University of New England

The Thermal Games Dung Beetles Play

A thesis submitted by

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ABSTRACT

Dung beetles are a group of coprophagous scarabs most well known for their reliance on dung as both a source of food and as a vital component of their reproductive cycle. Intense competition between sympatric dung beetles species has spurred the evolution of a variety of complex nesting behaviours; telecoprids move dung along the soil surface; endocoprids reproduce within the dung itself; paracoprids bury dung in tunnels beneath the soil surface. The act of breaking down and relocating dung facilitates numerous biotic and abiotic processes that shape the environment and aid in maintaining ecosystem function. As a result of their ubiquity, the unique niche they inhabit, diverse range of species and life history strategies that have evolved, and strong ties to the biotic and abiotic processes in their surrounding habitat dung beetles have been the subject of research across the globe. We conducted a review of 1463 peer reviewed papers concerning dung beetles with the aim of elucidating geographical, temporal, and methodological patterns of research in order to identify gaps in the literature to better inform future research. All continents, with the exception of Antarctica, have a strong body of literature that has been shaped by the composition and structure of the local dung beetle assemblage, the ecosystems they inhabit, and the cultural and societal influences of its human inhabitants. Paracoprids were the most studied functional group overall with *Onthophagus* being the genus most frequently used in manipulative experiments, present in 10.3% (151) of the publications assessed. The species *Onthophagus taurus* is the most studied dung beetle, being one of the few species that has been the subject of genetic research, featuring in 4.5% (66) of studies. The most studied aspects of dung beetles are their ecology and behaviour, with relatively little work conducted on their genetics and physiology, although interest in these fields is increasing. Field surveys are the most frequently utilised method across the literature being used in 456 (31.2%) studies, followed by laboratory experiments (29.1%), and field experiments (13.5%). Studies of dung beetle communities across environmental gradients were also popular, particularly in South America where the focus is often on their use as a biological indicator of environmental health along disturbance gradients. Across the 1463 studies 75 types of dung were used, however the majority of studies utilised dung from livestock such as cattle (39.8%), pigs (6.6%), sheep (5.5%), and horses (4.2%). Human dung (16.9%) and carrion (9.8%) were also amongst the most frequently used types of dung, with most use occurring in South America and North America (primarily Mexico) where they have been shown to be an effective bait for a wide range of species. Studies assessing two or more types of dung are in the minority, accounting for only 22.4% of the publications assessed. Many studies failed to include important information such as the location where the study took place, a description of the habitat, the type of dung used, and whether the dung used was sourced from an animal that had been chemically treated.

Dung beetles are strongly influenced by their thermal environment with sympatric species utilising thermal niche partitioning to reduce competition. Climate change models indicate that by 2070 temperature in Australia will increase by 0.4°C-4.5°C in coastal areas and 1.1°C-5.7°C further inland. Rising temperatures and increasingly variable thermal regimes will likely alter the community dynamics of Australia's dung beetle fauna, which it is reliant upon for the provision of a number of ecosystem services. The aim of this research was to determine the physiological tolerances of an Australian dung beetle community and to measure the metabolic responses of these species to thermal changes within these limits to determine whether thermal niche specialisation is driven by metabolic adaptations. Seven species of dung beetle (*Onthophagus australis*, *O. granulatus*, *O. binodis*, *Aphodius fimetarius*, *Euoniticellus intermedius*, *E. fulvus*, and *Sisyphus rubrus*), belonging to three functional groups, were retrieved from baited pitfall traps placed in grazed fragmented pasture ecosystems at Armidale and Bingara, NSW, Australia. Thermal limit respirometry was used to measure the CT_{min}, CT_{max}, $\dot{V}CO_2$ ml h^{-1}, and the thermal sensitivity of their metabolic rate to changes in temperature using a dynamic temperature protocol to subject individual beetles to either increasing (25°C-60°C) or decreasing temperatures (25°C- -15°C). The telecoprid *Sisyphus rubrus* had the highest CT_{max} (53.1°C ±0.6**)** and a stable metabolic rate when exposed to high temperatures. The endocoprid *Aphodius fimetarius* was the most tolerant to low

temperatures with a CT_{min} of -2.5°C ±2.0, while still having a CT_{max} of 43.6 ±1.3°C. Relative to the other species assessed it had a high metabolic rate across its entire thermal window, indicative of a thermal generalist. The paracoprids *E. fulvus* (CT_{min}: 9.1°C ±2.4; CT_{max}: 49.9°C ±1.4) and *E. intermedius* (CT_{min}: 8.3°C ±1.8; CT_{max}: 49.1°C ±1.9) have adapted to operate at higher temperatures than the onthophagine paracoprids which have thermal traits intermediary to the cold tolerant *A. fimetarius* and heat tolerant species.

Given the central role of temperature in the evolution and ecology of dung beetles, and the ecosystem functions they provide, understanding the capacity of species to respond to climate change is required to understand the response of communities and implement effective management strategies. Dung beetles of the species *Onthophagus binodis* were collected from the University of New England's Kirby SMARTFARM, NSW, Australia. Beetles were placed in custom built temperature controlled chambers and soil temperature data used to program chambers with a variable temperature regime based on the natural regime experienced by the sampled population in the field. The chambers were programmed to simulate increased temperatures based on climate change warming estimates of +0°C, +2°C, +3.5°C, and +5°C. Every 10 days two beetles from each chamber were removed and dynamic thermolimit respirometry performed to measure the CT_{max}, $\dot{V}CO_2$ ml h^{-1}, thermal sensitivity of metabolic rate, and the intercept of the regression of metabolic rate and temperature. Changes in the nesting behaviour of *O. binodis* were also assessed by recording the number of brood balls produced, brood ball burial depth, and brood ball size. Brood balls were then reared at 25°C and the experiment repeated with the second generation. *Onthophagus binodis* did not display the capacity for physiological plasticity under the simulated warming scenarios, with no significant effect of warming on CT_{max} (46.43°C ±1.68), $\dot{V}CO_2$ ml h^{-1} (32.28 ±8.84), thermal sensitivity (2.51 ±0.63), or the intercept of the MR-T line (-0.13±0.41). Warming regimes had no effect on brood burial depth (9.12cm ±7.02). We found evidence for a potential density dependent effect of temperature on brood ball production in the second generation: beetles from the +5°C treatment producing significantly more

broods than the +0°C control. When brood ball production was low beetles from the +5°C treatment produced broods with a significantly greater volume than +0°C controls.

DECLARATION

I, Zac Hemmings, certify that the ideas, experimental work, results, analyses, and conclusions reported in this dissertation are entirely my own effort, except where otherwise acknowledged. I also certify that the work is original and has not been previously submitted for any other award, except where otherwise acknowledged.

Zac Hemmings

18/09/2018

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Signature of Candidate Date

ENDORSEMENT

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Signature of Supervisor/s Date

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CHAPTER ONE

GENERAL INTRODUCTION

1.1 – Insects and climate change

Insects are ubiquitous. Their ability to adapt to new environments over relatively short time-scales has allowed them to colonise every continent and become the dominant group in most terrestrial and freshwater ecosystems. As a result of this adaptability insects are the most abundant and diverse group of animals on the planet, with many species having evolved to excel in narrow ecological niches (Grimaldi and Engel 2005, Forister, Novotny et al. 2015). The potential for a species to maintain a population in a changing environment, or to colonise a new habitat or niche, can only be realised when existing biological processes, and the abiotic conditions that drive them, allow.

The response of insects to climate change has been extensively studied, however the majority of this work focuses on a limited number of species and primarily concerns changes in distribution, with studies rarely addressing physiological and behavioural responses (Andrew, Hill et al. 2013). Changes in species distribution in response to climate change have historically been modelled by extrapolating future distributions using climate data from the species current range (Gillson, Dawson et al. 2013). Species distribution models that account for the physiological limitations of species, known as mechanistic models, have a number of advantages over traditional correlative models, namely: allow for the formulation of predictive hypotheses (Evans, Diamond et al. 2015); do not require presence/absence data; can be extrapolated to novel climates (Buckley and Kingsolver 2012); and provide an understanding of the mechanisms underlying species distribution (Kearney and Porter 2009). While mechanistic models are a promising technique for predicting range shifts of species due to climate change, they require extensive, species specific, data that can be difficult and time-intensive to obtain (Fischer, Thomas et al. 2014, Singer, Johst et al. 2016). The synthesis of traditional, correlative, climate change models with physiological data can give a more accurate understanding of species response to climate change (Buckley, Waaser et al. 2011).

Insects adapt to changes in their environmental temperature physiologically, behaviourally and ecologically, however thermal regimes are becoming increasingly perturbed as a result of climate change (Houghton, Ding et al. 2001). In this regard it is not only the ability to adapt to long-term changes in mean temperature that is vital, but also the capacity for short-term adaptation to increased climatic variability (Sinclair, Vernon et al. 2003). Climate change is expected to have the greatest impact on insect species living at low latitudes, as tropical species often live closer to their physiological tolerances, despite larger increases in temperature expected at higher latitudes (IPCC 2007, Deutsch, Tewksbury et al. 2008, Diamond, Sorger et al. 2012). Studies such as these have been criticised for their use of broad scale climate data, such as mean temperature, gathered from weather stations. Weather station data is rarely an accurate reflection of the microclimatic conditions experienced by insects (Andrew, Hart et al. 2013) and long-term measures of change such as mean annual temperature fail to encapsulate the selective pressure of increasingly variable thermal regimes (Vorhees, Gray et al. 2012). The accuracy of climate change models is therefore reliant on the availability of fine-scale microclimate data as well as a thorough understanding of species thermal biology. Understanding species thermal biology allows for important insights into the impact of climate on the ecology, distribution, and evolution of a species (Janzen 1967, Chown and Terblanche 2006, Ghalambor, Huey et al. 2006) which is becoming increasingly important with the changing thermal regimes in many environments caused by the modification of habitat structure (Nobre, Sellers et al. 1991, Groom, Meffe et al. 2006), as well as the impact of anthropogenic climate change (Dale, Joyce et al. 2001, Theurillat and Guisan 2001, Bale, Masters et al. 2002, Crick 2004, Parmesan 2006).

1.2 – Thermal biology

The effects of temperature on organisms can be seen at every scale, from the molecular level, where it dictates the rate of biochemical reactions necessary for life (Stockbridge, Lewis et al. 2010), to the ecosystem level where changes in temperature alter species interactions and affect ecosystem function (Dunson and Travis 1991, Memmott, Craze et al. 2007, Traill, Lim et al. 2010). Temperature exerts a strong influence on insects, their small body size and limited ability for physiological thermoregulation means they lack the capacity to maintain body temperatures substantially different from ambient conditions, making them particularly susceptible to changes in environmental temperature (Stevenson 1985). Many important biological traits, such as metabolic rate (Nespolo, Lardies et al. 2003), fecundity (Gotthard, Berger et al. 2007), development time (Bayoh and Lindsay 2003), body size (Tun-Lin, Burkot et al. 2000), and phenology (Ellwood, Diez et al. 2012) are temperature dependent.

The ability of an organism to perform under temperature induced stress can be described using a thermal performance curve (Huey and Slatkin 1976, Huey and Kingsolver 1989). Thermal performance curves plot the physiological activity of an organism against temperature, where the slope of the curve describes how sensitive the organism is to changes in temperature (Figure 1.1). Temperatures at which performance is reduced to zero indicate the organisms' physiological tolerances. As body temperature approaches these tolerances performance drops and animals become thermally stressed. T_{opt} represents the optimum body temperature for the organism, at this point activity is greatest. Thermal performance curves are often asymmetric, with the optimum temperature laying closer to the upper thermal tolerance (Angilletta Jr, Niewiarowski et al. 2002).

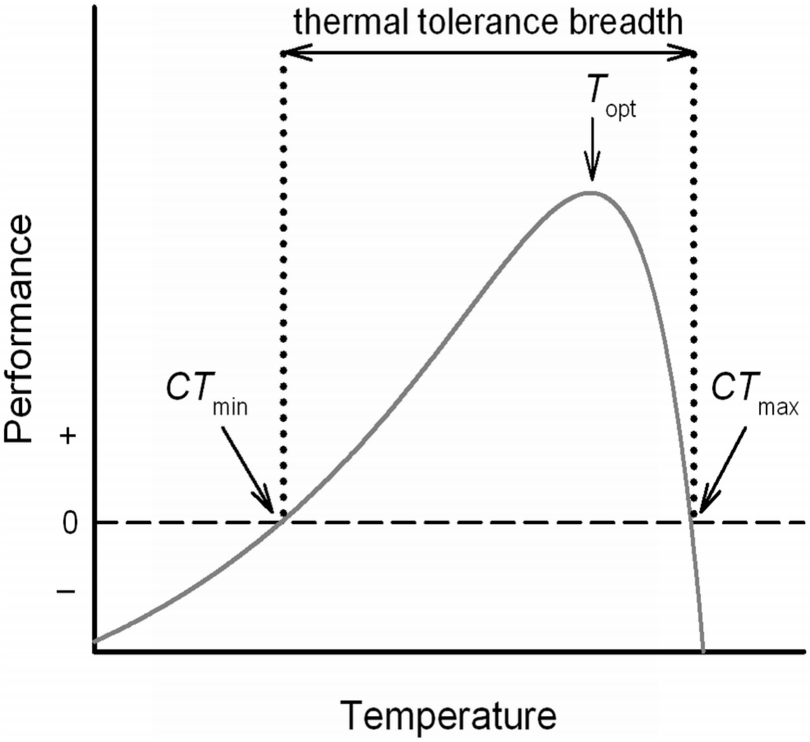


Figure 1.1. A common thermal performance curve displayed by ectotherms. The thermal optimum (T_{opt}) is the body temperature that maximises performance. The performance breadth is the range of body temperatures that allows for performance above a certain level. From Krenek, Petzoldt et al. (2012).

1.2.1 – Thermal specialisation

Thermal performance curves represent a conglomerate of physiological and genetic traits, upon which selection pressures act. Thermal generalists are capable of activity over a wide range of temperatures, displaying broad curves with shallow slopes, while thermal specialists display high performance over narrow temperature ranges and have correspondingly steep slopes (Figure 1.2) (Angilletta 2009). Thermally stable environments favour the evolution of specialist species as the cost of thermoregulation in these environments is minor, whereas both generalist and specialist species can arise in variable environments, dependent on the cost of thermoregulation (Angilletta Jr, Bennett et al. 2006). Thermal specialists have high levels of performance within their thermal niche and are thought to be more resilient to short-term variation in temperature due to greater metabolic plasticity, which is required to account for stochastic temperatures. Generalists are capable of modest activity over a wide range of temperatures and may lack plasticity owing to the evolutionary cost of maintaining it in a variable environment (Verberk, Bartolini et al. 2016). Should generalist species lack such plasticity they will be unable to respond to rapid changes in climate by shifting their thermal niche (Seebacher, White et al. 2015).

Thermal niche differentiation allows for the coexistence of species that would otherwise compete, with the spatio-temporal distribution of species dictated by the habitats thermal regime; generalists foraging when temperatures are unfavourable for more specialised species. Thermal niche partitioning has been observed in grasshoppers (Gillis and Possai 1983), fish (Wehrly, Wiley et al. 2003), fruit flies (Duyck, David et al. 2006), ants (Lessard, Dunn et al. 2009), and dung beetles (Verdú, Arellano et al. 2006). To understand the physiological basis of a species thermal performance curve requires knowledge of its thermal tolerances, metabolic rate and its sensitivity to temperature, and its capacity for physiological plasticity (Seebacher, White et al. 2015, Verberk, Bartolini et al. 2016).

1.2.2 – Thermal tolerance

Exposure to high temperatures can disrupt cell structure and function, and damage proteins (Hochachka and Somero 2002), resulting in reduced fertility (Jørgensen, Sørensen et al. 2006), muscular contractions (Krebs and Thompson 2005), and synaptic damage (Robertson 2004). Insects appear to have limited capacity to evolve increased resistance to high temperature due to low heritability and a lack of adaptive genetic variation (Kristensen, Overgaard et al. 2015). This is thought to be due to the physiological limitations that must be overcome in order to compensate for its destabilising effect on membranes and proteins (Angilletta 2009). The low levels of variation observed in the upper thermal tolerances (Hoffmann, Chown et al. 2012) of insects is cause for concern as this may reflect an inability to adapt to rising temperatures.

Insects are similarly limited by exposure to low temperatures, often being susceptible to temperatures well above their freezing point (MacMillan and Sinclair 2011). The biological processes underpinning susceptibility to low temperatures differ from, and are often independent of, those dictating performance at high temperatures (Sinclair and Roberts 2005). As temperature declines the activity of insects decreases until they enter a chill coma, a state of neuromuscular paralysis, and become entirely unresponsive (Mellanby 1939, Findsen, Pedersen et al. 2014). Entry into a chill coma is thought to be a result of an inability to maintain ion and water homeostasis and the subsequent loss of cell membrane potential, however, the exact mechanism remains unclear (Andersen, MacMillan et al. 2015, Andersen, Folkersen et al. 2017). While chill comas are a temporary state, with function returning as body temperature rises, there is evidence to suggest that there is ongoing physiological damage (Kelty, Killian et al. 1996, Rinehart, Yocum et al. 2000).

1.2.3 – Metabolic rate

Metabolic rate is a measure of an organisms current energy use, and therefore a reflectance of their energy needs. An organism's energy budget must meet the needs of both maintenance costs, which is the energy required to sustain the basic functions needed to sustain life, and the substantial energy required for activity. Basal metabolic rate, that is metabolic rate measured during inactivity, is positively correlated with sustained energy output. Individuals possess a finite-amount of energy they are able to allocate to the various functions required for survival, therefore there are often compulsory trade-offs between competing functions (Wootton 1993, Kotiaho, Alatalo et al. 1998). When an organism is unable to meet its energy requirements and falls into a deficit it must divert energy away from maintenance costs and towards competing functions. Metabolic rate is therefore a case of trade-offs with species with high basal metabolic rates often utilising cost-intensive behaviours and foraging strategies resulting in less energy being devoted to physiological functions such as reproduction and development (Nespolo, Roff et al. 2008). Higher resting metabolic rates are correlated with a higher proportion of energy invested into activity and costly behaviours such as signalling (Reinhold 1999).

The metabolic rate of insects can vary with age (Hack 1997, Piiroinen et al. 2010), size (Lighton and Fielden 1995), activity (Bartholomew and Lighton 1985), sex, feeding status, reproductive status, time of day (Terblanche et al. 2004), and temperature (Niitepõld 2010, Le Lann et al. 2011), leading to high intra-population variability (Stevens et al. 2010). Body size is the largest source of both inter and intra-species variation in metabolic rates as larger organisms possess more metabolic machinery. The relationship between metabolic rate and body mass can be expressed as M^b, where b is a scaling exponent normally less than one, and M is the mass of the organism. Thorax and abdomen size were the strongest predictors of residual metabolic rate in nymphs of the sand cricket (*Gryllus firmus*) (Nespolo, Castañeda et al. 2005), likely because these are the site of important biological functions such as locomotor and respiratory organs.

Metabolic rate has been found to be both heritable and repeatable (Marais and Chown 2003, Nespolo, Lardies et al. 2003). Metabolism is central to the evolution of life-histories as the fundamental concept of conversion of resources to reproduction is inextricably linked to fitness (Arnqvist, Dowling et al. 2010). Metabolic rate has been correlated with altered levels of fitness in a number of invertebrates; higher metabolic rates were associated with increased dispersal capacity in European butterflies (Niitepõld et al. 2009), lower metabolic rates were associated with higher survival in garden snails (Artacho and Nespolo 2009), lower metabolic rates were associated with higher survival in mealworm beetles due to an increased prevalence of anti-predator behaviour (hiding in a layer of bran) (Krams et al. 2013), it was found that a lower resting metabolic rate was selected for as individuals with higher rates were more bold and therefore were predated upon more often, showing that metabolic rate is correlated with behaviour (Biro and Stamps 2010, Krams et al. 2013). Climate change is expected to result in significant increases of the metabolic rates of ectotherms and the plasticity of thermal traits is expected to be an important factor governing the response of populations (Seebacher, White et al. 2015).

Temperature directly affects the metabolism of insects and is an important determinant of metabolic rate (Brown, Gillooly et al. 2004). The sensitivity of the metabolic rate of ectotherms to changes in temperature is a defining characteristic as their lower capacity for thermoregulation means performance is strongly influenced by changes in ambient temperature; it is therefore a pivotal trait in understanding species response to climate change (Angilletta 2009, Clusella-Trullas, Blackburn et al. 2011). The thermal sensitivity of the metabolic rate of insects is expected to be greater at low temperatures due to the relationship between temperature and the biochemical reactions that drive metabolism (Schmidt-Nielsen 1997). Species with high thermal optimums are expected to have higher performance than cool temperature species, owing to the fundamental effect of temperature on the processes underlying performance as reaction rates increase with temperature (Kingsolver and Huey 2008). In insects, this pattern has been observed with warm-adapted insects having higher performance than cold-adapted (Frazier, Huey et al. 2006). The relationship between metabolic rate and temperature was unaffected by gender, age, feeding status, pregnancy, or acclimation in *Glossina morsitans morsitans* (Terblanche, Jaco Klok et al. 2005). Species from thermally variable environments tend to have decreased thermal sensitivity (Williams, Marshall et al. 2012).

1.2.4 – Plasticity

Phenotypic plasticity refers to the capacity of individuals of a single genotype to express multiple phenotypes in response to environmental stimuli (Whitman and Agrawal 2009, Verdú 2011). Nearly all traits can be subject to phenotypic plasticity whether they be morphological, physiological, or behavioural (Schlichting and Pigliucci 1998, Moczek 2009). Extreme examples include the development of distinct life stages seen in holometabolous insects (Yang 2001), and the adult polyphenism seen in insects (Reynolds 2013), while more subtle examples come in the form of changes to underlying physiology. Plasticity can act as a buffer against environmental variation during development or allow adaptive changes to specific stimuli, in highly variable environments plasticity can reduce variation in fitness from one generation to the next (Nijhout 2003, Snell-Rood, Van Dyken et al. 2010). When an individual is subject to a plasticity-inducing stimuli multiple traits can be affected, such as increases in both metabolic rate and upper thermal tolerance when subjected to extreme temperatures (Precht 1973). Plasticity of this form is known as acclimatisation (Whitman 2009).

Acclimatisation (acclimation when referring to controlled experiments) is a form of phenotypic plasticity that allows an individual to adaptively alter its thermal physiology in response to temperature (Whitman and Agrawal 2009). Acclimation differs from typical hardening responses both in the time taken to induce change and the severity of thermal stress required (Schulte, Healy et al. 2011). Hardening takes place within minutes or hours of exposure to stressful but non-lethal temperature, acclimation generally takes days and is a response to changes in temperature within their normal thermal range (Hoffmann, Sørensen et al. 2003). The ability to alter physiological traits such as thermal tolerance and metabolic rate can allow species to expand their ecological niche or allow them to maintain functionality while persisting in their current niche in the face of climate change, however it is uncertain whether these traits are labile enough to overcome the most extreme global warming estimates (Sørensen, Kristensen et al. 2016).

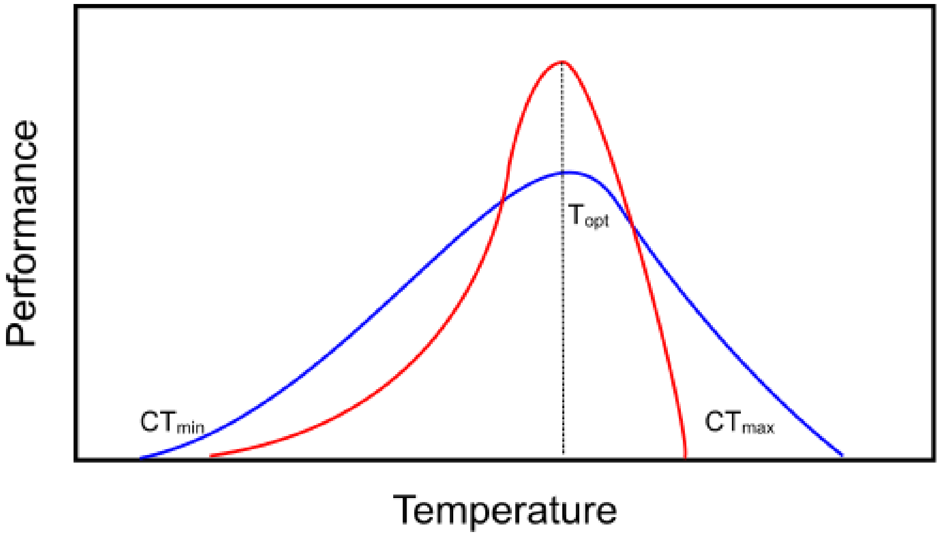


Figure 1.2. Thermal performance curves of a thermal generalist (blue) and thermal specialist (red). Specialists have high peak performance, limited to a narrow range of temperatures; generalists have lower peak performance but are capable of operating across a broader range of temperatures.

1.3 – Measuring thermal physiology

1.3.1 – Critical thermal limits

When measuring the critical thermal limits of an animal in a laboratory setting two assumptions are made: that the values observed reflect the maximum temperature that the animal can tolerate before they suffer permanent physiological damage; and that the average thermal tolerance of a number of individuals is representative of the entire population (Ribeiro, Camacho et al. 2012). A number of different traits are used when measuring an insect's thermal tolerances, such as: the upper and lower lethal temperatures (Sinclair, Terblanche et al. 2006); $CT_{min/max}$ (Cowles and Bogert 1944, Lutterschmidt and Hutchison 1997); knock down temperature; supercooling point; and chill coma recovery time (Castañeda, Lardies et al. 2005). The most commonly used measures of thermal tolerance are $CT_{min/max}$ and the upper (ULT) and lower (LLT) lethal temperatures. The ULT and LLT are determined by a time-temperature interaction (Somme 1999) and are measured using the static method of applying temperature stress. The static method involves subjecting the animals to a constant temperature for varying durations (or varying temperatures for the same duration) and observing the survival rate of the animals (Terblanche, Deere et al. 2007). Determination of $CT_{min/max}$ utilises the dynamic, or ramping, method whereby animals are initially subjected to a non-stressful temperature which is then gradually increased until an endpoint is reached (Cowles and Bogert 1944, Cokendolpher and Phillips 1990, Santos, Castaneda et al. 2011). Dynamically altering the temperature provides the opportunity to study an organism's response to changes in temperature in a manner that is ecologically relevant.

