent differences in their behavioural responses to an open-field environment. These individual differences were substantially repeatable over the three months of measurements, which comprises the average expected lifespan of feral house mice (Pocock *et al.,* 2004). These results are important because they i) demonstrate that a large proportion of variation (~ 47%) in key

behavioural traits occurs among individuals, ii) that these among individual differences are highly repeatable over a mouse's natural lifetime, and iii) that some behavioural responses are correlated, which suggest the presence of consistent behavioural syndromes.

4.5.1 Effects of season, time and sex on mean expression of behaviour

The 69 individuals were measured in five groups that were staggered so that the OFTs took place throughout the year, which helped to account for the effect of season. A result of this staggering was that the first hour of the OFT (1700 h-1800 h) overlapped with the strongest shift in photoperiod over the seasons. Consequently, for some experiments the first hour of the OFT took place during dusk, which preliminary studies showed was when the colony were most active. As I found no effect of these groups on the main behavioural variables this indicates that the time of year the individuals were measured over had no effect on their behavioural responses. This was expected as the test area was illuminated for the entire experiment so the photoperiod each individual experienced did not vary between tests.

Over the course of the three runs the population appeared to become less risk averse. During the first hour of the OFT, I found evidence that run had a significant effect on all the main behavioural characteristics, except for distance moved. It has been proposed that based on the trade-off between current and future reproduction, individuals with higher future expectations in respect to reproductive success (e.g. sub-adults) would be more risk averse than those with lower expected future reproductive success (senescing adults; Wolf *et al.,* 2007). As younger individuals have not yet had the chance to reproduce they have more to lose by adopting risky behaviour in their natural habitat, therefore being more risk averse in their early life is more likely to lead to increased survival and reproductive opportunities. After maturation, there may be a selective advantage for males that invest in actively locating breeding females, and for females that invest more towards discovering food resources and nest sites in their home range. This hypothesis has been supported in other OFT studies that have shown that younger mice are shyer than older mice (Schuster *et al.,* 2017) and in dogs where boldness decreases with age (Starling *et al.,* 2013). My observations that the mice were less risk averse after the first run may also reflect habituation to an increasingly familiar environment in the OFT resulting from repeated exposure. Habituation to a novel environment can arise with repeated tests

(Grove and Thompson, 1970; Archer, 1973). Alternatively, the mice may have become more habituated to captivity as the first OFT occurred within two weeks of capture. Captivity has been shown to result in similar behavioural changes in captive-reared mice compared to wild populations, however the effect of captivity on wild-derived individuals is less often studied (Jones *et al.,* 2017). However, habituation also occurs in the wild and is believed to affect behaviour. As animals get accustomed to the specific variables in their home range (i.e. learn the location of hiding places and food sources) they can display more bold behaviour (Schuster *et al.,* 2017).

Our results showed an effect of sex on some of the behavioural response of the mice in the first hour of the OFT suggesting that male and female mice may vary in how they cope with a risky environment. Many studies using the OFT and other behavioural experiments (e.g. light-dark box) to measure behaviour have found no differences between sexes whilst others have observed consistent sex differences, and others still have only found an effect of sex during particular life stages (Courtney Jones *et al.,* 2017; Schuster *et al.,* 2017). I observed that in general, over the first hour of the OFT, females emerged from the dark chamber earlier, entered the foraging area quicker, covered greater distance and spent less time in the dark chamber compared to males. Additionally, when females were active in the arena they had lower levels of thigmotactic behaviour (percentage of time active in edge zone) than males, which is supported in the literature as male murine rodents are frequently recorded as being shyer than females (Donner and Lowry, 2013; Schuster *et al.,* 2017). Rodents often display sex-typical fear responses, whereby female rodents tend to adopt an active avoidance response faster than males in reaction to fear evoking stimuli. Whereas male rodents are more likely to respond to a fearful situation (e.g. novel environment) by freezing and remaining immobile, female rodents are more likely to adopt an escape behaviour (Archer, 1975; Blizzard *et al.,* 1975).

These results may imply that female dispersal behaviour in mice are under even stronger selection than male dispersal. Perhaps because, in the wild female mice dominate females born in later litters and supress the ability of younger females to reproduce within their natural deme. Due to their short lifespan in the wild, it is necessary that females reproduce as early as possible, so younger females are inclined to leave their natal deme to increase their chances of reproduction. Females are more likely than males to be

accepted into a non-native deme (Oakeshott, 1973; Gerlach, 1990; Voslajerova Bimova *et al.,* 2015). Increased ambulation in females compared to males in the OFT, as shown here, have been observed in numerous studies (Valle, 1970; Archer, 1975; Tatem *et al.,* 2014; Kokras and Dalla, 2014; Tucker *et al.,* 2017) but is not a widespread occurrence across all rodent species or among studies on house mice specifically (Schuster *et al.,* 2017). Inconsistent responses relating to the effect of sex on behaviour in the OFT may indicate that there are no consistent sex differences in behavioural traits. On the other hand, they are likely to be heavily influenced by variations in methodological conditions (e.g. experiment length, illumination levels, time of day, pretesting conditions) between studies that can alter the outcome variables. Additionally, it is quite likely that any sex differences would not be stable across all species. It is not known why we observe activity differences between males and females, although it has been speculated that they may reflect variations in foraging strategies in the wild or could have a hormonal basis. For example, there is evidence that oestrogen regulates open field activity in female rats as ovariectomies in female rats have shown to cause a decrease in ambulation in the OFT, whilst castration of males has no effect on their OFT activity (Blizard *et al.,* 1975). Not all the behavioural differences between sexes were consistent across all runs. Some of the behavioural response became more pronounced with time (e.g. latency to emerge from dark chamber and latency to enter foraging area), which could be a result of life stages or indicate possible differences in how males and females response to captivity, an understudied effect that is not understood.

4.5.2 Effects of OFT duration on mean expressions of behaviour

The higher activity of *M. musculus* shown within the first hour compared with the 15 hour experiment was expected as the first hour of the OFT overlapped with the time of day the colony was normally most active (early evening). The effects of run on measured behavioural responses became less significant as experimental time increased (i.e. in the 15-hour compared with the one hour experiment), demonstrating that longer measurement durations produce less variability. There were fewer sex differences in the population's behavioural responses over the 15h OFT compared to the first hour. This was expected, as has already been noted, female rodents are often shown to have increased levels of ambulation in the OFT (Valle, 1970; Archer, 1975; Tatem *et al.,* 2014; Kokras and Dalla, 2014; Tucker *et al.,* 2017). Sex only affected the distance moved over the 15h OFT

whereby females travelled further than males, as was shown in the first hour. This highlights how specific experimental conditions, such as measurement duration, can have significant effects on the outcome variables and bias measurement if individuals show temporal activity patterns differently.

4.5.3 Behavioural syndromes

Through repeatedly measuring multiple behavioural variables in a modified OFT I was able to quantify boldness and exploration in each individual. In the first hour of the OFT all the measured behavioural variables, except for initial mass and faeces produced, were correlated with each other and these correlations were strongest in the first run. The strongest relationships ($r > 0.60$) were between behavioural variables that were closely related, and I believed contributed to the same "personality trait". For example, latency to emerge from the dark chamber and latency to first enter the central foraging zone were strongly correlated and both these variables are important indicators for how bold an individual was. There was also a strong negative relationship between distance travelled and the percentage of overall time spent in the dark chamber, which together provide a good indication of activity. The percentage of time active spent in the edges (thigmotaxis) is frequently used as an indication of exploration, and though this variable was positively correlated with the latency variables, this relationship was much weaker than between the variables representing boldness. The same was true for the latency measurements and distance moved or percentage of time spent in the dark chamber. The first principal component from run one explained the most variance compared to the subsequent runs with the amount of variation explained by the first principal component decreasing gradually over the experimental period.

Within each run, the behavioural variables that underpinned the personality traits of boldness and exploration were not able to be separated by the PCA in the first hour of the OFT. Bolder individuals (emerged from dark chamber earlier) were also more thorough explorers as they covered more distance and spent less time in the dark chamber. Although boldness and exploration were both represented by PC1_h, for simplicity I referred to PC1_h as an index of boldness as the behavioural traits often used as measures of boldness (latency to emerge from dark chamber and latencies to enter exposed central and foraging zones) were the strongest contributors to this principal component. In the

first hour of the OFT there was a weak tendency for females to be bolder than males, though this sex difference was not significant. This provides additional support to the idea that differences in consistent behaviour (personality traits) are usually independent of sex.

The high positive correlation between boldness and exploration over the first hour of the OFT indicate the presence of slow-fast behavioural syndrome (animal personality) as was hypothesised and is in line with other studies on animal personalities (Koolhaas *et al.,* 1999; Sih *et al.,* 2004). Similar behavioural axes between boldness and exploration/activity subjected to antagonistic selection pressures are commonly observed in small mammals (Mazue *et al.,* 2015; Schuster *et al.,* 2017). These behavioural syndromes may be maintained by disruptive selection in stochastic environments where contrasting behavioural types provide increased fitness within relatively small intervals of the other (Sih *et al.,* 2004). Boldness is associated with risk taking behaviour and increased predation risk in *M. musculus* and exploration has a significant role as food resources are found heterogeneously through space and time (Bowers *et al.,* 1993, Reale *et al.,* 2007). Whilst highly explorative and bold individuals experience higher rates of predation, they also may be more likely to discover novel food resources or nesting sites and be more likely to survive environmental changes compared to less explorative and risk averse individuals. *Mus musculus* live in variable environments in which the fitness advance for different behavioural responses frequently change. For examples, population sizes increased dramatically during the summer and usually peak in autumn and then show significant reduction over winter (Gomez *et al.,* 2008). Bolder and more explorative mice may have a fitness advantage in higher competitive conditions, such as during periods of high population density. At such times fast behavioural types are more likely to achieve access to food resources, nesting areas and mating partners. Consequently, when populations have decreased (i.e. over winter and spring) slow behavioural types may have the fitness advantage as their lower activity levels provide a reduced risk of predation as wlel as conserving energy. It has also been suggested that such slow-fast relationships between boldness and exploration may be maintained through more complex associations with physiological traits or other life-history mechanisms that have all co-evolved together (Reale *et al.,* 2010). The observed combination of exploration and boldness as traits within a behavioural syndrome supports other studies on small mammals and rodents (Reale *et al.,* 2010; Lantova *et al.,* 2011; Dammhan, 2011; Herde and Eccard, 2013). Therefore, it can

be assumed that boldness and exploration are very common personality axis in small mammals.

There were less significant correlations between the main measured behavioural variables over the 15h OFT there were than seen in the first hour. As in the first hour, the strongest relationships among the behavioural variables were between variables that contributed to a single personality trait (e.g. boldness). In contrast to the first hour, the behavioural variables over the 15h OFT that indicated boldness (e.g. latency to emerge from dark chamber) were not correlated with the behavioural variables I believed underpinned exploration (e.g. distance moved, percentage of active time spent in the centre, edges and food zones). The PCAs from the 15h OFT runs had two principal components that explained the variation in the behavioural variables. The first component represented boldness whist the second component was used as an index of exploration. Therefore, over the entire 15h OFT boldness and exploration were not tightly associated in a behavioural syndrome as had been expected. I may not have detected a behavioural syndrome if the syndrome structure was fairly weak or because a behavioural syndrome composed of these traits did not exist. Over the entire 15h OFT there was an insignificant propensity for females to be more thorough explorers.

4.5.4 Rank order stability

In some species bolder individuals have been shown to display less variability in their behavioural responses then shyer individuals, which tend to be more responsive to cues in the environment (Bell *et al.,* 2009). For example, higher rigidity in the rank order of bolder individuals, compared to shyer individuals, has been observed in sticklebacks (Jolles *et al.,* 2014) and similar results have been shown in rodent species (Dochtermann and Jenkins, 2007). Alternatively, in mouse lemurs shyer individuals appear to be less variable in their behavioural responses indicating species specific differences (Verdolin and Harper, 2013). In the first hour of the OFT I found no difference in the consistency of rank order over all runs between the boldest and shyest individuals. Over the 15h OFT I did observe a difference in consistency between the boldest and shyest individuals whereby the bolder individuals appeared to show increased behavioural plasticity (higher variation in their rank change between OFT). Perhaps this effect was not seen in the first hour as the PCA for this period consisted of multiple personality traits (boldness and exploration) that may

have partially obscured the effect of boldness. The most thorough explorers in the population also showed reduced behavioural plasticity compared to the more superficial explorers. These results indicate significant ecological and evolutionary consequences as they show some individuals are limited in their ability to adapt to changing environmental conditions. Less bold (risk averse) individuals may be less capable of decoupling behavioural traits in different situations, constricting their capability to adapt to environmental change, and therefore demonstrating behavioural traits in situations that would seemingly be ill-suited (Dall *et al.,* 2004). As the opposite pattern, with bolder individuals showing less variation in their behavioural traits has been recorded in many species, it is likely that there are important species-specific differences in rank order stability that justify further study (Dochtermann and Jenkins, 2007; Bell *et al.,* 2009; Jolles *et al.,* 2014).

Individual variation in rank change between the second and third OFT were slightly less than between the first and second OFT, implying that individual boldness was more consistent between the last two OFT experiments compared to the first two. It is unknown whether this is due to habituation to the experiment and/or captivity, or development changes in the mice as they matured from sub-adults in the first run to adults in the second OFT. An individual's personality ranking in the first hour of the first OFT could not be used to predict how that individual's rank would change over the three experimental runs.

4.5.5 Repeatability

A central component of animal personality is that behavioural traits are repeatable over at least part of the individual's lifetime. As hypothesised, I found that individuals were consistent in all their behavioural responses from the OFT over the three runs. Specifically, individual rankings for boldness and exploration were repeatable in individual mice over a significant portion of their natural expected lifespan. Additionally, repeatability estimates did not differ between the boldness and exploratory measurements. The repeatability estimates for these behavioural traits were between 0.46 and 0.48 (Table 4.7), which is within the range of what has been recorded for behavioural traits in similar studies on rodents (Herde and Eccard, 2013; Schuster *et al.,* 2017) and other animals (Dingemanse *et al.,* 2002; Bell *et al.,* 2009; Patrick *et al.,* 2013). This high repeatability indicate there is

consistent variation in the behavioural responses among individuals and provides strong support for the repeatable nature of personality traits that has been shown in multiple taxa (Dingemanse *et al.,* 2002; Martin and Reale, 2007; Dhellemmes *et al.,* 2016**)**. As body mass and run (an indicator of age) were accounted for in the model, individual variations in the behavioural responses are not explained by body condition, age or individual experience at the time of measurements. A high repeatability may reflect possible high heritability of these traits (Boake, 1989; Martin and REale, 2007). However, some of these individual differences in behaviour could be attributed to environmental variables (e.g differences in resource abundance) in an individuals' home range that can alter its personality early in life (Martin and Reale, 2007).

I observed no differences in individual repeatability between the sexes for either boldness or exploration. Few studies have looked at sex differences in repeatability of behaviours over the length of an organism's natural expected lifespan. Studies that have touched upon the topic have produced conflicting results depending on the behavioural trait and species in question. In field crickets (*Gryllus integer*) boldness was found to be repeatable across life stages in females but not males (Hedrick and Kortet, 2011) whilst in other species males have been shown to have higher behavioural repeatability than females (Nakagawa *et al.,* 2007; Bell *et al.,* 2009; Dammhahn, 2012). Regarding research where males have been shown to display higher behavioural repeatability it has been proposed that females select males that exhibit consistent behaviour as reliable cues of potential mate quality (Schuett *et al.,* 2010). My results add to the evidence that any sex differences in behavioural repeatability cannot generalised across taxa and are likely to be species-specific (Dingemanse *et al.,* 2002; Schuster *et al.,* 2017).

The main individual behavioural variables (Table 4.7) were also all significantly repeatable though some differences in repeatability estimates between males and females were found, whereby females were slightly more repeatable in their latency to emerge from the dark chamber and latency to enter the foraging enclosure over the entire 15 hour OFT. This observed difference in repeatability between the sexes was very minor. These results contrast with other research that often show behavioural traits that indicate boldness, such as latency to emerge from dark chamber and enter central area in OFT, to be more repeatable in males than in females (Dammhahn, 2012). Females may be more repeatable

in their foraging effort, which is heavily influenced by their latency to emerge from dark chamber and enter the foraging area, because reproduction is more energetically costly for females than males (Schuett and Dall, 2009).

Generally, repeatability estimates for individual behavioural variables were higher over the 15 hour OFT than just the first hour. Considering the whole population this temporal difference in repeatability was never significant; however, for females specifically the repeatability of some of the main individual traits (percentage of time active spent in the edges and foraging area) were significantly more repeatable over the entire OFT than just the first hour.

All individuals included in this study were estimated to be sub-adults (5-8 weeks) during the first run and mature adults (over 8 weeks) for the subsequent two runs. These findings show that the behavioural traits are repeatable across life history stages, although as only one measurement was taken whilst the mice were sub-adults it is not possible to tell whether the strength of repeatability of the behavioural traits varied across life history stages as has been shown in other studies (Petelle *et al.,* 2013; Class and Brommer, 2016). As the ages of the individuals within this study were an estimate, it cannot be conclusively determined that individuals from different life stages were not included in this study, which if so, is likely to have affected the repeatability estimates.

4.5.6 Conclusion

In this study I observed consistent individual differences in boldness and exploration over a significant portion of the natural expected lifespan of house mice. Consistency of behaviour is an important condition if trying to use captive behaviour to predict responses in a natural environment. Although these results provide strong evidence for behavioural consistency in mice it should be noted that it is likely that the repeatability of the measured behavioural responses may vary when measured over longer periods. Recent studies have suggested that personality traits can go through a senescent decline in the wild or become less repeatable during sexual maturation and other significant stages of ontogeny (Stamps and Groothuis, 2010; Class and Brommer, 2016). Changes in the repeatability of behaviour over an individual's lifetime does not oppose the idea of animal personality but emphasises the importance of measuring behaviour across multiple life

history phases to understand how consistent behaviour is over an individual's entire lifetime. Long-term consistency in behavioural traits are predicted to have greater ecological and fitness consequences than short-term behavioural consistency (Luttbeg and Sih, 2010). Therefore, further research on the topic of consistent individual differences necessitate the use of more longitudinal studies to observe the consistency of behaviour over an individual's entire expected lifespan in the wild including multiple developmental stages.

I also observed some evidence for a behavioural syndrome linking boldness and exploration, but this was only evident from the behaviour variables measured over the first hour of the OFT, rather than the entire 15h experiment. Behavioural syndromes are believed to have crucial ecological impacts as the predicted correlations between behavioural traits and the limited behavioural plasticity may create trade-offs that limit the ability of a species to cope with a rapidly changing environment (Sih *et al.,* 2004). Despite the recognition that animal personality and behavioural syndromes may have significant interactions with ecological processes, there have been very few studies that have evaluated the expression of consistent behavioural traits and behavioural syndromes between laboratory and wild populations. The significance and need to incorporate longitudinal studies in natural conditions should not be overlooked.

Few studies have researched consistent individual differences and repeatability of the behavioural response of wild derived house mice to a novel situation in as substantial way as performed in the current study. My use of a modified OFT allowed us to measure multiple behavioural traits in a more ecologically relevant context than traditional OFTs enabling us to draw out different behavioural traits within a single context. Most research on consistent behavioural responses on mice have used laboratory strains of *M. musculus* but the relevance of their behaviour to that of wild derived *M.musculus* is frequently called into question (Fonio *et al.,* 2006). To understand the existence, maintenance and ecological consequences of behavioural differences in wild populations it is crucial to have an in-depth understanding of the interspecific differences within wild populations and be aware of how consistent different behavioural strategies are as has been described here.

Table 4.1. Abbreviations and definitions of behavioural variables measured from house mice (*Mus musculus*) during open field tests.

Behavioural variable	Abbreviation	Definition
Food consumed	Food	Amount of seed (g) consumed over the course of the entire experiment.
Faeces produced	Faeces	Amount of faeces (g) produced over the course of the entire experiment.
Initial body mass	Mass	Body mass (g) recorded immediately prior to the start of the experiment.
Change in body mass	Mass.Change	Difference in body mass recorded immediately prior to the open field experiment and then again after the end of each run.
Latency to emerge	Lat.Emerge	Time taken (s) from the start of the experiment to first emerge from the dark chamber.
Latency to foraging area	Lat.Food	Time taken (s) from the start of the experiment until first entry into the foraging zone.
Latency to enter centre	Lat.Centre	Time taken (s) from the start of the experiment until first entry into the central zone.
Time in dark chamber	Perc.Dark	The percentage of the whole experiment spent inside the dark chamber.
Time in edges	Perc.Edges	The percentage of total time active (outside of the dark chamber) spent within the corners, and edges of the field, and top of dark chamber, and sides of the dark chamber.
Time in foraging area	Perc.Food	The percentage of total time active (outside of the dark chamber) spent within the foraging zone.
Time in centre	Perc.Centre	The percentage of total time active (outside of the dark chamber) spent within the central zone.
Distance moved	Distance	Total distance (m) moved over the entire experiment.

Table 4.2. Descriptive statistics for behavioural characteristics of wild caught house mice (*Mus musculus*) during the first hour and whole 15 hour run over three open-field experimental runs. Mean ± standard deviation (SD) shown

Variable		First Hour						Whole Run (15 hours)					
		Females			Males			Females			Males		
		n	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD
Mass (g)	Run 1	36	14.36	1.95	31	16.05	2.35	-	-	-	-	-	-
	Run 2	36	14.35	1.71	31	16.68	2.27	-	-	-	-	-	-
	Run 3	36	14.95	1.81	31	16.96	2.02	-	-	-	-	-	-
	All	108	14.55	1.85	93	16.56	2.23	-	-	-	-	-	-
Mass.Change	Run 1	-	-	-	-	-	-	36	-0.63	0.61	31	-0.85	0.66
	Run 2	-	-	-	-	-	-	36	-0.29	0.67	31	-0.52	0.78
	Run 3	-	-	-	-	-	-	36	-0.39	0.76	31	-0.48	0.79
	All	-	-	-	-	-	-	108	-0.44	0.69	93	-0.62	0.74
Faeces (g)	Run 1	36	0.16	0.11	31	0.18	0.08	-	-	-	-	-	-
	Run 2	36	0.13	0.09	31	0.17	0.14	-	-	-	-	-	-
	Run 3	36	0.15	0.09	31	0.2	0.13	-	-	-	-	-	-
	All	108	0.15	0.1	93	0.18	0.12	-	-	-	-	-	-
Distance (m)	Run 1	36	125.62	92.31	31	73.02	62.81	36	1008.18	686.46	31	677.37	401.36
	Run 2	36	157.51	123.21	31	85.24	62.58	36	1031.04	860.84	31	582.33	320.60
	Run 3	36	123.75	82.69	31	90.80	68.27	36	987.91	797.60	31	659.74	428.22
	All	108	135.83	101.4	93	82.91	64.30	108	1009.18	777.98	93	639.74	384.14
Perc.Dark (%)	Run 1	36	74.44	19.52	31	83.25	15.44	36	82.78	11.71	31	85.65	10.19
	Run 2	36	62.46	22.12	31	78.56	17.05	36	79.66	11.89	31	86.37	7.70
	Run 3	36	68.40	20.64	31	71.94	22.30	36	81.56	8.42	31	84.36	8.96
	All	108	68.38	21.19	93	77.97	18.85	108	81.33	10.77	93	85.46	8.97
Perc.Edges (%)	Run 1	36	83.33	10.26	31	86.90	14.55	36	67.78	12.32	31	59.81	16.10
	Run 2	36	74.93	14.64	31	78.56	17.05	36	63.54	17.06	31	58.46	15.07
	Run 3	36	76.03	18.99	31	78.81	14.81	36	62.19	18.23	31	58.47	16.37
	All	108	78.07	15.37	93	82.90	12.39	108	64.28	16.08	93	58.91	15.70

Variable		First Hour						Whole Run (15 hours)					
		Females			Males			Females			Males		
		n	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*	Mean	SD
Perc.Food (%)	Run 1	36	7.89	6.96	31	5.39	6.77	36	25.54	11.93	31	31.34	13.21
	Run 2	36	16.49	12.68	31	9.10	7.76	36	29.15	18.40	31	33.66	11.48
	Run 3	36	16.23	19.08	31	15.21	12.80	36	30.28	19.11	31	34.26	15.35
	All	108	13.56	14.26	93	9.85	10.20	108	28.32	16.76	93	33.08	13.35
Perc.Centre (%)	Run 1	36	8.70	4.98	31	7.70	5.66	36	7.17	2.75	31	6.67	2.61
	Run 2	36	8.58	4.10	31	8.05	4.54	36	7.36	3.91	31	6.10	3.02
	Run 3	36	7.74	4.20	31	6.54	3.60	36	7.53	5.08	31	6.23	6.45
	All	108	8.34	4.42	93	7.43	4.68	108	7.35	3.99	93	6.33	3.09
Lat.Emerge (s)	Run 1	36	661.08	668.11	31	865.87	745.96	36	1341.03	3371.82	31	865.87	745.96
	Run 2	36	400.97	352.41	31	690.69	696.22	36	1392.03	4160.30	31	707.67	690.40
	Run 3	36	330.50	332.79	31	564.10	599.86	36	330.50	332.79	31	564.10	599.86
	All	108	464.18	492.97	93	708.83	687.80	108	1034.22	3135.35	93	714.23	685.91
Lat.Food (s)	Run 1	36	1652.14	1494.49	31	1978.77	1460.17	36	3508.05	6893.62	31	5297.81	8742.82
	Run 2	36	1064.46	1014.84	31	1466.13	1163.03	36	3127.27	9431.18	31	1815.23	2487.12
	Run 3	36	1156.08	1203.3	31	1455.93	1207.78	36	2509.24	6463.69	31	2162.19	4292.66
	All	108	1288.82	1266.28	93	1637.41	1295.18	108	3048.19	7567.50	93	3091.74	5952.40
Food eaten (g)	Run 1	-	-	-	-	-	-	36	1.04	0.69	31	0.89	0.49
	Run 2	-	-	-	-	-	-	36	1.44	0.60	31	1.28	0.55
	Run 3	-	-	-	-	-	-	36	1.43	0.65	31	1.62	0.88
	All	-	-	-	-	-	-	108	1.30	0.67	93	1.26	0.72

Table 4.3. The effects of sex and run, and their interaction, on the performance and behavioural characteristics of *M. musculus* from mixed-effect linear models (Fixed effects: Sex*Run+Body Mass, random effect: ID) and ANOVA test results. Data for behavioural characteristics were analysed for the first hour and over the entire 15 hour experimental run separately. *P*-values highlighted in bold indicate significance ($P < 0.05$).