Table 1.1. Measures of thermal tolerance commonly used in ectotherms. Adapted from Terblanche, Hoffmann et al. (2011)

Endpoint Measured	**Description**	**Method of Stress Application**
Survival	Survival following stress	Static/Ramping
Loss of activity or movement	Inactivity or loss of righting response	Ramping
Recovery	Ability or time to recover a trait e.g. fertility, locomotion	Static
Recovery temperature	Temperature at which an animal recovers locomotor activity after application of a stressful temperature	Ramping
Knockdown Temperature	Temperature at which the animal is knocked down	Ramping
Knockdown Time	Time to knockdown following stress	Static/Ramping
Spiracular failure	Sudden change in metabolic rate	Ramping
Supercooling point	Exothermic reaction associated with latent heat of crystallization	Static/Ramping

1.3.2 – Thermal limit respirometry

Thermal limit respirometry, a method developed by Lighton and Turner (2004), provides an objective and unambiguous endpoint for measuring the $CT_{min/max}$ of an insect. Thermal limit respirometry is achieved via flow through respirometry which allows for the indirect observation of aerobic metabolism by analysing changes in the composition of the air stream created by an organism (Davis, Chown et al. 2000). In the case of insects CO_2 is the most commonly used measure of metabolic rate as it can be scrubbed from the air with relative ease and allows for greater precision than measurements of O_2 (Schilman 2017). The volume of CO_2 ($\dot{V}CO_2$) emitted is an indirect measure of metabolic rate, with larger volumes corresponding to higher metabolic rates. Changes in the pattern of CO_2 emissions can indicate changes in physiological state or stress. By combining respirometry with traditional temperature ramping protocols the physiological response of insects to temperature stress can be empirically assessed. Insects display a characteristic pattern of CO_2 emissions as they approach CT_{max} (Figure 1.3). In the equilibration phase metabolic rate remains steady; as temperature begins to increase metabolic rate rises; $\dot{V}CO_2$ plateaus as the insect approaches death; muscle control is lost and spiracular activity ceases, this is the CT_{max}; CO_2 levels begin to drop before a large burst of CO_2 is released in what is known as the post-mortal peak (Lighton and Turner 2004). Studies have shown that spiracular and motor failure occur simultaneously in insects at their thermal maximum (Galante and Cartagena 1999, Folk et al. 2007). The post-mortal peak is the result of continuing metabolic activity within the mitochondria, which may have a higher thermal tolerance than the animal itself (Heinrich, Gray et al. 2017). This phenomenon has so far been observed exclusively in insects (Klok, Sinclair et al. 2004).

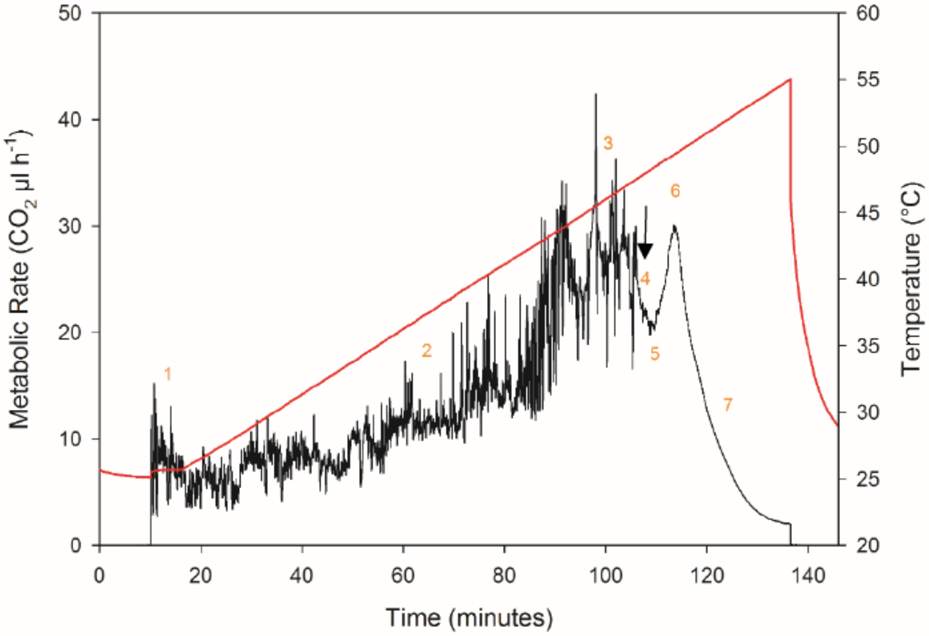


Figure 1.3. Thermal limit respirometry performed on the dung beetle *Onthophagus binodis*. (1) Volume of CO_2 is stable through equilibrium phase. (2) Volume of CO_2 increases as temperature begins to climb. (3) Maximum volume of CO_2 reached, the pre-mortal plateau. (4) Spiracular activity plateaus. The black arrow indicates the critical thermal maximum (5) Pre-mortal valley. (6) post-mortal burst of CO_2 released, known as the post mortal peak. (7) Decay as CO_2 drops. The red line is the temperature experienced by the animal.

1.4 - Dung Beetles

The term dung beetle refers to species belonging to the order Coleoptera that use vertebrate dung as a food source. Dung beetles are a relatively small group of scarabs with ~8000 species identified from three taxonomic groups, the family Scarabaeidae (~5000 species), subfamily Aphodiinae (~1800 species) and the subfamily Geotrupinae (~300 species). Despite the relative scarcity of dung beetle species when compared to other insects they are often found in great abundance (Hanski and Cambefort 1991). Species from a range of other taxa, such as Hybosoridae and Trogidae, are known to utilise faeces at some stage of their lives however they are not considered true dung beetles. Dung beetles are ubiquitous, being found on every continent with the exception of Antarctica, however the largest diversity of species can be found in Africa (Hanski and Cambefort 1991). This is due to the high diversity of vertebrates, and therefore dung types, present which is strongly correlated with dung beetle species diversity (Davis and Scholtz 2001).

Like all insects dung beetles are strongly influenced by their environment. In temperate environments temperature is the most significant factor limiting activity, whereas at lower latitudes where temperatures are stable, rainfall and soil moisture define activity (Hanski and Cambefort 1991). Both moisture and temperature structure mediterranean communities as they are characterised by cold (relative to the tropics) winters and dry summers (Hanski and Cambefort 1991). Species exhibit preferences for both soil moisture and soil type, with the choice a compromise between suitable moisture for the larvae and ease of construction for the adults. Some species mitigate the strong effect of soil moisture on larval mortality (Sowig 1995, Sowig 1996) by altering their nest structure in moist and dry soils (Barkhouse and Ridsdill-Smith 1986).

Environmental temperature is a key factor in shaping the spatio-temporal distribution of dung beetles. Northern European dung beetle assemblages are thought to be the result of climate patterns during the last glacial maximum, with post-glacial dispersion constrained by the thermal tolerance of species (Hortal, Diniz-Filho et al. 2011). The influence of temperature on community structure depends on the spatial scale at which it is considered, with mean and maximum temperatures predicting richness at large scales and minimum temperatures dictating richness at the regional scale (Lumaret and Jay-Robert 2002). Daily flight patterns of dung beetles have been observed to correlate with ambient temperature in the Ivory Coast (Krell, Krell-Westerwalbesloh et al. 2003, Krell-Westerwalbesloh, Krell et al. 2004), Mexico (Verdú, Arellano et al. 2007), and Argentina (Giménez Gómez, Lomáscolo et al. 2017). The body temperature of small species tends to conform to their environment but large species (>1.98 g) have a limited capacity to maintain body temperatures significantly higher than ambient, with temperature excesses of up to 15°C observed (Bartholomew and Heinrich 1978, Verdú, Arellano et al. 2006). This limited endothermy plays a role in thermal niche partitioning in dung beetle communities (Verdú, Arellano et al. 2006, Verdú, Alba-Tercedor et al. 2012), allowing nocturnal and crepuscular species to be active (Caveney, Scholtz et al. 1995).

The intense speciation of dung beetles has resulted in many species being habitat specific, filling niches along temperature and moisture gradients (Bell, Moritz et al. 2007). The specialisation of individual species to these abiotic factors results in community structure displaying a broad range of responses to environmental disturbances including (Spector 2006) smaller body size, lower species diversity, and reduced activity in disturbed environments, resulting in a loss of ecosystem function (Andresen 2003, Horgan 2005). Climate change induced range shifts have already been observed in species (Menéndez, González-Megías et al. 2014), the effects of further increases in temperature amplified by anthropogenic degradation of habitats is expected to alter the structure and dynamics of dung beetle communities across the globe (Larsen 2012). Seasonal variation in the habitat association of species suggests that climate change could result in altered community interactions (Menendez and Gutierrez 2004).

1.4.1 – Feeding

The mouthparts of adult dung beetles are adapted for sucking and lapping dung liquids and small dung particles that contain microorganisms originating from the gut of the source animal, it is these microorganisms that provide adult beetles with most of their nutrients (Matthews and Halffter 1966, Aschenborn, Loughnan et al. 1989, Doube 2014). When feeding they use setal labral and labial pads to concentrate dung particles for ingestion (Hata and Edmonds 1983). In contrast to adults the mouthparts of larval beetles are adapted for breaking down the solid and fibrous components of the dung (Matthews and Halffter 1966). In order to find food, dung beetles rely on olfactory and tactile sensillae on their antennae (Dethier 1947). Beetles often search for food whilst in flight, however some species will perch with their antennae exposed waiting to detect dung volatiles (Scholtz, Davis et al. 2009).

Most dung beetle species can be considered generalists and will feed on any available dung, some however are specialists that will only use dung of a particular age, texture, moisture, fibre content or from a particular species (Hanski and Cambefort 1991). Dung produced by different animals has different nutritional qualities and therefore dung from an animal that is suitable for one species may not be suitable for another (Gittings and Giller 1998). Generalist species still have their preferences, which can vary depending on a number of factors. Dung beetles from North America display a preference for omnivore dung over herbivore or carnivore dung (Fincher, Stewart et al. 1970) whereas beetles from India prefer cow dung to human dung (Mittal and Bhati 1998). Dormont, Epinat et al. (2004) found that out of 39 species 13 showed a clear preference for cow dung over horse dung. A small number of dung beetles have been found to feed on resources other than dung such as millipedes (Larsen, Lopera et al. 2009), fruit (Halffter and Halffter 2009), or carrion (Ratcliffe 2013).

1.4.2 – Reproduction, nesting behaviour, and bi-parental care

Dung plays a key role in the reproductive cycle of dung beetles. Nest building and provisioning of resources are not unknown amongst the Coleoptera but few species take it as far as dung beetles. Dung beetles belonging to the Aphodiinae live freely inside the dung (Yoshida and Katakura 1986). Members of the Geotrupidae will provision tunnels with leaf litter or twigs (Scholtz, Davis et al. 2009). Unique among beetles, members of the subfamily Scarabaeinae will provision their burrows with sausage shaped lumps of dung (Scholtz, Davis et al. 2009). Just as there is a wide variety of food preference among dung beetles they have also developed a number of different strategies for nesting and provisioning. These nesting strategies can be broken down into three broad groups with further subdivisions based on body size (Figure 1.4)(Doube 1990). Paracoprid beetles, called tunnelers, bury dung in the soil below the dung. One or both of the parents will dig a tunnel and form a brood mass at the end in which a single egg is placed with multiple eggs from the same parent being separated by soil. Telecoprids, known as rollers, remove a portion of the dung and move it away from the source. The dung is formed into a ball and one or both of the parents will roll it away and bury it in a shallow tunnel before laying an egg in it. Endocoprids, called dwellers, reproduce inside the dung. These beetles do not produce brood balls with eggs being laid directly into the dung (Halffter and Edmonds 1982). Although virtually all dung beetle can be grouped into one of these categories they do not represent shared phylogeny, with nesting patterns being driven by ecological requirements (Hanski and Cambefort 1991).

The bi-parental care and provisioning displayed by many dung beetle species, particularly in the genus *Onthophagus*, is rare amongst other insects (Hunt and Simmons 1998). Its evolution is a result of the temporary nature of dung and the inability of larvae to seek new sources (Gardner and Smiseth 2011). Nesting behaviours such as these are often seen in animals that inhabit environments with high levels of competition (Scott 1990). This is a significant investment of time and energy; however survival of the larvae is entirely dependent on a well-constructed brood as it is both a refuge and the sole source of food (Simmons and Ridsdill-Smith

2011). Within brood are hollow chambers which the mother lines with her saliva which contains endosymbionts that are transmission to the egg (Estes, Hearn et al. 2013). After hatching the larvae feed on the brood mass, undergoing three instars. Pupation of larvae is triggered by depletion of the brood ball (Shafiei, Moczek et al. 2001). Larval weight at the time of pupation determines whether males will present a horned or hornless morph (Moczek 1998). This intricate behaviour results in structural changes to the soil below the dung altering the flow and availability of energy in the surrounding area.

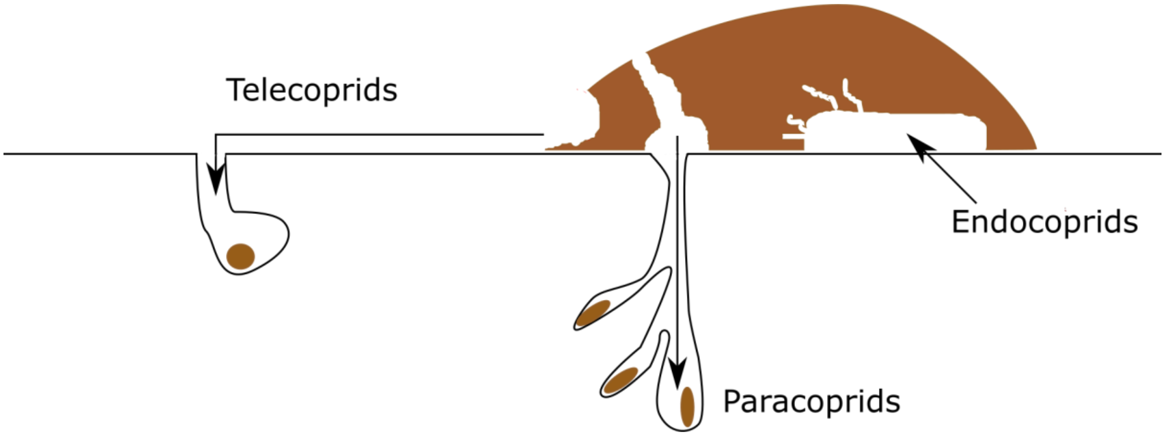


Figure 1.4. Dung beetle functional groups: Telecoprids gather dung and transport it away from the source along the soil surface; paracoprids burrow into the dung and create brood balls in tunnels beneath the soils surface; endocoprid do not form brood balls, rather the young live freely within the dung source.

1.4.3 – Ecosystem services provided by dung beetles

Dung beetles provide a number of key ecosystem functions and to some extent can be considered ecosystem engineers (Boze 2012). When an ecosystem function provides a direct benefit to humanity, be it economical, ecological or otherwise, it is deemed to be an ecosystem service (De Groot, Wilson et al. 2002). Dung beetles have been found to significantly contribute to nutrient cycling (Bertone, Green et al. 2006, Yamada, Imura et al. 2007), bioturbation (Bang, Lee et al. 2005), plant growth (Kabir, Howlader et al. 1985, Lastro 2006), plant nutritional content (Bang, Lee et al. 2005), secondary seed dispersal (Andresen, Feer et al. 2005), pest control (Bornemissza 1970, Fincher 1975), soil hydrology (Brown, Scholtz et al. 2010), soil structure (Bornemissza 1976), reduced pasture fouling (Horgan 2001), and altering the greenhouse gas emissions profile of dung (Slade, Riutta et al. 2016). Their contribution to these processes has direct economic value estimated in the millions of dollars (Losey and Vaughan 2006, Beynon, Wainwright et al. 2015).

Dung beetles' potential to slow the pace, and mitigate the effects, of climate change are, perhaps, worth more than any monetary value they can be ascribed. Aeration of dung due to dung beetle activity disrupts anaerobic microbial activity, modifying the greenhouse gas emission (GHG) profile (Slade, Riutta et al. 2016). Typical of this is a decrease in CH_4, increase in CO_2, and sporadic spikes of N_2O (Penttilä, Slade et al. 2013, Hammer, Fierer et al. 2016). As CH_4 is a more potent GHG than CO_2 there is an overall reduction in GHG of approximately 7% over the lifespan of a dung pat, however scaling this to include emissions for the entire livestock production cycle dung beetle activity reduces emissions produced by meat and dairy industries by less than 1% (Slade, Riutta et al. 2016). Burrowing activity in dung beetles can improve soil water retention and increase plant growth by 280% under drought conditions (Johnson, Lopaticki et al. 2016) which are expected to become more frequent and severe (IPCC 2007). Dung beetles have been intentionally introduced into a number of other countries for the purpose of provisioning ecosystem function and services including; Brazil (Miranda, Santos et al. 2000), Hawaii (Markin and Yoshioka 1998), North America (Anderson and Loomis 1978) and Australia (Bornemissza 1960).

1.4.4 – The dung beetle fauna of Australia

The modern dung beetle fauna of Australia is composed of endemic species, primarily found in closed environments, and the more commonly encountered exotic species introduced by the Australian government over the course of several decades. The native dung beetle fauna of Australia is unique in that they are the only assemblage, outside of Madagascar, to have evolved without access to animals that drop large dung pats (Adrian and Scholtz 2001). It has been suggested that Australia once housed large bodied dung beetle species, similar to those found in Africa, but that these were lost due to the extinction of Australia's mega fauna, cutting off their food source (Doube 2018).

The modern day assemblage is primarily composed of small bodied species that have evolved to feed on dry, pellet-like, marsupial dung (Matthews 1971). Therefore when large mammals were re-introduced to Australia alongside European colonisation the native fauna showed little interest in this foreign resource. Those native species that could utilise the large, moist, livestock dung lacked the abundance to adequately process it (Tyndale-Biscoe 1994). Dung was produced far quicker than the native beetles could process it resulting in the loss of grazing land via fouling, and a large increase in dung breeding flies. In 1960 George Bornemissza proposed the introduction of exotic dung beetle species that had co-evolved with animals that produced large dung (Bornemissza 1960). The project received funding by the CSIRO and Australia's Dung beetle project began (Bornemissza 1976).

The exotic species chosen for introduction were selected for their broad distributions, climatic tolerances, high fecundity, and ability to efficiently bury cattle dung (Bornemissza 1976). Climate matching between the beetles native range and climatic regions of Australia was used provide comprehensive coverage of the nation's major livestock grazing. This method has proven to be accurate with most introduced species reaching he limits of their expected climatic distribution in Australia (Duncan, Cassey et al. 2009). Indeed, many of the introduced populations out-performed populations in their native range (Hanski and Cambefort 1991). A strict quarantine system was put in place to prevent the introduction of disease and parasites (Tyndale-Biscoe 1996). Forty three species sourced from Hawaii, Africa, and Europe were reared in sufficient numbers for release. Twenty three of these species remain established as of 2007 with the highest diversity observed on the central-east coast of Australia (Edwards 2009).

The program as a whole has been considered a resounding success, in stark contrast to similar programs such as that of the infamous Cane Toad (Shine 2010). In response to the foreign dung beetles populations of the pest fly *Musca vetustisima* were drastically reduced. This has been credited for the rising adoption of European café culture and al fresco dining which are prominent aspects of Australia's identity today (Doube 2018), thereby shaping the culture of modern civilisation much as they did with ancient Egyptians. Despite these successes there remain geographical and temporal gaps in dung beetle activity, particularly in regions characterised by winter-rainfall, and introductions are ongoing (Doube 2014). Two new species, *Onthophagus vacca* and *Bubas bubalus*, have been identified as candidates for Australia's southern region with trial releases underway (Doube 2018).

1.5 – Thesis Overview

Dung beetles have been extensively studied throughout history, with particular focus on their life-history, the influence of abiotic factors on distribution and community structure, provision of ecosystem services, value as bio-indicator species, and developmental plasticity. While it is recognised that temperature plays an important role in structuring dung beetle communities, there appears to be a dearth of information concerning their physiological response to temperature. In Australia, where the ecology of dung beetle fauna is well studied as a result of the CSIRO Australian Dung Beetle Project, physiological data is even more lacking. While climate models for introduced species have proven to be reliable the addition of physiological data to distribution models can increase accuracy. The introduction of dung beetles is ongoing and climate change may result in altered species interactions resulting in loss of ecosystem function. Understanding the response of Australian dung beetles to temperature and the role of thermal physiology in community structure will allow for data-based management strategies going into the future. At a global-level study of their thermal physiology can provide important insights into the physiological processes that drive the evolution of thermal specialists and the role of metabolic adaptation in highly competitive ectotherms.

Chapter Two: Dung beetles have a long history as a source of cultural and religious symbolism, and as the focus of significant scientific interest. Extensive research has been conducted to explain the processes that have driven the evolution of such a diverse range of species, and morphologies, in such a unique and competitive niche. While the published literature on dung beetles is plentiful relatively few studies appear to ask whether the success and diversification of dung beetles has a physiological basis. Evidence for thermal niche specialisation among dung beetles comes primarily in the form of correlations between ambient temperature and foraging behaviour. To better elucidate the trends and patterns of research and to identify areas where it is lacking we conduct a review of 1463 peer reviewed papers pertaining to all aspects of dung beetle research. We use network graphs as a means to visualise and explore the relationships between factors and methods that are common in the field such as; the type of dung used; The methodology employed in the study; study location; dung beetle functional group; and the responses that were assessed.

Chapter Three: Telecoprids have been observed to utilize thermal partitioning throughout the day to minimise competition between conspecifics. Whether dung beetles from other functional groups and communities utilize a similar mechanism is unknown. We use thermal limit respirometry to compare the CT_{min}, CT_{max}, $\dot{V}CO_2$ ml h^{-1}, and thermal sensitivity of dung beetle species from a cool-temperate community at Armidale, NSW, Australia. The temperature dependence of organisms' metabolic rate is increasingly being recognised as a crucial trait in understanding their thermal ecology with a higher metabolic rate being correlated with increased performance. The energetics of dung beetles have been implicated as a driving force behind the ecological patterns observed in the field, however the role of metabolism in determining community structure and competitive interactions is unexplored. We provide a methodological template that allows simultaneous and objective measurement of multiple thermal traits which will prove useful in identifying species for introduction into specific climatic regions and elucidating activity patterns in the field.

Chapter Four: The effects of climate change have already been observed in montane dung beetle communities. Phenotypic plasticity is a potential mechanism through which species can mitigate the effects of altered thermal regimes. We examine the thermal physiology, nesting behaviour, and potential for plasticity of the dung beetle *Onthophagus binodis,* a common paracoprid species in pastures across Australia. Using microclimate data and temperature control chambers we expose beetles to four levels of warming based off of climate change estimates to determine whether the species can mitigate the effects of climate change via (1) physiological or (2) behavioural adaptation. By measuring CT_{max}, VCO_2 ml h^{-1}, and thermal sensitivity we answer (1) while (2) is answered by measuring changes in brood ball burial depth, production, and volume. Changes in nesting behaviour has implications for both the provision of ecosystem function due to changes in dung burial rates, and for the population dynamics of the species as the size of brood ball determines the morph, and thereby behaviour, of male beetles.

CHAPTER TWO

WHERE HAVE WE BEEN AND WHERE ARE WE GOING? DUNG BEETLE RESEARCH ACROSS THE WORLD

2.1 – Abstract

Dung beetles are a diverse and widespread group with a robust literary history dating back to ancient Egypt. They are important drivers of processes within ecosystems, mediating a number of vital functions such as nutrient cycling and seed dispersal. As a result of their unique behaviour, strong linkages to the environment, and dazzling array of morphologies dung beetles are well-studied with a large number of publications relative to the size of the taxa. We conducted a review of the dung beetle literature in order to illuminate historical trends, identify knowledge gaps and areas in need of further study. Using the scientific literature databases Scopus and Google scholar, combined with papers made available by the Scarabaeinae Research Network, we examine the temporal, spatial, methodological patterns of 1463 publications and the responses they aim to assess: utilizing network graphs to visualise the relationships between these factors. Publication rates were relatively low prior to 1974 after which it began to rapidly increase. The majority of these studies focus on the ecology (61%) of dung beetles using data gathered via field surveys. Behaviour is also a favoured topic (29%) alongside morphology (24.8%) with physiology (16.5%) and genetics (6.5%) remaining relatively understudied, although research in these fields is increasing, likely due to the increased availability and reduced cost of the equipment required. While all major continents, excepting Antarctica, are well represented there was a disturbing tendency of publications to fail to state the location where the study took place, or from where the dung beetles were sourced, with “Not Stated” being the continent with the second highest number of publications at 200. A similar pattern emerged when looking at the types of dung

used in these studies 419 publications, over a quarter of the studies assessed, failing to specify the type of dung used in the study or to maintain the beetles.

2.2 – Introduction

> “*It is six or seven thousand years since the Sacred Beetle was first talked about. The peasant of ancient Egypt, as he watered his patch of onions in the spring, would see from time to time a fat black insect pass close by, hurriedly trundling a ball backwards. He would watch the queer rolling thing in amazement, as the peasant of Provence watches it to this day*” – Jean-Henri Fabre (Fabre 1925)

Dung beetles have been a subject of interest to scientists and natural philosophers for centuries. During the 19th century they captured the imagination of famed entomologist Jean-Henri Fabre, in his typical prose he describes the behaviour and life cycle of these scavenger beetles detailing the lives of the “Sacred beetle”, “Spanish Copris”, and “the Sisyphus”, noting the care with which they provide for their young, a trait most uncommon amongst insects (Fabre 1925). Given this long-held fascination one may surmise that the dung beetles are a large and prolific group; however they are a relatively small group with approximately 8000 described species, comprising ~2% of described beetle species and ~0.8% of described insect species, with among the lowest fecundity of any insect group (Hanski and Cambefort 1991). Most recent estimates are that there are 1,013,825 species of insect described (Stork 2018), 387,000 of which are beetles. The term dung beetle is colloquially used to describe any beetle found inhabiting dung, however among the scientific community the term is denotes scarab beetles belonging to the family Geotrupidae, and the subfamilies Aphodiinae and Scarabaeinae; with members of the Scarabaeinae often referred to as “true” dung beetles. The majority of these species rely on the dung of vertebrates, as both a source of food and vital component of their reproductive cycle. There has been significant global interest in dung beetles relative to the size of the group, with hundreds of peer-reviewed articles, three books (Hanski and Cambefort 1991, Scholtz, Davis et al. 2009, Simmons and Ridsdill-Smith 2011), and an untold amount of grey literature.

2.2.1 – Why are dung beetles studied

Scientific interest in dung beetles can be attributed to the myriad adaptations that have evolved as a result of the unique ecological niche they inhabit. As obligate coprophages they are one of two insect groups, alongside the Dipterans, that feed on and actively break down dung, doing so on a far larger scale than any other group (Losey and Vaughan 2006, Holter 2016). Processing and relocation of dung facilitates a number of vital ecosystem functions including; nutrient cycling, bioturbation, and seed-dispersal (Nichols, Spector et al. 2008). Dung beetles are a widespread group with representatives inhabiting both xeric and mesic ecosystems, from warm tropical forests and savannahs to hot deserts and temperate rangelands (Hanski and Cambefort 1991), however, it is their potential to compliment livestock systems and pastures via increases in productivity and decreased management costs that captures the imagination of policy makers (Herrero and Thornton 2013, Beynon, Wainwright et al. 2015). Livestock grazing systems cover ~26% of the planets ice free land area (Steinfeld, Wassenaar et al. 2006) with livestock estimated to consume 4.7 billion tons of biomass per anum, excreting 60-95% of the nutrients present in the original plant matter (Wilkinson and Lowrey 1973). The breakdown of this excrement by dung beetles facilitates the movement of organic matter and nutrients through the soil profile, increasing plant biomass in livestock systems which is subsequently passed on to the livestock themselves (Doube 2018). This process can be controlled and supplemented to improve results or for a more targeted effect, such as the integration of biochar into cattle feed, which is subsequently moved into the soil (Joseph, Doug et al. 2015).