Response variable	Period	Fig.	df	Run		Sex		Run × Sex	
				F_2	*P*	F_1	*P*	F_2	*P*
Performance characteristics									
Food	Whole run	2A	132	30.74	**< 0.001**	0.18	0.674	2.13	0.123
Faeces	Whole run	--	132	1.01	0.365	0.27	0.604	0.39	0.676
Mass.Change	Whole run	2B	132	19.75	**< 0.001**	2.21	0.142	0.91	0.405
Mass	Whole run	2C	132	9.15	**< 0.001**	20.28	**< 0.001**	1.46	0.237
Behavioural characteristics									
Lat.Emerge	First hour	3A	130	5.99	**0.003**	5.73	**0.020**	0.302	0.583
Lat.Food	First hour	3B	115	6.68	**0.002**	6.72	**0.012**	0.36	0.701
Perc.Dark	First hour	3C	126	7.49	**< 0.001**	68.26	**0.008**	3.15	**0.046**
	Whole run	10C	132	1.46	0.240	1.22	0.272	1.80	0.169
Perc.Edges	First hour	3D	126	8.65	**< 0.001**	7.16	**0.009**	2.14	0.122
	Whole run	10D	132	3.24	**0.042**	2.61	0.111	2.02	0.137
Perc.Food	First hour	3E	127	20.10	**< 0.001**	3.82	0.372	0.59	0.441
	Whole run	10E	132	3.36	**0.038**	2.41	0.100	0.13	0.880
Distance	First hour	3F	127	2.44	0.091	9.33	**0.003**	2.04	0.135
	Whole run	10F	127	0.10	0.909	5.04	**0.028**	0.33	0.723

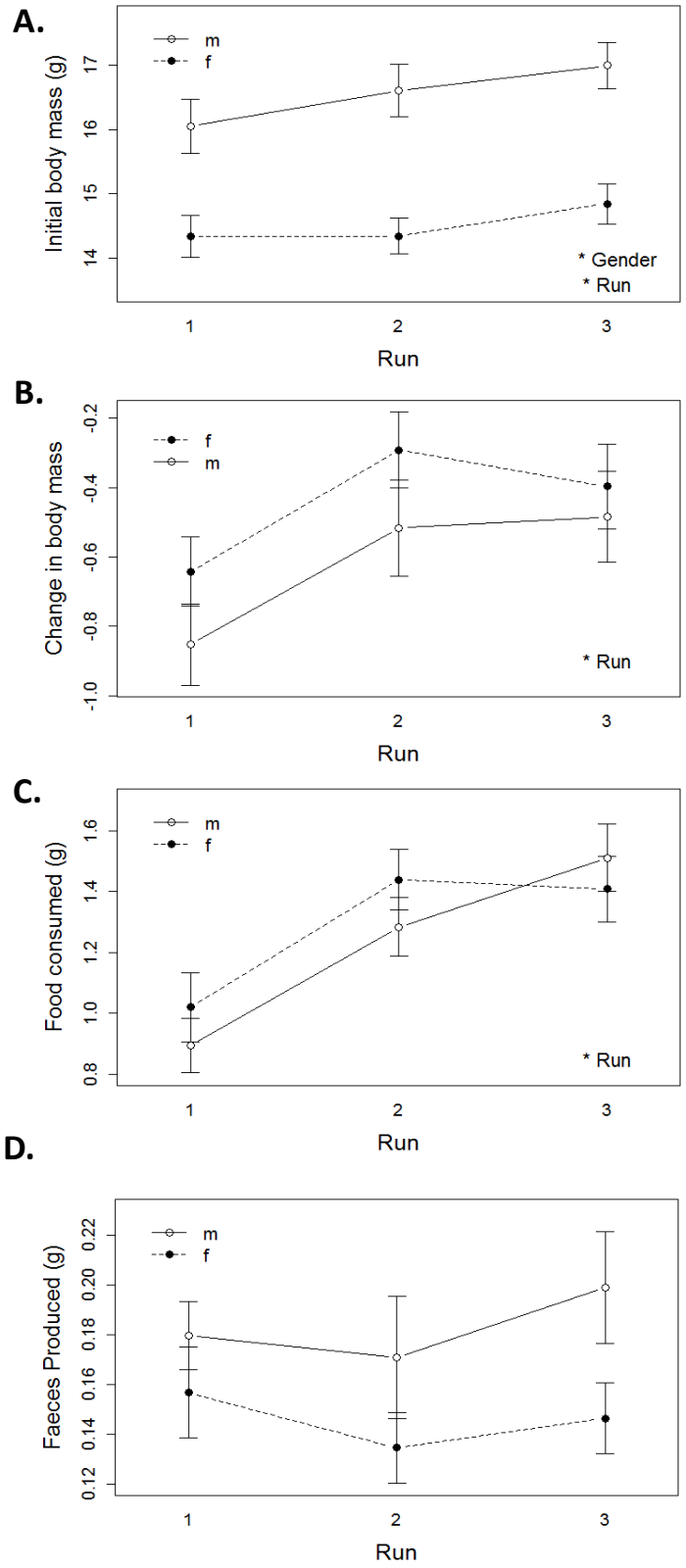


Figure 4.2. Effects of run and sex (males: open circles, females: closed circles) on initial body mass (A), body mass change (B), food consumed (C) and faeces produced (D) in *Mus musculus* over the entire 15 hour experiment. Shown are means ± SE. Significant variables from mixed models displayed by *($P < 0.05$). See table 3 for statistical analyses.

E.

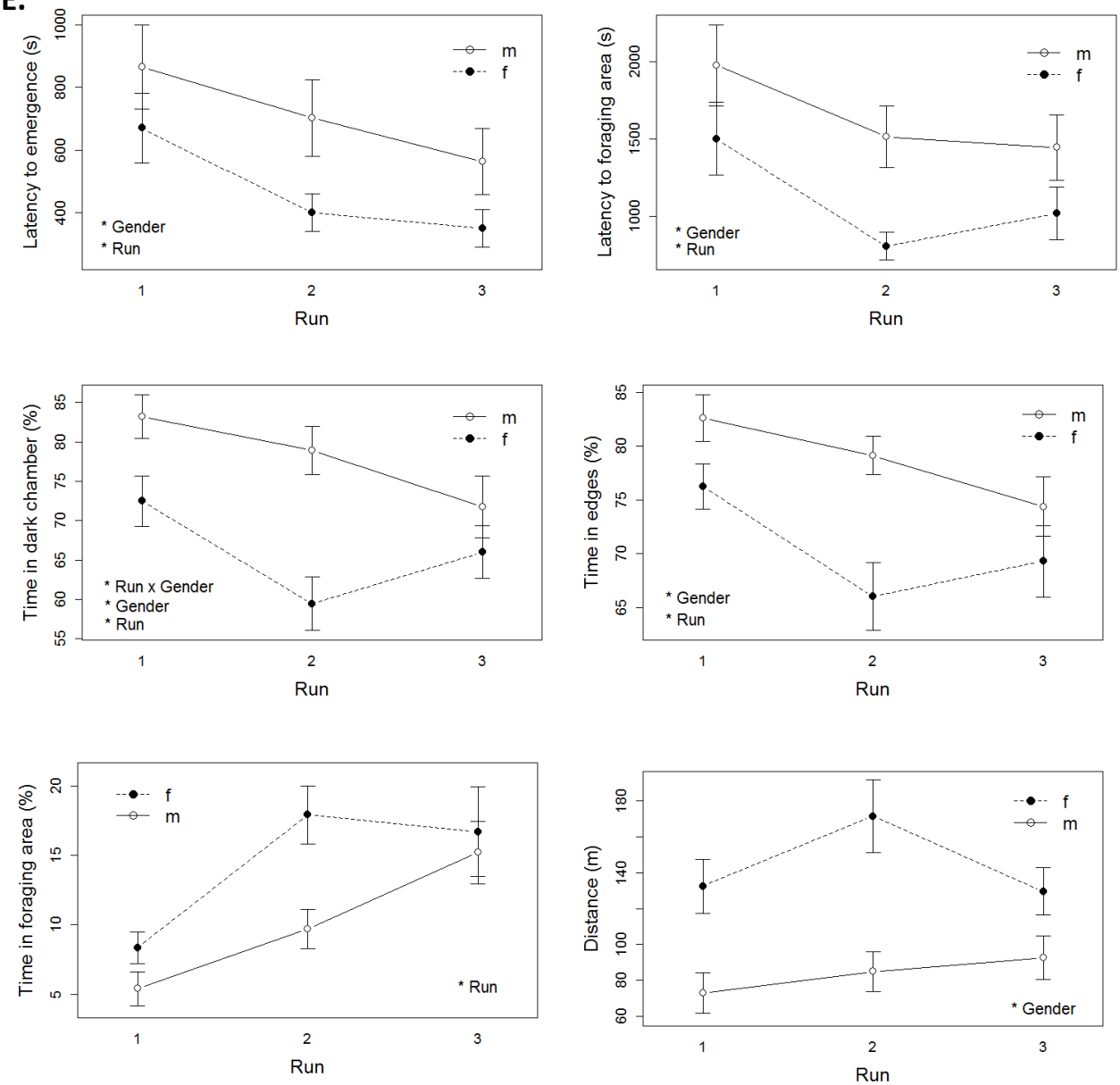


Figure 4.3. Effects of run and sex (females: closed circles, males: open circles) on latency to emerge (A), latency to enter foraging area (B), percentage of overall time in the dark chamber (C), percentage of active time in corners and edges (D), percentage of active time in foraging area (E) and distance moved (F) within the first hour of the open field test in *Mus musculus* . Shown are means ± SE. Significant variables from mixed models displayed by *($P < 0.05$). See table 3 for statistical analyses.

Table 4.4. Correlation coerfficients (*r*) and *P* values of the relationships between measured behavioural variables in the first hour of all three runs in *Mus musculus*. Significant results indicated in bold ($P < 0.05$).

	Run 1		Run 2			
Variables	*r*	*P*	*r*	*P*	*r*	*P*
Lat.Emerge & Food	-0.23	0.063	-0.06	0.650	-0.15	0.220
Lat.Emerge & Mass	0.10	0.410	-0.00	0.970	0.02	0.890
Lat.Emerge & Mass.Change	**-0.26**	**0.033**	-0.10	0.410	-0.17	0.170
Lat.Emerge & Distance	**-0.33**	**<0.01**	**-0.42**	**<0.01**	**-0.35**	**<0.01**
Lat.Emerge & Perc.Dark	**0.31**	**0.010**	**0.40**	**<0.01**	**0.42**	**<0.01**
Lat.Emerge & Perc.Edges	**0.25**	**0.043**	**0.35**	**<0.01**	**0.35**	**<0.01**
Lat.Emerge & Perc.Food	**-0.32**	**<0.01**	**-0.28**	**0.024**	**-0.37**	**<0.01**
Lat.Emerge & Lat.Food	**0.60**	**<0.01**	**0.61**	**<0.01**	**0.68**	**<0.01**
Lat.Emerge & Perc.Centre	-0.12	0.330	**-0.26**	**0.044**	**-0.27**	**0.031**
Lat.Emerge & Lat.Centre	**0.78**	**<0.01**	**0.71**	**<0.01**	**0.83**	**<0.01**
Food & Mass	-0.03	0.820	**-0.37**	**<0.01**	-0.00	0.970
Food & Mass. Change	**0.63**	**<0.01**	**0.64**	**<0.01**	**0.67**	**<0.01**
Food & Distance	**0.39**	**<0.01**	0.14	0.280	**0.34**	**<0.01**
Food & Perc.Dark	**-0.42**	**<0.01**	-0.24	0.053	-0.22	0.074
Food & Perc.Edges	**-0.39**	**<0.01**	**-0.35**	**<0.01**	**-0.28**	**0.024**
Food & Perc.Food	**0.49**	**<0.01**	**0.37**	**<0.01**	**0.41**	**<0.01**
Food & Lat.Food	**-0.30**	**<0.01**	**-0.30**	**0.016**	**-0.26**	**0.038**
Food & Perc.Centre	**0.35**	**0.003**	0.05	0.720	**0.34**	**<0.01**
Food & Lat. Centre	**-0.34**	**0.004**	**-0.25**	**0.048**	-0.23	0.063
Mass & Mass.Change	**-0.34**	**0.005**	**-0.55**	**<0.01**	**-0.35**	**<0.01**
Mass & Distance	**-0.26**	**0.032**	-0.24	0.053	-0.14	0.270
Mass & Perc.Dark	0.14	0.270	0.18	0.150	0.03	0.790
Mass & Perc.Edges	**0.34**	**0.006**	**0.28**	**0.025**	0.09	0.490
Mass & Perc.Food	-0.22	0.079	-0.24	0.054	-0.04	0.760
Mass & Lat.Food	0.18	0.140	0.24	0.058	0.15	0.230
Mass & Perc.Centre	-0.21	0.096	-0.09	0.470	**-0.26**	**0.038**
Mass & Lat.Centre	0.16	0.210	0.22	0.087	0.20	0.110
Mass.Change & Distance	**0.43**	**<0.01**	**0.26**	**0.042**	**0.36**	**<0.01**
Mass.Change & Perc.Dark	**-0.41**	**<0.01**	**-0.29**	**0.021**	**-0.37**	**<0.01**
Mass.Change & Perc.Edges	**-0.42**	**<0.01**	**-0.43**	**<0.01**	**-0.36**	**<0.01**
Mass.Change & Perc.Food	**0.43**	**<0.01**	**0.46**	**<0.01**	**0.48**	**<0.01**
Mass.Change & Lat.Food	**-0.26**	**0.032**	**-0.50**	**<0.01**	**-0.42**	**<0.01**
Mass.Change & Perc.Centre	**0.28**	**0.022**	0.21	0.096	**0.32**	**<0.01**
Mass.Change & Lat.Centre	**-0.34**	**0.005**	**-0.44**	**<0.01**	**-0.29**	**0.02**
Distance & Perc.Dark	**-0.87**	**<0.01**	**-0.84**	**<0.01**	**-0.77**	**<0.01**
Distance & Perc.Edges	**-0.67**	**<0.01**	**-0.56**	**<0.01**	**-0.38**	**<0.01**
Distance & Perc.Food	**0.81**	**<0.01**	**0.51**	**<0.01**	**0.37**	**<0.01**
Distance & Lat. Food	**-0.70**	**<0.01**	**-0.64**	**<0.01**	**-0.62**	**<0.01**
Distance & Perc.Centre	**0.62**	**<0.01**	**0.30**	**0.015**	**0.61**	**<0.01**
Distance & Lat.Centre	**-0.60**	**<0.01**	**-0.67**	**<0.01**	**-0.55**	**<0.01**
Perc.Dark & Perc.Edges	**0.57**	**<0.01**	**0.58**	**<0.01**	**0.42**	**<0.01**
Perc.Dark & Perc.Food	**-0.80**	**<0.01**	**-0.53**	**<0.01**	**-0.46**	**<0.01**
Perc.Dark & Lat.Food	**0.62**	**<0.01**	**0.57**	**<0.01**	**0.58**	**<0.01**
Perc.Dark &Perc.Centre	**-0.41**	**<0.01**	-0.23	0.07	**-0.25**	**0.046**
Perc.Dark & Lat.Centre	**0.51**	**<0.01**	**0.55**	**<0.01**	**0.45**	**<0.01**
Perc.Edges & Perc.Food	**-0.74**	**<0.01**	**-0.91**	**<0.01**	**-0.87**	**<0.01**
Perc.Edges & Lat. Food	**0.58**	**<0.01**	**0.60**	**<0.01**	**0.49**	**<0.01**
Perc.Edges &Perc.Centre	**-0.61**	**<0.01**	**-0.39**	**<0.01**	**-0.28**	**0.024**
Perc.Edges & Lat.Centre	**0.46**	**<0.01**	**0.57**	**<0.01**	**0.400**	**<0.01**
Perc.Food & Lat.Food	**-0.71**	**<0.01**	**-0.65**	**<0.01**	**-0.56**	**<0.01**
Perc.Food & Perc.Centre	**0.61**	**<0.01**	**0.27**	**0.032**	0.230	0.062
Perc.Food & Lat.Centre	**-0.57**	**<0.01**	**-0.57**	**<0.01**	**-0.410**	**<0.01**
Lat.Food & Perc.Centre	**-0.60**	**<0.01**	**-0.41**	**<0.01**	**-0.470**	**<0.01**
Lat.Food & Lat.Centre	**0.88**	**<0.01**	**0.93**	**<0.01**	**0.830**	**<0.01**
Perc.Centre & Lat.Centre	**-0.49**	**<0.01**	**-0.40**	**<0.01**	**-0.48**	**<0.01**

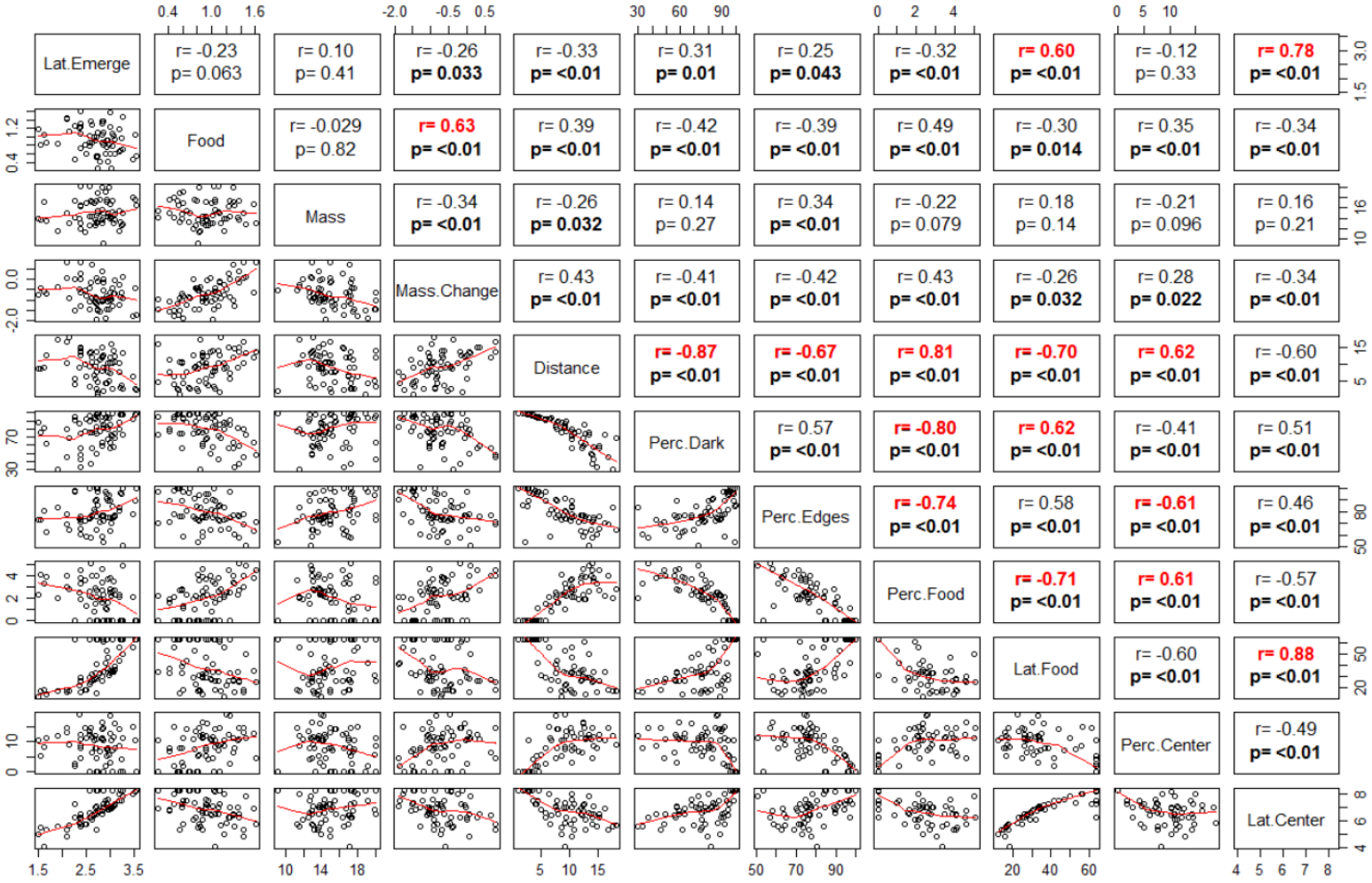


Figure 4.4. Correlation matrix of measured variables for *Mus musculus* in the first hour of run one. Scatter plots are shown in the bottom left of the graph and corresponding *r* and *P* values displayed in the top right panels. *r* values highlighted in red represent robust correlations (i.e. values above ±0.6). See Table 4.1 for variable descriptions.

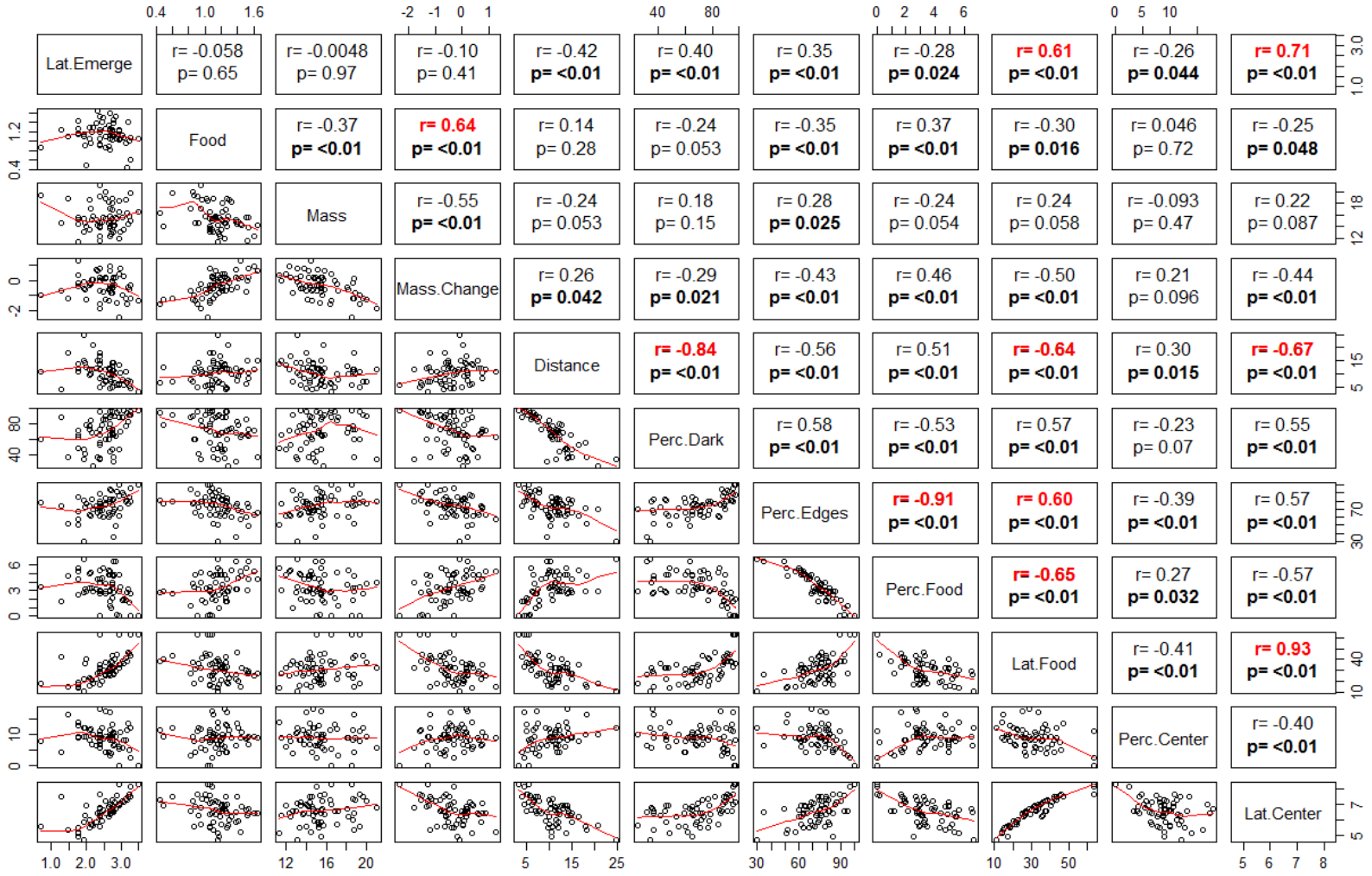


Figure 4.5. Correlation matrix of measured variables for all individuals in the first hour of run two. Scatter plots are shown in the bottom left of the graph and corresponding *r* and *P* values displayed in the top right panels *r* values highlighted in red represent robust correlations (i.e. values above ±0.6). See Table 4.1 for variable descriptions.