Not only are these functions key to the continued existence of many habitats, the provision of these functions also provide a number of services that directly benefit humans such as; enhancing plant growth, bio-control of pest species, dung removal, and increased water infiltration (Nichols, Spector et al. 2008). Dung beetles are unlikely to become invasive pests in the traditional sense as they are limited by the local supply of dung, competing only with other dung beetle species and with dung dwelling invertebrates such as dipterans, which themselves are often viewed as pests to be managed (Bornemisszza 1976, Hanski and Cambefort 1991). The management of local dung beetle communities and the introduction of species to provide ecosystem functions that are lacking provide an avenue for land managers and conservationists to reap ecological and financial benefits with little risk of unwanted flow-on effects within the ecosystem. Societies changing perception of the risk of climate change has driven the study of ecologically sound methods of mitigating its impacts.

The reasons that such a small group is so extensively covered across a broad range of literature types can be attributed to not only their scientific and ecological value but also to their societal value, particularly societies changing perception of climate change, and the increasing focus on conservation and ecologically sustainable management practices. Agriculture is a major contributor of anthropogenic habit modification and producer of greenhouse gases, accounting for 18% of anthropogenic emissions (Steinfeld, Gerber et al. 2006). Post excretion microbial activity within the dung causes the release of CO_2, NH_3, N_2O, and CH_4 (Clemens and Ahlgrimm 2001), resulting in soil acidification, eutrophication, ozone depletion, and strengthening the green-house effect of the atmosphere resulting in an increase in mean global temperature and perturbed weather patterns. There has been increasing evidence that dung beetles alter the profile of greenhouse gasses released into the atmosphere by breaking up dung and disrupting the anaerobic conditions required by gas producing microbes (Penttilä, Slade et al. 2013, Iwasa, Moki et al. 2015, Slade, Riutta et al. 2016, Piccini, Arnieri et al. 2017). Recent evidence has also suggested that activity by tunnelling dung beetle species reduces the impact of drought conditions on plant growth by increasing soil water retention, providing a potential avenue for biological mitigation of the effects of climate change (Johnson, Lopaticki et al. 2016).

2.2.2 – Interactions with the other fauna

Due to the strong commensal relationship between dung beetles and vertebrates there is often extensive co-evolution between the two groups, with dung beetle communities inextricably linked to the historic and contemporary structure of the associated vertebrate community (Bogoni, Graipel et al. 2016). The availability of multiple types of dung in an ecosystem is strongly correlated with the tribal diversity of the associated dung beetle community, with abiotic processes having a stronger influence on diversity at the generic and species levels (Davis and Scholtz 2001). The decline of large bodied dung beetles in Europe has been proximally tied to the extinction of local megafauna and the subsequent loss of large, wet, dung as resource (Schweiger and Svenning 2018), with a similar situation thought to have occurred in Australia (Doube 2018). Changes in the composition of the vertebrate community can have strong effects on dung beetles of a particular functional group; The density of deer was associated with an increase in the abundance of small bodied dung beetle species (<10mm), whereas the abundance of large bodied species (>10mm) was unaffected (Iida, Soga et al. 2018); In Panama areas of forest dung beetle communities differed between areas with no hunting and areas where monkeys were hunted; hunted fragments showing decreased species diversity, with the abundance of nocturnal beetles negatively correlated with the abundance of mammals (Andresen and Laurance 2007). Flow-on effects from this can further influence the community, as the presence/proportion of larger more dominant species of dung beetle influences the structure of smaller species via size-asymmetric competition (Horgan and Fuentes 2005).

The planet is currently experience levels of anthropogenic disturbance previously unseen (Newbold, Hudson et al. 2015). The modification of ecosystems by humans has increased at a rapid pace with only inhospitable and geographically isolated areas remaining pristine. The use of bio-indicator taxa to monitor the health of ecosystems has gained prominence among ecologists and conservationists. Dung beetles have proven to be an ideal bio-indicator due to their well described diversity, strong links to environmental processes, global distribution, reliance on other organisms in the community, and the ability to monitor changes in dung beetle communities over time easily and at little financial cost (Spector 2006).

2.2.3 – Aims

The purpose of this review is not to provide a comprehensive analysis of results from the literature as there are a number of books that summarise the current state of knowledge and provide excellent synthesis (Hanski and Cambefort 1991, Scholtz, Davis et al. 2009, Simmons and Ridsdill-Smith 2011). Rather, we use social network analysis (SNA) to visualise historical and geographical patterns in methodology and research focus to elucidate patterns of research and determine, broadly, how the field has developed; identify areas where further research is required; determine whether concepts that are frequently cited in the literature are based on widely replicated studies from a diverse range of species and assemblages, and whether the generalisation of these concepts to all dung beetles is valid; what geographical areas dominate the field; and whether the nature of dung beetle research changed over time. Given the increasing pace at which the world's environments are changing and the increasing demand for ecologically sustainable practices, and expectations that dung beetles will satisfy some of these demands, it is vital that there is a comprehensive body of literature so that researchers and policy makers are able to effectively address these issues.

2.3 – Methods

2.3.1 – Literature selection and coding

The intent of this review was to examine publishing trends in the peer-reviewed literature on dung beetles; we therefore conducted our initial literature search using broad search terms in order to get the most complete picture of the field. A literature search was performed on the scientific databases SCOPUS (https://www.scopus.com) and Google scholar (https://scholar.google.com.au/) using the search terms “dung beetle”, “Scarabaeinae”, “Aphodiinae”, and “Geotrupinae”. A large number of papers were also made available courtesy of the Scarabaeinae Research Network. As we were assessing the publication rate by year and therefore only wanted to include complete years papers were only included if they had been accepted for publication prior to the end of 2017.

The review was limited peer-reviewed articles published in recognised scientific journals; textbooks were excluded as these are primarily a synthesis of journal articles; grey literature represents a vast body of work that can be extremely valuable and easily overlooked, however its decentralised nature and the difficulty obtaining this information makes it essentially impossible to include in this review without introducing significant biases owing to varying levels of availability across the globe. For example in Australia alone there are an estimated 5400 Landcare groups, many of which have surveyed or otherwise utilised the local dung beetle fauna. Inclusion of literature produced by these Landcare groups would artificially increase the number of Australian publications relative to other countries. Furthermore, the scientific rigour of publications produced by these groups is uncertain and may represent prejudices rather than facts. This is not to say that all, or even most, of the literature produced by these groups is inaccurate, to the contrary resources such as the dung beetle pocket guide (Edwards 2015) are extremely useful to scientists and laymen alike.

Papers were initially reviewed for inclusion based on the presence of search terms in the title, abstract, or keywords. The search process resulted in several papers that did not include the search terms in the title, abstract, or keywords. Papers in which the inclusion of dung beetles was ambiguous due to a lack of search terms (i.e. terms not present but the abstract reasonably implies that dung beetles are a core topic in the paper) were assessed on a case-by-case basis (Figure 2.1). For example, none of the search terms appear in the title or abstract of Snell-Rood, Troth et al. (2013), however the abstract clearly indicates that the study species is a dung beetle of the genus *Onthophagus*, therefore this paper was included in the initial literature search. Citations and full-text manuscripts were downloaded for the papers deemed suitable after the initial search. Articles written in languages other than English were not included due both to the difficulty of finding these articles to begin with and the logistics of translating hundreds of papers written in an unknown number of languages. Given that the most major journals require articles to be submitted in English, and the relative lack of non-English citations present in the literature the exclusion of non-English articles is unlikely to change the interpretation of this review. If an English copy of the original manuscript was unavailable then the paper was removed from consideration. Only papers studying extant species were considered. A study was deemed fit for inclusion if removal of all data pertaining to dung beetles would significantly alter the conclusions of the study or the methodology utilised in the publication was specifically designed to include dung beetles. For example Nummelin (1998) is included in the database as dung beetles comprise one of four taxa studied and there is obvious intent to differentiate them from related taxa (i.e. other beetles).

In total 1992 publications were assessed, of which; 186 were discarded due to being in a language other than English; 233 publications were not included as they focused on either extinct dung beetle species or non-dung beetle taxa; a full-text copy was not accessible for 110 publications. The final library (Hemmings 2018) was comprised of 1463 papers published between 1933 (Lindquist 1933) and the end of 2017. From these we extracted a range of information pertaining to the geographical and methodological aspects of the study as well as general publication details (Table 2.1). Furthermore, we marked each study according to the specific traits assessed (Table 2.2). Each publication assessed at least one of these traits, with many assessing multiple traits simultaneously.

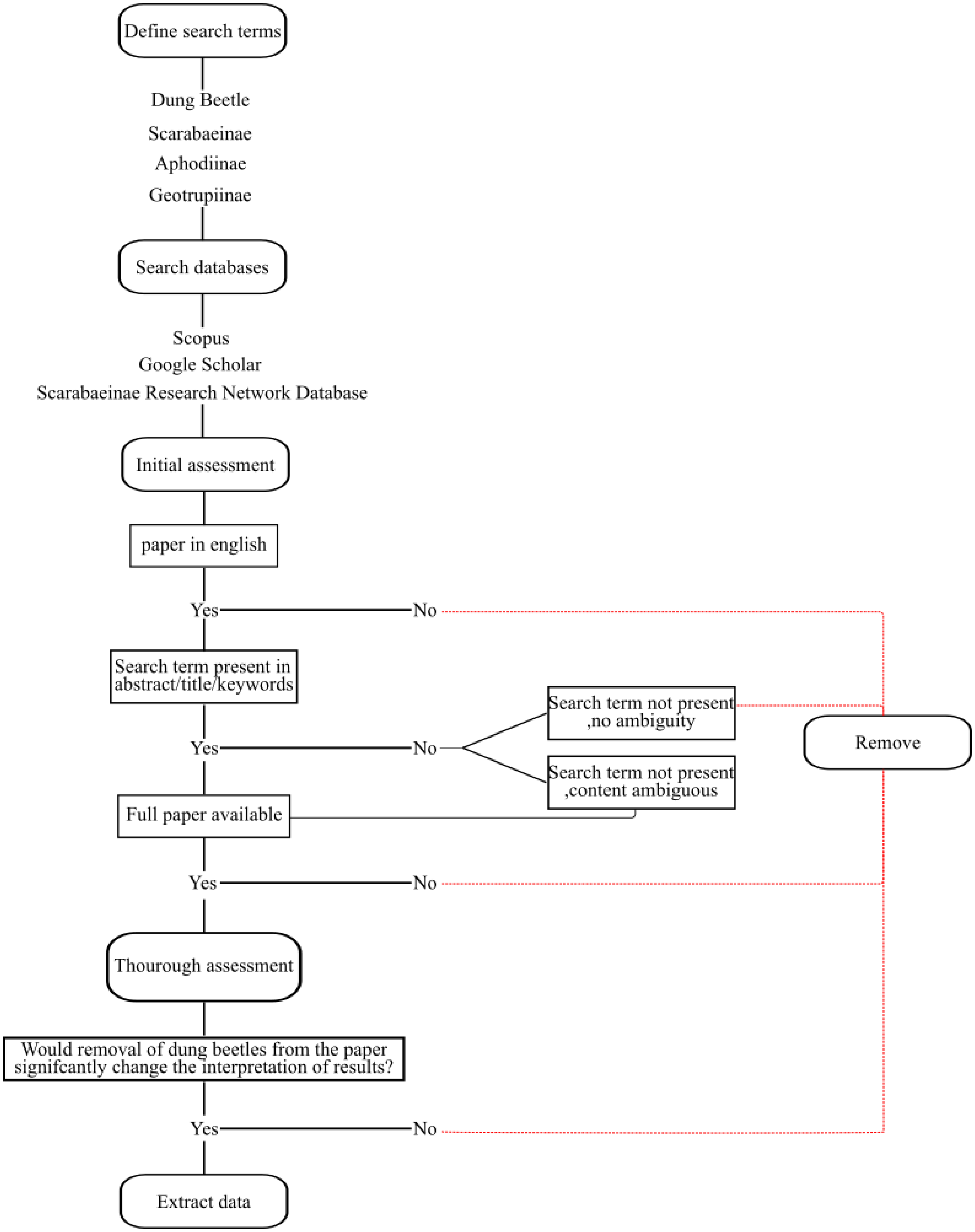


Figure 2.1. Flow diagram of literature assessment methodology.

Table 2.1. Summary of the information extracted from 1463 published scientific papers accessed via Scopus, Google Scholar, or the Scarabaeinae Research Network library using the keywords; dung beetle, scarabaeinae, aphodiinae, or geotrupiinae.

Information	**Description**
Authors	Surname of all authors
Date	Date article was published
Journal	Journal in which article was published
Volume	Journal volume
First Page	Page number of paper
Country	Country from which the study population was sourced*
Continent	Continent on which the aforementioned country is located
Location	Specific site where the study was performed
Species	Species examined in the paper. If more than 10 species are included, as is often the case with surveys, then number of species given
Dung Type	Type of animal dung used in the study
Habitat	Specific habitat as written in study (e.g. spruce forest, alpine grassland)
Type of Study	Fundamental methodology utilised in the study (e.g. survey, review, laboratory)

*Country refers to the country from which the individuals/population observed in the study were collected and is not necessarily the country in which the author(s) or their institution resides.

Table 2.2. Key for category of factors assessed. Studies may address multiple factors.

Factor	Description
Abundance	Study aims to assess abundance of dung beetles across temporal and/or spatial gradients
Phenology	Study examines life-cycle events in relation to inter-annual variation in climate
Distribution	Examines the current distribution of species and/or changes in their distribution, including elevation
Physiology	Examines the physiological mechanisms underpinning their behaviour and ecology such as thermal tolerance, respiration, digestion
Behaviour	Examines behaviour, choices, and preferences in relation to biotic and abiotic factors
Interaction	Examines the effect of dung beetle activity on biotic and abiotic components of their environment, often associated with studies of ecosystem function/services
Genetics	Study focuses on genetic diversity of beetles, multi-generational studies of genetic inheritance, and forces driving the evolution of species, in both the past and present
Assemblage Composition	Study specifically addresses questions concerning the structure of an assemblage, beyond a simple survey of abundance
Development	Study examines either the developmental process of species or the effect of a particular stimuli on development e.g. the effect of drenches
Survival	Addresses questions on factors that influence survival of larval and adult beetles
Body Weight	Specifically addresses body weight or biomass
Morphology	Morphology is a major focus of the study
Dung Usage	Compares and contrasts the different ways in which dung beetles utilise dung
Other Life-History Trait	Study addresses a specific life-history trait unrelated to the above categories
Non-Specific	Study topic does not fit into any of the above categories

2.3.2 – Network analysis

Network analysis is used to study the relationships between entities in order to elucidate the structure of the network(s) to which they belong. The technique is frequently utilised in the social sciences, being used to study the relationship between social entities, however their use is not limited to the study of people. They have been applied to the study of livestock grazing movements, the spread of disease (Hamede, Bashford et al. 2009), ecology (Poulin 2010), and evolution (Papp, Pál et al. 2004, Proulx, Promislow et al. 2005, Farine, Garroway et al. 2012).

Networks are composed of entities, known as nodes, which represent the subject of the study; for example in a study looking at the incidence of disease spread by livestock each node may represent a farm. Networks can be used to represent any type of relations between discrete objects (Newman, Barabási et al. 2006). Traditional social networks represent the interactions of a population as a graph in which individuals are represented as nodes or vertices with interactions between individuals represented by edges. Edges can be weighted to represent the strength of an interaction and can either be directed, if the behaviour is directional (e.g. grooming behaviour), or undirected. The relations can be symmetric in that any object can act as both recipient and donor of whatever passes between objects, or asymmetric, when one-way relations exist between one subset of objects and another (Poulin 2010).

Our intention was to utilise network analysis to visualise and clarify the links between the aforementioned factors (Table 2.2) and the meta-information extracted from the 1463 papers in the database (Table 2.1). The formulation of new hypotheses, and the experiments used to test them, are driven by the findings of past research. The ecology of dung beetles is influenced by an array of biotic and abiotic factors such as soil type, temperature, and habitat structure, which are rarely uniform across small spatial scales, let alone on the scale of countries and continents. Conducting controlled experiments in a laboratory or glasshouse setting allows variables such as these to be accounted for and, dependent on the stringency of the controls, greatly increase the generalizability of results.

2.4 – Results and Discussion

2.4.1 – Temporal trends

Publication rate was relatively low until 1974, with only one or two studies published a year (Figure 2.4.1). Post-1974 the publication rate increases to 10+ papers a year until a dramatic rise in the mid-1990s. One potential explanation for the sudden increase in publications is the Australian Dung Beetle Project. This project ran from 1965 to 1985 (Bornemissza 1976) and was an international undertaking with the purpose of identifying species of dung beetle suitable for introduction to Australia. Research centres were established in Australia, South Africa, and France, dedicated to understanding the abiotic factors that constrained and facilitated the distribution of species (Tyndale-Biscoe, Wallace et al. 1981, Ridsdill-Smith, Weir et al. 1983, Ridsdill-Smith and Kirk 1985, Barkhouse and Ridsdill-Smith 1986, Kirk and Ridsdill-Smith 1986, Ridsdill-Smith 1986, Davis 1987, Tyndale-Biscoe 1988, Kirk and Wallace 1990); dung beetles role in burying dung and reducing pest populations, particularly dung breeding flies (Bryan 1973, Bryan 1976, Hughes, Tyndale-Biscoe et al. 1978, Wallace, Tyndale-Bidcoe et al. 1979, Fay and Doube 1983, Doube 1986, Matthiessen, Hall et al. 1986, Ridsdill-Smith and Matthiessen 1986, Doube and Huxham 1987, Doube, Macqueen et al. 1988, Ridsdill–Smith and Matthiessen 1988); and monitoring the establishment of species once introduced to Australia (Hughes 1975, Hughes 1975). The resulting publications helped shape our modern understanding of the group including the classification of various functional groups (Doube 1990), sexual selection (Cook 1987, Cook 1988, Cook 1990, Cook 1993), and competition (Doube and Giller 1988, Giller and Doube 1989).

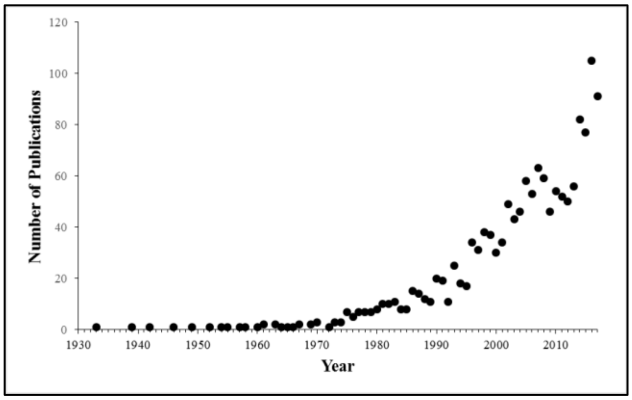


Figure 2.4.1. The number of publications per year concerning dung beetles from 1933 to 2017.

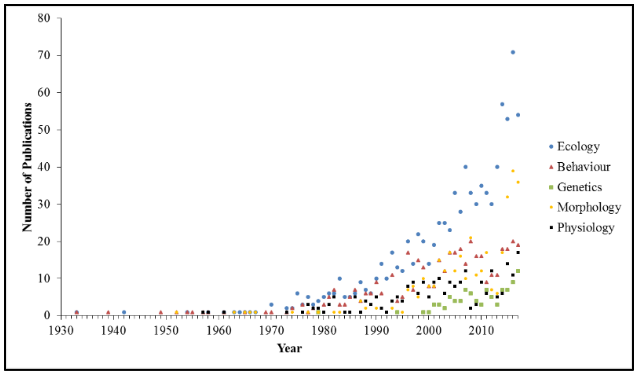


Figure 2.4.2. Number of publications per year concerning the ecology, physiology, genetics, behaviour, or morphology of dung beetles.

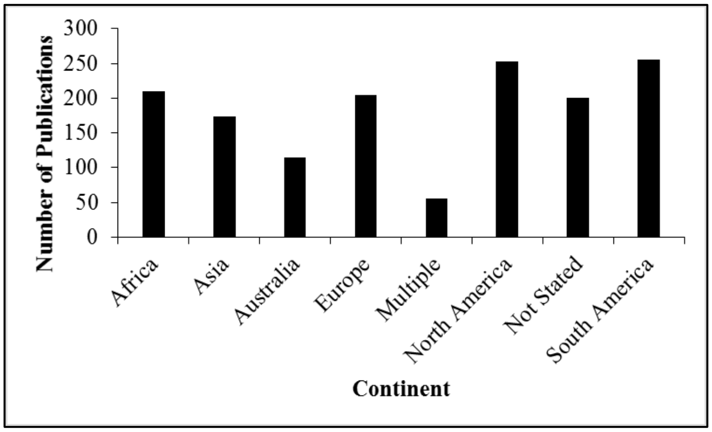


Figure 2.4.3. Number of publications concerning dung beetles from regions across the globe. Publications that did not specify a location where the study was performed or failed to specify where beetles were sourced from were recorded as ‘Not Stated’.

Publication rates rose from 17 papers per year in 1995 to 34 papers in 1996, after which the rate does not drop below 30 a year. The general trend of increasing publication volume is no doubt prevalent in all fields of scientific literature, with research flourishing due to the increasing accessibility of the internet and availability of computing power, the greatly enhanced ability to communicate and coordinate across larger spatial scales, and as a simple function of increasing population resulting in a larger pool of researchers (e.g. Andrew, Hill et al. (2013)). These effects can be seen more clearly when breaking down publications into the broad fields of study that encompass them; whether they focused on the studying the ecology, physiology, morphology, behaviour, or genetics of dung beetles (Figure 2.4.2). Behavioural, ecological, and morphological studies dominated the field prior to the 1980s while physiological and genetic studies, which often require specialised equipment or techniques, were infrequent. Studies of ecology and behaviour still dominate the field with 898 (61.4%) publications having a significant ecological component and 425 (29.0%) a significant behavioural component. This is unsurprising as the ecology and behaviour of species can be readily studied at little monetary cost with diversity and variation in community composition allowing for a large number of similar studies to be conducted whilst still gaining important insight into the dynamics that mould the structure of dung beetle communities.

An area of research that has helped to shape this trend is the use of dung beetles as a biological indicator group (Spector 2006, Syaripuddin, Sing et al. 2015). In tropical ecosystems dung beetles (along with birds) were among the most effective indicators of ecosystem health whilst also having among the lowest standardized survey costs (Pineda, Moreno et al. 2005, Gardner, Barlow et al. 2008). A quantitative review of 33 studies across a gradient of disturbance levels found that community richness, abundance, and dung beetle body mass was negatively correlated with the level of habitat modification (Nichols, Larsen et al. 2007). These findings are supported by a number of other studies that have found that species richness and assemblage composition are positively correlated with the size of forest fragments (Feer and Hingrat 2005). In contrast, recent studies found that species richness was similar across all levels of modification even in areas that have been restored for 10 years (González-Tokman, Cultid-Medina et al. 2018), highlighting that it is vital that researchers do not rely on a sole metric, such as abundance or species diversity, as this can result in a misleading impression of habitat health (Audino, Louzada et al. 2014). Dung beetles have been proposed as a superior bio-indicator compared to birds and butterflies however recent research suggests that butterflies may be a better taxon owing to the ease of DNA barcoding protocols relative to dung beetles and the lack of barcode reference libraries available for them (Syaripuddin, Sing et al. 2015). This highlights an area in which dung beetle research has fallen behind other groups: genetics.

Genetic studies on dung beetles are rare and are predominately focused on studying single species; out of the 95 genetic studies in the database 25 (26.3%) looked at more than five species, 13 looked at between 2-5 species (13.7%), and 57 (60.0%) looked at only a single species. Many of these single species studies address the genetics of sexual selection (Kotiaho, Simmons et al. 2003, Simmons and García-González 2008, Simmons, House et al. 2009, Garcia-Gonzalez and Simmons 2011, Almbro and Simmons 2014), sperm competition, parental care (Hunt and Simmons 2000, Hunt and Simmons 2002, Kotiaho, Simmons et al. 2003, Gardner and Smiseth 2011), and phenotypic plasticity (Moczek 1998, Moczek 1999, Moczek, Hunt et al. 2002, Simmons and Kotiaho 2002, Kishi, Takakura et al. 2015). Dung beetles have become a model organism for studying the evolution of horns (Emlen 1994, Emlen and Nijhout 2000, Emlen and Philips 2006, Emlen 2008, Warren, Vera et al. 2014). Beetles from the speciose genus *Onthophagus* are particularly favoured for these studies, being used in 33 of the 95 (34.7%) genetics studies. Despite the species diversity present among dung beetles and their apparent proclivity for behavioural and morphological divergence (Macagno, Moczek et al. 2016) few studies have attempted to address genetic differentiation between populations (Sole, Bastos et al. 2008, Beckers, Anderson et al. 2015, Macagno, Beckers et al. 2015). A surprising amount of genetic work has been conducted on Madagascar owing to the isolation and diversity of its endemic fauna (Monaghan, Balke et al. 2005, Orsini, Koivulehto et al. 2007, Wirta and Montreuil 2008, Wirta, Orsini et al. 2008, Wirta 2009, Wirta, Viljanen et al. 2010, Miraldo, Wirta et al. 2011). Of the 95 papers devoted to genetics 11 originate from Madagascar and detail the evolution and radiation of its dung beetles (Monaghan, Balke et al. 2005, Orsini, Koivulehto et al. 2007, Hanski, Wirta et al. 2008, Wirta and Montreuil 2008, Wirta, Orsini et al. 2008, Wirta 2009, Wirta, Viljanen et al. 2010, Miraldo, Wirta et al. 2011, Montreuil, Viljanen et al. 2014). Similar studies in other locations could prove beneficial; Australia in particular is promising owing to the history of controlled introductions across geographically distinct areas of the country (Duncan, Cassey et al. 2009). While the phylogeography of some of some tropical endemics has been tested (Bell, Yeates et al. 2004, Bell, Moritz et al. 2007) these species are rarely encountered and have a limited distribution, studies focusing on the much more common and economically important introduced species may provide insight into the divergence and evolution of species introduced into a new habitat in. Large scale phylogenetic work has been done (Philips, Pretorius et al. 2004, Forgie, Philips et al. 2005, Forgie, Kryger et al. 2006, Sole and Scholtz 2010, Mlambo, Sole et al. 2011, Mlambo, Sole et al. 2015) including studies into the origin of nesting and food relocation behaviour with results indicating that rolling behaviour may have evolved multiple times (Ocampo and Hawks 2006).

The physiology of dung beetles remains understudied in comparison to their morphology, ecology, and behaviour, accounting for 242 (16.6%) of all studies. The patterns of publication follow a similar trend to other fields, in that the number of publications addressing this subject is rising rapidly (Chown, Gaston et al. 2004, Roscoe and Hinch 2010, Andrew, Hill et al. 2013, Sheldon and Tewksbury 2014, Macagno, Beckers et al. 2015, Sugiyama 2015, Birkett, Blackburn et al. 2018, Hemmings and Andrew 2018), it reveals a weakness in the foundational literature, particularly because of the strong links found between the spatio-temporal structure of dung beetle communities and the response of individual species to temperature (Verdú, Díaz et al. 2004, Verdú, Arellano et al. 2007, Verdú and Lobo 2008, Verdú, Alba-Tercedor et al. 2012). The visual physiology of certain dung beetle species appears to be unique among the animal kingdom. The African dung beetle

Scarabaeus nigroaeneus orientates itself using the light from Milky Way, ignoring any landmarks (Dacke, Nordstrom et al. 2002, Baird, Byrne et al. 2012, Dacke, Baird et al. 2013, Dacke, Byrne et al. 2013, Dacke, el Jundi et al. 2014). Diurnal beetles possess neurons attuned to the sun to orientate them and navigate, whereas the neurons of nocturnal beetles are capable of attuning themselves exclusively to polarised light when light intensity drops at dusk allowing them to orientate using the Milky Way (el Jundi, Smolka et al. 2014, el Jundi, Warrant et al. 2015).