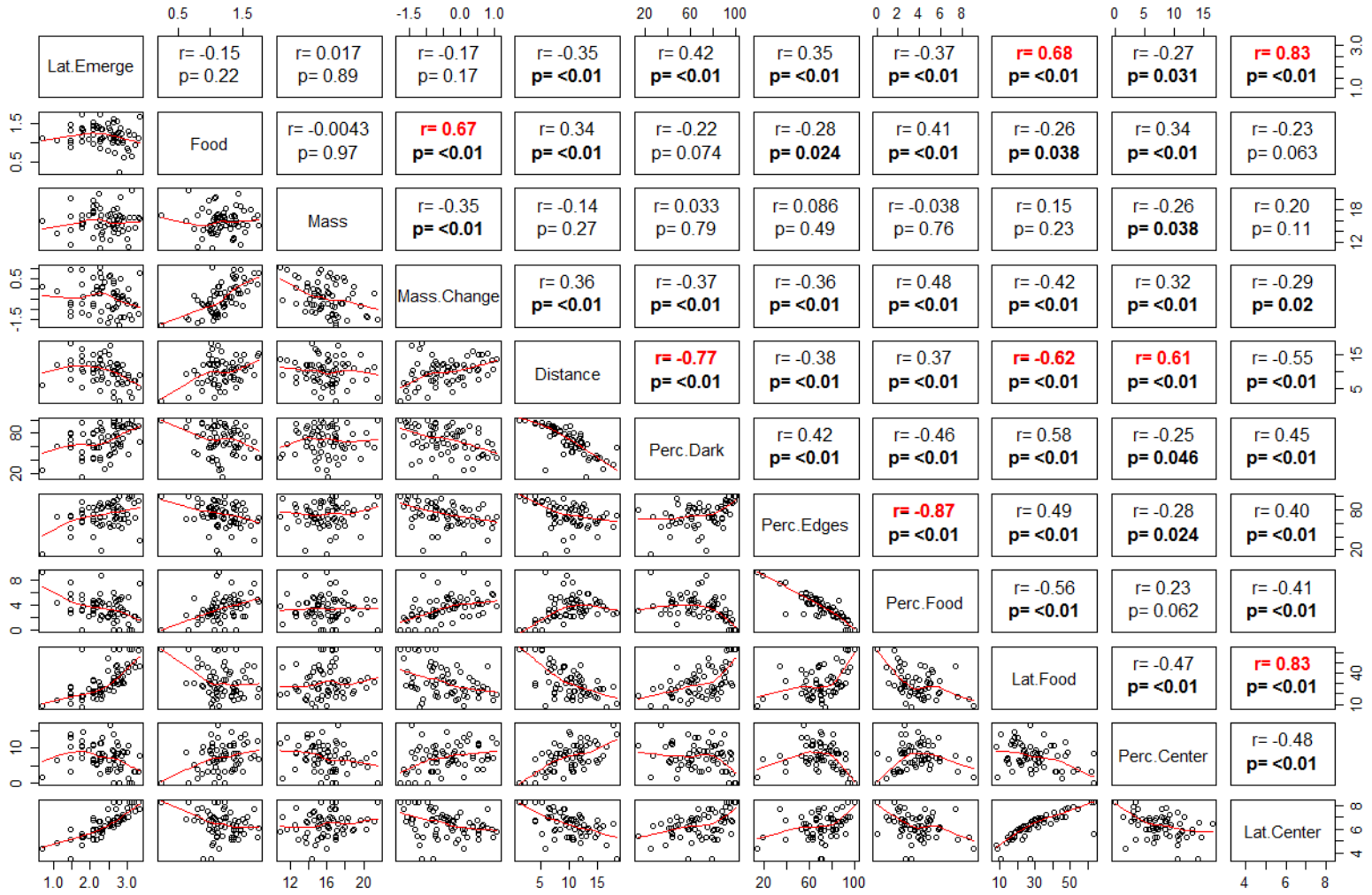


Figure 4.6. Correlation matrix of measured variables for all individuals in the first hour of run three. Scatter plots are shown in the bottom left of the graph and corresponding *r* and *P* values displayed in the top right panels *r* values highlighted in red represent robust correlations (i.e. values above ±0.6). See Table 4.1 for variable descriptions.

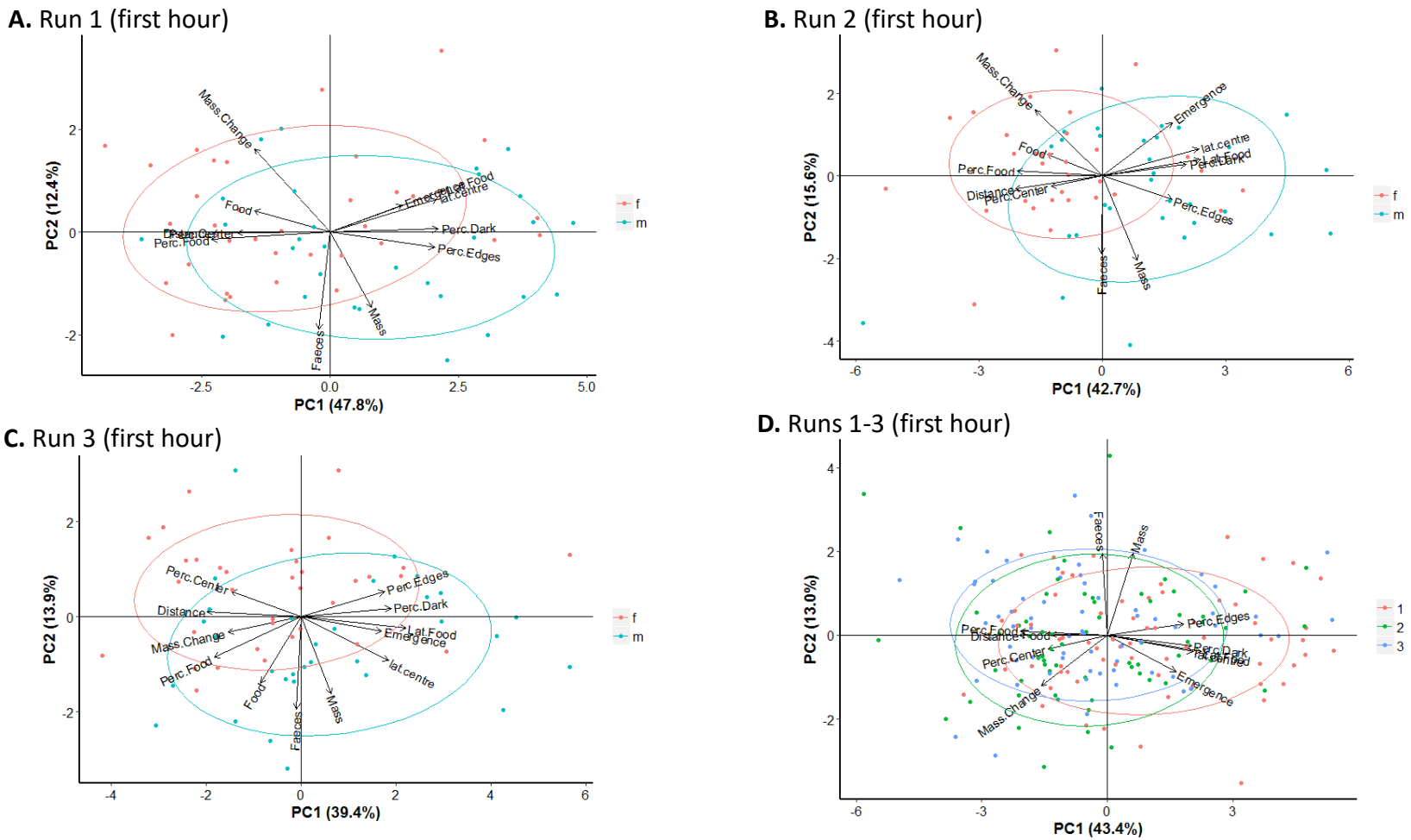


Figure 4.7. Biplots of the contribution of the measured variables to the first and second most explanatory principal components (over the first hour) of runs one (A), two (B) and three (C), and all runs combined (D) for *Mus musculus* . The percentage of total variance explained is shown in brackets for each principal component. Black lines represent the contribution of each measured variables to the principle components, with line length representing the strength of the contribution. Variables with arrows pointing in similar directions were positively correlated, whereas variables with arrows pointing in opposite directions were negatively correlated. Similarly, individuals in similar positions on the plot exhibited similar variation in the derived behavioural variables. Plots and ellipses (representing 68% of the predicted data) were coloured according to sex (A, B & C) and run (D). For variable descriptions see Table 1.

Table 4.5. The contribution of the measured variables of *Mus musculus* from the principal component analysis (PCA) to principal components one and two in the first hour and entire 15 hour experiment. For variable description see Table 1.

Behavioural variable	PC1_1h			PC2_hour			PC1_15h			PC2_15h		
	Run			Run			Run			Run		
	1	2	3	1	2	3	1	2	3	1	2	3
Faeces	-0.04	0.00	-0.02	-0.60	-0.51	-0.59	-0.06	-0.11	-0.11	0.03	-0.03	0.15
Lat.Emerge	0.23	0.28	0.30	0.17	0.35	-0.09	0.34	0.36	0.32	-0.01	-0.01	0.03
Food	-0.24	-0.22	-0.15	0.13	0.14	-0.43	-0.35	-0.42	-0.35	0.25	-0.10	0.12
Mass	0.13	0.14	0.11	-0.47	-0.56	-0.50	0.19	0.08	0.08	0.28	0.27	0.34
Mass.Change	-0.24	-0.27	-0.28	0.51	0.44	-0.10	-0.33	-0.31	-0.35	0.18	-0.12	0.01
Distance	-0.37	-0.35	-0.36	0.00	-0.08	0.03	-0.29	-0.15	-0.10	-0.43	-0.54	-0.49
Perc.Dark	0.34	0.34	0.34	0.02	0.08	0.06	0.30	0.24	0.23	0.33	0.42	0.39
Perc.Edges	0.33	0.28	0.32	-0.09	-0.16	0.16	0.20	0.26	0.37	-0.46	-0.37	-0.25
Perc.Food	-0.37	-0.34	-0.33	-0.04	0.03	-0.26	-0.26	-0.32	-0.38	0.50	0.41	0.34
Lat.Food	0.36	0.40	0.40	0.26	0.11	-0.07	0.37	0.43	0.40	0.09	-0.08	0.14
Perc.Centre	-0.29	-0.21	-0.27	-0.01	-0.07	0.16	-0.17	0.05	-0.01	-0.25	-0.37	-0.49
Lat.Centre	0.33	0.39	0.33	0.20	0.18	-0.28	0.41	0.38	0.36	0.05	-0.01	0.14
Eigenvalues	*2.39*	*2.26*	*2.19*	*1.22*	*1.37*	*1.28*	*1.91*	*1.89*	*2.00*	*1.50*	*1.57*	*1.57*

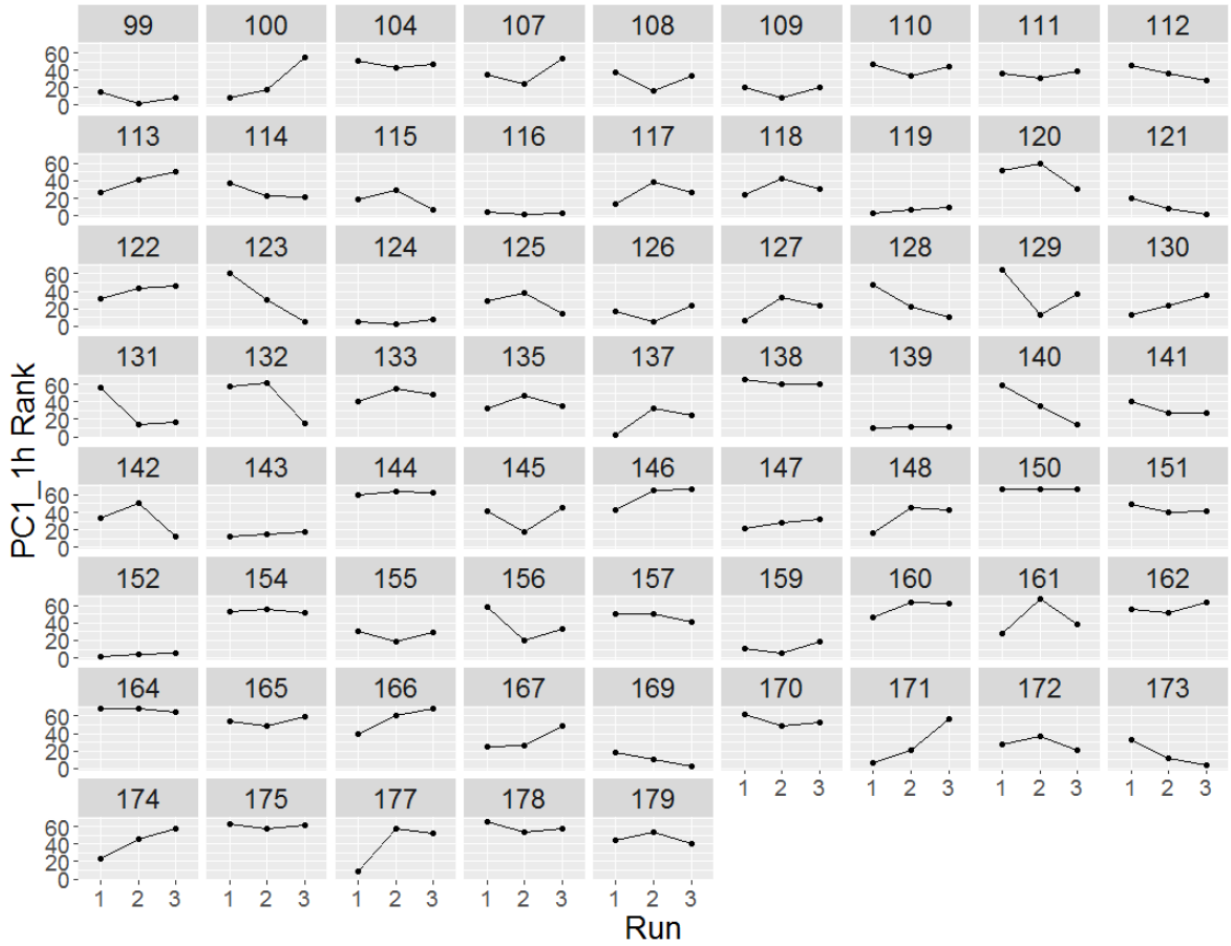


Figure 4.8. Plots showing the PC1 rank of *Mus musculus* from the first hour of the open-field test as a function of the run for each individual (numbered in each panel).

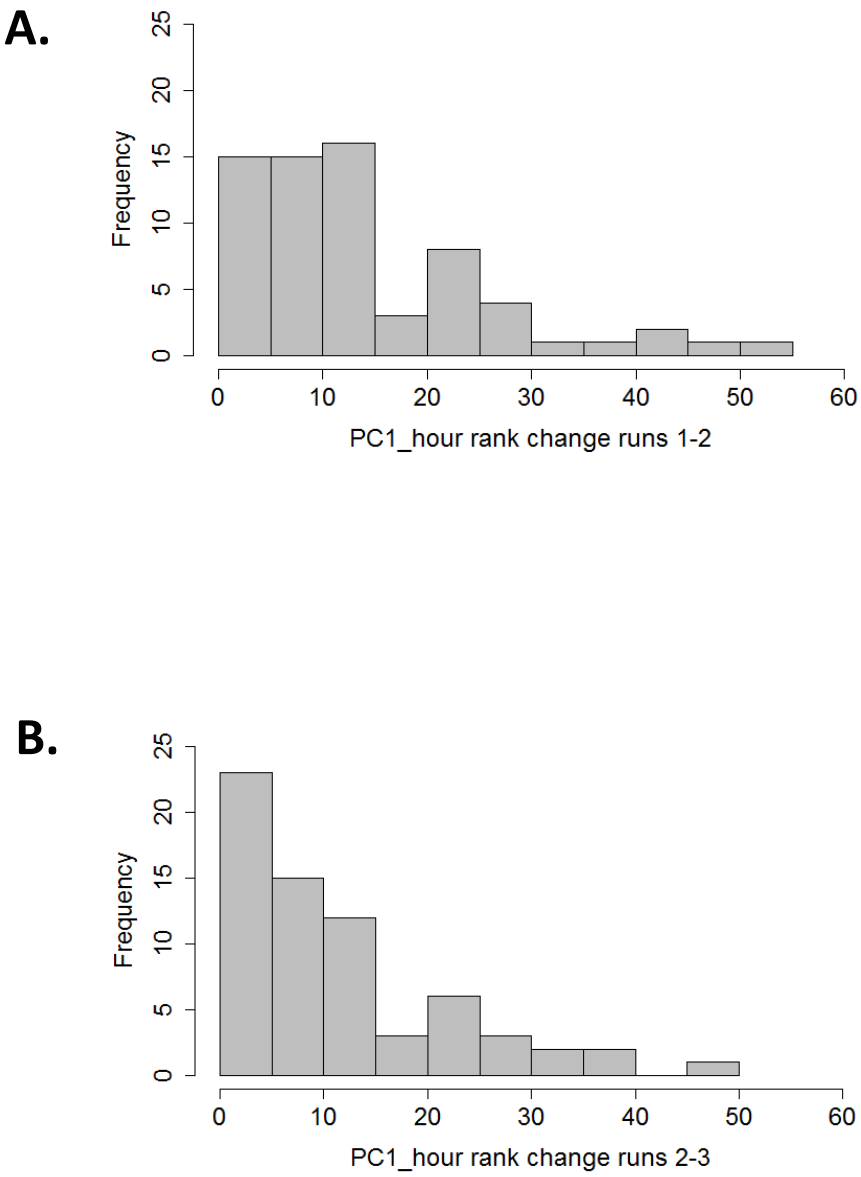


Figure 4.9. Comparing *Mus musculus* individuals' PC1_1h rank change for the entire population between consecutive open field test runs one and two (A) and two and three (B).

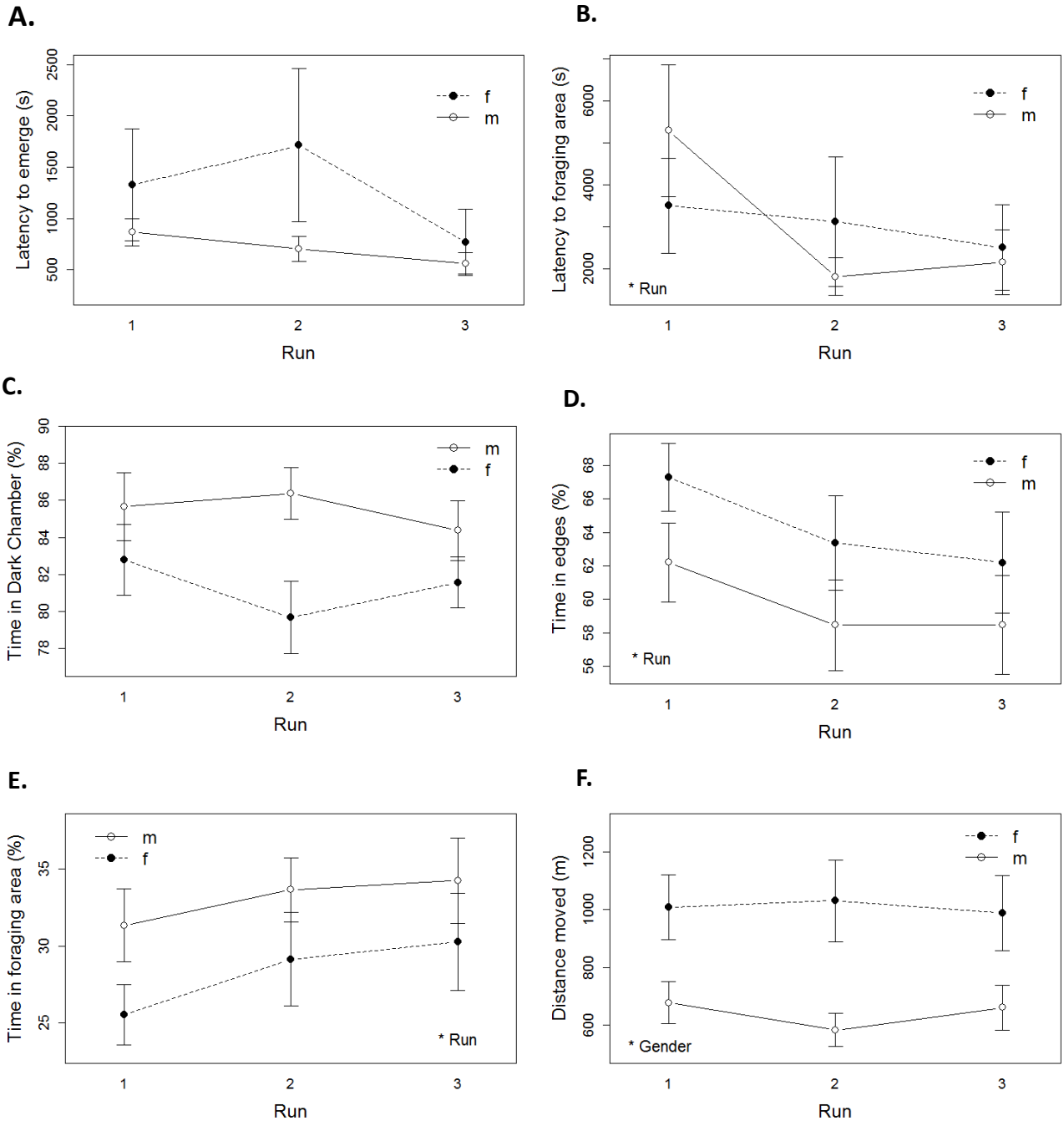


Figure 4.10. Effects of run and sex (females: closed circles, males: open circles) on *Mus musculus* latency to emerge (A), latency to enter foraging area (B), percentage of overall time in the dark chamber (C), percentage of active time in corners and edges (D), percentage of active time in foraging area (E) and distance moved (F) over the entire 15 hour open field test experiment. Shown are the mean ± SE. Significant variables from mixed models displayed by *($P < 0.05$). See table 3 for statistical analyses.

Table 4.6. Correlation coefficients (*r*) and *P* values of the relationships between measured behavioural variables of *Mus musculus* from the whole open field test expeirment over all three runs. Significant results indicated in bold ($P < 0.05$).