Dung beetle communities utilise thermal niche partitioning to reduce competition between sympatric species, without an understanding of the fundamental aspects of species' thermal biology we will be unable to predict how communities will respond to altered temperature regimes as a result of climate change (Traill, Lim et al. 2010). The strong, and sometimes peculiar, response of dung beetles to their thermal environment has been observed to take a variety of forms; during the cooler reproductive months the Iberian dung beetle *Thorectes lusitanicus* incorporates acorns into its diet, improving their immune system, ovarian development, and tolerance to low temperatures (Verdú, Lobo et al. 2007, Verdú, Casas et al. 2010, Verdú, Numa et al. 2011, Verdú, Casas et al. 2013); While transporting a brood the telecoprid *Scarabaeus lamarcki* utilises the cooler microclimate at the top of the brood ball for respite from the hot soil, periodically climbing on top of the brood (Smolka, Baird et al. 2012). Mean temperature has been found to influence the iridescent colouration of the exoskeleton of the African *Gymnopleurus humanus* with low temperature associated with blue or green individuals (Davis, Brink et al. 2008), whether this is true for other species is unknown.

Much of the early physiological work looks at temperature excess and flight time in Onitine dung beetles from Africa. However, surprisingly few papers attempt to address basic thermal physiology questions such as determining the thermal tolerances of the species in a community and measuring metabolic rate. Krell, Krell-Westerwalbesloh et al. (2003) hypothesised on the energetics of the various functional groups, theorising that endocoprids were adapted for heterogeneous environments, telecoprids for open, exposed, environments, and paracoprids to a middle ground between these two. Telecoprids require energy from the sun to fuel the metabolism required for their energy intensive method of dung collection.

2.4.2 – Network graphs and geographical trends

One of the key purposes of a literature review is to identify gaps in the literature and areas for futures research. Methods to make this process easier and reduce the effects of author bias are therefore beneficial in advancing a field. The use of network graphs to visualise the data is a promising technique that may aid this process. The use of network graphs allows the reader to see both what HAS and what HAS NOT been studied, unlike traditional graphs. The purpose of graphs and figures is to allow the reader to quickly and easily compare the values of a particular factor across a number of groups. This purpose is defeated when there are many factors of interest as the amount of information can quickly become overwhelming. By deciding on factors of interest *a priori* authors can prevent themselves from falling into the trap of designing their literature review and database around only what HAS been covered in the literature, as once the process of identifying and reading papers begins it can be difficult to divorce oneself from the concepts that they are immersing themselves in (Page, McKenzie et al. 2014, Haddaway, Woodcock et al. 2015).

The primary benefit of visualising this sort of data using network analysis over more traditional methods is that it provides the reader with a better understanding of the relationships between entities, at the expense of some degree of quantativity. For example, the information contained within the first network graph presented here (Figure 2.4.4.) could also be presented in a more conventional stacked histogram, which would inform the reader on the exact number of studies performed on each continent using each methodology. The problem, however, is that these graphs must show data for 16 methodologies across eight continents (six plus not stated and multiple), a lot of data to be easily conveyed. Furthermore, many combinations of continent and methodology have no publications recorded meaning that readers are either exposed to a large number of data points along the horizontal axis with no values, or the horizontal data points for each continent will not match, frustrating attempts to glean information.

The network Figure 2.4.4 does not make quantitative data as accessible to the reader as a traditional graph, however by looking for patterns between the nodes and edges we are able to identify areas of interest and narrow the scope. In Figure 2.4.4 we have mapped the continents as nodes (Australia, Africa, Asia, North America, South America, Europe, Not stated, and Multiple) as well as the various methodologies used in the publications. The size of each node has been mapped to correspond to the number of edges connected to it. The lines linking the nodes (edges) represent whether the publication used endocoprids, telecoprid, or paracoprids, with the width of the line indicating a greater number of publications looked at this group. This figure can be interpreted as such; the greater the number of methodologies used and the greater the number of functional groups addressed in these publications the greater the size of the node.

North America has the greatest number of publications followed by Africa, Europe, South America, Asia, and then Australia (Figure 2.4.3). Antarctica is not represented in the literature, being the sole continent with no native or introduced dung beetles. The dung beetle fauna of Mexico is well studied, contributing 110 of the 253 total publications from North America, especially considering the large number of Spanish publications that were not included in this review. Studies which looked at populations from multiple continents accounted for only 3.8% of all publications and are predominately field and greenhouse experiments (Figure 2.4.4). Surprisingly 13.7% of papers did not include information on where the study took place or where the experimental population was sourced from. While it may be safe to assume that the study took place at the same locale as the authors institution, that such vital and easily added information is missing from such a large proportion of publications is baffling and hints at a troubling tendency of authors and reviewers to exclude this information if it does not appear to be directly relevant to the publication. ‘Physiology’ and ‘genetic’ studies, which account for 58 and 28 of the ‘Not Stated’ studies respectively, tend to involve laboratory experiments, are disproportionately more likely to fail to state the location of the study. Of the 200 papers that failed to state a location 123 contained a significant laboratory experiment portion.

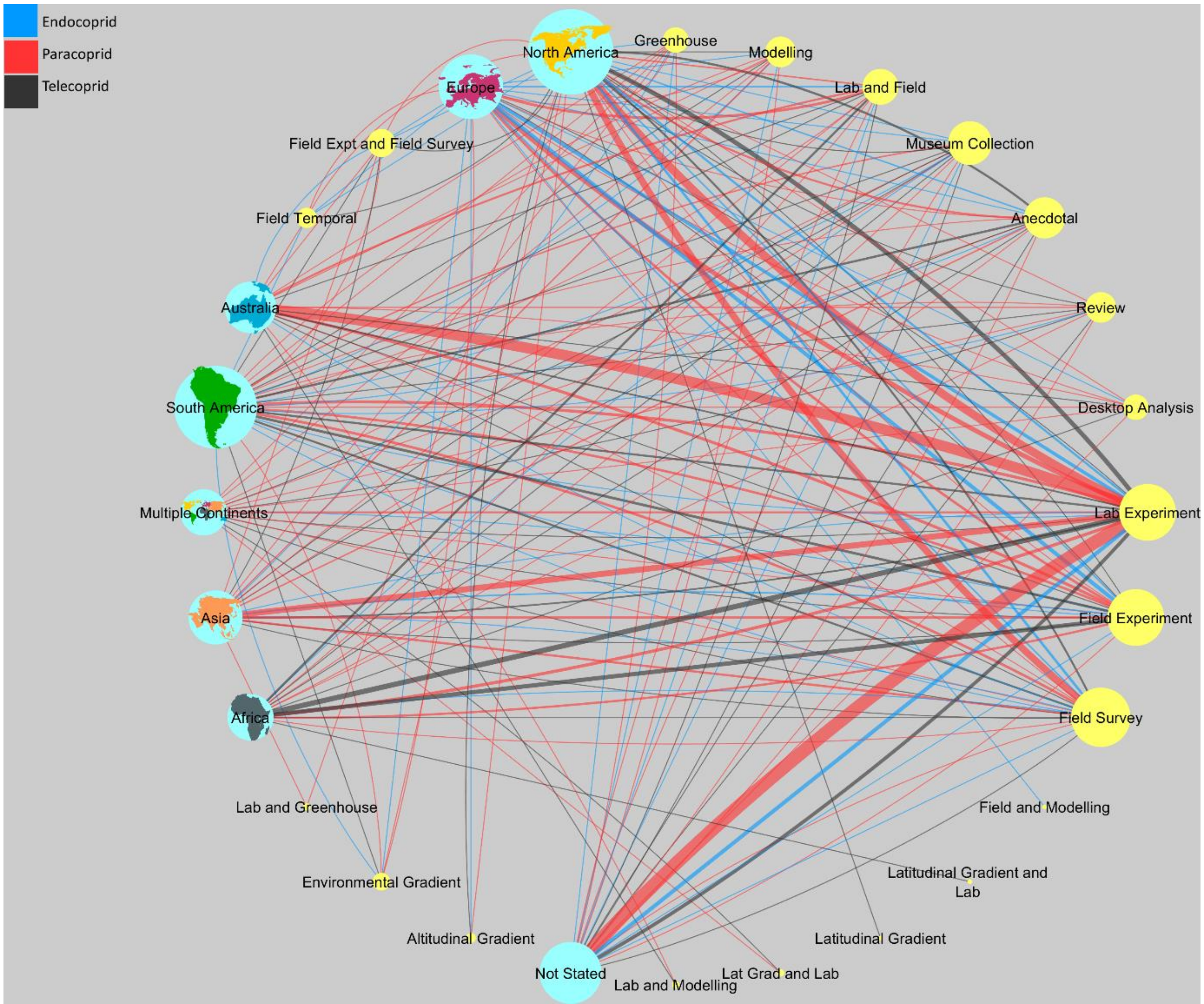


Figure 2.4.4. Network graph showing the relationship between continents (blue circles) and the type of experiment performed (yellow circles) on Telecoprids (black line), Endocoprids (blue line), and Paracoprids (red line). The larger the edge line connecting two nodes the higher the number of publications, ranging from 1 (thinnest line) publication to 68 publications (thickest line). The size of each node is based off of the number of edges connected to it with larger nodes being connected to more edges. A large node therefore represents either a continent where studies have been conducted using a diverse range of methodologies or a methodology that has been used on many continents

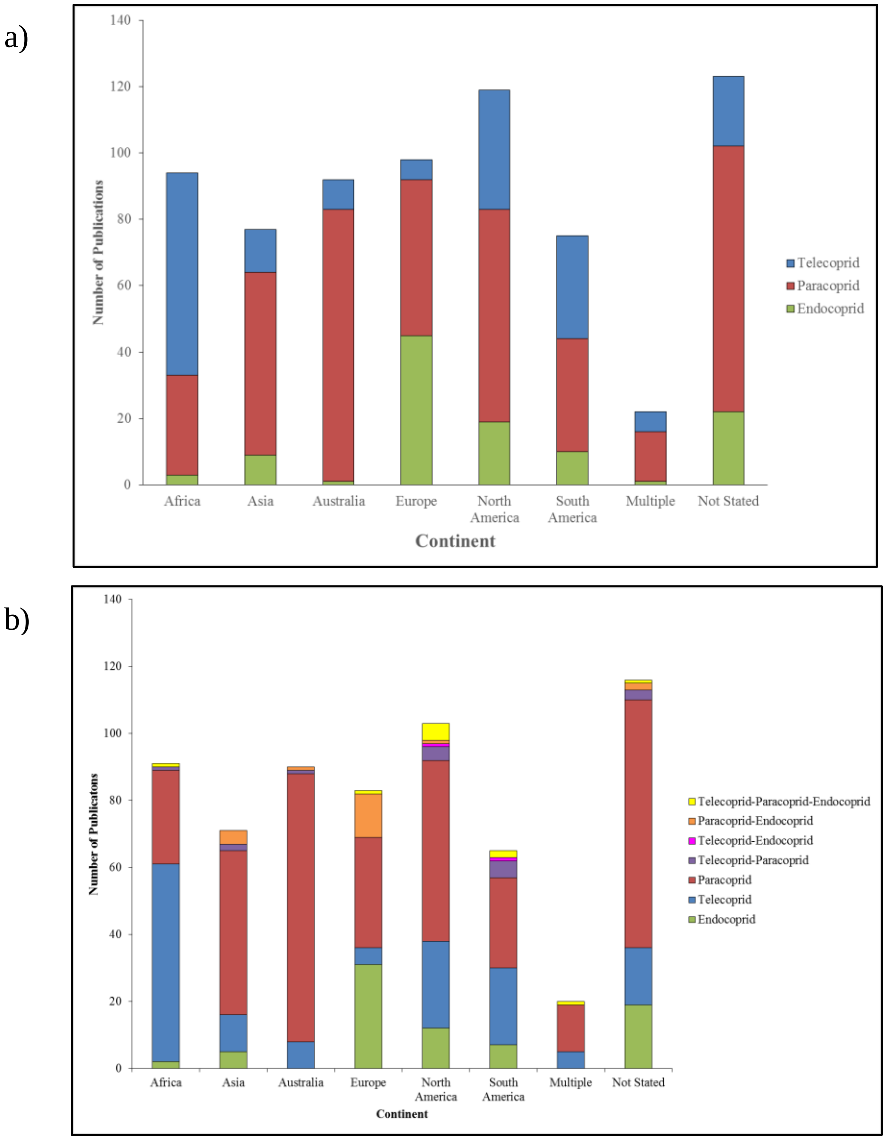


Figure 2.4.5. Number of publications with a) specific reference to a Telecoprid, Paracoprid, or Endocoprid species (a single study may contain reference to multiple functional groups) and b) with specific reference to Telecoprids, Paracoprids, Endocoprids, or any combination within a single study. Studies utilising simple surveys are not included in this data.

2.4.3 – Functional groups and multispecies studies

While surveys can provide valuable information concerning the distribution, behaviour, and phenology of the functional groups the combination of a large number of papers using this method and the possibility that individuals of a particular functional group may be surveyed by simple chance means it is likely that certain functional groups will appear overrepresented relative our knowledge of the group. When not considering studies solely utilising field surveys paracoprids are the most studied group, with large (pink) edges running from Australia, North America, Asia, and Europe. Smaller (pink) lines emerge from South America and Africa, indicating that a lower proportion of the studies conducted on these continents investigated paracoprids (Figure 2.4.4). When looking at the breakdown of publications addressing each of the functional groups it is clear that; paracoprids are indeed the most studied group (Figure 2.4.7), comprising the largest proportion of studies on all continents; telecoprids are the second most studied group, however the number of publications devoted to them varies greatly across continents; relatively few publications address endocoprids and these are mostly limited to Europe and North America (Figure 2.4.5). Few publications incorporate multiple functional groups in the same study, with most that do being conducted in the Americas or Europe (Figure 2.4.5). Multi-group studies in Europe are dominated by the co-study of paracoprids and endocoprids, whereas North and South America show a much more balanced distribution with most multi-group studies looking at all three groups, or a combination of paracoprids and telecoprids (Figure 2.4.5.b).

It is important to view these patterns of research in the context of the biogeography of dung beetles, as the choice of focal species is driven by access to a species for research and the potential application of the research to the community in question. In the case of Australia this is likely to be driven by the natural history of the continent and the introduction of non-native dung beetle fauna that are predominately paracoprids (Edwards 2009). Australia’s native dung beetle fauna remains mostly a mystery, they are mostly limited to xeric and woodland environments and are therefore rarely encountered and of little economic interest (Matthews 1971, Monteith 2015). Similarly, while telecoprids are present in Europe, many of the areas with a warmer climate are dominated by paracoprids, whereas endocoprids, particularly the Aphodiinae, are most abundant in colder regions where other groups have difficulty with the low temperatures, such as Northern Europe (Hanski and Cambefort 1991). Outside of Europe and North America research on endocoprids is scarce. They are traditionally viewed as less competitive than either telecoprids or paracoprids and thought to provide less in the way of ecosystem services than either group (Krell, Krell-Westerwalbesloh et al. 2003, Krell-Westerwalbesloh, Krell et al. 2004, Batilani-Filho and Hernandez 2017). However given how little these groups have been studied it would appear that this conclusion may be without basis; there is recent evidence that endocoprids contribute more to these processes than previously thought, and there may be synergistic interactions between the activity of endocoprids and paracoprids (Manning, Slade et al. 2016, Sands and Wall 2017, Verdú, Lobo et al. 2018).

The majority of research conducted on the provision of ecosystem services has utilised paracoprids, with work on telecoprids primarily focused on their role as secondary seed dispersers in South America, and comparatively little research into the role of endocoprids in maintaining ecosystem function. Not only must there be increased focus on studies utilising multiple functioning groups, species diversity has also been found to be an important factor in the provision of ecosystem services, therefore the study of dung beetle communities and the factors that influence their structure is critical. It has become evident that the interaction of multiple species can alter the provision of these services and provide redundancy for these services should species be lost from the assemblage (Sánchez, Sarzetti et al. 2012, Manning, Slade et al. 2016), therefore multi-species studies are required if we are to apply this knowledge to the field. Given this, it is vital that spatial and temporal variation in the structure of communities be considered when applying research conducted from one area as a solution to a problem in other areas, which have distinct communities.

The study of a single species was most prevalent (631), followed by studies that looked at more than five species (613), together comprising 85.0% of all publications (Figure 2.4.9). This general pattern held across all continents, fields of research, and through time (Figure 2.4.9, 2.4.11, 2.4.10).When removing studies that relied on a survey methodology the proportion of multi-species studies drops dramatically with single species studies comprising 55.9% of the remaining 984 publications. Over half of all ecological studies address over five species, however after removing studies utilising surveys this number drops to 33.4% (Figure 2.4.11). This is likely a function of surveys being frequently used to study abundance and community composition and the nature of surveys in that they are likely to find more than five species unless the assemblage is depauperate.

The most studied genus by far is *Onthophagus*, having nearly as many publications devoted to it as the next five next most studied genera combined, constituting 10.3% of all publications assessed (Figure 2.4.8). *Onthophagus* is a model organism for the study of the evolution of sexual dimorphism and the development of horns (Moczek 2011, Kijimoto, Pespeni et al. 2013). Nearly half of the studies published on *Onthophagus* focus specifically on the species *Onthophagus taurus*, with 66 of the 151 studies utilising this species. There have been extensive efforts to provide a comprehensive expressed sequence tag database for this species (Choi, Kijimoto et al. 2010). Somewhat surprisingly *Aphodius* was the second most studied genus, despite endocoprids as a group being the least studied. This imbalance is likely due to telecoprids and paracoprids being the dominant functional group in most ecosystems, providing a multitude of genera to study whereas *Aphodius*, typically dominant at higher latitudes where temperatures are too cold for other groups, is a widely distributed and frequently encountered genus (Hanski and Cambefort 1991, Wassmer 1994).

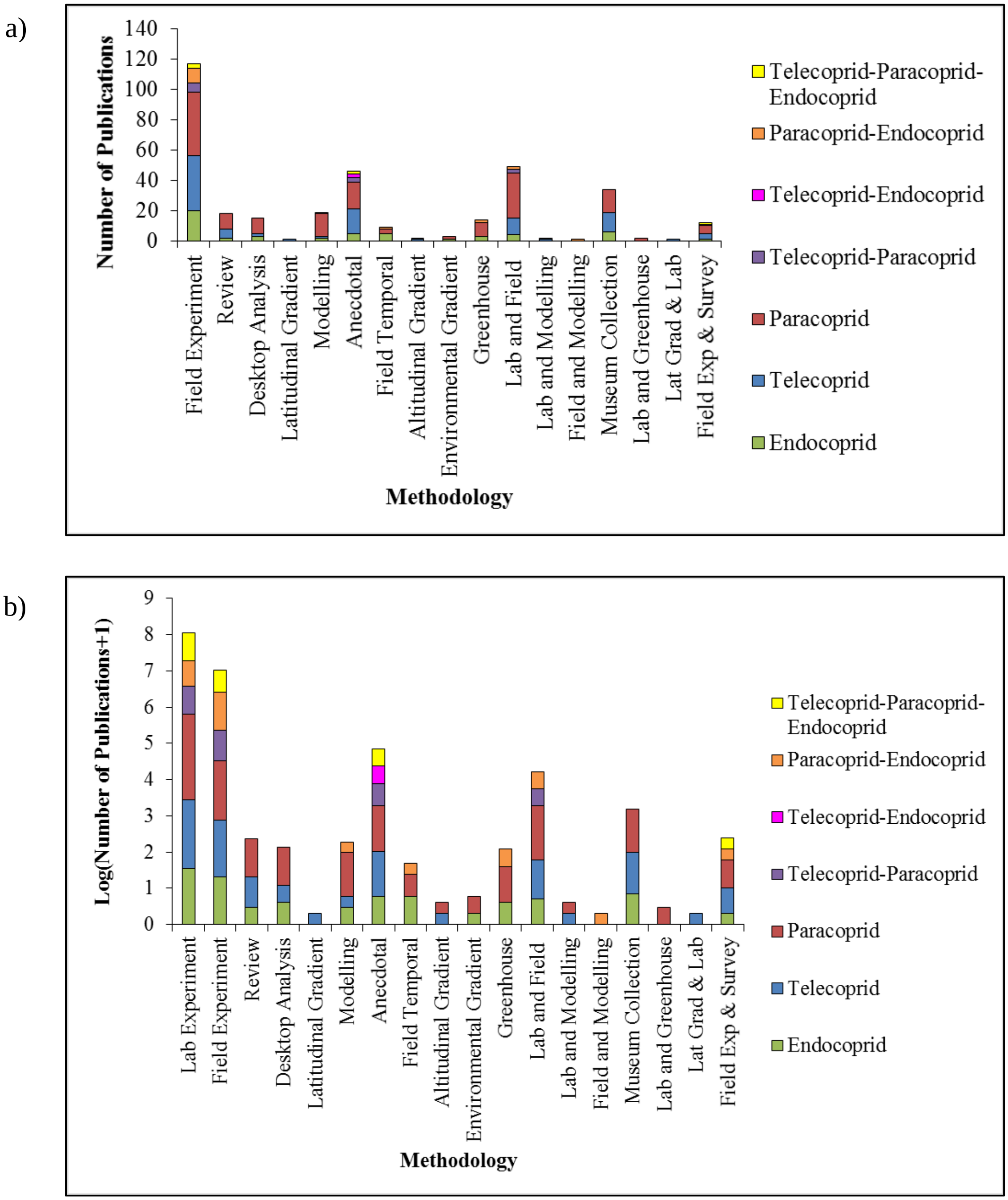


Figure 2.4.6. Types of studies performed on the different functional groups, excluding studies that exclusively utilised surveys. a) Excludes laboratory studies as there were a large number making interpreting the graph difficult and b) Including laboratory experiments with number of publications+1 log transformed.

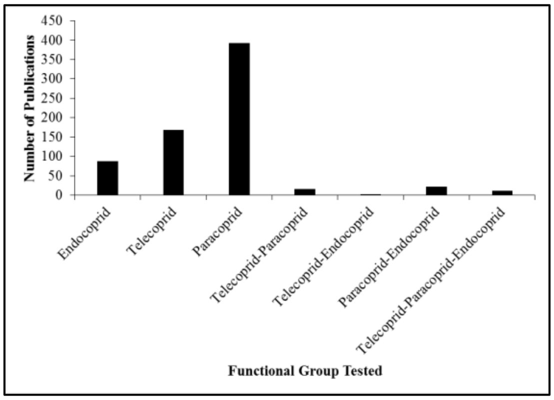


Figure 2.4.7. Number of experiments performed on endocoprids, telecoprids, and paracoprids, excluding studies that exclusively utilised surveys. Studies may have assessed multiple functional groups in the same publication.

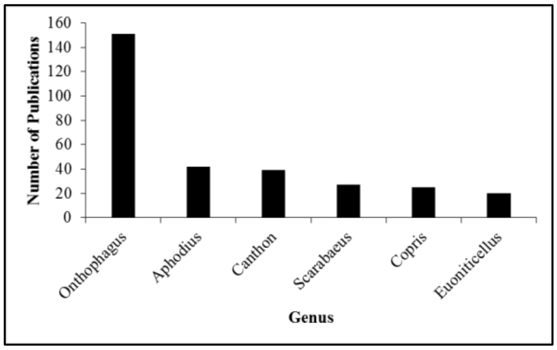


Figure 2.4.8. The most studied genera among publications that examined a single species (n=631). Genera are listed in descending order of number of publications: *Onthophagus* (n=151), *Aphodius* (n=42), *Canthon* (n=39), *Scarabaeus* (n=27), *Copris* (n=25), *Euoniticellus* (n=20).

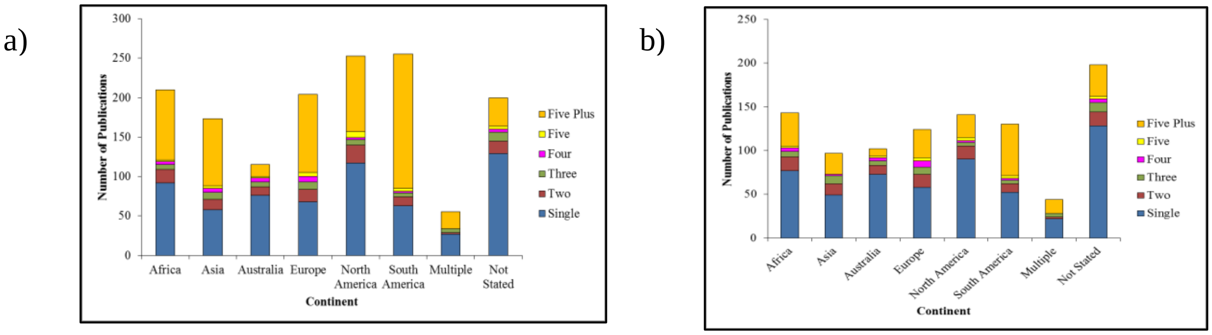


Figure 2.4.9. Number of publications from each continent that studied one, two, three, four, five, or more dung beetle species in the same publication a) across all studies contained within the database and b) excluding publications whose methodology was limited to a survey.