	Run 1		**Run 2**		**Run 3**	
Variables	*r*	*P*	*r*	*P*	*r*	*P*
Lat.Emerge & Food	**-0.26**	**0.03**	-0.15	0.23	-0.23	0.06
Lat.Emerge & Mass	0.10	0.41	-0.11	0.37	-0.05	0.70
Lat.Emerge & Mass.Change	**-0.32**	**<0.01**	-0.13	0.28	-0.20	0.11
Lat.Emerge & Distance	-0.22	0.08	-0.11	0.40	-0.02	0.87
Lat.Emerge & Perc.Dark	0.21	0.08	0.15	0.22	0.23	0.06
Lat.Emerge & Perc.Edges	0.19	0.13	0.02	0.88	**0.32**	**<0.01**
Lat.Emerge & Perc.Food	-0.24	0.05	-0.10	0.43	**-0.30**	**0.01**
Lat.Emerge & Lat.Food	**0.46**	**<0.01**	**0.64**	**<0.01**	**0.58**	**<0.01**
Lat.Emerge & Perc.Centre	-0.07	0.57	0.12	0.34	-0.06	0.60
Lat.Emerge & Lat.Centre	**0.74**	**<0.01**	**0.82**	**<0.01**	**0.84**	**<0.01**
Food & Mass	-0.03	0.80	**-0.26**	**0.04**	-0.01	0.97
Food & Mass. Change	**0.63**	**<0.01**	**0.62**	**<0.01**	**0.68**	**<0.01**
Food & Distance	0.20	0.10	**0.34**	**<0.01**	**0.26**	**0.04**
Food & Perc.Dark	**-0.32**	**<0.01**	**-0.43**	**<0.01**	**-0.37**	**<0.01**
Food & Perc.Edges	**-0.56**	**<0.01**	-0.24	0.06	**-0.59**	**<0.01**
Food & Perc.Food	**0.56**	**<0.01**	**0.35**	**<0.01**	**0.66**	**<0.01**
Food & Lat.Food	**-0.28**	**0.02**	-0.21	0.09	**-0.49**	**<0.01**
Food & Perc.Centre	0.07	0.57	-0.09	0.50	-0.05	0.67
Food & Lat. Centre	**-0.38**	**<0.01**	**-0.28**	**0.02**	**-0.33**	**<0.01**
Mass & Mass.Change	**-0.33**	**<0.01**	**-0.50**	**<0.01**	**-0.35**	**<0.01**
Mass & Distance	**-0.32**	**<0.01**	-0.23	0.07	-0.16	0.19
Mass & Perc.Dark	012	0.06	0.20	0.11	0.18	0.14
Mass & Perc.Edges	-0.04	0.74	-0.06	0.61	0.02	0.87
Mass & Perc.Food	0.10	0.43	0.14	0.28	0.05	0.67
Mass & Lat.Food	**0.31**	**<0.01**	0.02	0.84	0.17	0.16
Mass & Perc.Centre	**-0.27**	**0.03**	-0.20	0.11	**-0.38**	**<0.01**
Mass & Lat.Centre	0.23	0.06	0.01	0.91	0.17	0.16
Mass.Change & Distance	0.14	0.26	0.15	0.24	0.14	0.26
Mass.Change & Perc.Dark	-0.14	0.25	-0.20	0.11	**-0.26**	**0.03**
Mass.Change & Perc.Edges	**-0.39**	**<0.01**	-0.07	0.59	**-0.50**	**<0.01**
Mass.Change & Perc.Food	**0.39**	**<0.01**	0.11	0.38	**0.56**	**<0.01**
Mass.Change & Lat.Food	**-0.28**	**0.02**	**-0.27**	**0.02**	**-0.43**	**<0.01**
Mass.Change & Perc.Centre	0.13	0.30	-0.12	0.35	-0.03	0.83
Mass.Change & Lat.Centre	**-0.41**	**<0.01**	**-0.31**	**0.01**	**-0.33**	**<0.01**
Distance & Perc.Dark	**-0.78**	**<0.01**	**-0.82**	**<0.01**	**-0.71**	**<0.01**
Distance & Perc.Edges	-0.01	0.95	**0.29**	**0.02**	0.08	0.54
Distance & Perc.Food	-0.06	0.62	**-0.40**	**<0.01**	-0.14	0.25
Distance & Lat. Food	**-0.28**	**0.02**	-0.02	0.87	**-0.27**	**0.03**
Distance & Perc.Centre	**0.43**	**<0.01**	**0.42**	**<0.01**	**0.50**	**<0.01**
Distance & Lat.Centre	**-0.24**	**0.05**	-0.17	0.18	-0.19	0.13
Perc.Dark & Perc.Edges	0.13	0.34	**-0.27**	**0.03**	0.17	0.17
Perc.Dark & Perc.Food	-0.05	0.66	**0.32**	**<0.01**	-0.12	0.35
Perc.Dark & Lat.Food	**0.27**	**0.03**	0.07	0.55	**0.36**	**<0.01**
Perc.Dark &Perc.Centre	**-0.34**	**<0.01**	**-0.25**	**0.04**	**-0.30**	**0.01**
Perc.Dark & Lat.Centre	**0.26**	**0.04**	0.17	0.17	**0.29**	**0.02**
Perc.Edges & Perc.Food	**-0.97**	**<0.01**	**-0.86**	**<0.01**	**-0.93**	**<0.01**
Perc.Edges & Lat. Food	**0.32**	**<0.01**	0.18	0.14	**0.35**	**<0.01**
Perc.Edges &Perc.Centre	**-0.28**	**0.02**	0.16	0.21	0.08	0.54
Perc.Edges & Lat.Centre	**0.28**	**0.02**	0.11	0.36	**0.27**	**0.03**
Perc.Food & Lat.Food	**-0.36**	**<0.01**	**-0.26**	**0.04**	**-0.40**	**<0.01**
Perc.Food & Perc.Centre	0.10	0.43	**-0.38**	**<0.01**	**-0.28**	**0.02**
Perc.Food & Lat.Centre	**-0.33**	**<0.01**	-0.19	0.13	**-0.27**	**0.03**
Lat.Food & Perc.Centre	-0.08	0.51	**0.28**	**0.02**	-0.12	0.33
Lat.Food & Lat.Centre	**0.80**	**<0.01**	**0.84**	**<0.01**	**0.79**	**<0.01**
Perc.Centre & Lat.Centre	0.02	0.85	0.20	0.11	-0.11	0.36

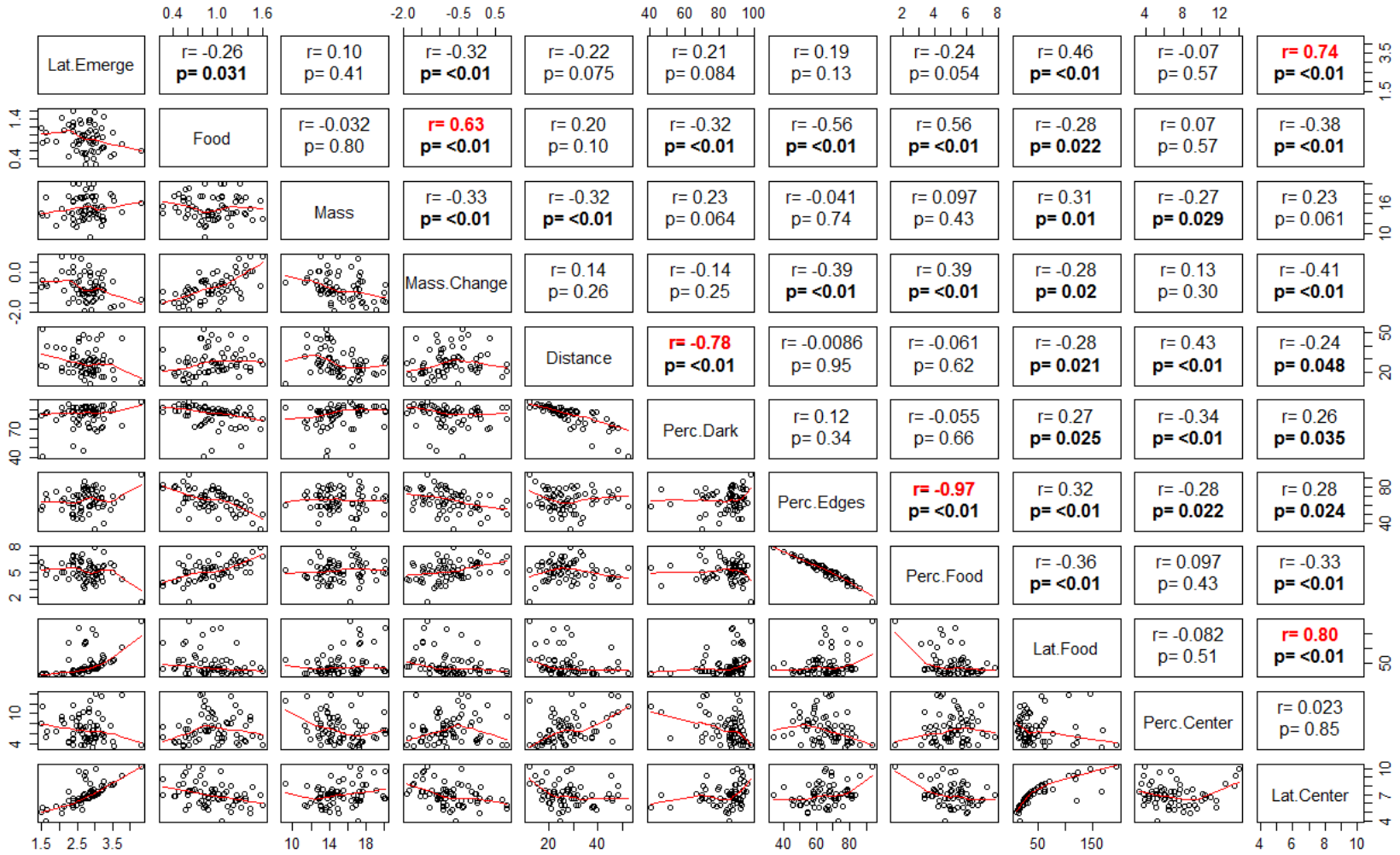


Figure 4.11. Correlation matrix of measured variables for all individuals from the whole experiment in run one. Scatter plots are shown in the bottom left of the graph and corresponding *r* and *P* values displayed in the top right panels. *r* values highlighted in red represent robust correlations (i.e. values above ±0.6). See Table 4.1 for variable descriptions.

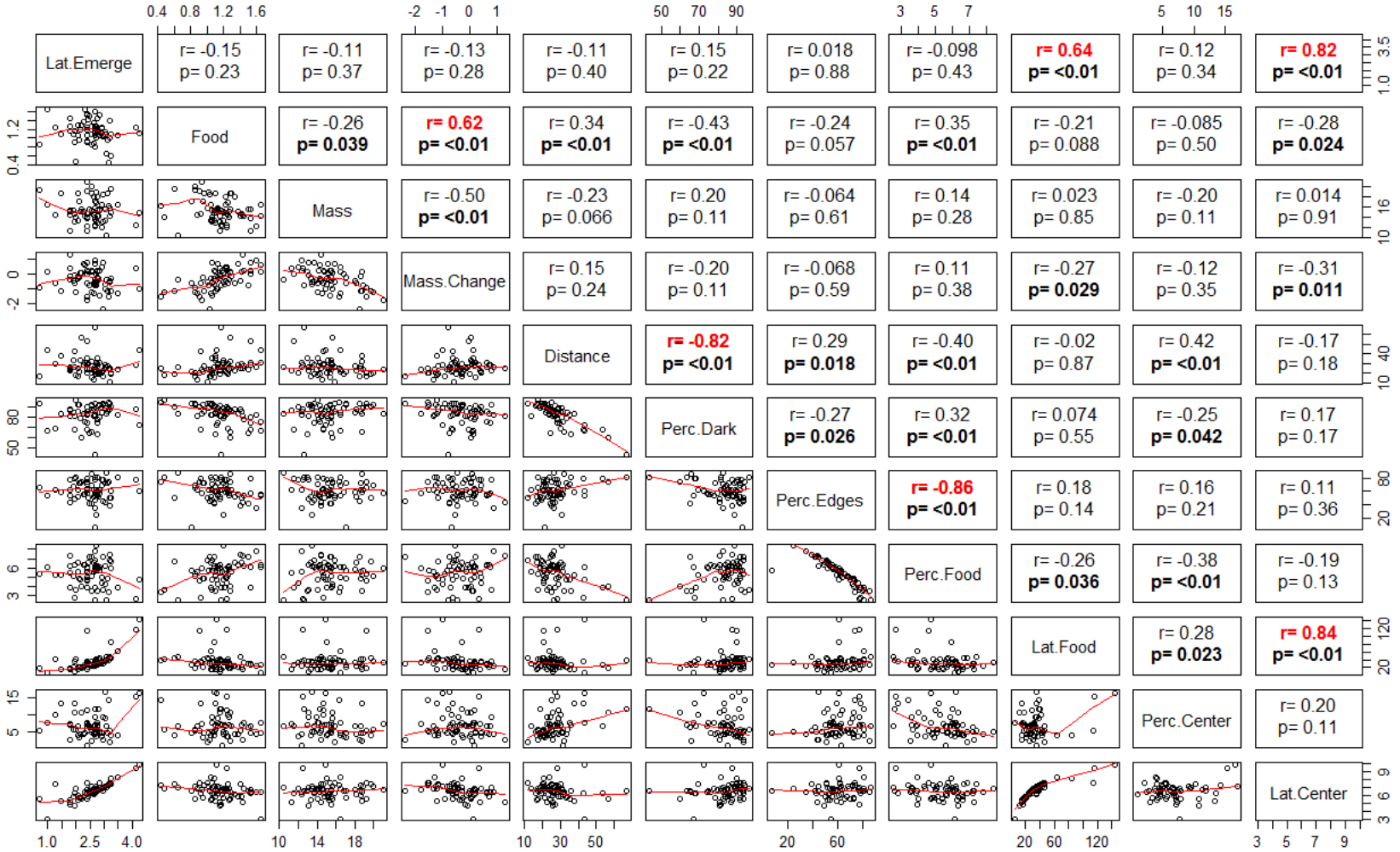


Figure 4.12. Correlation matrix of measured variables for all individuals from the whole experiment in run two. Scatter plot are shown in the bottom left of the graph and corresponding r and p values displayed in the top right panels. *r* values highlighted in red represent robust correlations (i.e. values above ±0.6). See Table 4.1 for variable descriptions.

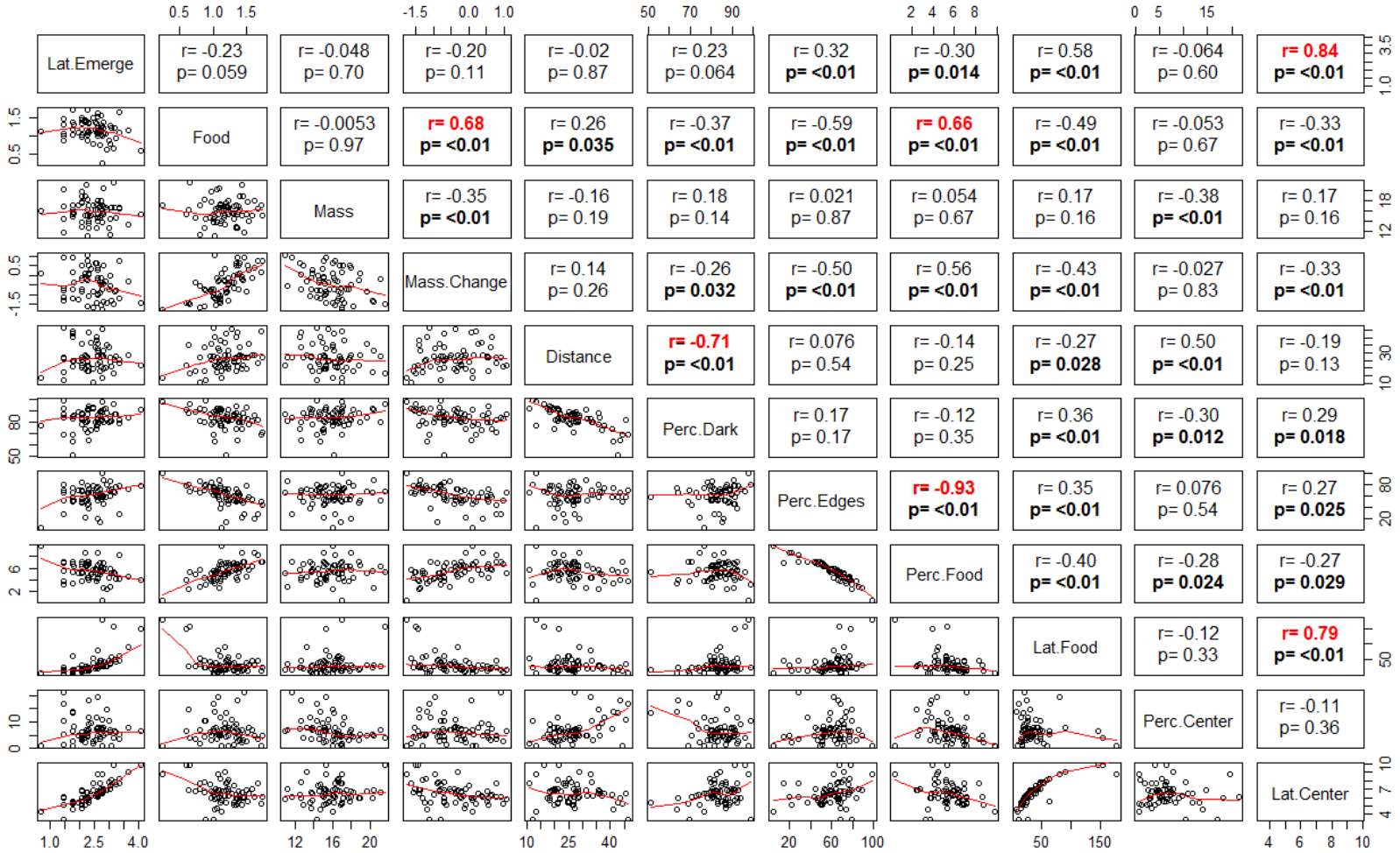


Figure 4.13. Correlation matrix of measured variables for all individuals from the whole experiment in run three. Scatter plot are shown in the bottom left of the graph and corresponding r and p values displayed in the top right panels. *r* values highlighted in red represent robust correlations (i.e. values above ±0.6). See Table 4.1 for variable descriptions.

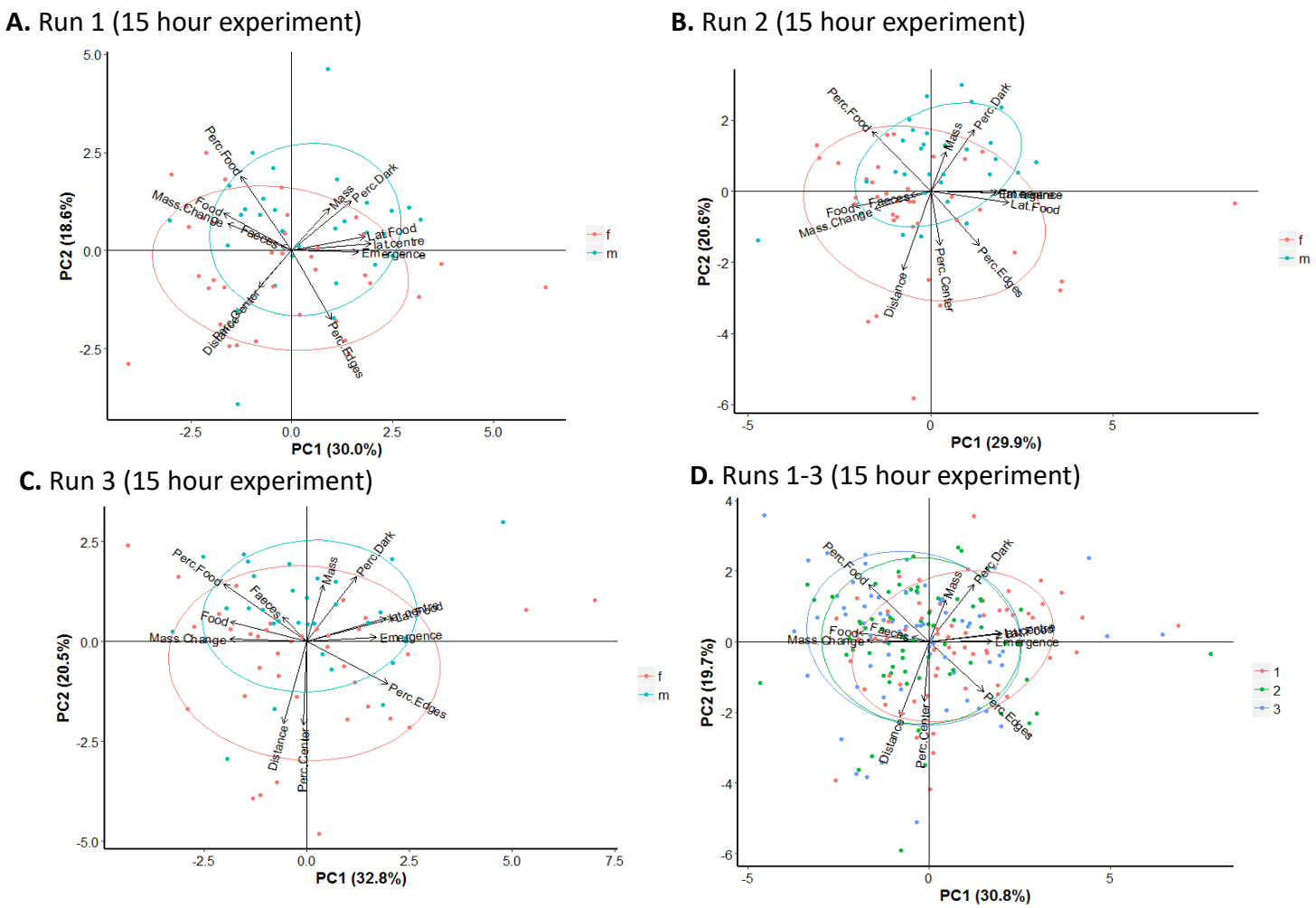


Figure 4.14. Biplots of the contribution of the measured variables to the first and second most explanatory principal components (over the whole experiment) for runs one (A), two (B) and three (C) and all runs combined (D). The percentage of total variance explained is shown in brackets for each principal component. Black lines represent the contribution of each measured variables to the principle components, with line length representing the strength of the contribution. Variables with arrows pointing in similar directions were positively correlated, whereas variables with arrows pointing in opposite directions were negatively correlated. Similarly, individuals in similar positions on the plot exhibited similar variation in the derived behavioural variables. Plots and ellipses (representing 68% of the predicted data) were coloured according to sex (A, B & C) and run (D). See Table 1 for variable descriptions.

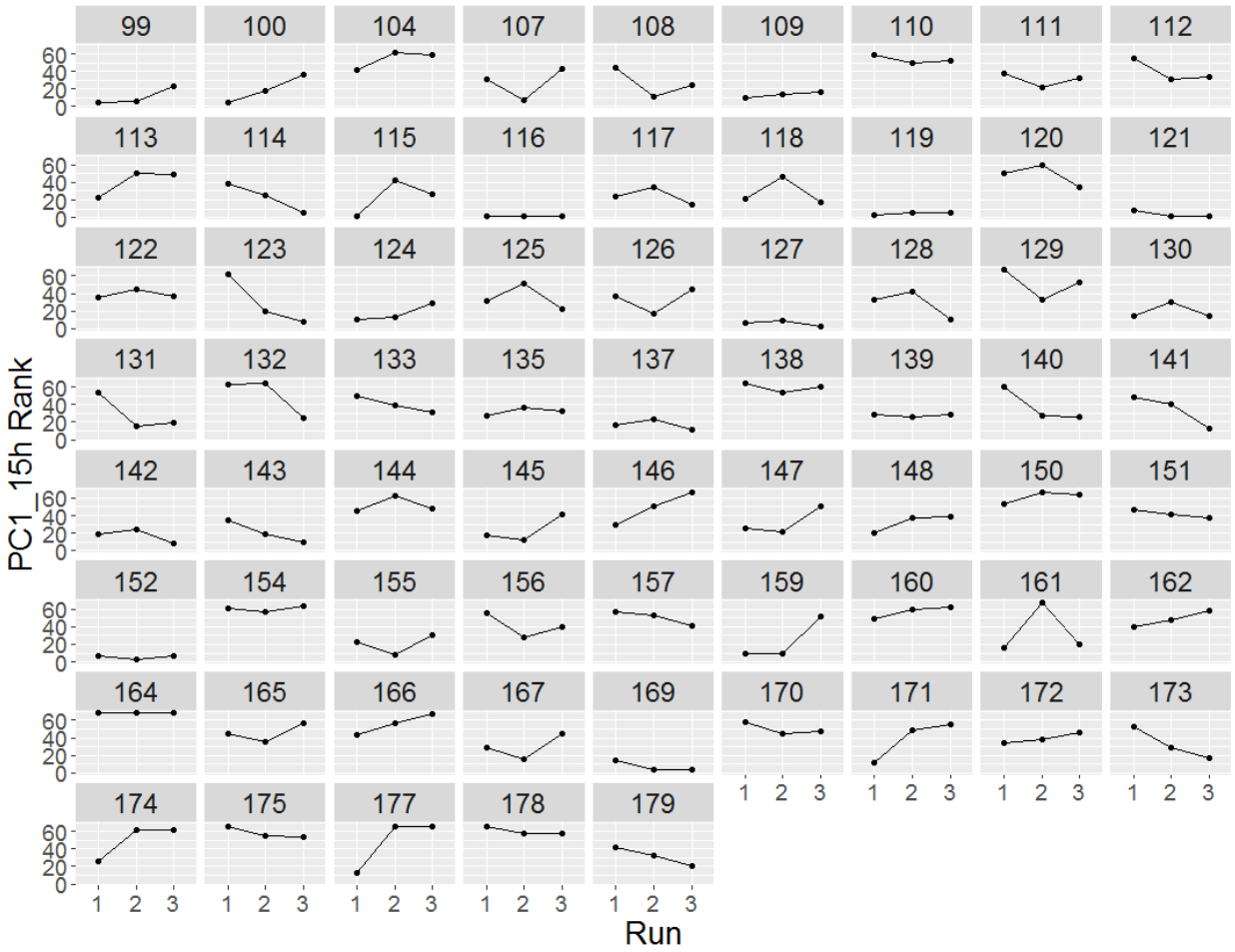


Figure 4.15. Plots showing the PC1 rank from the whole open field test as a function of the run for each individual *Mus musculus* (numbered in each panel).

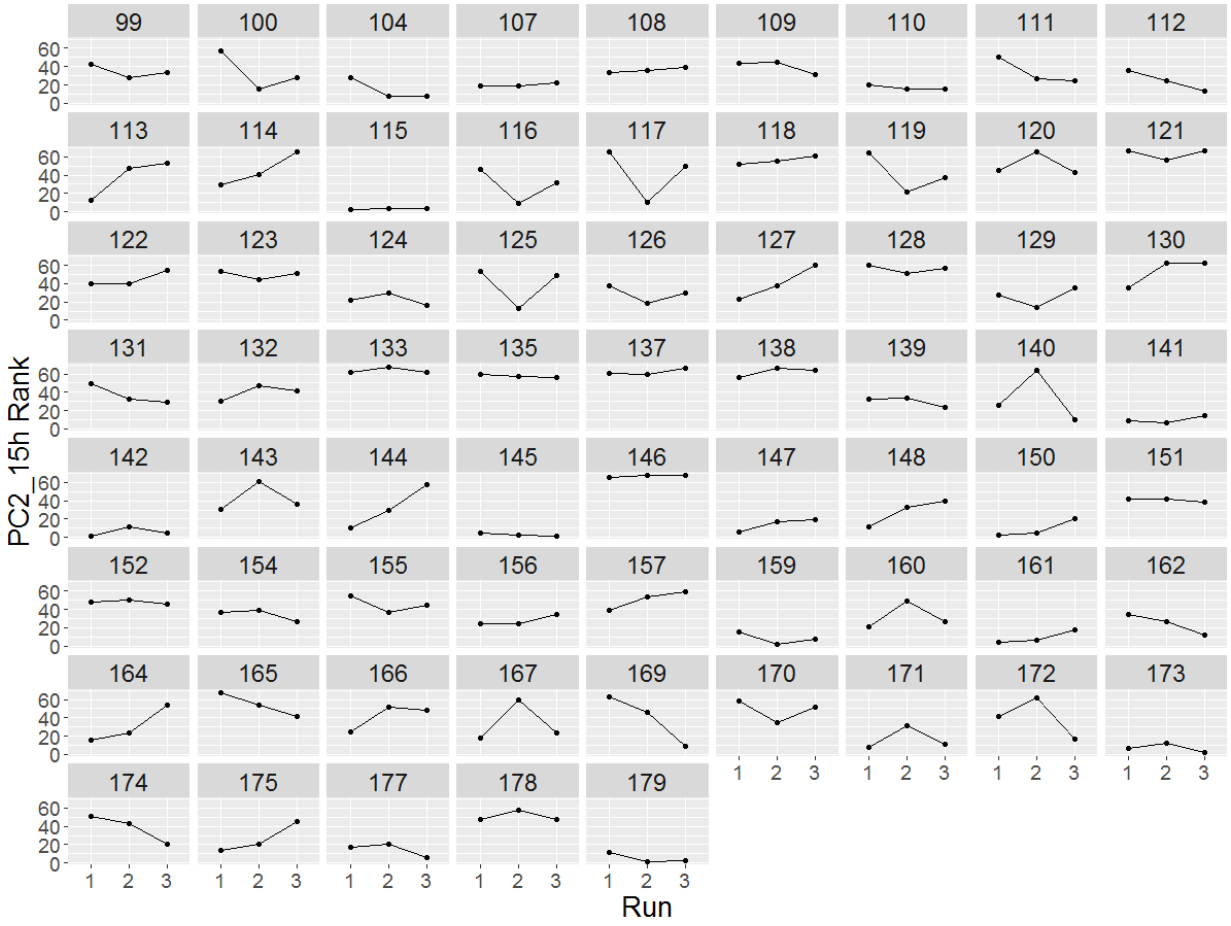


Figure 4.16. Plots showing the PC2 rank from the whole open field test as a function of the run for each individual *Mus musculus* (numbered in each panel).