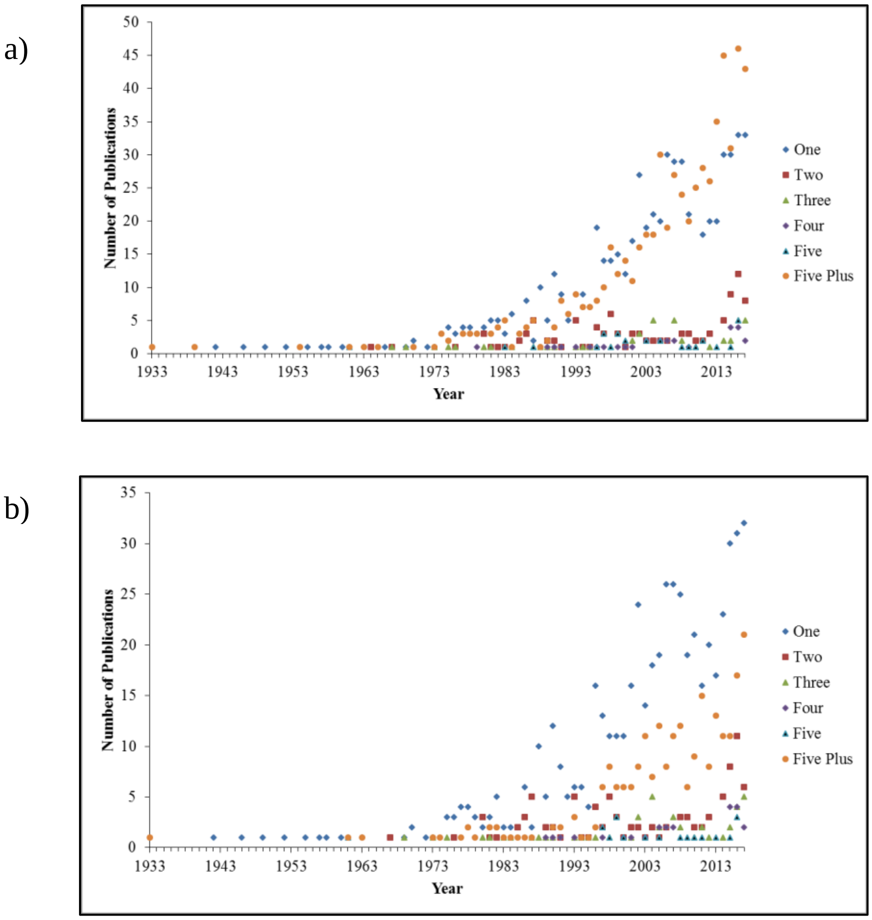


Figure 2.4.10. Number of publications and the number of species examined in them for each year from 1933-2017. a) all studies in database and b) excluding publications whose methodology was a survey

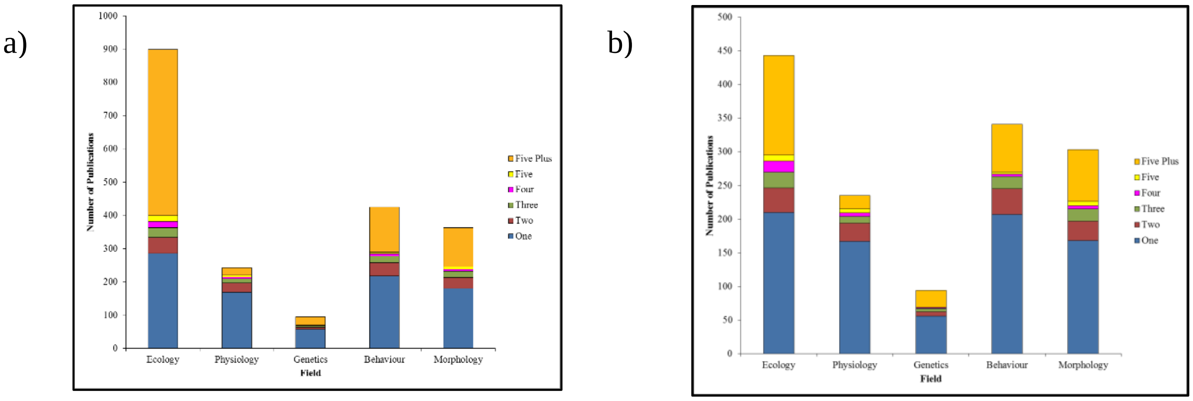


Figure 2.4.11. Studies in major fields that looked at number of species a) all studies in database and b) excluding publications whose methodology was a survey.

2.4.4 – Methodologies employed

Laboratory experiments, field experiments, and field surveys are the most utilised methodologies constituting 426 (29.1%), 197 (13.5%), and 456 (31.2%) publications respectively (Figure 2.4.12). This is unsurprising as laboratory and field experiments are broad categories representing number of possible methodologies. That surveys are the most widely used method also unsurprising, as has been mentioned previously surveying dung beetle populations is cheap and can provide a wealth of information concerning abundance, distribution, and assemblage composition. Dung beetles community structure is strongly influenced by the abiotic environment, therefore there is great potential to utilise surveys to examine the effects of factors such as ambient temperature, soil type, moisture, and plant cover on dung beetle communities. Indeed many studies utilise these methods when examining dung beetles as bioindicators, conducting surveys across land use disturbance gradients. This is exemplified by the high number of publications that use environmental gradients (Figure 2.4.13), which followed closely behind field studies with 172 (11.8%) publications, 83 of which also utilised a survey. The effects of environmental gradients on dung beetles has been studied relatively well on all continents with the exception of Australia, which lags behind in this respect with only three studies, two of which focus on tropical species (Hill 1995, Hill 1996) and another more recent paper that looks at the ratio of native to introduced species across a chronosequence of re-vegetation levels (Gollan, Reid et al. 2011) (Figure 2.4.12). Eighty four of the 425 publications on behaviour utilised a survey to gather data; surveys are useful in examining the behaviour of a wide variety of species in that multiple bait types can be utilised to analyse the preference of species within the community.

To our knowledge only two papers have directly assessed trap spacing and survey methodology (although a number of studies have addressed trap design and efficacy) (Larsen and Forsyth 2005), which estimates a minimum trap spacing of 50m and da Silva and Hernández (2015) which suggest a minimum spacing of 100m. Both of these publications were conducted in South American forests and are yet to be verified in other habitat types. There have been several publications that aim to provide guidance when designing surveys over wider geographical areas (Davis 2002), as there is a recognised bias in historical dung beetle surveys which can result in an incomplete understanding of the factors influencing the assemblage (Hortal, Jiménez-Valverde et al. 2008, Lobo 2008).

The effects of altitude on dung beetle communities has been widely studied (Figure 2.4.13), with altitude often being used as a surrogate for climate change or habitat modification (Menendez and Gutierrez 2004, Larsen 2012, Menéndez, González-Megías et al. 2014). Dung beetles have been used to test broad ecological theories such as such as the climatic variability hypothesis (Gaston and Chown 1999, Verdú 2011, Birkett, Blackburn et al. 2018) and Rapoport's rule (Herzog, Hamel-Leigue et al. 2013). Typically species diversity declines with decreasing altitude (Escobar, Lobo et al. 2005, Lobo, Chehlarov et al. 2007, Lobo, Gueorguiev et al. 2007), however there have been instances where this does not hold (Negro, Palestrini et al. 2011). The more cold tolerant Aphodiinae dominate the higher altitudes, with telecoprids and paracoprids moving from exposed pastures to covered woodland habitats where temperatures are warmer (Menéndez and Gutiérrez 1996, Menendez and Gutierrez 2004, Chamberlain, Tocco et al. 2015). Montane dung beetle communities in North America, specifically those in Mexico, are used to study theories of horizontal and vertical colonisation (Lobo and Halffter 2000, Escobar, Lobo et al. 2006). There is evidence to suggest that similar processes shape montane assemblages in Europe (Lobo, Gueorguiev et al. 2007) .

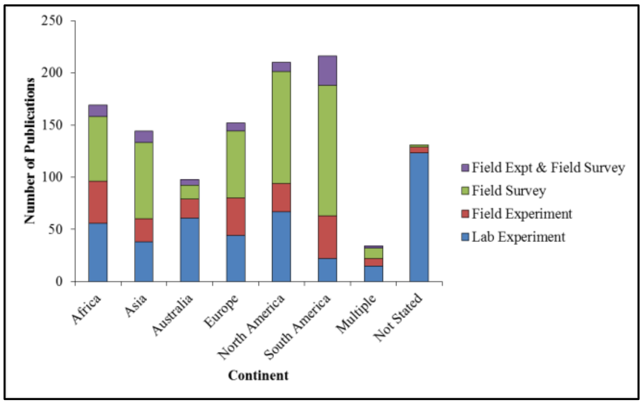


Figure 2.4.12. Number of publications whose methodology utilises experiments based in a laboratory, experiments conducted outside in natural conditions, and trapping surveys of a community or population.

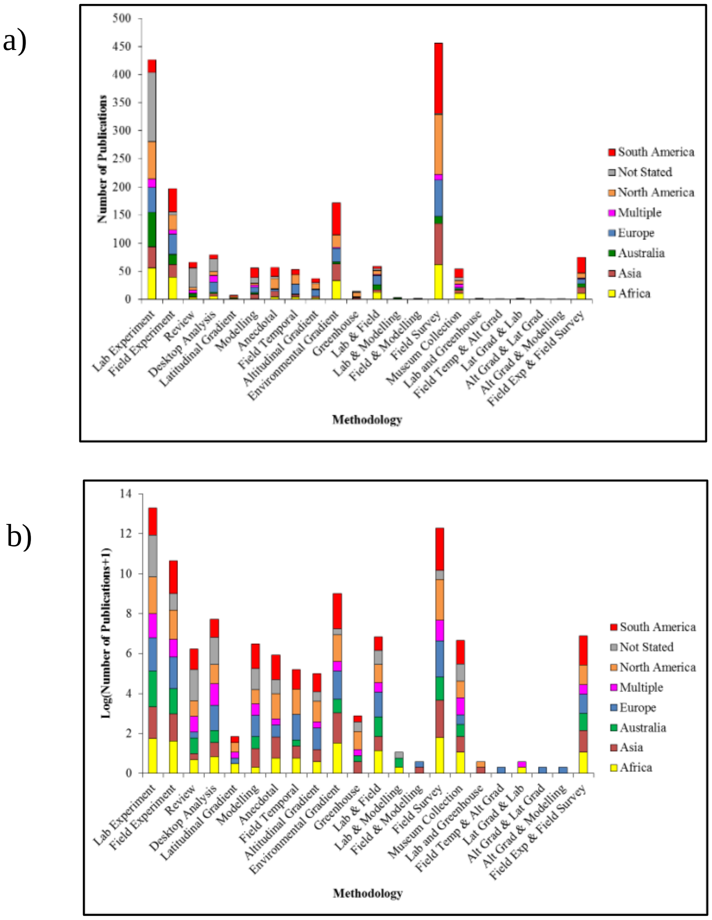


Figure 2.4.13. Number of publications concerning dung beetles from each continent according to the methodology utilised. Represented as a) Actual number of publications, and b) log transformed.

2.4.5 – Traits/responses assessed

The most assessed responses are abundance, behaviour, and assemblage composition. Again likely due to ease and low-cost of sampling dung beetles (Figure 2.4.14). These factors can be cheaply and easily assessed allowing valuable information to be gathered without diverting funding from more expensive aspects of a project. Abundance and assemblage composition were among the most studied responses across all continents, with the exception of Australia (Figure 2.4.15), accounting for 331 and 282 publications respectively. Behaviour was the second most studied response after abundance with 284 publications, followed by distribution and morphology.

Relative to these other responses genetics remain poorly studied. While it is only in recent years that the technology to conduct in-depth and comprehensive genetic studies has become cheap and readily available much of the dung beetle literature utilises a small complex of species belonging to the genus *Onthophagus*, primarily *O. taurus*. Those publications studying the development of horns and parental care have significant overlap with morphological and behavioural studies, as is to be expected given the topic. Recent studies on *O. taurus* have shown the remarkable evolutionary capacity of the genus, and potentially dung beetles in general, with recently separated populations diverging in nesting depth and tibial morphology (Macagno, Moczek et al. 2016), and ovarian development and fecundity (Macagno, Beckers et al. 2015). There is evidence to suggest that population of *O. taurus* in North America and Australia have diverged in terms of climatic preference (Silva, Vilela et al. 2016), whether there is a genetic basis to this is unknown, but such a study could prove fruitful.

Looking at the relationships between response, methodology, and field of study we can see that assemblage composition and abundance are often studied using surveys, as is distribution, although a lower proportion of distribution studies use this method, indicated by the thinner line (Figure 2.4.16). Genetic responses primarily use laboratory experiments, supported by a small number of surveys, desktop analysis, and the use of museum collections. Similarly, laboratory experiments are the primary method used by physiologists to study both survival and more general physiological concept such as gas exchange and thermal tolerance (Davis, Chown et al. 1999, Chown and Holter 2000, Davis, Chown et al. 2000, Duncan and Byrne 2002, Chown and Davis 2003, Duncan, Förster et al. 2010, Terblanche, Clusella-Trullas et al. 2010). The use of altitudinal gradients in the study of physiology is closely linked to the testing of broad physiological concepts involving elevation and thermal tolerances (Gaston and Chown 1999, Sheldon and Tewksbury 2014). The strong link between morphological publications and laboratory experiments is driven by the study of horn allometry and the study of weapons in sexual selection (Emlen and Nijhout 2000, Palestrini, Rolando et al. 2000, Moczek 2006, Pomfret and Knell 2006, Moczek 2011), which as previously mentioned often has significant overlap with studies of genetics and phenotypic expression.

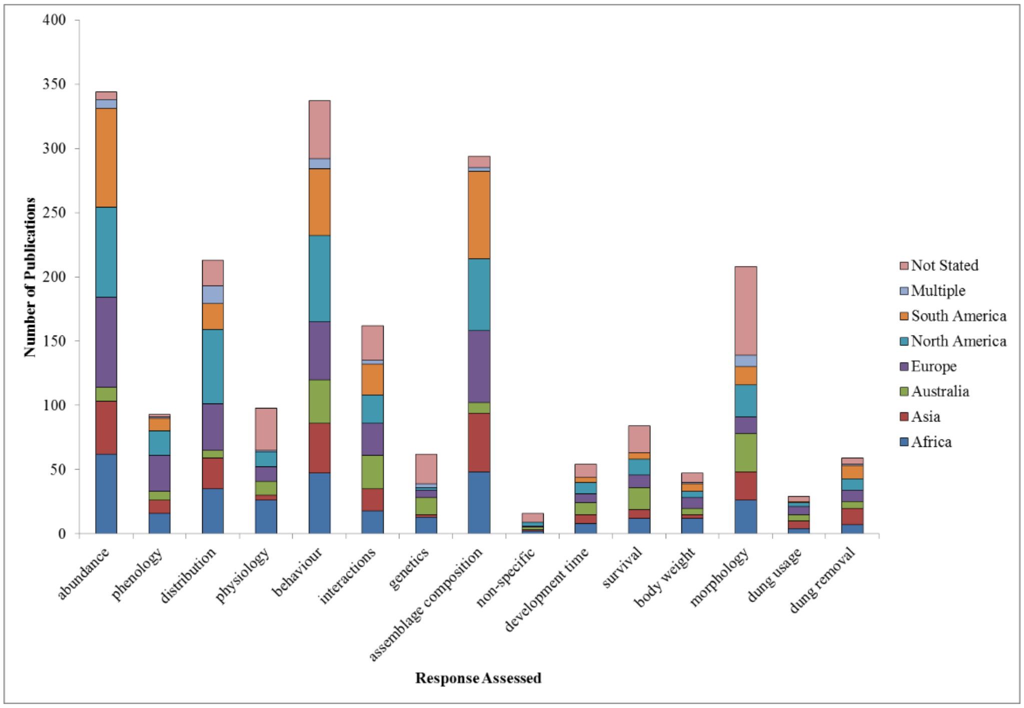


Figure 2.4.14. Number of publications that assessed responses, by continents (n=2100). Multiple responses may be assessed in an individual paper.

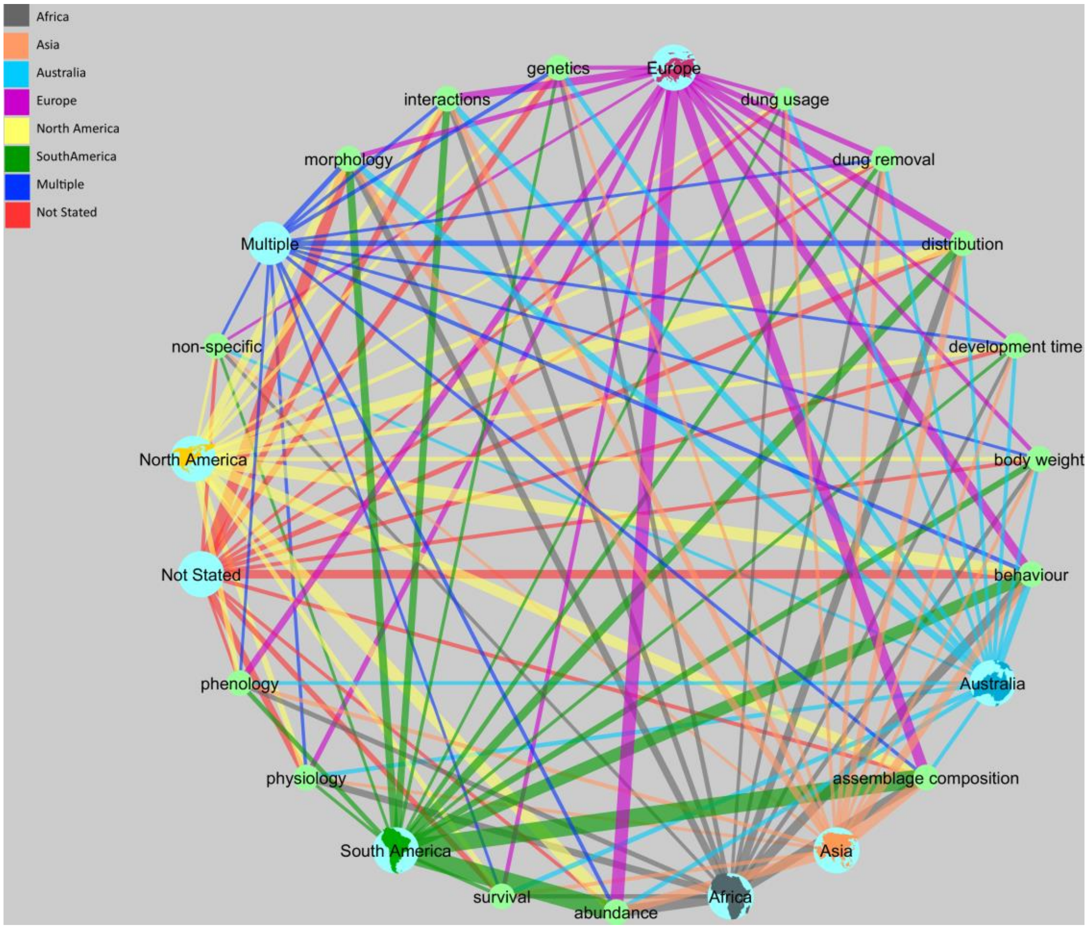


Figure 2.4.15. Network graph showing which responses (green circle) have been assessed on each continent. Edge size corresponds to the numbers of publications that have assessed a particular response on a particular continent, ranging from one publication (thinnest line) to 128 publications (thickest line). Edge colours are mapped to continent.

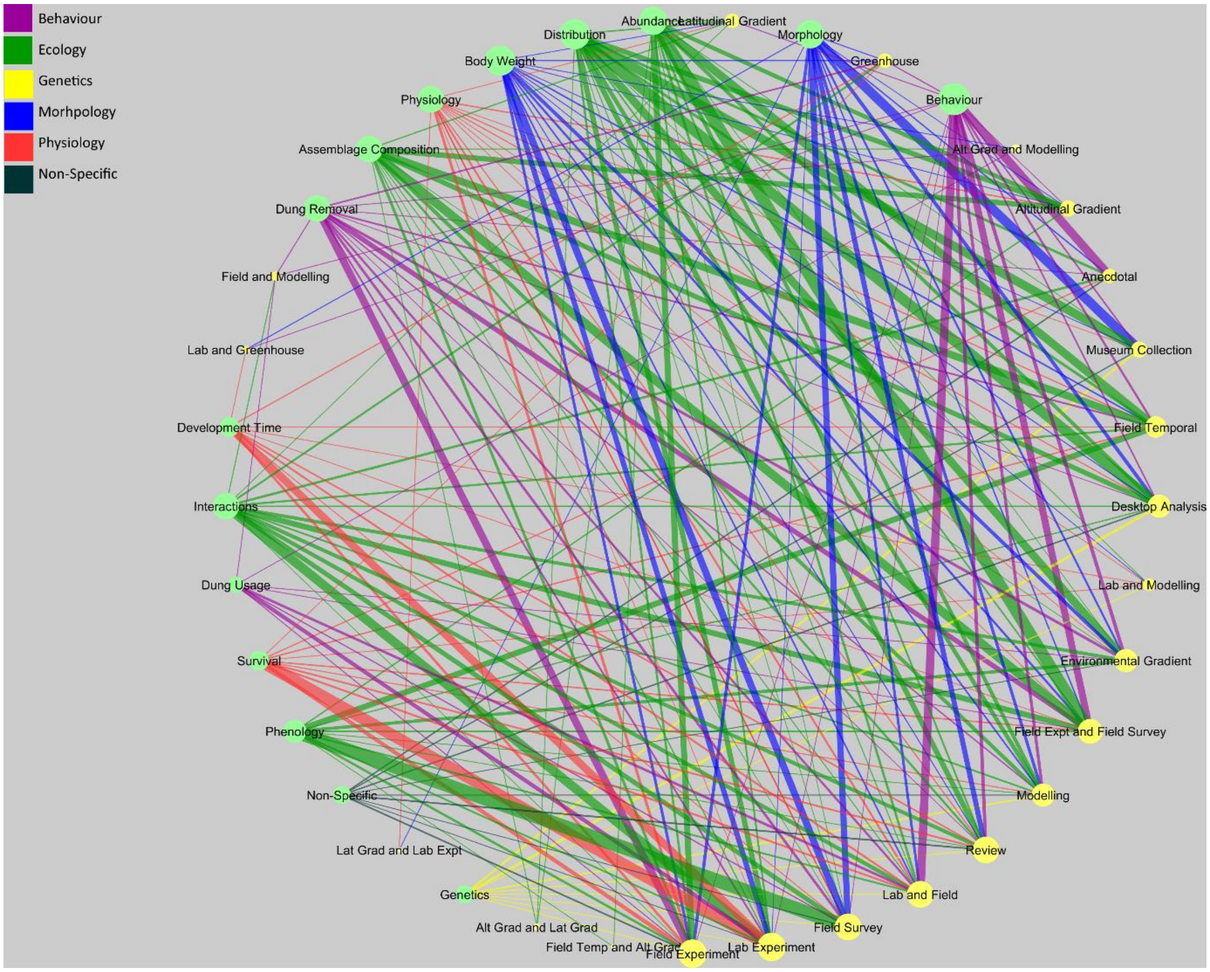


Figure 2.4.16. Relationships between experiment type (yellow circle), factors assessed (green circle), and field of study (edges). Edge size corresponds to the numbers of publications that have assessed a particular response using a particular method. Edge colours are mapped to continent. Edge size is mapped to number of publications, ranging from 1 publication (thinnest line) to 229 publications (thickest line).

2.4.6 – Use of dung and non-dung resources

The use of dung is the defining feature of dung beetles, although a minority use resources other than dung (Forgie, Grebennikov et al. 2002, Larsen, Lopera et al. 2006, Flechtmann, Tabet et al. 2009, Larsen, Lopera et al. 2009, Iannuzzi, Leal et al. 2013, Salomão, Lira et al. 2014), so it comes as no surprise that it is an area that has been heavily researched, although how dung beetles thrive on such a diet remain somewhat of a mystery (Holter and Scholtz 2007, Holter 2016). In the 1463 publications assessed we found 75 types of "dung" had been used (Figure 2.4.17). The number of individual species from which an attractant has been utilised is no doubt higher as a result of scenarios where a number of species are grouped under a common colloquial term (e.g. antelope), the fact that we were unable to access papers, and there were a large number in languages other than English. The use of many of these types of resources has been restricted to one or two continents where they have been utilised as an attractant when conducting surveys (Figure 2.4.18). Many are restricted to use in a handful of studies, with only 328 publications utilising two or more resources. For example Larsen, Lopera et al. (2006) utilises carrion from a number of species as well as dung from eight different animals, while Stavert, Drayton et al. (2014) assesses the dung of 16 species. Looking at the diversity of species omnivores are poorly represented relative to herbivores, however, in terms of the number of publications they are well represented with human and pig dung both being in the top five most used dungs (Figure 2.4.20). In comparative studies on the attractiveness of carnivore and herbivore dung carnivore dung is generally found to attract fewer species than herbivore dung, with omnivore, and specifically human, dung often attracting both the most diverse range of species and the highest abundance (Howden and Nealis 1975, Martín-Piera and Lobo 1996).

Cow dung was by far the most utilised with 582 (39.8%) publications, it is frequently used in surveys to assess abundance, distribution, community assemblage, and behaviour (Figure 2.4.17), with this trend holding true on all continents with the exception of South America (Figure 2.4.18, 2.4.19), where human dung is used in the majority of publications. Two hundred and forty seven publications used dung from humans, 138 of which were located in South America. Human dung was also well represented in North America and Asia with 47 and 38 publications respectively. Looking at Figure 2.4.17 we can see that human dung is often used to study assemblage composition in North and South America, with some studies from Asia using it in a similar manner. Far fewer publications use horse (61), pig (93), or sheep (80) dung.

Given the importance of dung to the behaviour and reproductive biology of dung beetles a startling number of papers failed to include information on the type of resource used in the study with ‘Not Stated’ being the second most prevalent resource type with 419 publications failing to state the type of dung used to maintain the beetles in the study. The dung from animals can differ greatly in the nutritional quality and water content, with the profile of volatiles released known to influence its attractiveness to different species of beetle (Paetel 2001, Dormont, Epinat et al. 2004, Dormont, Rapior et al. 2007, Dormont, Jay-Robert et al. 2010, Stavert, Drayton et al. 2014, Holter 2016). The quality and source of dung can have significant effects on the beetles themselves, with adult beetles altering brood ball size and abundance; the survival and weight of larvae (Macqueen, Wallace et al. 1986, Edwards 1991, Kishi and Nishida 2009), the development of horns in adults (Emlen 1997, Moczek 1998); thermal tolerance and ovarian development (Verdú, Casas et al. 2010). When the dung was collected is an important detail as the nutritional quality of herbivore dung is subject to seasonal variation. Dung quality is strongly correlated to rainfall, with dung of the highest nutritional quality being produced when rain is frequent, typically spring, and lowest when conditions are dry (Edwards 1991). Dung seasonality has been found to affect reproduction in the dung beetles *O. binodis* and *E. intermedius*, which produced significantly fewer brood balls when fed low quality dung (Macqueen, Wallace et al. 1986, Ridsdill-Smith 1986, Edwards 1991).

Perhaps even more troubling is the lack of information concerning the use of anthelmintics and other such chemicals on the animals from which the dung is sourced. While we did not specifically extract this information the fact that such a large number of papers did not mention resource type, combined with our anecdotal experience constructing this literature database, reveals that the majority of papers do not state whether the used in their study was sourced from chemical free stock or not. There is extensive literature on the effects of such chemicals on dung beetles (Wardhaugh and Rodriguez-Menendez 1988, Madsen, Overgaard Nielsen et al. 1990, Fincher 1992, Strong 1992, Ridsdill-Smith 1993, Krüger and Scholtz 1997, Krüger and Scholtz 1998, Krüger and Scholtz 1998, Floate, Colwell et al. 2002, Davis, Scholtz et al. 2004, Iwasa, Nakamura et al. 2005, Iwasa, Maruo et al. 2007, Webb, Beaumont et al. 2010) and while results are sometimes contrary there is evidence to suggest that the presence of such chemicals can influence larval development (Madsen, Overgaard Nielsen et al. 1990), survival (Hempel, Scheffczyk et al. 2006, Rosales, Martínez et al. 2012), olfaction and locomotion (Verdú, Cortez et al. 2015), fecundity (Rosales, Martínez et al. 2012), and the provision of ecosystem function (Beynon, Peck et al. 2012).