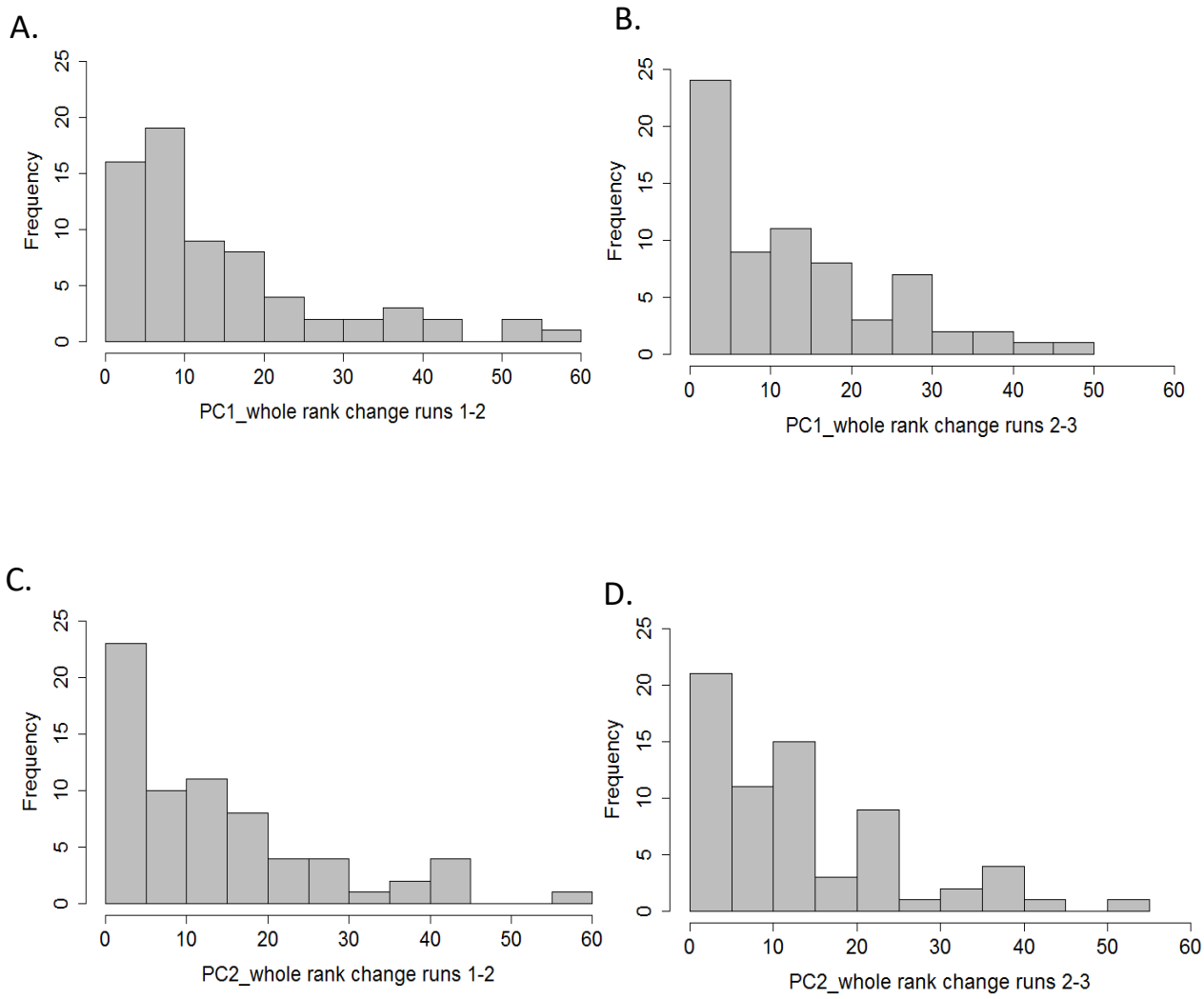


Figure 4.17. Comparing PC1_15h rank and PC2_15h rank change between consecutive open-field test runs one and two (A and C) and two and three (B and D) for *Mus musculus*.

Table 4.7. Repeatability estimates (*R*) of PCA rankings and measured behaivoural variables from open field tests with *Mus musculus*.

	Time period	Whole population		Females		Males	
		R	95% CI	*R*	95% CI	*R*	95% CI
PC1_1h_rank	First hour	0.48	0.34 – 0.63	0.50	0.26 - 0.67	0.47	0.30 - 0.64
PC1_15h_rank	15 hours	0.46	0.33 – 0.59	0.47	0.28 - 0.62	0.53	0.32 - 0.67
PC2_15h_rank	15 hours	0.46	0.32 – 0.58	0.42	0.25 - 0.59	0.40	0.18 - 0.56
Lat.Emerge	15 hours	0.42	0.26 – 0.53	0.55	0.31 – 0.68	0.18	0.10 – 0.30
Food	15 hours	0.45	0.33 – 0.62	0.46	0.28 – 0.63	0.50	0.25 – 0.64
Distance	First hour	0.37	0.22 – 0.47	0.36	0.15 – 0.53	0.37	0.21 – 0.55
	15 hours	0.50	0.33 – 0.61	0.48	0.28 – 0.65	0.57	0.36 – 0.71
Perc.Dark	First hour	0.39	0.22 – 0.51	0.42	0.23 – 0.59	0.33	0.09 – 0.52
	15 hours	0.35	0.24 – 0.49	0.25	0.15 – 0.46	0.64	0.41 – 0.77
Perc.Edges	First hour	0.31	0.19 – 0.43	0.23	0.09 – 0.38	0.53	0.34 – 0.66
	15 hours	0.46	0.32 – 0.59	0.60	0.39 – 0.73	0.21	0.07 – 0.43
Perc.Food	First hour	0.37	0.26 – 0.47	0.34	0.24 – 0.44	0.47	0.31 – 0.60
	15 hours	0.47	0.33 – 0.59	0.59	0.45 – 0.70	0.17	0.00 – 0.35
Lat.Food	First hour	0.36	0.22 – 0.49	0.40	0.21 – 0.58	0.27	0.10 – 0.46
	15 hours	0.46	0.35 – 0.57	0.74	0.57 – 0.84	0.10	0.06 – 0.21

Chapter 5

Correlating consistent individual differences in behavioural and physiological traits

5.1 Abstract

The pace-of-life syndrome hypothesis suggests that variation in single traits cannot be understood in isolation because suites of traits have co-evolved as integrated syndromes that optimise an individual's fitness depending on intrinsic and environmental conditions. The suggestion that variation in physiological, behavioural and life-history strategies has coevolved to form optimal syndromes provides a comprehensive explanation for the perplexing within-population variation seen in many key behavioural and physiological traits that seem likely to be under strong directional selection. To date, no study has properly integrated thermal physiology and thermoregulatory behaviour with other key traits as proposed by the POLS hypothesis. This is an important research topic because intraspecific variation in metabolic physiology may be a critical evolutionary mechanism for the persistence of populations in the face of environmental change. Despite its theoretical appeal, empirical research is needed to test the assumptions underlying the POLS hypothesis; that there are consistent differences in trait values among individuals and that there are correlations among traits across environmental contexts. Using wild caught house mice, *Mus musculus*, this study examined the relationship between behavioural and physiological traits. Behavioural traits were assessed using an open-field test (OFT) and metabolic traits were measured using open-flow respirometry. This study found strong evidence of repeatable differences in the measured metabolic and behavioural traits among individuals. Moreover, the main results demonstrated correlations between consistent individual differences in behavioural and metabolic traits. Bolder individuals exhibited lower levels of REE and had a weaker metabolic response to food withdrawal compared to shyer individuals. Additionally, more explorative individuals had higher levels of energy expenditure and displayed a stronger metabolic response to food withdrawal. Overall results provide empirical support for the POLS framework relating behaviour and physiology at the within population level in wild caught house mice, however the observed relationships were not always in the direction predicted. These

results indicate that associations between behaviour and metabolic traits vary in direction, strength and plasticity depending on the traits in question.

5.2 Introduction

Natural populations are comprised of individuals that consistently differ in their behavioural, physiological and life-history traits. Recently, there has been a surge of interest to determine what causes and maintains individual differences within populations, as well as their ecological implications and consequences for conservation issues (Reale *et al.,* 2010; Sih *et al.,* 2012). The pace-of-life syndrome (POLS) hypothesis provides a potential explanation for why individuals might display consistently different life-history strategies. It proposes that variation in single traits, such as metabolic rate, cannot be understood in isolation because suites of traits have co-evolved as integrated syndromes that optimise an individual's fitness depending on their intrinsic state and the surrounding environmental conditions (Biro and Stamps, 2008; Reale *et al.,* 2010). According to this hypothesis, within a species or population individuals can be characterised along a pace-of-life continuum ranging from slow to fast lifestyles. Individuals with fast pace-of-life traits (also known as pace-of-life syndromes) are predicted to be consistently associated with traits including boldness, higher metabolic rates, higher growth rates, but reduced survival (Biro and Stamps 2008; Smith and Blumstein, 2008). Slow characterised individuals, in contrast, are predicted to be associated with traits including shyness, lower metabolic and growth rates but a longer lifetime survival (Biro *et al.,* 2004; Stamps, 2007).

This idea of co-variation of multiple traits provides an explanation for the substantial intra-specific variation often seen in individual behavioural and physiological traits when there is apparent selection pressure towards a mean trait value. Basal metabolic rate (BMR), for example, often varies several-fold among individuals of the same population, but it is not understood why we find such large differences among individuals when it always seems valuable to conserve energy (Speakman *et al.,* 2004; Geiser *et al.,* 2014). While this variation has been partially explained by differences in body size or as reflecting behavioural differences during measurements, inter-individual variation in BMR within a single population is still not completely understood (Burton *et al.,* 2011). Such variation could be explained if it represents one component of a correlated suite of traits that provides an individual with increased fitness under specific conditions. Individuals exhibit

particular combinations of traits (i.e. pace-of-life syndromes) because these trait combinations provide viable strategies for achieving fitness depending on differences in intrinsic state, environmental conditions and frequency of other syndromes within the population (Wolf *et al.,* 2007). As a result, any environmental conditions that drive a specific life-history strategy could have knock-on effects on other associated traits. Different pace-of-life trait combinations are believed to be maintained in a population as they result in equal expected life-time fitness. The existence of individual diversity in the form of pace-of-life syndromes is thought to be important for maintaining population stability during times of environmental change (Reale, *et al.,* 2010).

Despite its theoretical appeal, further research is necessary to test the assumptions and predictions underlying the POLS hypothesis; that there are consistent differences in trait values among individuals and correlations among behavioural, physiological and life-history traits across environmental contexts. Though there has been much interest since it was suggested that consistent individual differences in animal behaviour (i.e. personality traits) are part of the pace-of-life syndrome, to date there has been little conclusive empirical evidence to satisfactorily explain the relationship between behavioural, physiological and life-history traits in rodents. A few models have been proposed to explain the potential relationship between animal behaviour and metabolism. The "increased-intake" (or "performance") hypothesis assumes energy expenditure reflects the cost of maintaining the "metabolic engine" and predicts a positive relationship between energetically demanding behaviours (e.g. increased activity) and metabolism (Drent and Daan, 1980, Burton *et al.,* 2011). This means bold, proactive individuals are expected to have a higher metabolism (Biro and Stamps 2010; Careau *et al.,* 2008a). Alternatively, the "compensation" hypothesis leads to contrasting predictions. This model assumes that there is a general fitness advantage of lower metabolism as the savings to the limited energy budget can then be allocated elsewhere. Therefore, the high energy requirements of bolder, proactive individuals will decrease the amount of energy available for other components (e.g. metabolism) of the fixed energetic budget, leading to a negative association between proactive behaviours and metabolism (Metcalfe *et al.,* 1995).

As of yet, empirical studies testing the POLS hypothesis have produced conflicting results (Le Galliard *et al.,* 2013; David *et al.,* 2015). Some research suggests behavioural traits are positively correlated with life-history traits (e.g. age of first reproduction) and metabolic rate (Gebczynski and Konarzweski, 2009; Biro and Stamps, 2010; Martins *et al.,* 2011; Careau and Garland, 2012). In contrast, other research has demonstrated that behavioural traits are negatively correlated with life-history traits and metabolic rate (Careau *et al.,* 2009). Whilst additional studies have found very weak or no significant relationships between consistent individual differences in behaviour and metabolism (Lantova *et al.,* 2011; Timonin *et al.,* 2011). These contrasting results highlight the importance of further research to clarify these interactions.

Basal metabolic rate is frequently used for determining an organism's minimum energetic cost of maintenance. It provides a standardised index of metabolic energy expenditure allowing for comparison among endothermic species and between studies. Basal metabolic rate is defined as the minimum rate of resting energy expenditure (REE) expressed by a non-reproducing mature endotherm measured during the normal inactive phase of its day cycle, when that individual is post-absorptive and resting in its thermoneutral zone (Kleiber, 1961; Mcnab, 1997). Many physiological ecologists hold concerns about the usefulness of BMR as an index of metabolic energy expenditure as it does not account for the challenges that animals face in their natural environment. Moreover, animals are often unlikely to experience the conditions required to measure BMR in the wild. This scepticism is particularly relevant to small endothermic animals because BMR fails to account for thermoregulatory effects on metabolism and the significant scope of daily and seasonal changes in metabolic rate, particularly in heterothermic species. Small individual differences in propensity to use torpor (i.e. a controlled and temporary reduction in body temperature and metabolic rate), for instance, can have a large effect on daily energy expenditures. So far, use of BMR as a sole index of energy expenditure has dominated studies of the ecological and evolutionary significance of variation in metabolic energy expenditure (Bouwhuis *et al.,* 2014).

While there are many benefits of a standardised protocol, such as BMR, we must be aware of its inherent limitations and the likelihood that BMR can be an inaccurate representation of an animal's metabolic energy expenditure in their natural habitat (Weathers, 1979; Speakman *et al.,* 1999; Mathot and Dingemanse, 2015). Progress to resolve the adaptive significance of energy expenditure and the POLS hypothesis requires a more critical, integrated approach to quantify the individual variation in metabolic energy expenditure and behaviour. To achieve this, holistic studies looking for links between animal behaviour and metabolic physiology should adopt multiple measures of metabolic rate to incorporate the large effects of thermoregulation and its effects on metabolism.

Reaction norms are used to demonstrate an organisms' capacity to adjust its phenotypic traits, such as metabolism, to changes in the surroundings (Petit and Vezina, 2014). They show the flexibility of a trait across an environmental gradient and provide an identifying trait value associated with physiological limits. Individuals with different intercepts and slopes have different phenotypes and phenotypic responses to changes in the surroundings (Terblanch *et al.,* 2009; Schaefer and Walters, 2010). Few studies have used reaction norms to see whether individuals differ in how their metabolic traits respond to changes in ambient temperature (T_a) or food availability (Careau *et al.,* 2014b). Neither have reaction norm trait values been incorporated into the POLs framework. A metabolic "reaction norm" approach that characterises individuals' energetic response to variation in environmental conditions (e.g. food restriction and change in T_a) will enable better defined and more realistic hypotheses about how consistent individual differences in metabolism relate to other key pace-of-life traits.

This chapter will assist in filling some crucial gaps in our understandings of the POLS hypothesis by incorporating measurements of both behaviour and metabolic rate that are more biologically relevant, informative and integrative, and through providing repeated measures of individuals kept under constant conditions to test for consistency. The objectives of this chapter were to determine whether individuals exhibit consistent and correlated differences in key behavioural (e.g. boldness and exploration)

and physiological traits, as suggested by the pace-of-life syndrome hypothesis, in wild caught house mice (*Mus musculus*). It was predicted that individuals that were more risk averse and associated with low exploration levels in the behavioural experiments would also have lower metabolic rates, whilst bolder and more explorative individuals would be associated with higher metabolic rates. These results were used to suggest how consistent individual differences in thermoregulatory metabolic responses to food availability could play an important role in the defining variation in pace-of-life syndromes. These results will help us understand the ecological significance of variation in key behavioural, physiological and life-history traits.

5.3 Materials and methods

Approval for all procedures in this experiment was granted by Western Sydney University's Animal Care and Ethics Committee and was carried out in accordance with federal standards for animal care and welfare (A10445; National Health and Medical Research Council, 2013).

5.3.1 Study animals and colony maintenance

Sixty-nine wild caught house mice (*Mus musculus*) from the same population were captured using Elliott aluminium live traps. All mice were trapped on private agricultural land in Wilberforce, NSW, Australia (GPS 33°33'40.779 S, 150°50'0.781 E). Trapping sessions took place over a one-night trapping session that took place at approximately three-month intervals between July 2015 and July 2016. The traps were set in fully shaded areas between 1700 h and 1800 h and baited with balls of rolled oats, honey and peanut butter. They were then checked the subsequent morning between 0700 h and 0800 h. Upon capture, mice were checked for reproductive status and measured. Females showing signs of pregnancy or lactation (exposed nipples) were immediately released at the capture site. The length of the animal was taken from the base of the tail to the nose tip (HB) and used to determine whether the individuals were juveniles (0-5 weeks old: HB < 64 mm), sub-adults (5 – 8 weeks old; 64 ≤ HB ≤ 71 mm) or adults (> 8 weeks old; HB > 71 mm; Newsome, 1969 and Singleton, 1983). Only sub-adults were included in this study to compare individuals of similar age and reduce the possibility of using senescing individuals. All other captured individuals were immediately released at the trapping location.

Following capture mice were transported to a rodent holding facility at Western Sydney University's Hawkesbury Campus (33°37'03.4 S 150°45'17.2 E) where they were weighed and treated topically with a drop of anti-parasitic agent (Ivermectin, 0.83 mg/mL) to the inter-scapula region. This anti-parasitic was then repeated weekly for three weeks. When mice were not being used for experimental measurements they were housed individually in clear standard mouse cages (1248 L Eurostandard Type II polysulfone cages with filter top lids; Techniplast, Italy) with *ad libitum* quantities of maintenance rodent pellets (Gordon's Specialty Stockfeeds P/L, Australia) and tap water. Each cage contained 500ml of Pura cob bedding substrate (Able Scientific, Australia), a handful of shredded paper and a cardboard tube for nesting material and environmental enrichment. The colony was checked daily and home cages were cleaned every three weeks. No experimental measurements were conducted within three days of an individual's cage being cleaned. The colony was housed in a single air-conditioned room where the T_a was maintained at 23 ± 2 °C and the mice experienced natural light cycles.

5.3.2 Data Collection

To determine the consistency and quantify each individual's metabolic energy expenditure and behavioural characteristics, three runs of metabolic and behavioural experiments, using open-flow respirometry (as described in detail in Chapter 3) and an OFT (as described in detail in Chapter 4), respectively, were carried out at one-month intervals. The respirometry measurements and OFTs were separated by at least seven days where the mice were left in their home cages and monitored with minimum disturbance. Each individual's first respirometry run started on their second day in captivity. For the behavioural measurements only two individuals at a time could be measured using the open-field apparatus and the order that individuals were tested within each of the three experimental runs was randomly assigned.

5.3.2.1 Measurement of metabolic traits

Mice were placed individually in unsealed "live-in" respirometry chambers (21 x 37 x 14cm), which matched in dimensions the size of their normal home cages. These chambers were housed inside a temperature controlled cabinet (Panasonic MIR-554) and their metabolic response to daily variation in temperature (1200 h to 2000 h: 31 °C, 2000 h to 0400 h: 20 °C and 0400 h to 1200 h: 15 °C) and alternate-day food withdrawal was

recorded continuously over six days (144 hours; hereafter termed a respirometry run). Food access was restricted during the first 24 hours of the respirometry run, and then for each alternate 24 hours of the experiment. Wheel access was restricted between 1200 h and 1700 h on non-food days by inserting a metal rod to block wheel rotations. The mice experienced a 12 h light – 12 h dark cycle where the lights were turned off at 1900 h and turned back on at 0700 h for each day of all respirometry runs.

Before the start of each respirometry run mice were weighed between 1400 h and 1500 h and then put into their respirometry chambers. At 1600 h the respirometry measurements commenced and on the last day of the respirometry run the experiment was stopped at 1600 h. After the end of the experiment mice were reweighed and returned to their home cages.

5.3.2.2 Measurement of behavioural traits

Individual behavioural traits were measured using a modified version of the standard protocol commonly used for open-field analysis. The OFTs for this study lasted for 15 hours, starting at 1700 h and running overnight until 0800 h the following morning. Immediately prior to the start of the experiment individuals were weighed and placed individually into an enclosed dark chamber (38 x 27 cm) located within the main arena (120 x 88 x 60cm) for 30 minutes. This 30-minute desensitisation period was used to reduce the effect of external stimuli on the individual's initial response. The dark chamber contained a toilet roll, a handful of shredded paper for bedding and access to water *ad libitum*. In the centre of the arena was a foraging tray (25 cm diameter) containing 6 g of seed ("Canary Mix") mixed in 1 L of sand. Dim illumination in the main arena was provided by a frosted incandescent light bulb mounted circa 120 cm above the floor of the centre arena (light level 35-55 lux as measured at the floor of the arena). All other lights in the test room were turned off for the duration of these behavioural tests.

The test commenced when the doorway barrier between the dark chamber and main arena was removed at 1700 h allowing free access between the two areas. For the duration of the 15-hour experimental period the observer left the testing room to avoid possible disturbance. During the experiment the arena was continuously recorded via an overhead digital camera that was connected via USB to a computer and later analysed

using video tracking software (Ethovision XT, Noldus Information Technology, Utrecht, The Netherlands).

After the completion of each test (0800 h) the mice were reweighed and returned to their home cage. Defecation was quantified by counting and weighing all faecal boles deposited during the test. Food consumption was recorded by sieving and weighing the remaining seed from the seed and sand matrix. Test room temperature, lighting and noise levels were consistent for all subjects. Ambient temperature over each test was recorded using two temperature-logging iButton devices (resolution: 0.0625 °C; Maxim Integrated, U.S.A) placed at ground level outside the open-field arena. At the end of each test the arena was cleaned with warm soapy water and a 75% ethanol solution to eliminate any residual odours.

5.3.2.3 Data Analysis

Metabolic measurements

Each experimental day was designated as starting at 1600 h and ending the following day at 1559 h. Additionally, each day was separated by the photo phase into an active (lights off to lights on; 1900 h – 0700 h) and rest (lights on to lights off; 0700 h – 1900 h) phase. Daily energy expenditure (DEE) was calculated by averaging an individuals' energy expenditure over each experimental day the mouse was in the respirometry run. Resting energy expenditure (REE) was calculated daily by combining average energy expenditure over the late active (0000 h-0700 h) and early resting phases (0700 h-1200 h). Average energy expenditures (AEEs) were calculated daily for each of the three temperatures by averaging the energy expenditure over each temperature period and resting metabolic rates (RMR) were calculated daily for each temperature by averaging the lowest consecutive 12- minute period within each temperature period (excluding the first hour). Data collected between 1700 h and 1800 h were not included in these analyses as during this time the incubators were opened to confirm the status of the food access doors, remove the wheel block (if necessary) and check on the mice's welfare. Temperatures within the incubators took up to 50 minutes to stabilise during a temperature regime change. As a result, the hour immediately following each temperature change was excluded from analysis.

Behavioural measurements

For behavioural analysis the open-field arena was subdivided into six zones: corners, edges, dark chamber, top of dark chamber, central arena and foraging zone. An individual was determined to have entered a zone when the centre of its body had passed the zone border. The corners, edge of dark chamber, top of dark chamber and edges zones were grouped together and defined as the peripheral area to gain a total score of how much time each individual spend along the "edges". Ethovision behavioural analysis software was used to analyse each of the video recordings to calculate: the latency to first emerge from the dark chamber (seconds), latency to enter each zone (seconds), time spent in each zone (% of total recording time), time the individual was mobile (% of total recording time) and total distance travelled (cm) for each individual. The amount of time spent in each of the zones (% of total recorded time) was converted to percentage of time active (i.e. outside of dark chamber) in each of the zones. Additionally, the weight of faecal boli (g), amount of food consumed (g), initial mass (g) and mass change over the experiment were used to evaluate individual responses.

Statistical analysis

Statistical analyses were carried out within the R statistical interface v3.3.3 and RStudio 1.0.136 (R Core Team 2015; R Studio Team 2016; Bates *et al.,* 2015; Kuznetsova *et al.,* 2014).

The bi-variate relationships between the measured behavioural traits were examined with correlation matrices which calculated Pearson correlation coefficients and associated *P* significance. Data were analysed separately for the first hour of data and the entire 15-hour experiment. Separate principal component analysis (PCA) were carried out on the correlation matrix of behavioural variables to summarise the relationships between the multiple behavioural variables measured during the OFT within the first hour and over the entire 15 hours. The principal components explaining the highest contribution of individual behavioural traits were used as a composite behavioural measure, with each axis potentially representing a behavioural trait (Budaev, 2010). The measured variables with the largest loadings were used to interpret the behavioural trait that each principal

component represented. The PCA scores were then used to rank the individuals for each principal component within the first hour and over the entire 15-hour OFT.