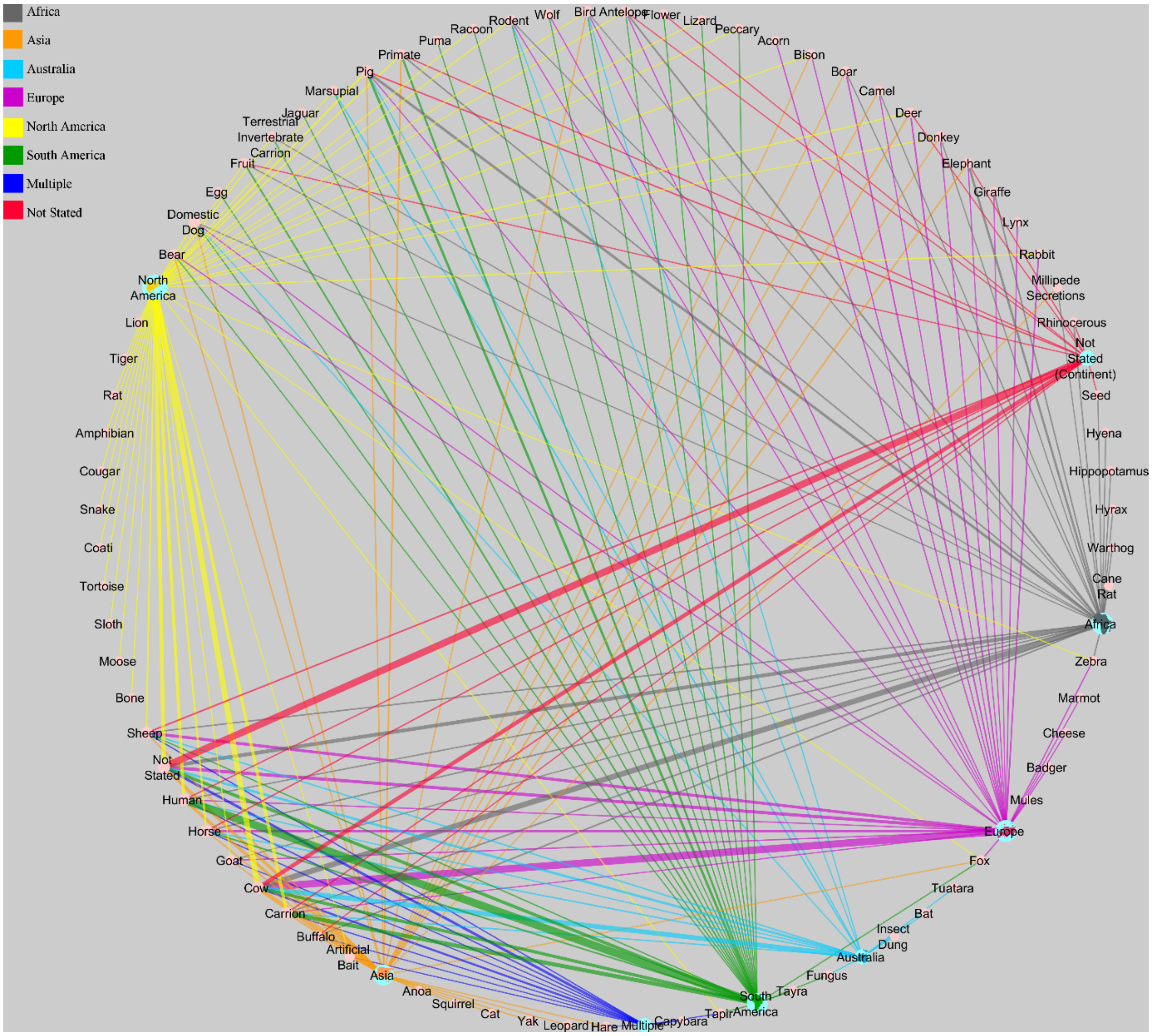


Figure 2.4.17 Network graph of the types of resources used in all publications in the database. Edge size is linked to the number of publications that used that dung type on that continent; Edge colour is mapped to continent.

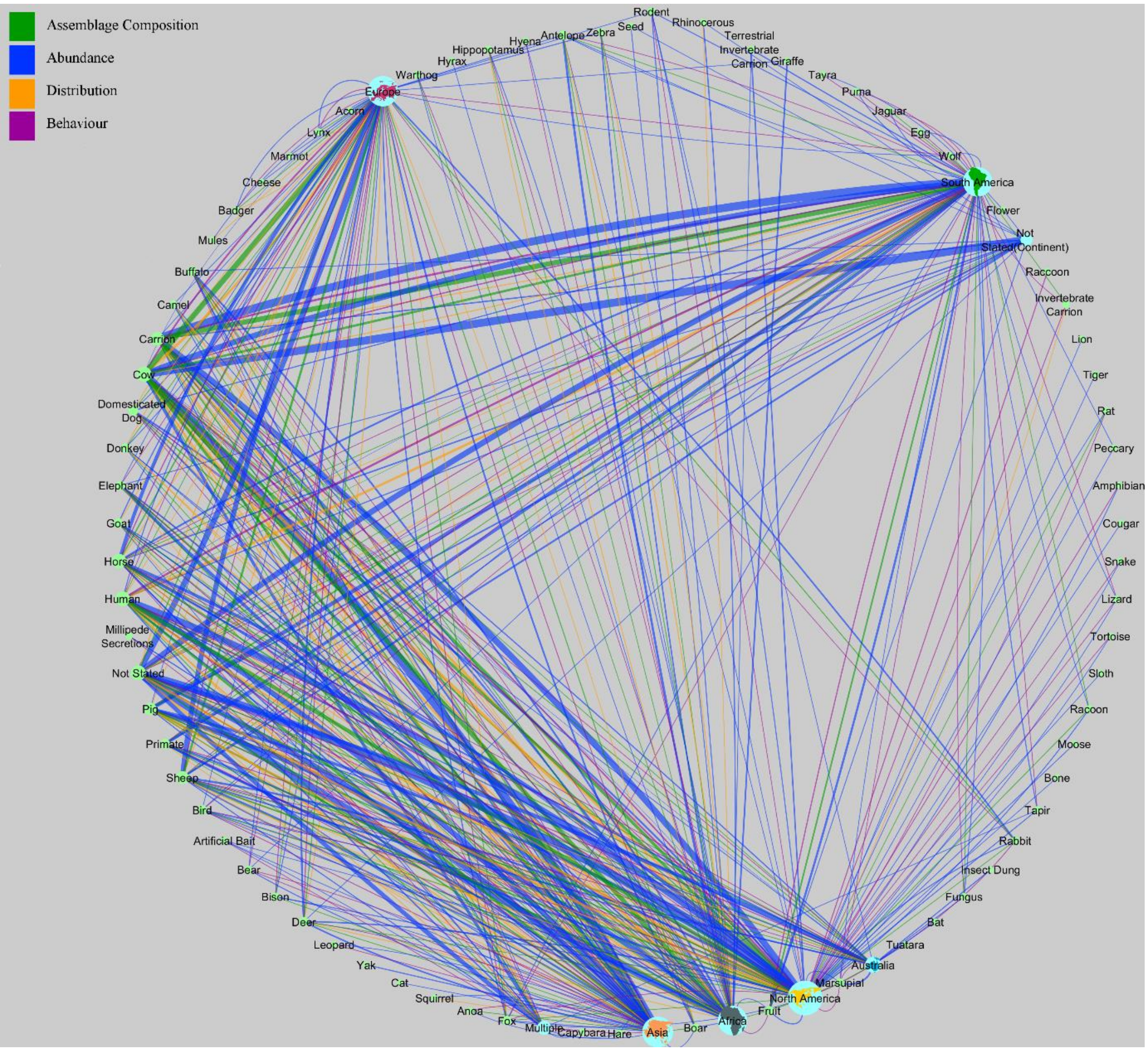


Figure 2.4.18 Network graph of resource types used in surveys and continent. Edge colour is mapped to the response being assessed.

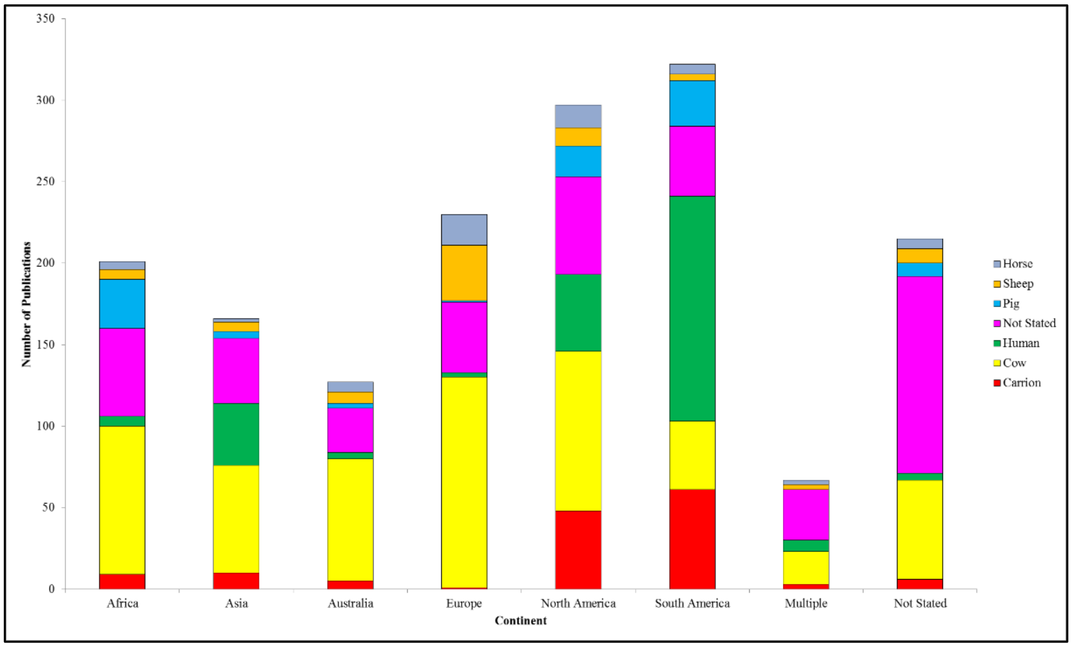


Figure 2.4.19. Number of publications that used the top five most used resource types. Studies may use resources from multiple animals.

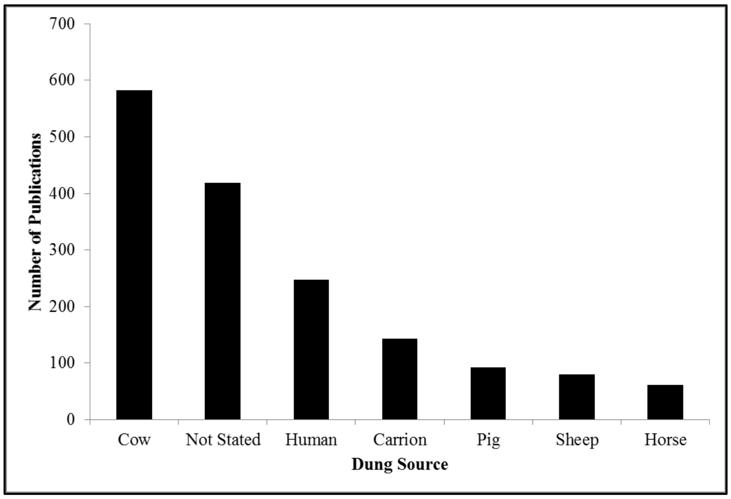


Figure 2.4.20. The top five most used resource types across 1463 studies. Studies may use resources from multiple animals.

2.4.7 – Conclusions

As evidenced by climbing publication rates scientific interest in dung beetles is increasing, while there is a strong body of literature originating from all continents the focus of each of these bodies differs in the functional groups studied, resource types used, methodologies utilised, and responses assessed. These patterns are influenced by the biogeography of the area the present day structure of dung beetle assemblages. Studies from South America and North America (primarily Mexico) largely focus on how the structure of the local dung beetle assemblages have been influenced by biogeography and how these communities respond to changes in the biotic and abiotic processes the compose their current habitat. The role of dung beetles as bio-indicators and facilitators of ecosystem regeneration is often studied by conducting a field survey across an environmental gradient and assessing how abundance and assemblage composition are influenced by habitat structure. Paracoprids are the most studied group, both overall and on each continent, with the exception of Europe and Africa where endocoprids and telecoprids were the most studied groups respectively.

Many of the publications included in this review failed to provide vital information such as the type of food resource used, whether the animal the dung was sourced from had been treated with chemicals, and the location where the study was performed or experimental population was sourced. The strong links between dung beetles and their abiotic environment has been consistently supported and is the basis for their use as bio-indicators. Looking to the future we propose that the following information be included to standardise the reporting of important metrics easier as well as the synthesising and analysis of data from across multiple continents. Given the lack of studies performed across multiple continents, and the prohibitive cost of performing such experiments, we propose that the following information should be included in all publications, both to provide as much relevant information as possible when interpreting the results of the experiment, and to give future desktop and meta-analysis as much information as possible; the animal from which the food resource was sourced for the experiment; whether the dung source had been treated with chemicals such as anthelmintics, the use of which has been shown to influence reproduction and development; the functional group the studied species belong to, although this is generally well-reported already; whether the species is nocturnal or diurnal as this can influence competitive interactions and provision of ecosystem function via asynchrony; the location where the experiment was performed and the origin of the experimental populations; a brief description of the habitat of this location such as mean annual temperature range, rainfall, and habitat structure, as this gives insight into whether the species is a closed or open habitat specialist or a habitat generalist; the body size of the species as larger dung beetles provide increased ecosystem function relative to smaller species, are generally more competitively dominant, and may have the capacity for limited endotherm (if above 1.98g)

Few manipulative laboratory and field studies assess multiple functional groups and while the number of studies utilising multiple species is increasing over time, the majority of laboratory and field studies still utilise a single species. Dung beetle species exist in a world of intense competition for resources, a factor which must be considered when conducting such experiments, especially in situations where the response of a species is altered by the presence of another, as is the case for the provision of many ecosystem services. Going forward studies utilising multiple species simultaneously will be vital. The studies looking at the physiology and genetics of dung beetles make up a minor fraction of the literature. While the number of publications in these fields is increasing it is important that physiology and genetics are given consideration when describing broad ecological and behavioural patterns. The thermal niche partitioning observed in various dung beetle communities suggests that the thermal biology of species may be an important factor in determining the spatio-temporal structure of dung beetle communities, yet there has been surprisingly little research. In chapters three and four we address this lack of research by performing a comparative analysis of the thermal biology of a dung beetle community from a temperate Australian pasture ecosystem and assessing the capacity of the dominant species in the area, *Onthophagus binodis*, to mitigate the effects of climate change via phenotypic and behavioural plasticity.

CHAPTER THREE

A COMPARATIVE ANALYSIS OF THE THERMAL TOLERANCE AND METABOLIC RATE OF DUNG BEETLES FROM A TEMPERATE PASTURE ECOSYSTEM IN AUSTRALIA

3.1 – Abstract

The thermal biology of insects influences their distribution, community structure, and species interactions. Their capacity to tolerate extremes of temperature dictates their potential geographic and temporal range, whilst metabolic rate and its sensitivity to temperature determines their realized range. Sympatric dung beetle species utilise thermal niche differentiation to minimize interspecies competition for dung, a sparse and ephemeral resource. We use thermolimit respirometry to measure the CT_{min}, CT_{max}, metabolic rate and its thermal sensitivity of seven dung beetle species from three feeding guilds. The CT_{min} of species ranged from -2.51°C for *A. fimetarius* to 9.07°C for *Euoniticellus fulvus*. The telecoprid species *Sisyphus rubrus* had the highest CT_{max} (52.86°C) while the native paracoprid *Onthophagus australis* had the lowest (43.03°C). When exposed to downward ramping temperatures (starting temperature: 25°C, rate: 0.2°C min^{-1}) *E. intermedius* (0.73ml h^{-1}), *E. fulvus* (0.79ml h^{-1}) and *S. rubrus* (0.76 ml h^{-1}) had the lowest metabolic rates and *O. binodis* the highest (1.63ml h^{-1}). When subjected to increasing temperatures (starting temperature: 25°C, rate: 0.2°C min^{-1}) these three species, along with the smaller bodied *A. fimetarius* (2.95ml h^{-1}) and *O. granulatus* (2.97ml h^{-1}) had metabolic rates higher than *O. binodis* (2.49 ml h^{-1}) and *O. australis* (2.31ml h^{-1}). The sensitivity of metabolic rate to changes in temperature was calculated for all individuals by regressing log-transformed CO_2 vs. temperature; below 25°C *A. fimetarius* (0.69) and *O. binodis* (0.81) were significantly less sensitive than *E. fulvus* (1.34), *E. intermedius* (1.21), and *S. rubrus* (1.28); above 25°C *A. fimetarius* (0.51), *O. granulatus* (0.56)

were the least sensitive, significantly lower than *O. australis* (0.89), *E. fulvus* (0.89), and *E. intermedius* (0.96), which were the most sensitive. *Euoniticellus fulvus*, *E. intermedius*, and *S. rubrus* appear to be adapted to warmer climates, performing poorly at low temperatures relative to other beetles, while the endocoprid *A. fimetarius* is a generalist capable of operating over a wide thermal window; we provide a template for assessing multiple thermal traits of a dung beetle community, and provide physiological evidence for thermal niche differentiation within these communities.

3.2 – Introduction

Temperature is a fundamental limiting factor in shaping an organisms life history (Clarke 2003). Its effects are pervasive, influencing the behaviour, distribution, and interactions of species, thereby shaping the structure of ecosystems and the pace of the processes that maintain them (Winder and Schindler 2004, Jamieson, Trowbridge et al. 2012). Habitats do not represent a static thermal environment; rather they are composed of a matrix of thermally distinct microhabitats which can experience large changes in temperature over short periods of time (Scheffers, Brunner et al. 2013, Kleckova, Konvicka et al. 2014, Baudier, Mudd et al. 2015). The response of populations to their thermal environment is driven by variation in the thermal biology of individuals, dictating the structure of communities, how resources are partitioned, and thereby the flow of energy through the ecosystem (Addo-Bediako, Chown et al. 2002). Key traits of species' thermal biology are their capacity to tolerate a range of temperatures and the relationship between temperature and metabolic rate (Tattersall, Sinclair et al. 2012).

The range of temperatures at which an organism can function is constrained by the critical thermal minimum (CT_{min}), representing the minimum temperature that a species can tolerate before activity ceases, and the critical thermal maximum (CT_{max}), the temperature at which irreparable physiological damage occurs. These traits appear to be underpinned by independent processes, with the CT_{min} of insects showing a greater degree of inter- and intra-specific variation (Addo-Bediako, Chown et al. 2000, Sunday, Bates et al. 2011). Furthermore individuals are able to plastically adapt one without affecting the other (Anderson, Hoffmann et al. 2005). These thermal tolerances constrain the fundamental niche breadth of a species and are strongly correlated with species latitudinal range (Calosi, Bilton et al. 2010). In ectotherms thermal window breadth is positively correlated with distribution and is thought to facilitate range expansion (Hidalgo-Galiana, Sánchez-Fernández et al. 2014). As insects are unable to maintain steady body temperatures via metabolic heat their biological machinery must be able to operate at a range of temperatures (Gaitán-Espitia, Belén Arias et al. 2013).

At the fundamental level temperature alters the rate of the chemical reactions that drive the biological processes necessary for life, consequently insects display a broad range of metabolic responses to their thermal environment (Irlich, M et al. 2009). Key tenets of thermal physiology are that basal metabolism increases with temperature in ectotherms and that variation exists in their short and long-term responses (Ege and Krogh 1914, Portner, Bennett et al. 2006). Historically our understanding of insect metabolism was based on a mechanistic understanding of the physical laws that underpin it. That is, metabolic rate scales as a function of body mass and body temperature (Gillooly, Brown et al. 2001). However it has become clear that there exist physiological mechanisms that allow insects to regulate their metabolic rate independent of body mass and temperature, and that metabolic rate is an adaptive trait upon which selection pressures act (Gudowska, Schramm et al. 2017). This is reflected in the significant inter- and intraspecific variation observed in the metabolic rate of insects (Addo-Bediako, Chown et al. 2002, Nespolo, Lardies et al. 2003, Messamah, Kellermann et al. 2017) as well as correlations with behaviour (Biro and Stamps 2010), life-history (Reinhold 1999), and habitat preference (Betz and Fuhrmann 2001, Schmuki, Woodman et al. 2007).

Elevated metabolic rates have been correlated with increased performance in a number of traits including; locomotor and dispersal capacity (Krasnov, Khokhlova et al. 2004, Niitep, Mattila et al. 2011), mating and reproductive success (Le Lann, Wardziak et al. 2011), dominance when competing for resources (Hack 1997, Brown, Ross et al. 2003), and more aggressive behaviour (Gobin, Heinze et al. 2003). To meet the increased energy requirements, organisms with higher metabolic rates are generally more active foragers than their counterparts (Le Lann, Wardziak et al. 2011, Krams, Kivleniece et al. 2013), and may be better able to respond to rapid changes in their environment (Clarke and Fraser 2004).

The measurement of an individual's metabolic rate has been shown to be both repeatable (Marais and Chown 2003, Terblanche, Klok et al. 2004) and heritable

(Nespolo, Castaneda et al. 2007, Mattila and Hanski 2014) and can therefore be considered an adaptive trait subject to selection pressures (White, Schimpf et al. 2013). Artificial selection has been shown to influence metabolic rate in the fly *Drosophila melanogaster*, within 31 generations individuals selected for fast chill-coma recovery time also displayed an increased metabolic rate relative to flies selected for slow recovery (Williams, Szejner-Sigal et al. 2016). Metabolic adaptation has been implicated in both allowing species to persist in environments where their counterparts would be unable to function and in providing a competitive advantage (Chown and Gaston 1999, Addo-Bediako, Chown et al. 2002, Niitep, Mattila et al. 2011). Such metabolic adaptations often take the form of a change in the sensitivity of metabolic rate to temperature or temperature variation (Krasnov, Khokhlova et al. 2004). Thermal sensitivity can be used to describe this temperature-metabolic rate relationship: it is a measure of the change in the metabolic rate of an organism at different temperatures, with lower values indicating greater ability to maintain a steady metabolic rate during changes in temperature (DeVries 2013). It is clear that that metabolic rate has direct impacts on fitness, however the complex interplay between the evolutionary costs and benefits associated with maintaining an elevated or depressed metabolism mean that the nature of the relationship between an organisms metabolic rate and fitness appears to depend on the unique ecological context in which it is being studied (Burton, Killen et al. 2011).

Due to the ecological niche they occupy, dung beetles experience little competition from other taxa; however competition from other dung beetles is fierce with in-dung abundances reaching up to 16, 000 individuals in the span of a few hours (Anderson and Coe 1974). This intense competition has resulted in extensive speciation and niche partitioning between sympatric dung beetle species and the development of a diverse range of morphologies, behaviours, and life histories (Chesson 2000). The most striking example of this is the development of nesting strategies. Dung beetles can be grouped into three guilds depending on how they utilize dung for reproduction: Paracoprids dig tunnels below a source of dung from which they construct brood balls in which they lay eggs; Telecoprids remove a portion of the dung, form it into a ball, and roll it away from the source; Endocoprids reproduce inside the dung, these beetles do not produce brood balls with eggs being laid directly inside the dung source (Halffter and Edmonds 1982).

Temperature plays a primary role in determining dung beetle species diversity, habitat preference, and community structure (Verdú, Arellano et al. 2006). Variations in the microclimatic conditions of an ecosystem have direct impacts on dispersal and activity, with beetles tending to move from colder to warmer areas (Roslin, Avomaa et al. 2009) and remaining in dung pats for longer when temperatures are low (Vessby 2001). Species often utilize behavioural or physiological adaptations to widen their thermal window (Verdú and Lobo 2008, Smolka, Baird et al. 2012, Verdú, Alba-Tercedor et al. 2012, Andrew, Hill et al. 2013).

The thermal biology of dung beetles is an important determinant of their competitive ability as it dictates their access to dung and their ability to compete with sympatric species (Chown, Scholtz et al. 1995). Telecoprid assemblages utilize a wide range of thermal niches to avoid competition, when the thermal niches of species do overlap their trophic habits differ (Verdú, Arellano et al. 2007). It appears that some species may utilize metabolic compensation to reduce water loss in xeric environments (Sinclair, Vernon et al. 2003); however whether they invoke similar metabolic adaptations as a result of niche partitioning is unknown. Their reliance on dung, a highly contested and ephemeral resource, might necessitate such adaptations. The influence of habitat heterogeneity on the competitive hierarchy of a dung beetle assemblage in the Ivory Coast was assessed by correlating microhabitat temperature with the abundance of the various dung beetle guilds (Krell, Krell-Westerwalbesloh et al. 2003). It was posited that the structure of dung beetle communities is the result of trade-offs between competitive superiority and energetic constraints, with a competitive hierarchy based off the energetic costs of each guilds resource utilisation. Telecoprids are thought to have the highest energetic cost as they must roll dung away from the source, risking predation and exposure to the elements. Endocoprids were the least competitive and energetic group as they do not move dung away from the source but instead are better adapted to heterogeneous environments. Physiological evidence for such a hierarchy is likely to be reflected in the metabolic rate and thermal sensitivity of species rather than their $CT_{min/max}$. If the niche partitioning seen in dung beetles communities is a result of niche partitioning via temperature then species that are abundant when/where conditions are hot should have higher metabolic rates at these temperatures. Similarly those species that are most active when it is cold can be expected to have higher metabolic rates at low temperatures. A low thermal sensitivity indicates that metabolic rate is less responsive to changes in temperature, allowing the species to operate across the given temperature range with little change in performance. A high thermal sensitivity may indicate a sudden increase in metabolic rate upon reaching a critical temperature.

The aims of this experiment were to measure the traits relating to the thermal biology of a dung beetle community assemblage using dynamic temperature respirometry. We measured upper and lower thermal tolerance (CT_{max} and CT_{min}), metabolic rate ($\dot{V}CO_2$ ml h^{-1}) prior to their critical thermal limits, and the thermal sensitivity of the species. Given past research into how the thermal biology of dung beetles affects community structure and niche separation of different guilds we expect that the thermal traits of the species' studied will vary according to their ecological and thermal niches. Species with a larger thermal breadth and higher metabolic rate will be better adapted to disturbed, "higher pace", habitats that experience much greater acute variation in conditions. Telecoprids roll their brood balls across the soil surface where they are exposed to more intense and variable temperatures, we therefore expect *S. rubrus* to perform better at higher temperatures than either paracoprids or endocoprids. Following Krell, Krell-Westerwalbesloh et al. (2003) hypothesis that endocoprid make up for their lack of competitiveness by excelling in heterogeneous environments we expect *A. fimetarius* to have a relatively low thermal sensitivity, as this would indicate that it is able to forage in a thermally heterogeneous environment with minimal change in performance. We also expect that species that are more active during the colder parts of the year will have lower CT_{min} and have a higher metabolic rate in cooler environments relative to other species.

3.3 – Methods

3.3.1 – Study animals and site

This study was primarily focused on comparing the thermal biology of species from the same community. Accordingly, all members of *O. binodis*, *O. australis*, *O. granulatus*, *A. fimetarius*, and *E. fulvus* used in this study were collected from the site at UNE, Armidale (30.53°S, 151.62°E; 1079m ASL). *Sisyphus rubrus* and *E. intermedius* were included as a result of one-time opportunistic trapping at a cattle farm near Bingara, NSW (29.87°S, 150.57°E; 296m ASL) in January 2017 (Table 3.1). Identification of similar species was done using differentiating morphological features such as the shape of head ridges in the Onthophagines and leg spur shape among the *Sisyphus*. Due to logistical constraints and to avoid the confounding effect of inter-population variation respirometry was only performed on beetles from Bingara if that species was not caught at Armidale.