Separate linear mixed effects models (R package "lme4", "lmertest") were used to explain the variation in each of the metabolic traits to food availability and variation in RMR to T_a. All models included fixed effects of "temperature", "food", "run", "day" and a "sex by body mass" interaction. "ID" was included as a random effect to account for repeated measures within individuals and differences in mean responses among individuals in all models. Similarly, an additional random effect termed "series", which denoted a period of time (i.e. run) within which data were collected, and random slopes referring to the environmental condition for both individual and series identity were included in all models. Models initially included fixed effects of either "temperature" or "food" (i.e. environmental condition), "run", "day", "body mass", "sex" and a "sex and body mass" interaction. Terms that were not significant were not included in the final model. Linear mixed effect models were also used to explain the effect of "body mass", "individual" and "run" on PCA scores, within the first hour and whole experiment, over the three runs. All models included the PCA score as the dependent variable, a fixed effect of "body mass", a random effect of "individual" and a random slope for "run".

The multiple measurements of each individual's metabolic traits and PCA scores were used to estimate repeatability (*R*) over the three runs using the final mixed model (described above) for each response variable (Araya-Ajoy *et al.*, 2015). Repeatability is the proportion of total variance that could be attributed to among individual differences, after adjusting for any significant fixed effects, over the three runs (Falconer and Mackay, 1996). A semi-parametric bootstrap method ("lme4" package in R) was used to calculate the 95% confidence intervals (CI) for *R* from 100 simulations. When the CIs did not overlap with zero, the *R* estimate was considered significant.

Correlation matrices were used to examine bi-variate relationships between the metabolic traits and the indices of behaviour (PCA rankings) measured over the three respirometry runs and OFT runs respectively.

To assess how the various metabolic variables and metabolic responses to food availability explained the behavioural variables (PCA scores from first hour and whole experiment) the random effects for every individual were extracted from each of the reaction norm models. I then modelled separate linear models with each of the behavioural reaction norms as the dependent variable and each of the reaction norm components (intercept or slope) for every metabolic variable as the fixed effect.

5.4 Results

Sixty-nine mice underwent three runs of behavioural and respirometry measurements, however due to equipment error the data for nine individuals in the second run were not included in the following analyses.

5.4.1 Consistency of metabolic and behavioural responses

Individual's DEE, REE, resting metabolic rate at 15 °C (RMR_15), resting metabolic rate at 31 °C (RMR_31), average energy expenditure at 15 °C (AEE_15) and average energy expenditure at 31 °C (AEE_31) on food days ($R_{intercept}$) were found to be significantly repeatable over the three respirometry runs (Table 3.7). Estimates of the average individual response to food restriction (R_{slope}) for these metabolic measures were also found to be significantly repeatable over the three respirometry runs (Table 3.7). Individuals were significantly repeatable (95 % CI's non-overlapping with zero) over the three OFTs in their rankings for an index of boldness over the first hour (PC1_1h: males R = 0.47 and females R = 0.50), boldness over the entire duration of the OFT (PC1_15h: males R = 0.53 and females R = 0.47) and exploration over the entire OFT (PC2_15h: males (R = 0.40) and females (R = 0.42)) (Table 4.7). Additionally, among individual differences in the individual measured behavioural variables (e.g. latency to reach and percentage of experiment spent in various zones) were repeatable over the three runs for the population as a whole (Table 4.7).

5.4.2 Correlations between metabolic and behavioural traits

Simple bivariate correlations were used to look at the individual relationships between each of the metabolic traits measured over the three runs of respirometry and each of the three indexes of behaviour (i.e. boldness in first hour of the OFT, boldness over entire duration of the OFT and exploration over the entire duration of the OFT) from the three

OFT runs. Pearson correlation analyses showed weak to moderate associations ($r \leq \pm 0.30$) between the behavioural rankings and many of the mass specific metabolic measurements (Figs. 5.1 and 5.2). The relationships between the behavioural rankings and whole animal metabolic measurements were qualitatively similar. In general, metabolic measurements when food was available showed stronger correlations with the behavioural traits than metabolic measurements taken when food was restricted. The strongest relationships between behavioural and metabolic traits were observed between the rankings for exploration (pc2rank_15) with REE when food was available ($r = 0.30$) and DEE when food was available ($r = 0.28$). Specifically, more explorative individuals in the OFT had higher levels of REE and DEE on days where food was available.

5.4.3 Correlations between predicted individual reaction norms in behavioural and metabolic traits

The bivariate correlation analyses did not account for repeated measures. Therefore, to understand how the metabolic variables explained the behavioural variables, whilst simultaneously accounting for repeated measures (i.e. including individual as a random effect) and controlling for fixed effects of body mass, sex, run and respirometry day, linear mixed effects models were fitted separately to each of the behavioural and metabolic variables. Random effects were extracted from each of these reaction norm models and used to test the importance of individual difference from the population-mean metabolic reaction norm component (intercept or slope) as a fixed effect in explaining variation in the individual difference from the population-mean behavioural reaction norms (mean behaviour over the three runs) using separate linear models.

The predicted individual differences from the population mean in values of DEE, REE, AEE.Rest, RMR_15 and AEE_15 on days when food was available (i.e. predicted individual reaction norm intercepts) had a significant positive effect on the predicted individual differences from the population-mean behavioural reaction norms for both indexes of boldness (Table 5.1). Specifically, bolder individuals (i.e. those with lower reaction norm intercept values compared to the population mean) exhibited lower levels of energy expenditure, compared to the population mean, on days when food was available (Fig. 5.3). Predicted individual reaction norm intercept values for REE and AEE_15 explained the most variation in individual differences from population-mean behavioural reaction norms

for both indexes of boldness. The predicted individual differences in values of DEE, REE and AEE_15 from the population-mean slopes (i.e. predicted individual reaction norm slopes) in response to food availability had a significant negative effect on the predicted individual reaction norm intercepts for boldness. Shyer individuals (i.e. those with higher reaction norm intercept values) exhibited a stronger metabolic response to food availability compared to that population mean (i.e. had a lower reaction norm slope value; Table 5.1; Fig. 5.4). Predicted individual reaction norm slope values for REE values best explained the variation in individual differences from population-mean behavioural reaction norms for both indexes of boldness (Fig. 5.4). Overall, the best explanatory metabolic reaction norm component (intercept or slope) for boldness was the effect of the reaction norm slope for REE in response to food availability on behaviour over the first hour of the OFT (Table 5.4). In general, the reaction norm intercepts of the significant explanatory metabolic variables were slightly better predictors of boldness than the reaction norm slopes of the significant metabolic variables.

The predicted individual differences from the population-mean metabolic reaction norm components explained less of the variation in reaction norm responses for exploration than they did for boldness. Only the predicted individual differences from the population-mean in values of REE and AEE_15 on days when food was available (i.e. reaction norm intercepts) had a significant effect (negative) on the predicted individual differences from the population-mean behavioural reaction norms for the index of exploration. Specifically, individuals that were more explorative than the population mean (i.e. those with lower reaction norm intercept values) had levels of energy expenditure that were generally higher than the population mean (Fig. 5.5). The predicted individual differences from the population-mean in values of REE from the population-mean slopes in response to food availability was the only metabolic reaction norm slope to have a significant effect (positive) on the predicted reaction norm intercepts for exploration. More explorative individuals had a more pronounced response to food restriction (Fig.5.6). Overall, the reaction norm intercepts for REE and AEE.20 were the best explanatory metabolic reaction norm components for describing individual differences from the population mean behavioural reaction norm intercept for exploration. It should be noted, however, that the amount of variance explained is low with R2 less than 0.1.

The predicted individual differences from the population-mean for values of RMR in response to change in T_a (predicted individual reaction norm slope for thermal conductance) had a significant effect on the indexes of boldness in the first hour and over the entire OFT (Table 5.1). Shyer individuals exhibited a more pronounced response in their RMR to a change in T_a from 31 °C to 15 °C compared with the population mean (Fig. 5.7). The predicted individual reaction norm slope for the response of RMR to change in T_a did not have a significant effect on the index for exploration (Table 5.1).

5.5 Discussion

This study tested a recently proposed hypothesis that behaviour and metabolism are interlinked (Careau *et al.,* 2008b; Biro and Stamps, 2010; Reale *et al.,* 2010). Exploring the covariation of individual differences in metabolic and behavioural traits is a relatively new research paradigm and the present study is one of the first to incorporate the effects of thermal physiology with the POLS hypothesis. I found strong evidence of repeatable differences in metabolic and behavioural activity among individuals. Additionally, the main results demonstrated correlations between consistent individual differences in behavioural and metabolic response. In particular, analysis of individual reaction norms revealed that individuals that were bolder during behavioural testing also exhibited lower levels of REE when food was available during metabolic measurements. Bolder individuals also showed a weaker metabolic response (i.e. smaller change in REE) to food withdrawal compared to shyer individuals. In addition, more explorative individuals had higher levels of energy expenditure when food was available and displayed a more pronounced response to food restriction.

5.5.1 Consistency of metabolic and behavioural responses

For individual variation to be biologically meaningful it must be consistent, so this study started by demonstrating the statistical repeatability of the measured behavioural and metabolic traits. Often estimates of repeatability for metabolic traits are taken at a single T_a (Artacho and Nespolo, 2009; White *et al.,* 2013). For this study, I included metabolic traits from multiple temperatures (15, 20 and 31 °C) to reflect the large natural daily variations in T_a that mice would experience in the wild.

The findings that multiple components of individual's metabolic energy expenditure (including: DEE, REE, RMR_15, RMR_31, AEE_15 and AEE_31) and average individual response of these metabolic traits to food withdrawal were repeatable over the three respirometry runs has important ecological implications. The repeatability estimates for these metabolic traits (r = 0.49 – 0.64) were similar to those found in prior studies on the repeatability of metabolism in small rodents (Nespolo and Franco, 2007). Estimates of metabolic repeatability measured at 20 °C were not significant, likely because measurements at this temperature were taken during the mice's active phase and individual behavioural differences would invariably be associated with variation in metabolism.

Inter-individual behavioural responses to an unknown environment (i.e. rankings for boldness and exploration) were also highly repeatable (r = 0.46 – 0.48) over the three measurement periods and similar to behavioural repeatability estimates recorded in the literature (Bell *et al.,* 2009; Korpela *et al.,* 2011; Herde and Eccard, 2013; Schuster *et al.,* 2017). It thus appears that boldness and exploration are personality traits in *M. musculus* (Sih *et al.,* 2004). These results add to the growing field of research that show consistent individual differences in behavioural traits in many taxa, such as rodents (Koolhaus *et al.,* 1999; Montiglio *et al.,* 2012), reptiles (Carter *et al.,* 2012; Galliard *et al.,* 2013) and birds (Dingemanse *et al.,* 2002). Additionally, repeatability estimates did not differ between the boldness and exploratory measurements nor between sexes. Whilst some studies have shown that in other species males have higher behavioural repeatability than females, these results suggest that any sex differences in behavioural repeatability are not consistent across all taxa (Nakagawa *et al.,* 2007; Bell *et al.,* 2009; Dammhahn, 2012; Schuster *et al.,* 2017).

These results demonstrate that wild-derived house mice exhibit large and consistent individual differences in their measured behavioural and metabolic variables. These differences were repeatable over the three-month experiment, which comprises the average expected lifespan of feral house mice (Pocock *et al.,* 2004). Whilst repeatability estimates give some indication of the potential for natural selection to act on a trait, the meaning of repeatability patterns remains complex. Repeatability estimates are limited to the individuals and conditions under which the measured variables were collected. To

understand the relationship between natural selection and individual trait variation it is important that the suitable context and environmental conditions be examined (Brodie and Russel, 1999).

5.5.2 Correlations between metabolic and behavioural traits

Most studies to date have used BMR to explore a potential relationship between metabolism and personality (Careau and Garland, 2012). To properly understand covariation of metabolism and behaviour, a larger metabolic profile consisting of multiple different measures of metabolic rate is required. Weak to moderate ($r \leq \pm 0.30$) associations between behavioural rankings and the mass specific metabolic measurements of interest (DEE, REE, RMR_15, RMR_31, AEE_15 and AEE_31 on food and non-food days) were observed here. Stronger correlations existed between exploration and the measured metabolic traits compared to boldness and the metabolic traits. This could indicate that the associations between traits in a potential POLS framework are not all equally linked. It is possible that associations between particular traits are tighter and less flexible than between others.

The strongest associations were between exploration and both REE and DEE. More explorative individuals in the behavioural testing had higher levels of REE and DEE when food was available during the metabolic measurements. This supported the prediction that more explorative (i.e proactive) individuals would have higher levels of energy expenditure than individuals with a reactive lifestyle. The observed positive relationship between metabolism and behaviour agrees with the expectation that behaviour and metabolism can both be aspects of the POLS continuum (Biro and Stamps, 2010; Reale et al, 2010). Highly explorative individuals travel further and expend more energy in doing so, which could be reflected in their metabolism (Sih and Bell, 2008). These results support recent findings that metabolism and behaviour are positively related as seen in many different species (Careau and Garland, 2012; Sichova *et al.,* 2014).

The observed correlations between behaviour and metabolism were not always in my hypothesised direction. Boldness and the metabolism were weakly negatively correlated in this study with bolder individuals exhibiting lower levels of energy expenditure. This result did not support the prediction that bolder individuals would display higher levels of energy

expenditure, as proposed by the POLS hypothesis. The observed negative association between boldness and metabolism could reflect selection pressures that favour combinations of traits (Hayes *et al.,* 1992b). Food productivity and predictability are believed to be crucial selection pressures. It has been proposed that bolder individuals have a fitness advantage during periods of food shortage because they are more likely to enter novel areas to find food and their low metabolism would compensate for periods of food withdrawal, therefore leading to increased survival in poor conditions (Careau *et al.,* 2009; Bouwhuis *et al.,* 2014). To maintain variation, risk averse individuals are expected to have a fitness advantage over bolder individuals during period of benign conditions due to costs associated with boldness (e.g. increased risk of predation, increased risk of confrontation and injury from conspecifics) and low metabolism (e.g. slow growth and delayed onset of reproduction). In this proposed framework, we would expect correlational selection between boldness and metabolism whereby selection favours bold individuals with low metabolism or risk averse individuals with high metabolism (Careau *et al.,* 2009). *Mus musculus* have a high mass-specific energy demand and experience high predation risk when exploring. Natural selection on metabolism and behaviour is likely to be heavily influenced by variations in resource abundance (Careau *et al.,* 2009).

There are two main hypotheses to explain a potential link between metabolism and behaviour. These hypotheses relate to two energy allocation models – the "increased intake" model and the "compensation" model (described in section 5.2 above). The "increased intake" model hypothesises that a more energetically demanding lifestyle, as seen in proactive individuals (e.g. elevated levels of general activity, novelty seeking, aggression and boldness) should require larger than average organ systems and metabolically active tissues (i.e. a larger metabolic engine) to support this behaviour (Careau *et al.,* 2008b, 2009; Biro and Stamps, 2010). To date, the majority of studies exploring the relationship between behaviour and metabolism have produced support for a positive relationship between a measure of energy expenditure (e.g. BMR, RMR or SMR) and a behavioural trait (e.g. boldness and exploration) as suggested in the "increased intake" hypothesis (Nilsson, 2002; Biro and Stamps, 2010; Mathot and Dingemanse, 2015). The compensation model suggests that an organism has a limited amount of energy that can be allocated across competing energy demanding processes like REE and boldness. Metabolism and boldness are expected to be negatively associated as an organism with

higher levels of energy expenditure is consequently limited in the amount of energy it has left to spend on energetically expensive behaviours such as boldness. Results from this study do not clearly support either of these two models and instead indicate that the two models may act concurrently and the direction of the relationship between metabolism and behaviour is dependent upon the behaviour in question.

The observed correlations between the metabolic and behavioural traits remain in line with the hypothesis that variation in metabolism is associated with variation in behaviour. Despite this, the associations between metabolism and behaviour were not as strong as had been expected nor were they always in the predicted directions. Various factors may have contributed to our inability to detect stronger relationships between metabolism and behaviour. This study may be biased towards capturing more active individuals as when live-trapping with passive gear (e.g. Elliott and Sherman traps) you are more likely to catch more explorative, proactive individuals (Biro and Dingemanse, 2009). Additionally, respirometry can be stressful, leading to elevated measures of energy expenditure from higher activity levels, elevated breathing and heart rate (Careau *et al.,* 2008a). However, the use of live-in cages that were the same dimension to the mice's home cage minimised effects of stress and their associated metabolic artefacts. Individuals may well adopt different metabolic strategies (e.g. response of energy expenditure to food restriction) and these strategies, rather than individual measures of metabolism, could be strongly related to the observed behavioural traits.

5.5.3 Correlations between predicted individual reaction norms in behavioural and metabolic traits

The associations between the predicted individual reaction norm intercepts for the behavioural and metabolic traits were statistically stronger than the correlations observed between the individual behavioural and metabolic traits. Bolder individuals exhibited lower levels of energy expenditure (for DEE, REE AEE_Rest, RMR_15 and AEE_15) compared to the population mean and shyer individuals had higher levels of energy expenditure (for DEE, REE, AEE_Rest, RMR_15 and AEE_15). This mirrored the directions of the correlations observed between the individual behavioural and metabolic traits, and contrasts with the directional predictions of the POLS that boldness would be positively associated with energy expenditure. A potential explanation for this is that shyer

individuals experienced higher stress responses during measurements, hence displaying a long latency to emerge and inauthentic high metabolism. The potential effects of stress-related covariance when analysing the relationships between metabolism and behaviour warrants further empirical attention (Careau *et al.,* 2008; Careau *et al.,* 2019). This negative relationship supports the theory that organisms have a fixed amount of energy which results in a trade-off between competing pathways such as energy expenditure and energy-demanding behaviours (i.e. the compensation model; Careau *et al.,* 2008). The POLS hypothesis also predicts a positive relationship between energy expenditure and exploration which was supported in this study, whereby individuals that were more explorative had higher levels of REE and AEE_15 than less explorative individuals (Reale *et al.,* 2010; Careau and Garland, 2012). According to the increased-intake model more explorative individuals require a greater "metabolic machinery", which should be reflected in higher than average resting energy expenditure, to support their high activity levels (Nilsson, 2002). Furthermore, a positive feedback loop could exist where individuals with higher energy expenditure must explore their environment more often and travel further to search for food to support their high energy requirements (Biro and Stamps, 2010; Careau *et al.,* 2011).

To date, no study has determined whether among individual slope differences in behavioural and metabolic reaction norms explain the relationship between behaviour and metabolism. The only metabolic response (reaction norm slope) to have a significant effect on exploration was for REE. More explorative individuals showed a more pronounced decrease in their REE when faced with food withdrawal compared to less explorative individuals. When food was available more explorative individuals had higher levels of REE and it is likely that when food was withdrawn these individuals were more affected energetically thane than those with lower levels of REE. Consequently, individuals that generally had higher levels of REE may have been more likely to employ energy saving mechanisms like torpor to significantly reduce their high resting energy requirements that would normally be supported with the available food. In response to food withdrawal bolder individuals exhibited a weaker decrease in their energy expenditure for DEE, REE and AEE_15 whilst shyer individuals showed a stronger metabolic response for these traits. In general, bolder individuals had lower levels of energy expenditure than shyer individuals and presumably did not have to employ as marked a thermoregulatory response when

faced with food withdrawal as shyer individuals that needed to make more energy savings to support their energy expenditure.

This study is one of the first to report that individuals within a single population significantly differ in their metabolic response to food availability and change in T_a (thermal sensitivity). Additionally, these individual metabolic reaction norms are significantly related to behavioural responses in a novel environment. An important next step would to be to determine whether these individual differences in metabolic responses have an underlying genetic basis**.** Whether the proposed associations within different POLS strategies are due to genetic or environmental causes is currently not fully understood, although some studies have shown evidence of a genetic variance among traits (Careau *et al.,* 2011; Niemela *et al.,* 2013). If individual differences in reaction norms have a genetic base, natural selection can act on this part of individual phenotypes and bring evolutionary change among populations and species. Understanding the proximate mechanisms behind trait covariation patterns will help to make the biological interpretations about covariation of behavioural and physiological traits in a less speculative manner.

These results show how individuals' metabolic adjustments across an environmental gradient are associated with their behaviour. This approach, alongside investigating the patterns of covariation amongst single traits, provides a more comprehensive understanding of the complex relationship between behaviour and physiology than studies restricting themselves to looking at the relationship among individual traits. Future research should continue along this path. By including behavioural and physiological responses to biologically significant variables the results obtained will be more reflective of the trait relationships expressed in an animal's natural environment.

5.5.4 Conclusions

To comprehensively understand the relationship between behaviour and metabolism, and their role in a potential POLS framework, it is not sufficient to use single metabolic and behavioural traits. The associations between traits in a potential holistic framework of behavioural, physiological and life-history traits are likely to be more complex and less generalised than is often suggested. An in-depth approach incorporating all aspects of the

resting energy budget, behavioural traits measured in an ecologically relevant context and individual responses to biologically significant variables (i.e. reaction norms) is necessary to provide a more complete picture of trait covariation and move forward in the field.

This study takes an innovative and multidisciplinary approach to explore the patterns of covariation between behaviour and metabolism. It differs from previous studies in several key ways by: 1) measuring multiple components of the daily energy budget to obtain a complete metabolic profile, 2) incorporating individual metabolic adjustment (i.e. metabolic reaction norms) in response to key environmental variation (e.g. changes in food availability and T_a), 3) including long-term (6 days) metabolic measurements in live in respirometry cages that provided a minimum-stress environment, 4) measuring behavioural traits in a more ecologically relevant context than traditional OFTs and 5) repeating metabolic and behavioural measurements over the mice's expected natural lifespan.

By examining the relationships between multiple traits in individuals experiencing an array of natural ecological conditions, this study found evidence for i) significant individual consistency of multiple measures of behaviour and metabolism across biologically meaningful periods of the lives of wild derived house mice and ii) correlations among some behavioural and metabolic responses, although these were not always in the directions proposed by the POLS concept. Overall, results provide empirical support for some of the predictions of the POLS framework relating behaviour and energy metabolism at the within-population level in wild caught house mice. Despite these significant results, care should be taken not to simplify a complex reality and assume trait correlations based on the POLS hypothesis without examining the expected relationships directly (Reale *et al.,* 2010). It is probable that associations between behavioural and metabolic traits vary in direction, strength and plasticity, additionally developmental effects are believed to affect the patterns of trait correlations (Reale *et al.,* 2010; Careau *et al.,* 2014). I encourage more research to unravel the complexity surrounding the links between behaviour and physiology. This should include attempts to assess whether metabolic reaction norms are heritable and genetically correlated, to examine the adaptive role of trait correlations. Along with the current study, such research will improve our understanding of the physiological mechanisms that underpin animal behaviour and ecology and help us predict

how populations may cope with natural and human-induced environmental change (Barthed, 2015).

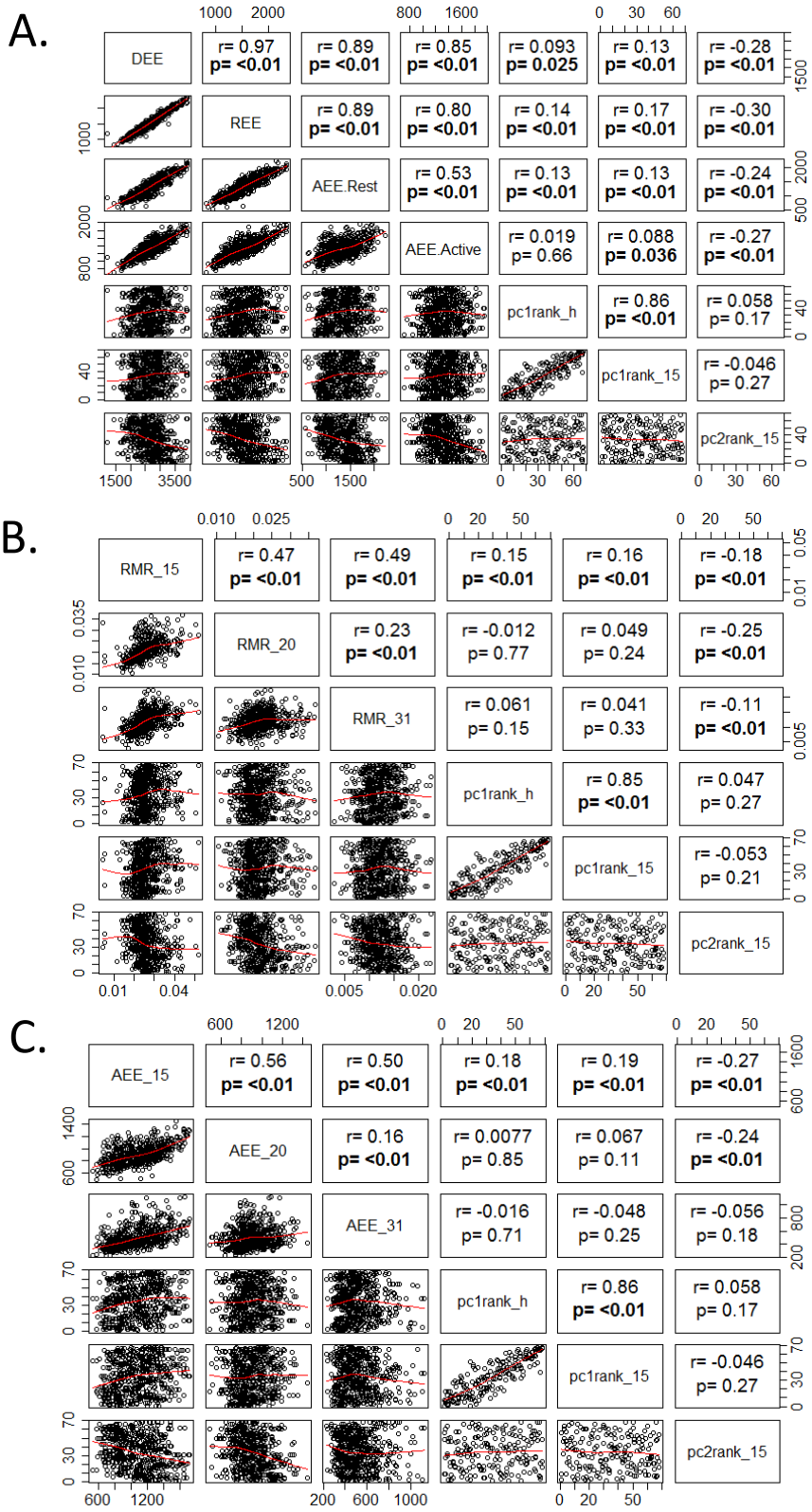


Figure 5.1. Correlation matrices of behavioural rankings (boldness in first hour (pc1rank_h), boldness over entire experiment (pc1_15) and exploration over entire experiment (pc2rank_15)) from open-field test experiments and mass specific metabolic traits from three respirometry runs on days where food was available (A: Daily energy expenditure (DEE (J day^{-1} g^{-1})), resting energy expenditure (REE (J/ 12h g^{-1}), average energy expenditure over rest phase (AEE.Rest (J/12h g^{-1})) and average energy expenditure over active phase (AEE.Active (J/12 h g^{-1})), B: Resting metabolic rates (W g^{-1}) at three temperatures (15 °C, 20 °C and 31 °C) and C: Average energy expenditure over each temperature). Scatter plots shown in the bottom left of the graph and corresponding *r* and *P* values displayed in the top right panels.

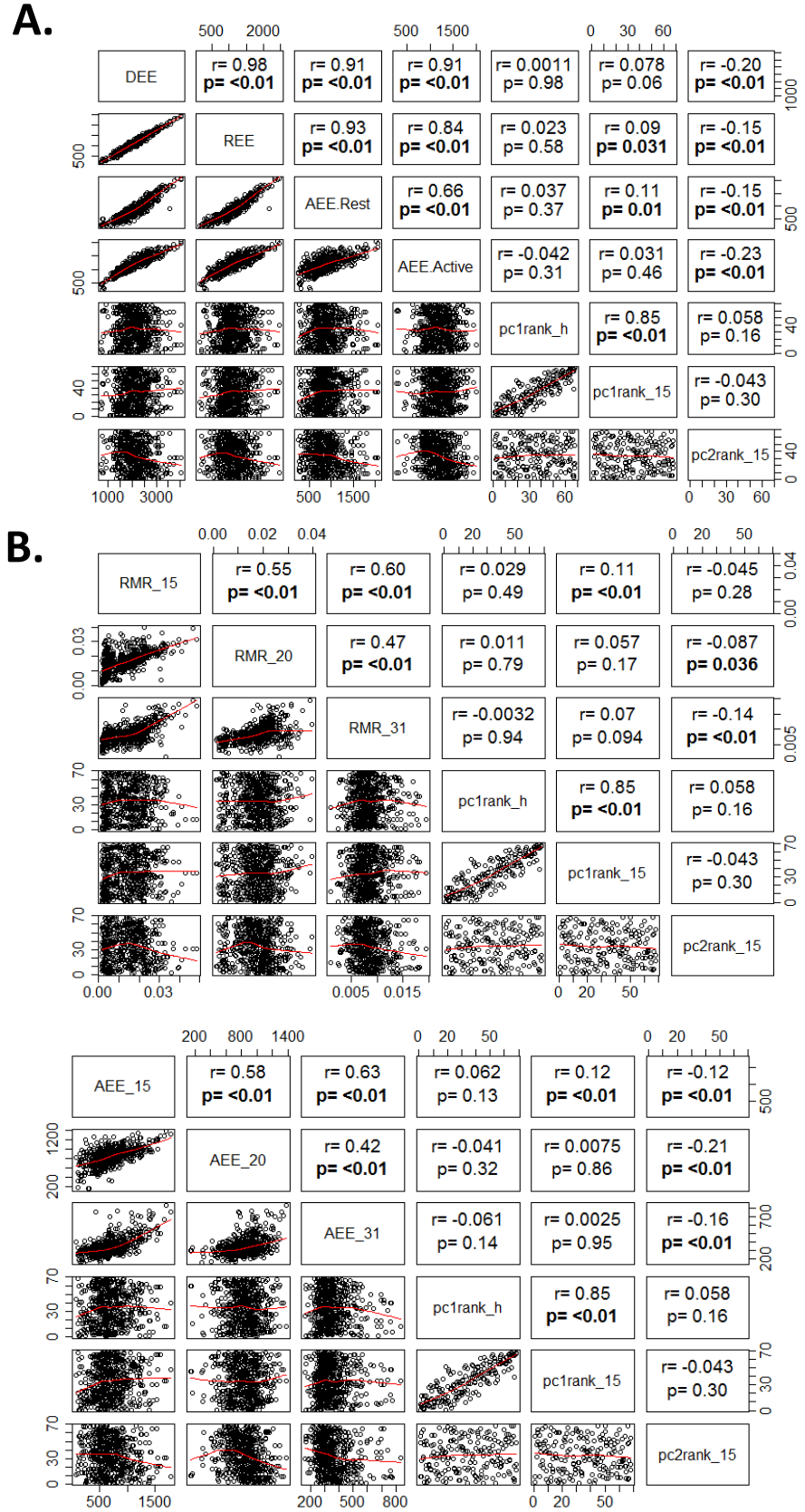


Figure 5.2. Correlation matrices of behavioural rankings (boldness in first hour (pc1rank_h), boldness over entire experiment (pc1_15) and exploration over entire experiment (pc2rank_15)) from open-field experiments and mass specific metabolic traits on days where food was not available from three respirometry runs (A: Daily energy expenditure (DEE (J day^{-1} g^{-1})), resting energy expenditure (REE (J/12h g^{-1})), average energy expenditure over rest phase (AEE.Rest (J/12h g^{-1})) and average energy expenditure over active phase (AEE.Active (J/12h g^{-1})), B: Resting metabolic rates (W g^{-1}) at three temperatures (15 °C, 20 °C and 31 °C) and C: Average energy expenditure over each temperature). Scatter plots shown in the bottom left of the graph and corresponding *r* and *P* values displayed in the top right panels.

Table 5.1. Responses of *M. musculus* to food availability and changes in ambient temperature. This includes the effects of A) the individual reaction norm intercept and slopes of daily energy expenditure, resting energy expenditure, average energy expenditure over the active and rest phase, resting metabolic rates at all temperatures and average energy expenditure at all temperatures in response to food availability and B) the individual reaction norm slope of resting metabolic rate in response to change in T_a on the predicted individual reaction norm intercept of the behavioural reaction norm intercepts.
* denotes significance ($P < 0.05$) from results extracted from a simple linear model with the behavioural reaction norms as the dependent variable and the metabolic reaction norm component (intercept or slope) as the fixed effect. β refers to the beta coefficient (i.e. estimates) from regression analysis.

		Boldness 1h (PC1_h)				**Boldness 15h (PC1_15)**				**Exploration 15h (PC2_15)**			
		Df	**β ± SE**	***F***	***P***	**df**	**β ± SE**	***F***	***P***	**df**	**β ± SE**	***F***	***P***
A) Response to food availability (intercept = food):													
DEE	**Intercept**	63	121.23 ± 53.82	5.138	**0.027***	63	132.81 ± 53.03	6.378	**0.014***	61	-73.77 ± 43.38	3.258	0.075
	Slope	63	-423.33 ± 187.95	5.073	**0.028***	63	-464.13 ± 185.20	6.281	**0.015***	61	257.61 ± 151.50	2.891	0.094
REE	**Intercept**	64	120.41 ± 40.84	8.694	**0.004***	64	125.33 ± 40.32	9.662	**0.003***	62	-71.56 ± 33.41	5.292	**0.025***
	Slope	63	-412.36 ± 127.02	10.538	**0.002***	63	-384.86 ± 127.48	9.115	**0.004***	61	171.70 ± 106.66	4.278	**0.043***
AEE.Active	**Intercept**	63	152.63 ± 91.08	2.808	0.099	63	222.07 ± 88.22	6.337	**0.014***	61	-118.85 ± 72.29	2.525	0.112
	Slope	62	-642.32 ± 371.73	2.986	0.089	62	-755.47 ± 365.81	4.265	**0.043***	62	-298.78 ± 299.32	0.893	0.348
AEE.Rest	**Intercept**	63	80.59 ± 37.19	4.697	**0.034***	63	78.20 ± 37.04	4.458	**0.039***	61	-54.08 ± 29.84	3.899	0.053
	Slope	63	-140.34 ± 64.48	4.737	**0.033***	63	-100.33 ± 65.26	2.364	0.129	61	47.08 ± 52.65	1.982	0.164
RMR.15	**Intercept**	64	142.47 ± 53.30	7.145	**0.010***	64	138.28 ± 53.15	6.768	**0.012***	62	-48.64 ± 44.24	1.444	0.234
	Slope	65	-112.19 ± 54.88	4.180	**0.045***	65	-47.25 ± 56.07	0.710	0.403	63	-9.06 ± 44.98	0.451	0.504

		Boldness 1h (PC1_h)				Boldness 15h (PC1_15)				Exploration 15h (PC2_15)			
		Df	**β ± SE**	***F***	***P***	**df**	**β ± SE**	***F***	***P***	**df**	**β ± SE**	***F***	***P***
A) Response to food availability (intercept = food):													
RMR.20	**Intercept**	64	167.48 ± 200.25	0.700	0.406	64	310.46 ± 196.55	2.495	0.119	64	310.46 ± 196.55	1.183	0.281
	Slope	65	-77.14 ± 275.55	0.078	0.780	66	39.65 ± 275.07	0.021	0.886	64	-479.83 ± 212.84	0.942	0.335
RMR.31	**Intercept**	61	117.54 ± 118.89	0.977	0.327	61	127.55 ± 117.94	1.170	0.284	59	-101.21 ± 93.81	2.216	0.141
	Slope	61	-247.67 ± 238.12	1.082	0.302	61	-219.67 ± 237.00	0.859	0.358	59	144.68 ± 188.86	1.695	0.197
AEE.15	**Intercept**	64	87.86 ± 29.42	8.917	**0.004***	64	86.34 ± 29.30	8.685	**0.005***	62	-52.76 ± 24.09	5.627	**0.021***
	Slope	64	-168.76 ± 57.34	8.663	**0.005***	64	-122.16 ± 58.82	4.314	**0.042***	64	51.58 ± 48.04	3.129	0.082
AEE.20	**Intercept**	63	143.22 ± 99.43	2.075	0.155	63	221.34 ± 96.52	5.259	**0.025***	61	-125.07 ± 78.67	2.386	0.127
	Slope	62	-289.39 ± 275.97	1.010	0.298	64	-486.66 ± 269.41	3.263	0.076	60	-238.70 ± 219.01	1.379	0.245
AEE.31	**Intercept**	62	-8.51 ± 70.96	0.024	0.879	62	-9.26 ± 70.54	0.022	0.83	60	-8.654 ± 56.14	0.018	0.893
	Slope	62	12.45 ± 103.74	0.014	0.905	62	13.53 ± 103.13	0.017	0.896	60	12.65 ± 82.07	0.018	0.878
B) Response of RMR to change in T_a from 31 °C (intercept) to 15 °C:													
Thermal conductance	**Slope**	62	-315.60 ± 129.20	5.968	**0.017***	62	-292.20 ± 104.90	7.359	**0.009***	61	53.79 ± 66.35	0.660	0.419

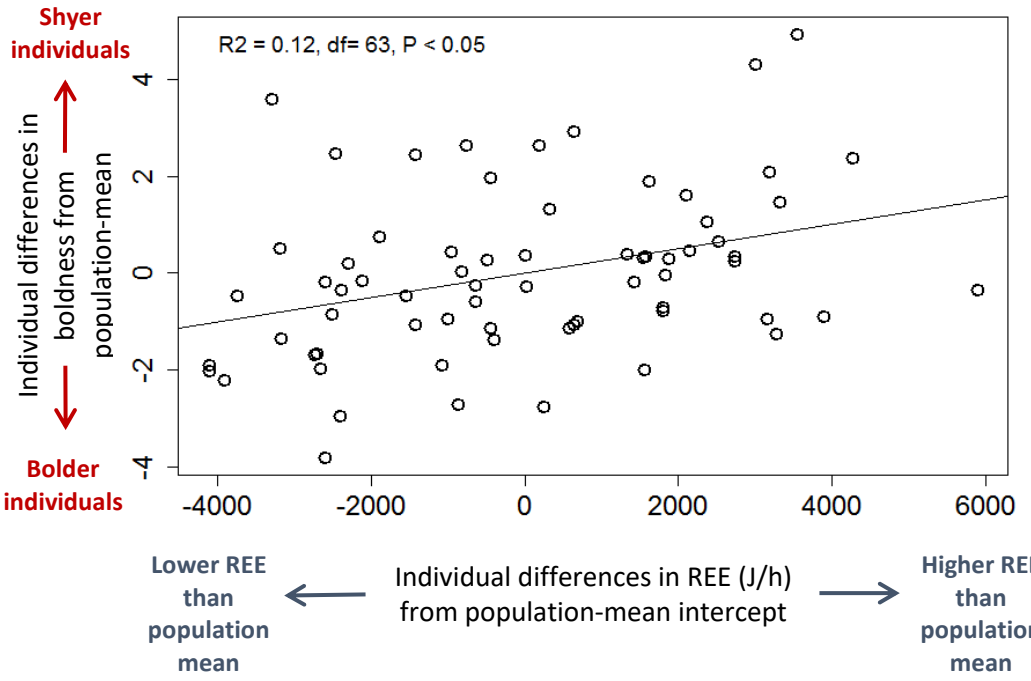


Figure 5.3. Effects of the predicted individual reaction norms for resting energy expenditure (REE) of *Mus musculus* when food was available on the predicted individual reaction norms for boldness over the first hour of the open-field experiment. Statistics shown in Table 5.1.

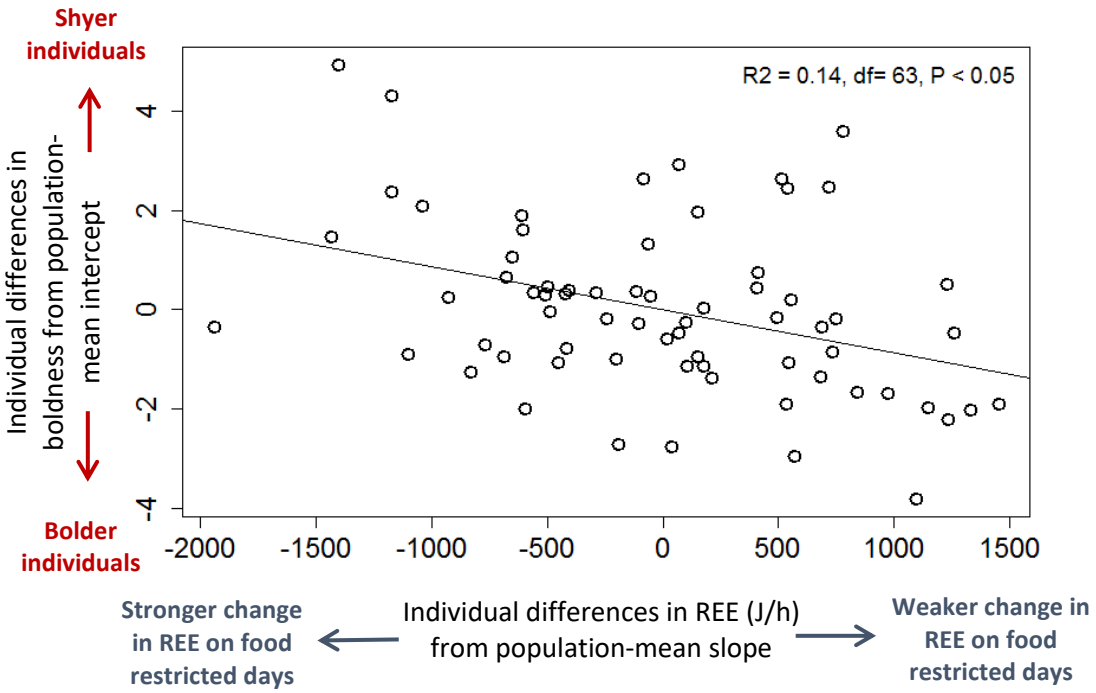


Figure 5.4. Effects of the predicted individual reaction norms for resting energy expenditure (REE) of *Mus musculus* in response to food restriction on the predicted individual reaction norms for boldness over the entire 15 hour open-field experiment. Statistics shown in Table 5.1.

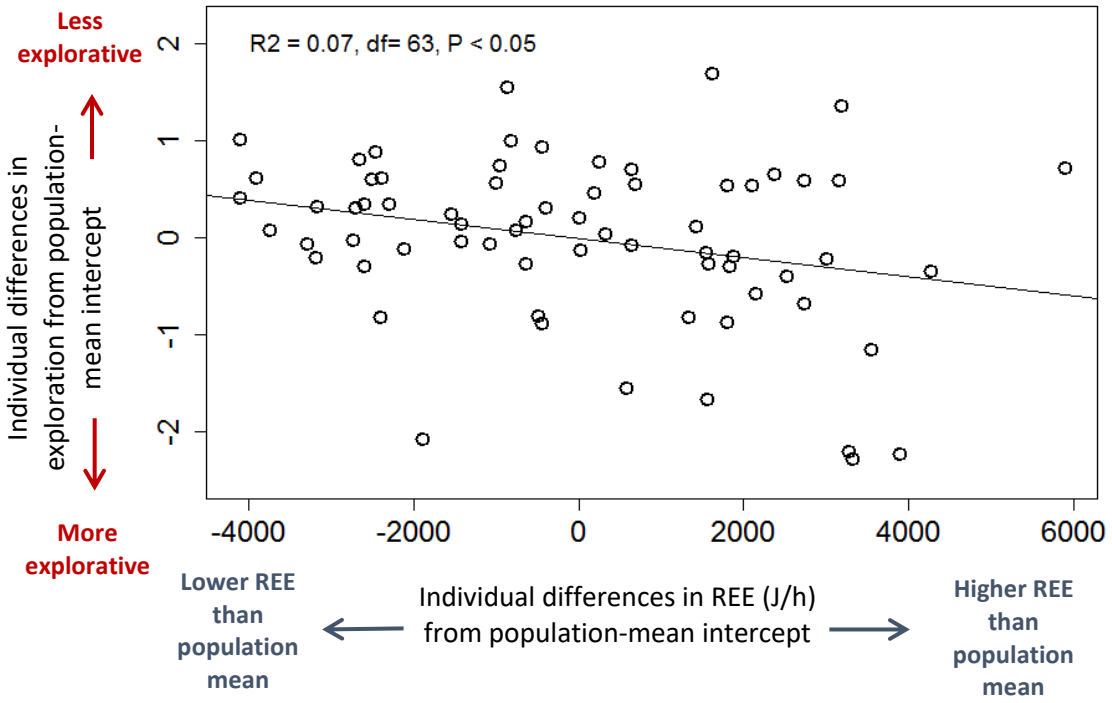


Figure 5.5. Effects of the predicted individual reaction norms for resting energy expenditure (REE) of *Mus musculus* when food was available on the predicted individual reaction norms for exploration over the entire 15 hour open-field experiment. Statistics shown in Table 5.4.

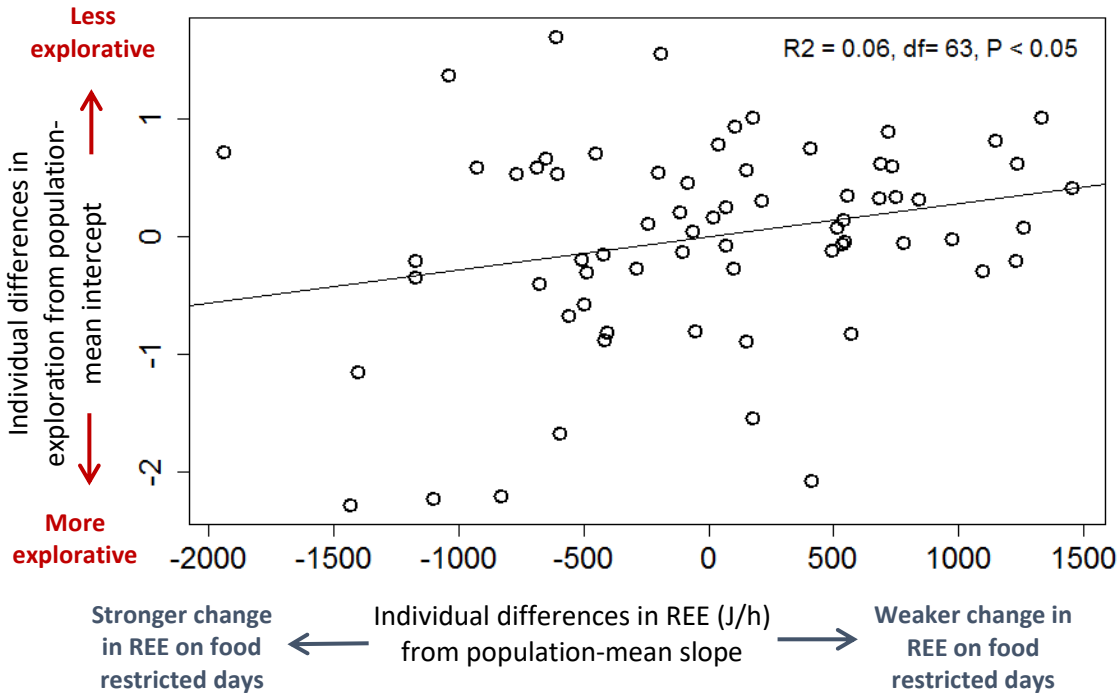


Figure 5.6. Effects of the predicted individual reaction norms for resting energy expenditure (REE) of *Mus musculus* in response to food restriction on the predicted individual reaction norms for exploration over the entire 15 hour open-field experiment. Statistics shown in Table 5.4.

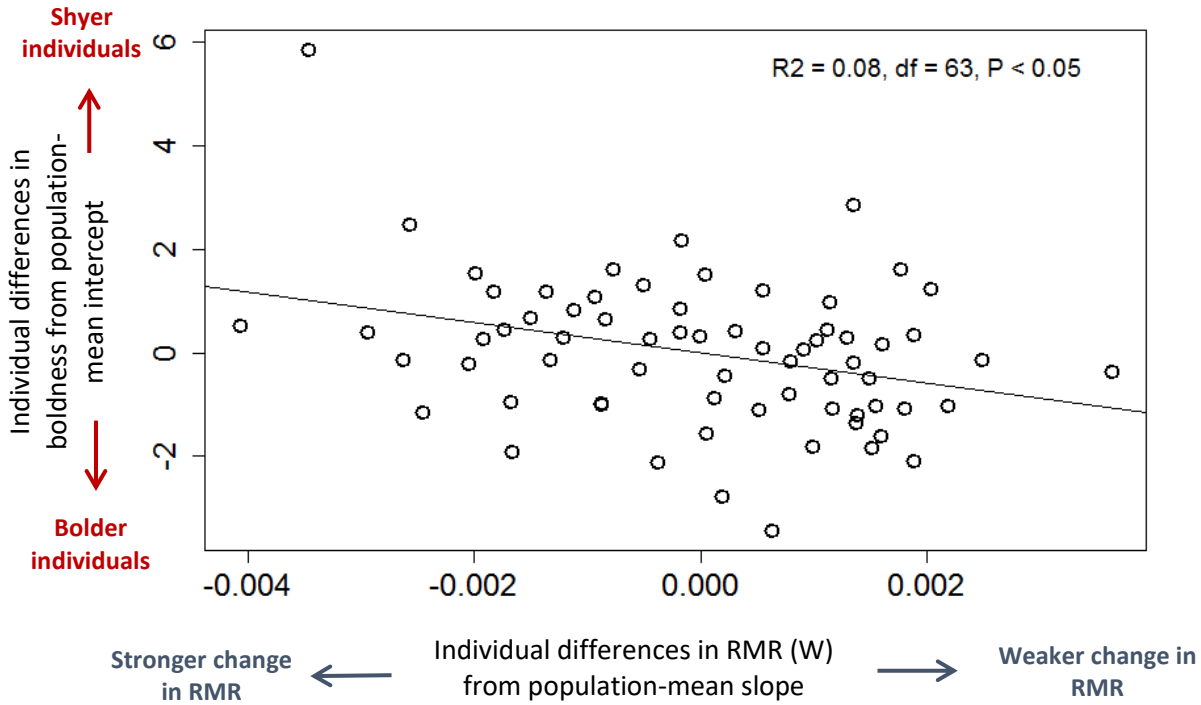


Figure 5.7. Effects of the predicted individual reaction norms for RMR of *Mus musculus* in response to change in T_a from 31°C to 15 °C on the predicted individual reaction norms for index of boldness over the first hour of the open-field experiment. Statistics shown in Table 5.4.

Chapter 6

General Discussion

6.1 General findings and synthesis

The overall objective of this thesis was to test for consistent individual differences and correlations among key behavioural (boldness and exploration) and physiological traits, as suggested by the pace-of-life syndrome (POLS) hypothesis, in wild caught *Mus musculus*. Initially, important research and knowledge gaps associated with the POLS hypothesis (chapter 1) and, more specifically, the need to incorporate thermal physiology with the POLS hypothesis (chapter 2) were identified. Empirically, this research aimed to investigate: (i) the impact of ambient temperature (T_a), dietary energy availability and time on the metabolic response in mice (chapter 3); (ii) the relationship between various components of the daily metabolic budget and daily energy expenditure (DEE) (chapter 3); (iii) the behavioural response of *M. musculus* to long term open-field tests (chapter 4); (iv) the repeatability (consistency) of measured metabolic and behavioural traits (chapters 3 and 4); and (v) whether individuals displayed correlated differences in key behavioural and physiological traits (chapter 5).