Armidale has a cool temperate climate with a mean annual temperature range of 7.8°C-20.4°C (Beaureu of Meteorology 2017). *Ad hoc* trapping took place from January-April 2017, ceasing when no dung beetles were found in the traps. All species sampled from pitfalls traps at UNE were included in this study with the exception of *Onitis pecuarius* as only two individual were caught, therefore they were excluded from all comparative analysis. Pitfall traps were baited with dung from cattle that had not been drenched within six months of collection, and left for 48 hours. Beetles were collected and kept in the laboratory until dynamic temperature respirometry was performed or four days had passed (Sheldon and Tewksbury 2014). Bingara has a warmer climate, with a mean annual temperature range of 10.2°C-26.2°C. Mean temperatures in January range from 18.1°C-33.6°C. Bingara experiences daily temperatures exceeding 30°C more than four times more frequently than Armidale (Bureau of Meteorology 2017).

Table 3.1. List of species used in this study. Mass is the mean live mass of the individuals used in this study. Site indicates whether collection of this species took place an Armidale or Bingara. Native indicates whether the species is native to Australia or was introduced. N is the number of individuals used.

Species	Mass (mg)	Site	Native	N
Onthophagus australis	99 5 ± 22 9	Armidale	Native	31
Onthophagus granulatus	30 6 ± 6 3	Armidale	Native	14
Euoniticellus intermedius	48 1 ± 13 2	Bingara	Introduced	17
Euoniticellus fulvus	43 6 ± 11 9	Armidale	Introduced	31
Aphodius fimetarius	27 6 ± 5 8	Armidale	Introduced	27
Sisyphus rubrus	72 9 ± 17 8	Bingara	Introduced	12
Onthophagus binodis	102 3 ± 29 9	Armidale	Introduced	20

3.3.2 – Respirometry

The terms CT_{min} and CT_{max} have been applied inconsistently in the literature. As the aim of this experiment was to measure the thermal biology of species in the context of their interactions and community dynamics we interpreted these traits with specific regard for their ecological relevance rather than the physiological mechanisms underpinning them. We define CT_{max} to be the maximum temperature an animal can tolerate before irreparable physiological damage (Terblanche, Klok et al. 2004). CT_{min} was defined as the temperature at which the animal enters into a state of neuromuscular paralysis (chill coma). Both of these endpoints can be determined by identifying the cessation of spiracular activity (MacMillan, Williams et al. 2012). When spiracular activity halts at low temperatures, CO_2 drops to near zero and levels out. However at high temperatures a large burst of CO_2 is emitted post CT_{max}, known as the post-mortal-peak. CO_2 ml h^{-1} at the apex of the post-mortal-peak can be over twice that of the individuals normal metabolic rate, likely deriving from aerobic metabolism in mitochondria (Heinrich, Gray et al. 2017).

Thermolimit respirometry was conducted using a push mode flow-through system (Andrew, Ghaedi et al. 2016)(Figure 3.1). An air pump (HB-40, Hiblow USA, Saline, MI, USA) was used to flow outside air through a scrubber column containing soda lime to remove CO_2 then a column containing silica gel and Drierite to remove H_2O. The airflow was then split and passed through two mass flow control valves (Side-Trak 840L, Sierra Instruments Inc., Monterey, CA, USA) connected to a mass flow controller (MFC-2, Sable Systems, Las Vegas, NV, USA) which regulated airflow to 200 ml min^{-1}, through two 15 ml glass cuvettes, and into a LiCor 7000 CO_2/H_2O infra-red gas analyser set to reference estimation mode. Reference estimation mode (REM) allows gas concentrations in both of the LiCor sampling cells to be measured independently, with the caveat that more frequent calibrations are required. Beetle movement was measured in each channel using separate activity detectors (AD2 Activity Detector, Sable Systems International, Las Vegas, NV, USA). Activity and gas concentration data was recorded every one second. To ensure accuracy of measurements while in REM 10 minute baselines were recorded with empty cuvettes before and after each assay. Each assay consisted of two beetles being run simultaneously. Feeding status may influence metabolic rate in insects (Terblanche and Chown 2007), therefore all animals were fasted for a minimum of 12 hours prior to the assay. Beetle weight was measured before and after performing dynamic temperature respirometry (hereafter referred to as DTR).

To perform upwards dynamic temperature respirometry (uDTR) cuvettes containing beetles were submerged in a Grant GP200/R4 programmable waterbath (resolution ± 0.1°C). The starting temperature was 25°C; this was maintained for five minutes to allow the internal temperature of the cuvettes to reach equilibrium with the surrounding water. After this equilibration phase temperature was increased by 0.2°C min^{-1} (Lighton and Turner 2004, Terblanche, Sinclair et al. 2005), stopping at 60°C. Temperatures inside the cuvettes were recorded every one second using a PicoTech TC-08 datalogger (resolution ± 0.025°C, accuracy ± 0.5°C; Pico Technology Ltd, Cambridge, UK) and a type T copper-constantan thermocouple. Downwards dynamic temperature respirometry (dDTR) was conducted as above with temperature decreasing 0.2°C min^{-1}, starting at 25°C and ending at -15°C. Temperature data from the datalogger and gas concentration data from the LiCor were combined in excel by matching the timestamps recorded by each program.

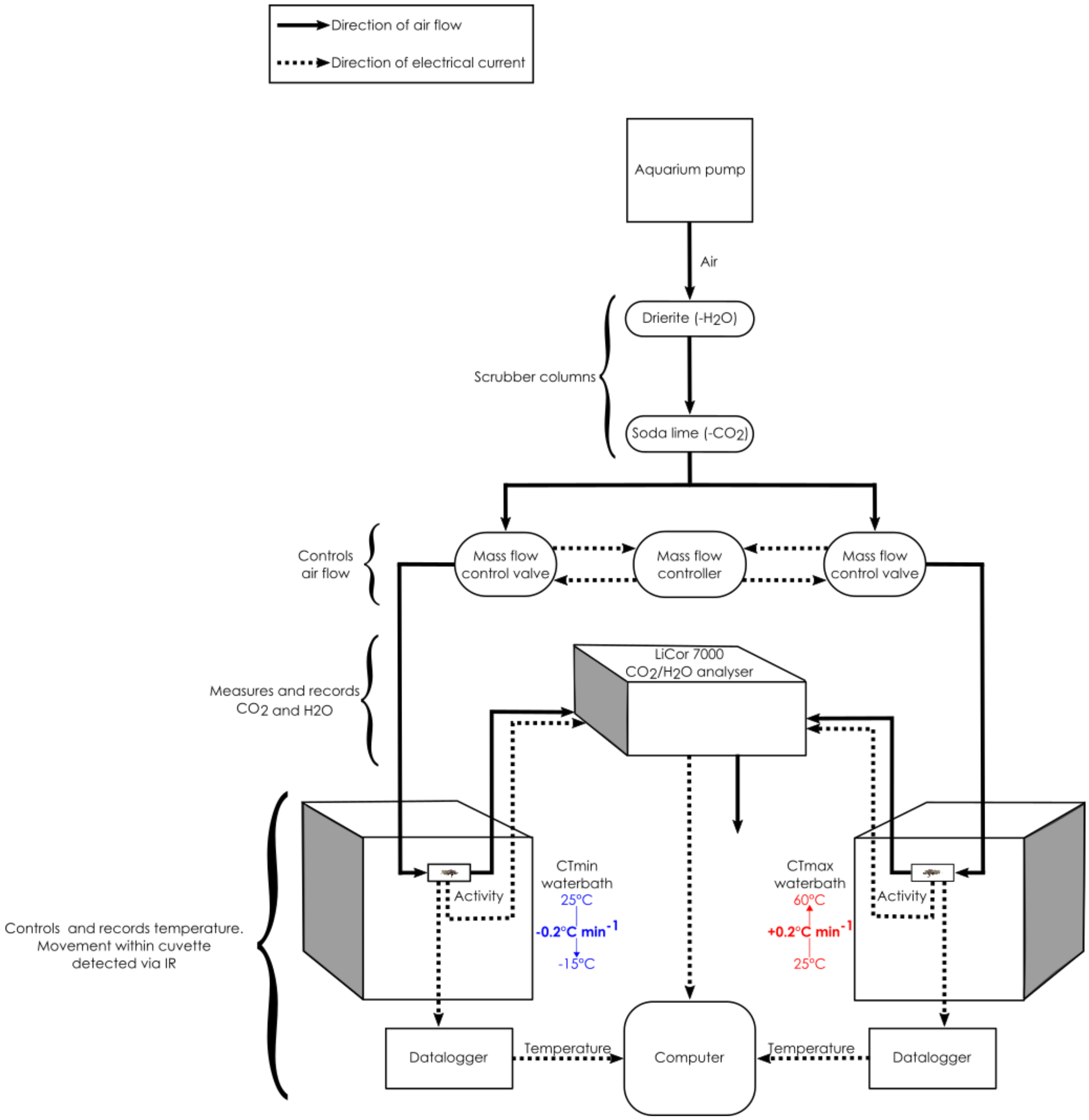


Figure 3.1. Schematic diagram of push mode flow-through respirometry system used in chapters three and four. An aquarium pump is used to push air through the system. H_2O and CO_2 are removed from the incoming airstream with Drierite and soda lime respectively. The airstream is split and mass flow control valves used to control flow rate. Air flows from the valves through the cuvette containing the animal, which is submerged in a temperature controlled waterbath, and into the LiCor where airstream composition is analysed.

3.3.3 – Data extraction

ExpeData data acquisition and analysis software (Emlen and Philips 2006) was used for all data extraction. Prior to data extraction CO_2 release rates were corrected for baseline drift and converted to CO_2 ml h^{-1}. $CT_{max/min}$ and $\dot{V}CO_2$ ml h^{-1} were extracted for all individuals. $CT_{max/min}$ was determined using the inflection point of the residuals of the absolute difference sum of CO_2 ml h^{-1} (Lighton and Turner 2004). $\dot{V}CO_2$ ml h^{-1} released prior to $CT_{min/max}$ was calculated by finding the integral of CO_2 ml h^{-1} vs. hours from immediately after the initial baseline to the individuals $CT_{min/max}$. All data was extracted using ExpeData macros and then verified manually.

Thermal sensitivity was calculated using the equation $TS=10^{(10*slope)}$ where TS is an individual's thermal sensitivity and slope is the slope of the least squares regression of its log10 transformed CO_2 ml h^{-1} vs. temperature inside its cuvette, giving the real time relationship between the two. From both dDTR and uDTR assays we were able to determine the thermal sensitivity of a species across its entire thermal window. Values reported for species are the mean ± SE for individuals of that species. This dynamic method of calculating thermal sensitivity yields similar estimates to traditional static methods such as Q_{10} (Lake, MacMillan et al. 2013). Individuals that displayed excessive activity were removed prior to analysis of $\dot{V}CO_2$ ml h^{-1} and thermal sensitivity.

3.3.4 – Analysis

$CT_{min/max}$, $\dot{V}CO_2$ ml^{-1} h^{-1}, and thermal sensitivity were analysed using generalized linear models in which they were the dependent variable, species was an independent variable, and log transformed weight was a covariate. Weight was not a significant predictor of CT_{min} or thermal sensitivity and was therefore dropped from the models. $\dot{V}CO_2$ ml^{-1} h^{-1} and thermal sensitivity for both dDTR and uDTR assays were modelled using a lognormal distribution. Models were initially run with a species*weight interaction to determine if the relationship of the dependent variable differed between species. No significant interactions were found so the term was dropped from all models. All post hoc analysis was performed using the least squares means and the Tukey-Kramer adjustment for unequal sample sizes.

3.4 – Results

3.4.1 – CT$_{min/max}$

There were significant differences of both CT$_{min}$ ($F_{7,61} = 99.83$, $p < 0.001$) and CT$_{max}$ ($F_{7,52} = 5167.98$, $p < 0.001$) among species while adjusting for weight ($F_{1,35} = 7.07$, $p = 0.012$). CT$_{max}$ increased with weight for all species ($F_{1,35} = 7.07$, $p = 0.012$). The endocoprid *Aphodius fimetarius* was the most tolerant to cold temperatures with a CT$_{min}$ of -2.51±0.50°C, statistically different (Appendix 3.1; Appendix 3.2) from all other species. The paracoprid species' *Euoniticellus fulvus* (CT$_{min}$=9.07±0.56°C) and *E. intermedius* (CT$_{min}$=8.3±0.76°C) were the least tolerant to cold temperatures and the second and third most heat tolerant species respectively, forming statistically distinct groups in both cases (Table 3.3). The telecoprid *Sisyphus rubrus* had the second lowest CT$_{min}$ (3.39±0.84°C) among species and a significantly higher CT$_{max}$ (52.9±0.21°C) than all other species (Appendix 3.3; Appendix 3.4). The three species belonging to the genus *Onthophagus* had similar CT$_{min}$ to each other; however, *O. australis* had a significantly lower CT$_{max}$ than the other *Onthophagus* species, and the lowest CT$_{max}$ overall (43.02±0.37°C). Intraspecies variation was more pronounced for CT$_{min}$ compared to CT$_{max}$ (Table 3.2).

Table 3.2. Summary of the key traits measured for eight species across both sites sampled (UNE), with two (*Euoniticellus intermedius* and *Sisyphus rubrus*) being exclusive to Bingara.

Species	CT$_{min}$ (°C) [1]	CT$_{max}$ (°C) [2]	VCO$_2$ ml h^{-1} dDTR [3]	VCO$_2$ ml h^{-1} uDTR [4]	TS dDTR[5]	TS uDTR[6]
Onthophagus australis	5 1±1 7	43 8±1 2	5 3±1 8	22 5±6 7	3 0±0 9	2 7±0 5
Onthophagus granulatus	4 5±2 6	44 9±0 8	3 1±1 6	11 3±5 2	2 6±0 7	1 8±0 4
Euoniticellus intermedius	8 3±1 8	49 1±1 9	2 3±1 0	19 6±9 5	4 0±1 2	2 6±0 5
Euoniticellus fulvus	9 1±2 4	49 9±1 4	2 0±0 8	20 1±6 2	3 9±0 9	2 5±0 5
Aphodius fimetarius	-2 5±2 0	43 6±1 3	2 8±1 2	10 3±4 1	2 4±0 6	1 8±0 3
Sisyphus rubrus	3 4±1 1	53 1±0 6	3 1±1 3	28 1±7 5	4 2±0 6	2 2±0 5
Onthophagus binodis	6 6±0 9	46 6±1 0	7 6±3 5	26 3±7 2	2 9±1 2	2 2±0 2

[1] CT$_{min}$ the temperature at which the beetle enters a chill coma,

[2] CT$_{max}$ the temperature at which spiracular activity ceases,

[3] VCO$_2$ ml h^{-1} is the volume of CO$_2$ ml^{-1} released prior to reaching CT$_{min}$ or CT$_{max}$,

[4] TS is the sensitivity of the rate of CO$_2$ ml^{-h} to changes in temperature from 25 C to CT$_{min}$ or CT$_{max}$.

[5] dDTR refers to a decreasing temperature change.

[6] uDTR refers to an increasing temperature change.

All values are the untransformed mean ± SD.

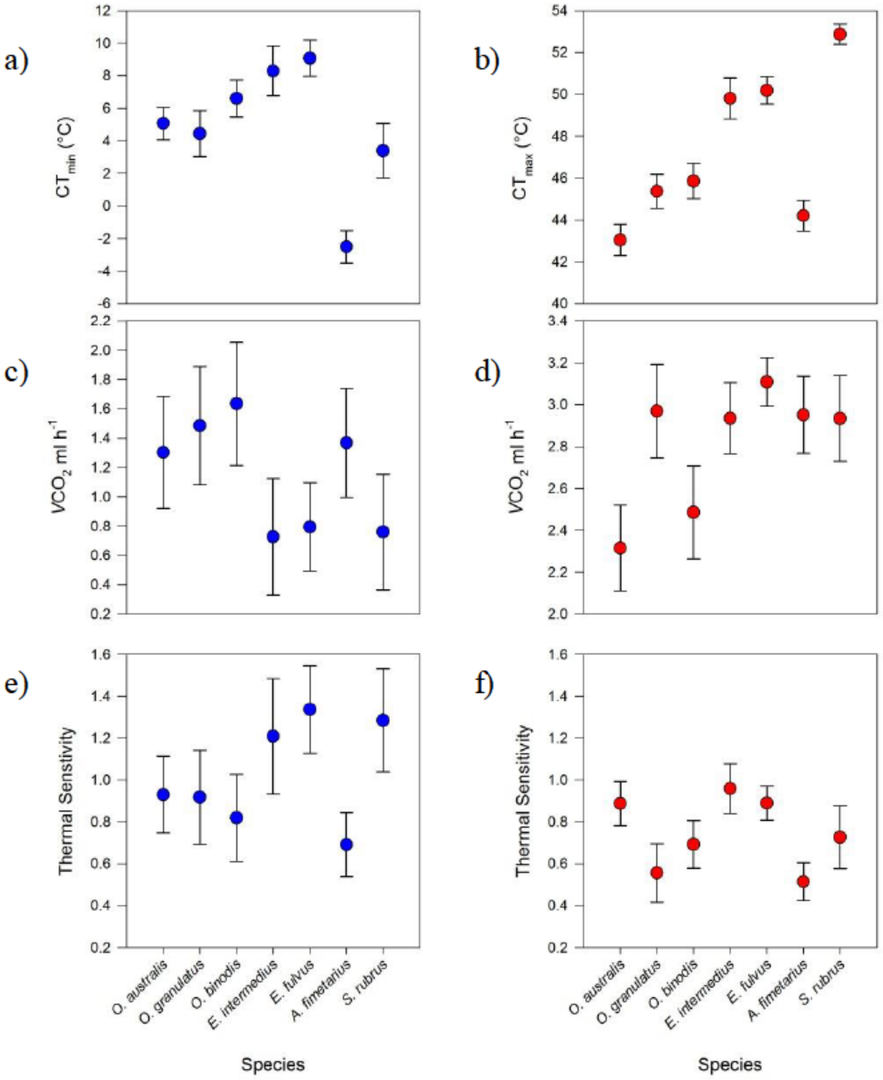


Figure 3.2. Thermal traits of seven species of dung beetle used in this study. Beetles were caught using pitfall traps baited with cattle dung. Values presented are least squares means with 95% confidence intervals a) CT_{min} b) CT_{max}, adjusted for live weight c) lognormal $\dot{V}CO_2$ ml^{-1} h^{-1}, adjusted for live weight, emitted between 25°C and the individuals CT_{min} while undergoing dDTR d) lognormal $\dot{V}CO_2$ ml^{-1} h^{-1}, adjusted for live weight, emitted between 25°C and the individuals CT_{max} while undergoing uDTR e) lognormal thermal sensitivity of metabolic rate between 25°C and CT_{min} while undergoing dDTR f) lognormal thermal sensitivity of metabolic rate between 25°C and CT_{max} while undergoing uDTR.

3.4.2 – $\dot{V}CO_2$ ml h^{-1}

There were significant differences in $\dot{V}CO_2$ ml^{-1} h^{-1} among species for both dDTR ($F_{7, 42} = 7.23$, $p < 0.001$) and uDTR ($F_{7, 87} = 7.40$, $p < 0.001$) assays while controlling for beetle weight (Figure 3.2). Log transformed weight was significantly correlated with higher $\dot{V}CO_2$ ml^{-1} h^{-1} for dDTR ($F_{1, 42} = 10.05$, $p = 0.003$) and uDTR ($F_{1, 87} = 88.28$, $p < 0.001$). The VCO_2 ml^{-1} h^{-1} of species were similar at low temperatures with differences only being detected between *O. binodis*, which had the highest $\dot{V}CO_2$ ml^{-1} h^{-1} during dDTR, and the two species with the lowest, *E. fulvus* ($t = 3.15$, $p = 0.044$) and *S. rubrus* ($t = 3.70$, $p = 0.01$). Adjusting for multiple comparisons there was a large but non-significant difference between *O. binodis* and the species with the third lowest $\dot{V}CO_2$ ml^{-1} h^{-1} *E. intermedius* ($t = 3.05$, $p = 0.\ 056$). There was greater interspecies variation in $\dot{V}CO_2$ ml^{-1} h^{-1} during uDTR. *Onthophagus australis* had a $\dot{V}CO_2$ ml^{-1} h^{-1} significantly lower than all species except *O. binodis* ($t = -1.56$, $p = 0.709$). *Onthophagus binodis* also had a similar $\dot{V}CO_2$ ml^{-1} h^{-1} to *A. fimetarius* ($t = -2.43$, $p = 0.199$) and *O. granulatus* ($t = -2.59$, $p = 0.141$). The five remaining species had similar $\dot{V}CO_2$ ml^{-1} h^{-1} with *E. fulvus* having the highest. As with thermal tolerance, there was more intra-species variation in dDTR compared to uDTR: $\dot{V}CO_2$ ml^{-1} h^{-1} during uDTR was nearly three times higher in *E. fulvus, E. intermedius,* and *S. rubrus* when compared to dDTR (Table 3.3).

3.4.3 – Thermal sensitivity

There were significant differences between the thermal sensitivity of species for both dDTR ($F_{7, 44} = 96.29$, $p < 0.001$) and uDTR ($F_{7, 88} = 207.10$, $p < 0.001$). In general CO_2 ml^{-1} was more sensitive when animals were subjected to dDTR rather than uDTR, indicating that changes in temperature below 25°C have a greater impact on CO_2 ml^{-1} than changes above. There were similar overall levels of interspecies variation for both dDTR and uDTR but the sensitivity of individual species differed between the two. In both cases *A. fimetarius* was the least sensitive to changes in temperature with a thermal sensitivity significantly different to that of the most sensitive species (Table 3.3). Similarly *E. fulvus* and *E. intermedius* were among the most sensitive species for both dDTR and uDTR. Compared to other species *S. rubrus* was more sensitive at colder temperatures than warmer ones where it was not-significantly different to other species. *Onthophagus granulatus* was also more sensitive at colder temperatures compared to warm. *Onthophagus australis* was among the least sensitive species using dDTR but among the highest using uDTR.

Table 3.3. Post hoc comparisons of the thermal physiology of seven species of dung beetle using downward dynamic thermal respirometry from 25°C – 15°C (dDTR) and upward dynamic thermal respirometry from 25°C – 60°C (uDTR). Values have been adjusted for multiplicity and covariates. $\dot{V}CO_2$ ml h^{-1} and thermal sensitivity were log transformed prior to analysis. Letters in columns represent significant differences between species. Species with the same letter are not significantly different.

	dDTR				uDTR			
Species	CT_{min} (°C)	$\dot{V}CO_2$ ml h^{-1}	TS	N	CT_{max} (°C)	VCO_2 ml h^{-1}	TS	N
Onthophagus australis	5 07±0 49 ^{cd}	1 30±0 19 ^{abc}	0 92±0 09 ^{abc}	13	43 04±0 37 ^{d}	2 31±0 10 ^{c}	0 89±0 05 ^{ab}	18
Onthophagus granulatus	4 45±0 71 ^{cd}	1 48±0 20 ^{abc}	0 92±0 92 ^{abc}	6	45 37±0 40 ^{c}	2 97±0 11 ^{abd}	0 56±0 07 ^{c}	8
Onthophagus binodis	6 60±0 56 ^{bc}	1 63±0 21 ^{ab}	0 81±0 10 ^{bc}	10	45 90±0 42 ^{c}	2 49±0 11 ^{bc}	0 69±0 06 ^{bc}	10
Euoniticellus fulvus	9 07±0 56 ^{a}	0 79±0 15 ^{ac}	1 34±0 10 ^{a}	7	50 18±0 32 ^{b}	3 11±0 06 ^{ad}	0 89±0 04 ^{ab}	24
Euoniticellus intermedius	8 28±0 76 ^{ab}	0 73±0 19 ^{abc}	1 21±0 14 ^{ab}	6	49 80±0 48 ^{b}	2 94±0 09 ^{ad}	0 96±0 06 ^{a}	11
Sisyphus rubrus	3 39±0 84 ^{d}	0 76±0 19 ^{ac}	1 28±0 12 ^{ab}	4	52 86±0 21 ^{a}	2 93±0 10 ^{ad}	0 73±0 07 ^{abc}	8
Aphodius fimetarius	-2 51±0 50 ^{e}	1 37±0 18 ^{abc}	0 69±0 08 ^{c}	12	44 20±0 36 ^{cd}	2 95±0 09 ^{abd}	0 51±0 05 ^{c}	15

3.5 – Discussion

The present day dung beetle fauna of Australia is composed of both a native assemblage of species and a number of exotic species introduced as part of the CSIRO's Australian Dung Beetle Project. Little is known concerning the biology and ecology of the 474 species of dung beetle endemic to Australia (Monteith 2015). Most native species have a proclivity for covered habitats and are predominately found in woodland, forest, or heathland habitats (Matthews 1971, Tyndale-Biscoe, Wallace et al. 1981). Some species', such as *O. granulatus* and *O. australis,* are associated with both pastures and woodlands (Hughes 1975). The low abundance of native species in pastures as well as their preference for marsupial dung, a much different resource to the large moist pats produced by cattle (Waterhouse 1974), resulted in unprocessed dung left sitting on pastures. This dung not only fouled pastures, reducing grazing capacity, but provided a perfect breeding ground for pests particularly populations of dung-breeding fly exploded. To control fly populations and improve soil quality the CSIRO sought to introduce exotic species that were better suited to processing livestock dung (Bornemissza 1976). From 1969-1984 populations of 43 species of exotic dung beetle were released as part of the initial program, 23 of which remain established (Tyndale-Biscoe 1996). The program exists to this day, albeit at a much reduced scale, with the preliminary release of two species successfully completed in 2017 (Doube 2018). The value of the ecosystem services provided by dung beetles in Australia is estimated to exceed AUD$600 million annually (Doube 2018). Recent research has shown that dung beetle activity can alleviate the effects of drought on plants, increasing growth by 280% under drought conditions (Johnson, Lopaticki et al. 2016). Australia is expected to see decreased precipitation and increased incidence of drought as a result of climate change (Fischer, Thomas et al. 2014), therefore a true estimation of their current and future value may in fact be much higher.

The current dung beetle assemblage is therefore decidedly unnatural in that native species have co-evolved and had the opportunity to carve an evolutionary niche, whereas recently introduced species were specifically chosen for their ability to rapidly bury dung and their tolerance to a broad range of climatic conditions. The fact that the majority of species recovered from pitfall traps were non-native paracoprids is unsurprising, most of the species introduced as part of the Australian Dung Beetle Project were paracoprids (Edwards 2009) with few native species becoming abundant in pastures; trapping in South Australian pastures only finding 1-2 native species per site (Ridsdill-Smith and Hall 1984, Tyndale-Biscoe 1994). *Euoniticellus fulvus*, *E. intermedius*, and *O. binodis* are among the most widely distributed and abundant pasture species in Australia and tend to dominate pasture ecosystems (Montes de Oca and Halffter 1998, Gullan and Cranston 2009, Simmons and Ridsdill-Smith 2011); the physiological data gathered in this study therefore has a more general application.