Organisms in their natural habitat experience fluctuating environmental, physiological and social conditions throughout their lifetime. Consequently, they experience a wide array of selective pressures. To enable individuals to respond appropriately to different contexts it might be expected that individuals would have very plastic phenotypic traits (Goulet *et al.,* 2017). On the contrary, individuals consistently show limited flexibility in their phenotypic traits and inter-individual differences in trait values (Sih, Bell and Johnson, 2004; Reale *et al.,* 2010). Anthropogenic impacts on environments often create ecological conditions that are evolutionary novel (Lawton, 1984) and population stability in environments experiencing rapid environmental change will largely depend on the phenotypic variation within a given population and degree of individual phenotypic plasticity (Sih *et al.,* 2012). A common consequence of environmental change is that energy availability is affected. To help predict how populations might respond to environmental change it is crucial to understand individual variation in key performance traits, how different aspects of

individual's daily energy budgets are associated and how these metabolic components respond to environmental variation.

The POLS hypothesis provides a unifying theory to understand the causes and maintenance of individual variation in key phenotypic traits. Empirical support for the POLS hypothesis has been mixed and inconclusive, and there is a need for more hypothesis driven research to test the assumptions that underlie the POLS hypothesis. To date, integration of energetics in the POLS hypothesis has relied almost exclusively on a single selected index of variation in metabolism; a standardised measure of resting metabolic rate (RMR) measured under a specific set of conditions termed standard (in ectotherms) metabolic rate (SMR) or basal (in endotherms) metabolic rate (BMR). Limiting ourselves to a single index of metabolism like BMR is not sufficient to accurately integrate metabolism with the POLS hypothesis. For instance, BMR does not provide any information regarding how individuals' respond energetically to changes in the environment (e.g. changes to food availability). To properly incorporate individual variation in metabolism with the POLS hypothesis it is important to move beyond the use of individual static traits like BMR, which oversimplify metabolic rate.

6.1.1 Impact of T_a, dietary energy availability and time on the metabolic response in M. musculus:

Metabolism has widespread impacts on an individual's energetic demand on their environment. We know that environmental variables such as food availability and thermal conditions (i.e. T_a) have significant effects on metabolism, but it is unclear how these effects vary among individuals (Metcalfe, 2016). Although laboratory strains of *M. musculus* can be an extremely useful model system in physiological ecology, it is crucial to have a comprehensive understanding of the thermal physiology of wild populations to make accurate inferences about adaption and evolutionary processes in the natural environment. *M. musculus* displayed standard physiological responses to variations in T_a and food withdrawal and exhibited a propensity to use torpor in the face of adverse conditions (low T_a and food withdrawal) (chapter 3).

Traditionally, respirometry experiments on small mammals like *M. musculus* do not last more than around six hours (Rosenmann and Morrison, 1974; Gorecki *et al.,* 1990; Selman

et al., 2001; Kristan and Hammond, 2003; Mathias *et al.,* 2004). Measurement duration has been shown to have a highly significant effect on metabolic measures (Hayes *et al.,* 1992a; Steffenson, 2002; Connolly and Cooper, 2014; Winwood-Smith and White, 2018); Cooper and Withers (2009) suggested that many of the BMR values reported in the literature for small marsupials may be overestimates as experimental durations involved are too short (less than four hours). This observation is likely to be applicable for many studies on small mammals. Clearly, the appropriate measurement duration is a vital element of experiments using respirometry to collect metabolic measures (Cooper and Withers, 2009) . My experimental set up enabled the respirometry system to run continuously over six days providing a continuous trace of metabolic rate for animals living under variable conditions of air temperature and food availability. This provided a detailed analysis of short-term (within run) temporal effects on metabolism. Metabolism was generally lowest on the first day of the experiment, during which time the mice were more likely to use torpor. Possibly, the lower metabolism recorded at the start of the experiment could be an artefact of stress. The respirometry chambers were designed to minimise stress. These were the same dimensions as the home cages and provided a familiar and more enriched environment than traditional respirometry chambers. Nevertheless, the handling and transfer into the respirometry chambers was likely to have been stressful which often has metabolic consequences that can last for hours (Hayes *et al.,* 1992a; Steffenson, 2002). This highlights how measurements of metabolism that are taken from short respirometry experiments probably do not provide an accurate reflection of an individual's normal state.

This thesis provides a unique look at torpor use in wild derived *M. musculus.* Male mice were more reluctant than females to enter torpor, but no sex-specific differences were detected in the metabolic characteristics of the torpor bouts. Often research on torpor in mice is carried out on laboratory strains (Ogilvie and Stinson, 1966; Hudson and Scott, 1979; Geiser and Baudinette, 1990; Dikie *et al.,* 2008; Swoap and Gutilla, 2009), which will not have experienced normal daily cycles in T_a during their early development and therefore do not represent an accurate reflection of state of wild mice (Chalfin *et al.,* 2014). Furthermore, many studies use stable temperatures to analyse torpor use (Holloway and Geiser, 1996; Swoap and Gutilla, 2009; McAllan *et al.,* 2012; Geiser *et al.,*

2014). T_a should mirror the natural conditions that mice would experience in the wild, as carried out in this thesis, to make ecologically relevant interpretations.

6.1.2 Relationship between metabolic measurements and DEE

Most studies trying to integrate metabolism into the POLS framework have used BMR (Careau and Garland, 2012). Using BMR to predict how metabolism is associated with life-history and behaviour depends on how measures of BMR are related to DEE (Bouwhuis *et al.,* 2014). In the present study, whilst traditional measures of BMR were not collected I did measure metabolic rate under the normal set of conditions that are required for BMR. The measurements taken were in post absorptive, non-reproducing, inactive individuals that were resting in their thermoneutral zone. Whilst these measurements were slightly lower than in many studies reporting traditional values of BMR in *M. musculus* (Geiser, 2004; Mathias *et al.,* 2004) they were within the range of BMR values cited in other studies for *M. musculus* and, therefore, concluded to be a fair representation of BMR (Degen *et al,* 1998; Johnston *et al.,* 2007). An important result in this thesis was that our representation of BMR (i.e. RMR at 31 °C on a food restricted day) was relatively poor at predicting DEE. RMR at 31 °C on non-food days did not include physiological mechanisms, like torpor, that are employed to adjust rates of energy expenditure to adverse environmental conditions (i.e. low temperature and food withdrawal). On the other hand, metabolic measurements (i.e AEE and RMR) at 15 °C did encompass individual thermoregulatory responses and were relatively more accurate for predicting DEE. In conclusion, studies aiming to integrate metabolism with the POLS hypothesis should ideally aim to use multiple measures of the daily metabolic budget, but if this is not feasible then it is advised to select a metabolic trait that incorporates adjustments in regulated body temperature as they provide a better representation of true energetic maintenance costs.

6.1.3 Behavioural response of M. musculus to long term open-field tests

Consistent differences in behavioural traits that are stable within individuals but vary among individuals are commonly reported (Reale *et al.,* 2007; Biro and Stamps, 2010; Carere *et al.,* 2013). Correlations between functionally distinct behavioural traits forming behavioural syndromes are also frequently observed (Koolhaas *et al.,* 2001; Sih *et al.,* 2004). These individual differences in behaviour are believed to affect fitness and have significant ecological consequences such as affecting how populations respond to

variations in their environment (Wolf and Weissing, 2012). In *M. musculus* some behavioural traits were correlated (chapter 4), suggesting the presence of a behavioural syndrome linking boldness and exploration. Specifically, bolder individuals also showed higher levels of exploration. This was only evident from the behavioural variables measured over the first hour of the OFT and not the entire 15 h test.

6.1.4 Consistent individual differences in metabolic and behavioural traits

A central component of the POLS framework is that individual differences in trait values are repeatable over the length of an organism's natural lifespan. Long-term studies that measure the consistency of multiple metabolic and behavioural traits over an individual's entire life expectancy are important (chapters 3, 4 and 5) as researchers sometimes assume that a metabolic or behavioural trait is consistent within individuals (Biro and Stamps, 2010). Overall, *M. musculus* show strong consistent differences in their behavioural responses to a novel environment. These results provide strong support for the repeatable nature of behavioural traits that have been observed in a wide range of species (Dingemanse *et al.,* 2002; Martin and Reale, 2007; Dhellemmes *et al.,* 2016).

Often studies estimate repeatability of metabolism at a single T_a, which means that any conclusions about the impact of natural selection on metabolism is restricted to that temperature (Careau 2014). Most individuals experience wide daily and seasonal fluctuations in temperature so there is limited evolutionary significance in getting repeatability estimates for single temperatures. It Is important for research to include multiple measures of resting metabolic measurements at various T_a (chapter 3) to allow the estimation of repeatability at each temperature and form more accurate evolutionary interpretations. The present study showed that *M. musculus* show consistent differences in multiple metabolic measures at various temperatures (15 °C and 31°C) over the expected lifespan of wild mice (Pockock *et al.,* 2004).

Few studies investigate effects of sex on repeatability of behavioural and metabolic measures, choosing instead to pool measures for the sexes (Schuett and Dall, 2009). In some species repeatability estimates for phenotypic traits have been observed to vary between sexes (Schuett *et al.,* 2010). For example, in grey mouse lemurs (*Microcebus murinus*), latency to emerge from a dark chamber in an OFT is more repeatable in males

than females (Dammhahn, 2012) and in field crickets (*Gryllus integer*) boldness across life stages is more repeatable in females (Hedrick and Kortet, 2011). Sex-specific natural selection is believed to create sex differences in behavioural repeatability (Schuster *et al.,* 2017). Males and females usually perform distinct roles in reproduction and are often exposed to their own specific selection pressures because of conspecific sexual competition or mate choice (Schuett and Dall, 2009). Due to this, it is often predicted that sexes should differ in their behavioural response and the repeatability of their behaviours. Males are often predicted to exhibit higher repeatability in behaviours that are indicated by a sexually selected trait because these traits are used by females to predict a potential mates' future behaviour (Bell *et al.,* 2009). However, studies often detect no sex differences in repeatability, for example, Dingemanse *et al.,* (2002) found no effect of sex on behavioural or metabolic traits between male and female *M. musculus*. Clearly, any differences in repeatability between sexes are not consistent across all species.

Most studies measuring the repeatability of metabolism gather estimates of repeatability for single metabolic traits (Bech *et al.,* 1999; Selman *et al.,* 2001; Labocha, *et al.,* 2004; Russell and Chappel, 2006). It is interesting to investigate whether metabolic responses to environmentally significant variables (e.g. food availability) are also repeatable traits. Reaction norms can be used to show an animal's capacity to adjust their metabolism to environmental variation (Terblanche *et al.,* 2008; Careau *et al.,* 2014). Here, *M. musculus* were significantly repeatable in their average individual responses to food availability (reaction norm intercepts) for most metabolic traits measured over the length of their natural expected lifespan (Pocock, 2004). The repeatability estimates for the average individual response to food withdrawal (reaction norm slope) provided lower estimates of repeatability compared to reaction norm intercepts. The reaction norm slope represents an individual's response to food withdrawal and the reaction norm intercept represents the individual's metabolic response to their normal state (i.e. food available). It is unsurprising that the repeatability of an individual's metabolic response on food days is more consistent than how the individuals respond to food withdrawal as energy expenditure to food restriction is intrinsically more variable because of the "sliding scale" of the reduction in T_b set-point. My thesis provides evidence of significant repeatability of multiple components of metabolic energy expenditure in *M. musculus*. In particular, high

individual consistency in DEE, REE and energy expenditure at 15 °C, relative to the total population variation was observed.

6.1.5 Correlations between metabolic and behavioural traits

Frequently studies investigating the relationship between metabolism and behaviour restrict themselves to looking at the trait values of single traits. Incorporating the metabolic responses of individuals to changing environments (i.e. change in energy abundance or T_a), as carried out in this study, is a key area for future research. A metabolic and behavioural "reaction norm" approach that characterise how individuals respond to changes in their environment will lead to better defined and more realistic hypotheses regarding how consistent individual differences in energy expenditure relate to key POLS traits.

Most studies investigating the associations between metabolism and behaviour have demonstrated a positive relationship between a measure of metabolic rate (e.g. BMR, SMR or RMR) and a behavioural trait (e.g. boldness or activity; Biro and Stamps, 2010; Mathot and Dingemanse, 2015). For instance, laboratory mice divergently selected on mass -corrected BMR were found to have a positive correlation between locomotor activity and BMR (Gebczynski and Konarzewski, 2009). Positive correlations between behaviour and various measures of metabolism (RMR, BMR and standard metabolic rate) were shown in 9 of 21 case studies in fish studies (Careau and Garland, 2014). Additionally, in a comprehensive analysis of 27 case studies ranging from invertebrates to mammals Biro and Stamps (2010) observed a positive relationship between behavioural (largely activity and aggression) measures and resting metabolic rates in 20 of these cases. Similarly, in this thesis more explorative individuals had higher levels of REE (chapter 5). However, the correlation between metabolism and personality is not equivocal in the literature. Negative relationships between metabolism and behaviour are also frequently described (Adriaenssens and Johnsson, 2011; Debecker *et al.,* 2016). Bouwhuis *et al.,* (2014) reported a negative relationship between exploration and BMR in female great tits (*Parus major*) whereby more exploratory individuals had lower BMR than less exploratory individuals. A comparison of 19 muroid species also reported exploration to be correlated with BMR (Careau *et al.,* 2009). In field crickets consistently bolder individuals were shown to have lower standard metabolic rates (Careau *et al.,* 2019). In this thesis a negative

association between boldness and metabolism, whereby bold individuals exhibited lower levels of REE than shy individuals, was also observed. Other studies find no significant relationship between behaviour and metabolism. For example, in root voles (*Microtus oeconomus*) no correlation was found between personality and BMR (Lantova *et al.,* 2011).

Two of the most notable hypotheses to explain associations between metabolism and behaviour are the "increased-intake" (or performance) model (Nilsson, 2002; Careau *et al.,* 2008a) and the "compensation" model. The "increased-intake" model assumes that an organism's capacity for an energetically demanding lifestyle (i.e. proactive) requires a larger "metabolic engine". It predicts a positive relationship been behaviour and metabolism. Alternatively, in the "compensation model" it is hypothesised that organisms have a fixed amount of energy that can be allocated amongst competing pathways and increasing investment in one pathway (e.g. metabolism) limits the amount that can be used in energetically costly behaviours (e.g. activity). Therefore, a negative association between metabolism and energy demanding behaviours, like boldness and exploration, are expected (Metcalfe *et al.,* 1995). Results from this study do not clearly support either of these two models and instead indicate there is no universal link between behaviour and metabolism, and the direction of any associations among behavioural and metabolic traits are dependent upon the traits in question.

The POLS hypothesis has produced an enormous amount of research interest in the last decade, with the key publications of the POLS hypothesis (Ricklefts and Wikelski, 2002; Wikelski *et al.,* 2003*;* Reale *et al.,* 2010) receiving an increasing number of citations every year (Dammhan *et al.,* 2018). Yet, empirical research testing the POLS hypothesis has produced ambiguous results, highlighted succinctly in Royaute's *et al.* (2018) meta-analysis on pace-of-life syndromes, hence, researchers in the field are encouraging a closer examination and review of the topic to develop a refined definition of POLS (Dammhan *et al.,* 2018)

The associations between metabolism and behaviour are clearly complex. There is reasonable concern that the traditional definition of the POLS hypothesis is too restrictive to suitably include all the potential associations (Dammhan *et al.,* 2018). Many additional factors (i.e. reproductive status, age, environmental conditions, conspecific density and

resource abundance) are likely to have a significant effect on the linkages among various behavioural, physiological and life-history traits and should be taken into consideration (Careau *et al.,* 2015). For instance, in wild great tits (*Parus major*) fluctuations in population density affect the relationship between exploration and survival. Specifically, compared to slow explorers, fast exploring individuals have higher survival rates in low density populations and lower survival rates in high density populations (Nicolaus *et al.,* 2016). Additionally, in wild brown trout (*Salmo trutta*) the associations between individual activity and growth rate is affected by resource abundance. Active trout have faster growth rates than inactive individuals when there is high food abundance but when food abundance is lower, active individuals will grow slower. Environmental differences among studies investigating the same traits may cause some of the disparities reported in the strength and direction of linkages among behaviour and metabolism (Adriaenssens, 2017).

Dammhahn *et al.,* (2018) proposed redefining POLS in a broader scope, forgoing the previous hypothesised directional relationship between different traits within a syndrome. In this new framework POLS is considered as "the suite of phenotypic traits (e.g. behavioural, morphological or physiological) associated with the life-history trade-off between current and future reproduction". These suites of correlated traits are believed to have coevolved in response to how organisms respond to trade-offs between current and future reproduction. For example, bold individuals are more likely to acquire resources (e.g. food, nest sites and mates) at the expense of survival (e.g. increased rates of predation; Wolf *et al.,* 2007; Reale *et al.,* 2010). Within a syndrome some phenotypic traits may be partially-independent, co-evolving as adaptations to alternative, associated, trade-offs. Consequently, not all phenotypic traits involved in the syndrome are believed to be directly associated (Aray-ajoy and Dingemanse, 2014; Dammhan *et al.,* 2018). This redefined definition fits the results observed in the present study. Because both boldness and exploration were assumed to represent "risky" behaviours, I expected both these traits to be related to metabolism in a similar way. Instead boldness and exploration were related to REE in opposite directions, thus it could be the case that these traits are indirectly linked within a syndrome whereby boldness and exploration are related to high and low REE respectively.

6.2 Limitations, cautions and further study

Support for the POLS hypothesis may be mixed, in part, because the predicted associations between key traits are frequently investigated at the phenotypic level, while the predictions of the POLS framework are predicted at the genetic level (Reale *et al.,* 2010). Experiments at the individual level that assume correlations between consistent behavioural and physiological traits reflect underlying genetic correlations risk conflating genetic and environmental sources of covariance among traits. This confusion can lead to unsuitable evolutionary or ecological interpretations (Royaute, 2015). While studies such as those presented in this thesis are crucial for investigating covariation of behavioural and physiological traits, selective breeding experiments, for example, could be used to establish the heritability of key traits and tease apart the genetic versus environmental causes of associations within pace-of-life strategies. Despite being a key assumption of the POLS hypothesis, whether the proposed association within different pace-of-life strategies have genetic or environmental causes is not well studied (Careau *et al.,* 2011). Some studies have shown evidence of a genetic association between trait combinations (Swallow *et al.,* 2009; Careau *et al.,* 2011; Niemela *et al.,* 2013). Further research incorporating multiple generations will assist in separating the additive genetic causes from phenotypic plasticity and assist in making appropriate evolutionary interpretations (Royaute *et al.,* 2015).

To date, empirical studies that have supported the POLS hypothesis are frequently conducted under captive conditions (Stamps 2007; Biro and Stamps, 2008; Adriaenssens and Johnsson, 2009), whereas those carried out on wild populations often fail to provide strong support for this hypothesis (Dingemanse *et al.,* 2004; Adriaenssens and Johnsson, 2009; Adriaenssens and Johnsson, 2013; Timonin *et al.,* 2011). Perhaps, the associations between behavioural, physiological and life-history traits are more variable in natural environments which often show substantial variations in resource abundance and competition levels (Adrianssens and Johnsson, 2009; Zavorka *et al.,* 2015). Whilst laboratory studies allow for animals to be closely monitored, they introduce their own problems by creating artificial environments which do not reflect real world scenarios. Moreover, studies have shown that associations between phenotypic traits in the wild are affected by habitat diversity and fluctuating environmental quality (Zavorka *et al.,* 2015). Experiments taking place outside of the laboratory and in an animal's natural habitat will provide useful insights and an ecologically realistic way of investigating associations among

phenotypic traits. For instance, semi-natural outdoor enclosures can reflect the challenges animals face in the wild (e.g. natural variation in environmental conditions) whilst allowing for the manipulation of key factors (e.g. food availability and predation risk).

A notable factor that may have contributed to an inability to detect stronger trait associations (chapter 5) is the possibility of capture bias in my study population. Specifically, the trapping method used in the experiments within this thesis may be biased towards trapping more active individuals. All mice used in this thesis were live trapped with Elliott and Sherman traps. When using passive gear, like live traps, more explorative and proactive individuals are more likely to be captured (Biro and Dingemanse, 2009). In fish it has been shown that there is a higher chance of catching bold fish compared to shy fish, with some of the shyest individuals never getting captured (Wilson *et al.,* 1993). Similarly, a study on Namibian rock agama (*Agama planiceps*) found bolder individuals entered traps significantly faster than shy individuals, leading to a higher trapping success for bolder individuals (Carter *et al.,* 2012). Trap response heterogeneity is commonly reported within populations of *M. musculus*, whereby some individuals are significantly trap-prone, and others trap-shy (Crowcroft and Jeffers, 1961; Drickamer, *et al.,* 1999). In the present studies, other sampling methods that possessed less bias, such as pit fall or bucket traps, were not feasible at our trapping site but could be useful to consider in future studies.

Whilst individual variation in key traits (e.g. boldness, exploratory behaviour and resting metabolic rates) have been widely documented by assessing individuals in isolation (Nespolo and Franco, 2007; Bell *et al.,* 2009; Dingemanse *et al.,* 2012; Carere *et al.,* 2013), surprisingly few studies have quantified individual variation in social settings (Aplin *et al.,*2015). Social network position is linked to fitness and where an individual fits in their social network can affect numerous aspects of life history (e.g. breeding and foraging success; McDonald, 2007; Boogert *et al.,* 2014). Individual differences in social behaviour (e.g. competitive interactions) may have some important implications for the evolution and maintenance of behavioural and physiological traits. However, little is known about how the position and stability of social relationships relate to other important performance characteristics. Whilst relationships between social behaviour and other

behavioural traits (e.g. exploratory behaviour) have been found (Snijders *et al.,* 2014), few studies have tested how laboratory measurements of solitary individuals are related to individual placement within a social hierarchy. Whether prior characterisation of individual differences and correlations among key behavioural and metabolic traits can be used to predict social rank would be a rewarding topic for further study.

Overall results in my thesis showed empirical support for associations between consistent individual differences in some behavioural and physiological traits, as is proposed by the POLS hypothesis. However, some of these associations deviated from the specific directions expected by the traditional POLS framework. This is a trend that has been frequently observed in empirical studies testing the POLS hypothesis (Niemela *et al.,* 2011; Zavorka *et al.,* 2015; Thomas *et al.,* 2016; Zavorka *et al.,* 2016). This indicates that some of the traits included in the POLS framework may not be as tightly linked as is sometimes implied. The POLS hypothesis is most useful in proposing a general picture of the expectations and for formulating hypotheses to be tested, rather than being used to draw conclusions based on single findings. Trait associations should not be assumed without examining the expected relationships directly. As stipulated in its conception, care must always be taken to avoid the temptation of using the POLS framework to generalise and simplify a complex reality (Reale *et al.,* 2010) as associations between behavioural, physiological and life-history traits most likely vary in direction, strength and plasticity.

6.3 Conclusions and future outlook

The prevalence and potential ecological importance of consistent individual differences in key traits makes studies into inter-individual variation crucial. For instance, the existence of individual variation in the form of alternative strategies might be important to the persistence of populations in the face of environmental change. It is important to test the assumptions and predictions of the POLS hypothesis as the POLS framework may explain why individuals often express significant differences in single traits even when there is apparently selection pressure towards a mean trait value. The work presented here provides a rigorous test of the POLS hypothesis in a wild-derived population and fills a clear gap in our knowledge about the ecology of wild *M. musculus.* The overall main findings of this work are summarised as follows:

- Metabolic traits at 15°C were more accurate as relative predictors of DEE than measurements at 31 °C, which lacked a thermoregulatory component
- Strong evidence of repeatable differences among individuals in multiple metabolic and behavioural traits were observed
- Correlations were observed between some metabolic responses to food availability and behavioural traits (boldness and exploration)
- The observed correlations between behaviour and metabolism did not always support the predictions of the POLS hypothesis. For instance, bold individuals had lower levels of REE.

Many of the empirical studies that have opposed the POLS hypothesis have investigated whether single behavioural, physiological or life-history traits are correlated with each other (Krams *et al.,* 2014; Montiglio *et al.,* 2014). Within this thesis, multiple behavioural variables, from which two behavioural traits were selected, and several components of the daily metabolic budget were used to analyse associations between numerous traits and provide a comprehensive analysis of potential linkages among behavioural and physiological traits. Additionally, metabolic responses (i.e. reaction norms) to significant environmental variables (e.g. food restriction) were incorporated and their relationship with behavioural traits investigated. Overall results provide empirical support for some of the predictions of the POLS hypothesis; that individuals exhibit consistent and correlated differences in behavioural and physiological traits.

To date, most empirical examinations of the associations between metabolism and behaviour have been carried out at interspecific level (Careau *et al.,* 2009) on domesticated species (Careau *et al.,* 2010) or lab-maintained animals (Huntingford *et al.,* 2010; Careau *et al.,* 2011). Complementing these laboratory studies with field studies to compare results observed within this thesis, in addition to the topics of further study mentioned above, would greatly enhance our understanding of intraspecific variation. Ongoing research is currently underway to determine: (i) how environmental variability in energy resources and predation risk affect within-population variation in metabolism and POLS traits and (ii) how detailed laboratory measurements characterising correlations among multiple behavioural and metabolic traits (chapters 3,4 and 5) relate to an individual's energetic response and fitness success in a semi-natural environment.

Incorporating measurements from semi-natural field enclosures will enhance the ecological relevance of results in this thesis by providing the opportunity to examine the integrative mechanisms allowing small mammals to cope with environmental change. This will provide an important applied aspect in terms of understanding the causes of vulnerability to environmental degradation and potential for population resilience to future environmental variability.

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