In a study of 26 South African dung beetle species across an elevation gradient reported a similar range of CT_{max} to those observed in this study, ranging from 40°C – 52°C (Gaston and Chown 1999). The African beetles were less cold tolerant than all but the least tolerant Australian species with CT_{min} ranging between 6°C and 22°C. Compared to the tropical dung beetles species *Dichotomius annae*, *Dichotomius satanas, Dichotomius colonicus, Canthon imitator,* and *Phanaeus quadridens* the species from the Australian temperate ecosystem were more tolerant overall (Sheldon and Tewksbury 2014)(Table 3.3). This is likely due to the thermal stability of their habitat, with temperate species experiencing a more variable temperature regime (Sunday, Bates et al. 2011). *Jekelius hernandezi* and *J. punctatolineatus* from the Iberian peninsula, had similar CT_{max} but far lower CT_{min} than even the most cold tolerant species in this study (Gallego, Verdú et al. 2016), *A. fimetarius* . It's generally acknowledged that there is greater variation in the CT_{min} of species than the CT_{max} (Bowler and Terblanche 2008). Differences in methodology such as the utilization of static or dynamic temperatures, rate of temperature change, and initial temperature, affect estimates of thermal tolerance therefore inter-study comparisons must be interpreted with caution (Terblanche, Deere et al. 2007). As beetles were collected from the field we were unable to control for the age of individuals. Age has been found to influence metabolic rate in the fly *Glossina pallidipes* (Terblanche, Klok et al. 2004), while a similar effect has not been observed in dung beetles this may influence the interpretation of results.

The CT_{max} of the beetles exceed the highest temperatures they would experience at either site. From this it appears that dung beetle communities are more heavily influenced by the minimum temperatures of their environment. In French dung beetle communities, mean minimum temperature was found to be the most influential factor affecting dung beetle species richness (Lobo, Lumaret et al. 2002). Furthermore the CT_{min} of the species tested are reflected in the global distribution of the genera to which they belong. In northern Europe dung beetle communities are dominated by *Aphodius*, with the ratio of *Aphodius* to Scarabaeinae species declining along a latitudinal gradient from north Europe, with a ratio of 95/0, to Morocco with a ratio of 38/57 (Mattila and Hanski 2014). In Spain the abundance of 11 species of dung beetle was found to be increasingly associated with more open habitats as altitude increased. Furthermore all *Aphodius* favoured cooler habitats compared to three species of *Onthophagus* (Menendez and Gutierrez 2004). *Euoniticellus* only begins to appear at lower altitudes, corresponding to higher temperatures with the limits of their current range expected to increase in response to climate change (Lumaret, Kadiri et al. 1992, Dortel, Thuiller et al. 2013).

Table 3.3. CT_{min} and CT_{max} of all species represented in the literature for which species specific data could be found. Species specific CT_{max} from Gaston and Chown (1999) could not be determined as species were pooled according to elevation

Species	**CT_{min} (°C)**	**CT_{max} (°C)**	**Environment**	**Source**
Dichotomius annae	12.1	39.3	tropical	Sheldon and Tewksbury (2014)
Dichotomius colonicus	12.9	41.3	tropical	-
Dichotomius satanas	13.3	37.5	tropical	-
Canthon imitator	15.0	42.8	tropical	-
Phanaeus quadridens	13.2	42.1	tropical	-
Jekelius hernandezi	-4.60	44.69	warm temperate	Gallego, Verdú et al. (2016)
Jekelius punctatolineatus	-8.72	47.19	warm temperate	-
Aphodius lapponum	-0.1	41.2	Grassland/heathland	Birkett, Blackburn et al. (2018)
Aphodius depressus	1.2	42.3	Grassland/heathland	-
Aphodius ater	-0.4	44.4	Grassland/heathland	-
Eucranium belenae	-	28.9*	Desert	Giménez Gómez, Lomáscolo et al. (2017)
Anomiopsoides cavifrons	-	44.2*	Desert	-
Anomiopsoides fedemariai	-	57.8*	Desert	-

*These values are not CT_{max} but thermal optimums calculated by observing beetle activity in the field. Ectotherms thermal optimum lies close to its CT_{max} so these should be indicative of their general tolerance to heat

Aphodiinae dung beetles favour cooler temperatures. A study conducted in northern England found the thermal tolerances of three species of *Aphodius* to be similar to that of *A. fimetarius* (Table 3.3). They are most abundant relative to other guilds in closed environments, at higher altitudes, and when present in open habitats are more active at night (Krell, Krell-Westerwalbesloh et al. 2003, Jay-Robert, Lumaret et al. 2008). The low slope of the metabolic rate vs. temperature relationship of *Aphodius fimetarius* indicates that it is able to maintain a higher metabolic rate at lower temperatures relative to other species (Chown, Haupt et al. 2016), supporting Krell, Krell-Westerwalbesloh et al. (2003) claim that Aphodiinae dung beetles are better able to handle changing microclimatic conditions. Our data matches anecdotal observations from the field, with *A. fimetarius* being retrieved from traps from April onwards. As temperatures cooled both its total and relative abundance increased. This activity further correlates with temperature data from a nearby weather station with April having a mean temperature of 17.9°C and temperatures not exceeding 10°C on some days (Bureau of Meteorology 2017). In dDTR assays *A. fimetarius* had among the highest metabolic rates of the species in this study, furthermore its low CT_{min} and the low slope of its MR-T relationship indicates that it is a more active forager during the cooler parts of the day when temperatures are below the CT_{min} of competitor species which have similar metabolic rates at low temperatures, such as the *Onthophagus* species, allowing them to seek dung before competitors are active. A stable metabolic rate would also be of benefit in disturbed, thermally heterogeneous, environments, allowing the beetle to forage over a range of microclimates without sudden changes of metabolic rate. It may be the case that *A. fimetarius* are present in abundances greater than trapping would suggest, however they maintain populations in the fragmented woodlands where temperatures are cooler, only foraging in pastures when temperatures are low enough. While conducting dDTR assays we observed some individuals of *A. fimetarius* to recover locomotor activity within minutes of being subject to temperatures of -15°C indicating the capacity to endure short, extreme, bouts of cold weather. While *A. fimetarius* did have a relatively high metabolic rate in comparison to other species at higher temperatures, its reproductive strategy makes it less competitive than the larger paracoprids, limiting its ability to effectively forage when these species are active. Endocoprids lay their eggs in the dung or at the soil-dung interface (Finn and Gittings 2003). When a large number of telecoprids or paracoprids become active they can find the dung and break it down before *Aphodius* have a chance to lay their eggs despite any metabolic advantage they may have. The capacity to sustain biological function over a wide range of temperatures involves bio-energetic trade-offs as energy is diverted from growth and reproduction (Clarke 2003). The capacity of *A. fimetarius* to maintain an elevated metabolic rate may therefore be balanced by its reproductive strategy, as endocoprids do not construct elaborate nests.

Paracoprids spend significant amounts of time in the soil below the dung where they are buffered from the full brunt of the greater climatic conditions (Shepherd, Prange et al. 2008). The thermal physiology of the two *Euoniticellus* species in this study clearly indicates a preference for warmer temperatures, being among the most tolerant to heat and least cold tolerant of all species studied. During autumn and winter, temperatures in Armidale frequently drop below the CT_{min} of *E. fulvus*, restricting activity and allowing less dominant species to contest dung. The CT_{max} of both *E. fulvus* and *E. intermedius* far exceeded the hottest air temperature recorded at Armidale and Bingara (Bureau of Meteorology 2017). Despite these species being sourced from sites with different climates there was no significant difference between any of their thermal traits, indicating a potential phylogenetic influence on their thermal physiology. A phylogenetic influence on the thermal traits of some dung beetle groups is further supported by the aforementioned similarity of *A. fimetarius* to three *Aphodius* species from England (Birkett, Blackburn et al. 2018). It also may be the case that thermal physiology such as that found in *Euoniticellus* is representative of a widespread generalist. *E. intermedius* is capable of long distance dispersal up to 480km a year, being able to tolerate high temperatures allows the day-flying beetle to forage and disperse across a range of habitats with varying thermal conditions (Montes de Oca and Halffter 1998). The *Onthophagus* species appear to inhabit an intermediate thermal niche between the cold tolerant *A. fimetarius* and the heat tolerant *Euoniticellus*. These beetles had a high metabolic rate that is relatively insensitive to changes in temperature below 25°C, at high temperatures their metabolic rate is the lowest of all species in the study. These beetles may therefore be most competitive at intermediate temperatures, at the edge of *E. fulvus* and *A. fimetarius* thermal window. The thermal traits among the Onthophagine species were much more variable than that of *Euoniticellus*. The genus *Onthophagus* is the most speciose insect genus on the planet with an estimated 2500 species (Tarasov and Solodovnikov 2011). They display high degrees of plasticity of morphological, behavioural, and developmental traits (Valena and Moczek 2012). This may drive a greater capacity for the divergence of species within the genus, creating a greater degree of thermal specialization; the plasticity of thermal traits in *Onthophagus* is addressed in chapter four.

The capacity of *E. intermedius* and *E. fulvus* to operate at higher temperatures than Onthophagine species is indicative of thermal niche partitioning similar to that observed in an African telecoprid dung beetle assemblage (Krell, Krell-Westerwalbesloh et al. 2003). High temperatures, rather than limiting their ability to forage, allow them to forage at times when other species are thermally stressed, thus minimizing competition. Evidence for thermal niche partitioning among species of *Onthophagus* was more limited with no difference in their response to temperatures below 25°C. However at temperatures above 25°C while there was no difference between *O. granulatus* and *O. binodis*, both species had significantly higher CT_{max}, metabolic rate, and a lower thermal sensitivity than *O. australis*. Evidence from the literature indicates that *O. binodis* appears to prefer slightly higher temperatures; *O. australis* does not produce brood balls at temperatures of 15°C or 30°C; *O. australis* maintained at 30°C died within five weeks, whereas *O. binodis* produces broods when experiencing a thermal regime of 29.8°C-35°C and survived for over 50 days. This is reflected in our results as *O. binodis* has a higher CT_{max} and slightly higher metabolic rate at temperatures above 25°C. The CT_{min} of *O. australis* is extremely similar to the estimated threshold temperature for development of 5.2°C (Tyndale-Biscoe and Walker 1992). *Onthophagus granulatus* is intermediary between these, with a CT_{max} similar to *O. binodis* but a greater metabolic rate (Table 2), and a developmental threshold temperature of 11.3°C. Brood production in *O. granulatus* occurs between 20°C and 30°C with production peaking at 25°C, albeit brood survival at 30°C is severely diminished (Tyndale-Biscoe, Wallace et al. 1981). The thermal biology of *O. granulatus* is similar to that of *A. fimetarius*, with a high metabolic rate that is insensitive to changes in temperatures, it may therefore fill a similar niche to this thermal generalist.

The CT_{min} of species may be correlated to their overwintering strategy with species that typically overwinter as adults being more cold tolerant. *Onthophagus australis*, *O. granulatus*, and *O. binodis* typically overwinter as inactive adults, with larvae experiencing high mortality during the cooler months (Tyndale-Biscoe, Wallace et al. 1981, Ridsdill-Smith 1986, Tyndale-Biscoe and Walker 1992). *Euoniticellus intermedius* and *E. fulvus*, the least cold tolerant species, overwinter as non-breeding adults in warmer areas while overwintering as larvae in cooler climates (Tyndale-Biscoe 1990, Hanski and Cambefort 1991). The introduced telecoprid *S. rubrus* overwinters as a breeding adult (Hanski and Cambefort 1991).

Dung beetle species selected for introduction were selected to minimize inter-species competition among themselves, however competitive interactions between native and introduced species were framed in the context of their respective proclivity for marsupial dung and it was thought unlikely that they would threaten native species with extinction as “They have evolved in the ecosystem and would probably have some competitive advantage over imported species that would enable them to survive” (Bornemissza 1976). While far from extinction *O. australis* and *O. granulatus* have been impacted by the introduction of foreign species (Tyndale-Biscoe and Vogt 1996). Given that species were chosen for introduction partly based on a wide distribution, indicative of broad climatic tolerances (Calosi, Bilton et al. 2008), it may even be expected that these species would come into direct competition with natives. Competitive exclusion such as this has been observed as a result of introduced dung beetles in Western Australia where the introduction of *O. binodis* reduced the abundance of the native *O. ferox* 97% within 10 years, with *O. taurus* subsequently overtaking *O. binodis* as the most abundant species (Simmons and Ridsdill-Smith 2011).

The current Armidale dung beetle community has been structured by both historical climate patterns and inter-species competition, however there has been insufficient time for them to adapt via evolution. Accordingly there is no reason to expect that *O. binodis* occupies a different niche to *O. australis* or *O. granulatus per se*. While *O. granulatus* favours slightly warmer environments compared to *O. australis* it appears that the thermal window of both these species overlaps with the more competitive *O. binodis*. The distribution of these three species in the states of New South Wales and Queensland are extremely similar suggesting that they have similar climatic tolerances (Tyndale-Biscoe 1990, Gullan and Cranston 2009). Prior to the introduction of *O. binodis* both of these native species were associated with very open woodlands, heathlands, and pastures (Matthews 1971). While *O. australis* can still be found in pastures it is less abundant than *O. binodis* and is more common in woodlands (Tyndale-Biscoe and Walker 1992). Similarly *O. granulatus* was found to be the most abundant species at many sites throughout the Hunter region of NSW in 1997-1998 (Bishop, Griffiths et al. 2000). Further surveys were carried out in the region in 2005 when Gollan, Reid et al. (2011) studied the abundance of native vs. introduced species across a Chrono-sequence of riparian vegetation. It was found that *O. binodis* was more abundant at unplanted sites compared to *O. australis* and *O. granulatus*; however at sites with revegetation native species were more abundant.

The suitability of dung beetles species for introduction to Australia was assessed by matching the climatic conditions across their native range to that of Australia's climatic regions. Climatic envelope models based on Australian climate data were highly accurate in predicting current distributions of *O. binodis*, however models run using data from their native range resulted in a poor fit, indicating that factors other than climate limit species distribution in their native habitat (Duncan, Cassey et al. 2009). The same phenomenon was observed following the introduction of the Cane

Toad to Australia wherein native toad populations were limited by competition and unable to realize their fundamental niche until introduced to an area lacking these limitations (Tingley, Vallinoto et al. 2014).

Little is known concerning Australia's native species and the ecosystem functions they provide. Much of Australia's native dung beetle fauna can be found in its rainforests where few, if any, introduced dung beetles are established. Introduced species do not share their proclivity for marsupial dung and there is evidence that Australia's native dung beetles have strong preferences for the dung of certain species (Wright, Williams et al. 2004, Vernes, Pope et al. 2005). Whether the loss of native species from a site would result in a significant loss of function requires further research (Coggan 2012). Should the microclimatic conditions of one habitat change and become unsuitable then species will be forced to compete in the same habitat. Dung beetle communities in Australia's north-eastern rainforests are strongly influenced by habitat structure with abundance, species richness, and biomass being lower in disturbed habitats relative to adjacent rainforest (Derhé, Murphy et al. 2016, Kenyon, Mayfield et al. 2016). A similar Australian study conducted by Gollan, Reid et al. (2011) examined community structure across a revegetation chronosequence and found no effect of habitat structure on overall abundance or species richness however the ratio of native-to-introduced species increased with chronosequence age. Spanish dung beetles were observed to alter their habitat associations along an elevational gradient, an analogue for climate change. The implication being that climate change may increase the incidence of dominant species in these refuges, not only increasing native vs. exotic competition but native vs. native competition, further marginalizing them (Menendez and Gutierrez 2004). Whether changing conditions as a result of climate change will force introduced species into environments currently inhabited by natives remains to be seen but seems likely given observed changes in the habitat association of *Onthophagus* and *Aphodius* species along an elevational gradient in Spain (Menendez and Gutierrez 2004).

Two telecoprid species are commonly found in Australian pastures, the exotic *S. rubrus* and *S. spinipes*. Only *S. rubrus* was retrieved from pitfall traps in Bingara. The thermal physiology of the species matched what was expected from a telecoprid species. When foraging during the day its high CT_{max} allows it to secure a small amount of dung and roll it away while being highly exposed to high temperatures. Its CT_{max} and high metabolic rate at temperatures above 25°C were similar to the paracoprid *E. intermedius* from the same site but was capable of maintaining a more stable metabolic rate across this temperature range. From this data it appears that there is overlap in the thermal niche of these species with competition between the species being minimized by life-history rather than thermal physiology. Telecoprids are most abundant in open habitats, rarely occupying forests and woodlands (Krell, Krell-Westerwalbesloh et al. 2003, Verdú, Arellano et al. 2007). Activity in tropical ball rollers is highly dependent on temperature, requiring ambient temperatures between 22°C and 26°C (Verdú, Arellano et al. 2007). A similar requirement may be reflected in the high sensitivity of *S. rubrus* to changes in temperature below 25°C. In the Monte Desert, Argentina, the thermal tolerances of three species were highly correlated with daily temperature fluctuations (Giménez Gómez, Lomáscolo et al. 2017). *Eucranium belenae*, the least tolerant species, was most only active in the morning with when the other species were less active. *Anomiopsoides cavifrons* was active across a broad range of temperature, overlapping with other species. *Anomiopsoides fedemariai* was the most tolerant and was most active at high temperatures. Activity patterns such as this may be characteristic of thermal niche partitioning in dung beetles, where what matters most is not the ability to forage at temperatures that others cannot, but the ability to forage better than others within narrow temperature ranges.

Environments in which the cost of thermoregulation is low favour the evolution of thermal specialists (Angilletta Jr, Bennett et al. 2006), while dung beetles often inhabit thermally heterogeneous environments they have a low cost of thermoregulation due to their use of dung. In many, ways their complete and utter dependence on dung frees them from many of the energetic costs associated with a typical life-history: beetles do not have to spend energy searching for mates or nesting sites, as the intense competition for dung means they will be incidentally encountered whilst foraging; once in a dung pat they are able to satisfy their fundamental needs for both food and reproduction, while protected from predators and extremes of temperature. This study is the first to our knowledge to assess the thermal traits of a dung beetle assemblage however there are a number of caveats when interpreting these results in a more general context. Foremost is the lack of species diversity in the study. A total of six species were captured from the Armidale site, five paracoprids and one endocoprid, and two from Bingara, a paracoprid and telecoprid. Study of more diverse communities with multiple representatives from each guild will better elucidate the role of thermal physiology in structuring dung beetle communities. We have identified significant differences in the thermal physiology of the species studied that correlate with the dung beetle thermal ecology literature.

3.7 – Appendix

Appendix 3.1. Post hoc differences between $\mathrm{CT}_{\mathrm{min}}$ least squares means of seven species of dung beetle captured from a temperate pasture ecosystem at Armidale, NSW. Adjusted for multiple comparisons using the Tukey-Kramer adjustment for unequal sample sizes.

species	**_species**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Adj P**	**Alpha**	**Lower**	**Upper**	**Adj Lower**	**Adj Upper**
Onthophagus australis	*Onthophagus binodis*	-1 53	0 75	61	-2 04	0 04	0 4019	0 05	-3 04	-0 02	-3 83	0 76
Onthophagus australis	*Aphodius fimetarius*	7 57	0 70	61	10 73	<0 01	<0 01	0 05	6 16	8 98	5 42	9 72
Onthophagus australis	*Euoniticellus fulvus*	-4 00	0 75	61	-5 32	<0 01	<0 01	0 05	-5 51	-2 50	-6 30	-1 71
Onthophagus australis	*Onthophagus granulatus*	0 62	0 86	61	0 72	0 47	0 99	0 05	-1 10	2 35	-2 01	3 25
Onthophagus australis	*Euoniticellus intermedius*	-3 21	0 91	61	-3 53	0 01	0 01	0 05	-5 04	-1 39	-5 99	-0 43
Onthophagus australis	*Sisyphus rubrus*	1 67	0 97	61	1 72	0 08	0 60	0 05	-0 26	3 62	-1 28	4 64
Onthophagus binodis	*Aphodius fimetarius*	9 10	0 75	61	12 10	<0 01	<0 01	0 05	7 60	10 61	6 81	11 40
Onthophagus binodis	*Euoniticellus fulvus*	-2 47	0 79	61	-3 10	<0 01	0 04	0 05	-4 06	-0 87	-4 90	-0 04
Onthophagus binodis	*Onthophagus granulatus*	2 15	0 90	61	2 39	0 02	0 22	0 05	0 35	3 96	-0 59	4 91
Onthophagus binodis	*Euoniticellus intermedius*	-1 68	0 94	61	-1 77	0 08	0 57	0 05	-3 57	0 21	-4 57	1 20
Onthophagus binodis	*Sisyphus rubrus*	3 21	1 00	61	3 19	<0 01	0 03	0 05	1 19	5 22	0 14	6 28
Aphodius fimetarius	*Euoniticellus fulvus*	-11 58	0 75	61	-15 38	< 01	<0 01	0 05	-13 08	-10 07	-13 87	-9 28
Aphodius fimetarius	*Onthophagus granulatus*	-6 95	0 86	61	-8 04	<0 01	<0 01	0 05	-8 68	-5 22	-9 59	-4 31
Aphodius fimetarius	*Euoniticellus intermedius*	-10 79	0 91	61	-11 84	<0 01	<0 01	0 05	-12 61	-8 96	-13 57	-8 01
Aphodius fimetarius	*Sisyphus rubrus*	-5 89	0 97	61	-6 06	<0 01	<0 01	0 05	-7 84	-3 94	-8 86	-2 92
Euoniticellus fulvus	*Onthophagus granulatus*	4 62	0 90	61	5 12	<0 01	<0 01	0 05	2 82	6 43	1 87	7 38
Euoniticellus fulvus	*Euoniticellus intermedius*	0 78	0 94	61	0 83	0 41	0 98	0 05	-1 10	2 68	-2 10	3 68
Euoniticellus fulvus	*Sisyphus rubrus*	5 68	1 01	61	5 64	<0 01	<0 01	0 05	3 67	7 70	2 61	8 75
Onthophagus granulatus	*Euoniticellus intermedius*	-3 83	1 03	61	-3 69	<0 01	<0 01	0 05	-5 91	-1 76	-7 00	-0 66
Onthophagus granulatus	*Sisyphus rubrus*	1 05	1 09	61	0 97	0 33	0 95	0 05	-1 13	3 24	-2 27	4 39
Euoniticellus intermedius	*Sisyphus rubrus*	4 89	1 13	61	4 33	<0 01	0 01	0 05	2 63	7 15	1 44	8 34

Appendix 3.2. Tukey-Kramer grouping for CT_{min} least squares means of seven species of dung beetle capture from a temperate pasture ecosystem at Armidale, NSW. Least squares means with the same letter are not significantly different. Alpha = 0.05.

Species	**Estimate**	**Grouping**	
E. fulvus	9.0733		A
			A
E. intermedius	8.2845	B	A
		B	
O. binodis	6.6017	B	C
			C
O. australis	5.0669	D	C
		D	C
O. granulatus	4.4450	D	C
		D	
S. rubrus	3.3878	D	
A. fimetarius	-2.5082		E

Appendix 3.3. Post hoc differences between $\mathrm{CT}_{\mathrm{max}}$, corrected for individual live mass, least squares means of seven species of dung beetle captured from a temperate pasture ecosystem at Armidale, NSW. Adjusted for multiple comparisons using the Tukey-Kramer adjustment for unequal sample sizes.

species	**_species**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Adj P**	**Alpha**	**Lower**	**Upper**	**Adj Lower**	**Adj Upper**
Onthophagus australis	*Onthophagus binodis*	-2 81	0 38	36 31	-7 32	<0 01	<0 01	0 05	-3 59	-2 03	-4 01	-1 61
Onthophagus australis	*Aphodius fimetarius*	-1 16	0 62	59 55	-1 85	0 07	0 52	0 05	-2 41	0 093	-3 07	0 75
Onthophagus australis	*Euoniticellus fulvus*	-7 14	0 54	70 08	-12 99	<0 01	<0 01	0 05	-8 23	-6 04	-8 81	-5 47
Onthophagus australis	*Onthophagus granulatus*	-2 32	0 64	47 16	-3 61	<0 01	0 01	0 05	-3 62	-1 02	-4 31	-0 33
Onthophagus australis	*Euoniticellus intermedius*	-6 75	0 64	65 54	-10 55	<0 01	<0 01	0 05	-8 03	-5 47	-8 70	-4 81
Onthophagus australis	*Sisyphus rubrus*	-9 82	0 36	31 48	-26 98	<0 01	<0 01	0 05	-10 56	-9 08	-10 97	-8 68
Onthophagus binodis	*Aphodius fimetarius*	1 65	0 66	60 78	2 49	0 02	0 18	0 05	0 32	2 98	-0 36	3 67
Onthophagus binodis	*Euoniticellus fulvus*	-4 32	0 58	70 57	-7 36	<0 01	<0 01	0 05	-5 49	-3 15	-6 11	-2 54
Onthophagus binodis	*Onthophagus granulatus*	0 48	0 68	47 27	0 72	0 47	0 99	0 05	-0 87	1 85	-1 60	2 58
Onthophagus binodis	*Euoniticellus intermedius*	-3 94	0 67	70 47	-5 86	<0 01	<0 01	0 05	-5 28	-2 60	-5 98	-1 89
Onthophagus binodis	*Sisyphus rubrus*	-7 01	0 41	37 66	-16 94	<0 01	<0 01	0 05	-7 84	-6 17	-8 29	-5 72
Aphodius fimetarius	*Euoniticellus fulvus*	-5 98	0 43	55 18	-13 74	<0 01	<0 01	0 05	-6 85	-5 10	-7 31	-4 64
Aphodius fimetarius	*Onthophagus granulatus*	-1 16	0 45	55 79	-2 57	0 01	0 1542	0 05	-2 07	-0 25	-2 54	0 21
Aphodius fimetarius	*Euoniticellus intermedius*	-5 59	0 57	53 28	-9 79	<0 01	<0 01	0 05	-6 74	-4 45	-7 34	-3 84
Aphodius fimetarius	*Sisyphus rubrus*	-8 66	0 46	37 78	-18 70	<0 01	<0 01	0 05	-9 60	-7 72	-10 10	-7 22
Euoniticellus fulvus	*Onthophagus granulatus*	4 81	0 47	70 05	10 18	<0 01	<0 01	0 05	3 87	5 75	3 38	6 25
Euoniticellus fulvus	*Euoniticellus intermedius*	0 38	0 56	31 62	0 68	0 50	0 99	0 05	-0 76	1 53	-1 38	2 15
Euoniticellus fulvus	*Sisyphus rubrus*	-2 68	0 40	40 5	-6 56	<0 01	<0 01	0 05	-3 50	-1 85	-3 95	-1 41
Onthophagus granulatus	*Euoniticellus intermedius*	-4 43	0 60	62 9	-7 38	<0 01	<0 01	0 05	-5 63	-3 23	-6 26	-2 60
Onthophagus granulatus	*Sisyphus rubrus*	-7 49	0 49	40 54	-15 22	<0 01	<0 01	0 05	-8 49	-6 50	-9 02	-5 97
Euoniticellus intermedius	*Sisyphus rubrus*	-3 06	0 53	41 89	-5 72	<0 01	<0 01	0 05	-4 14	-1 98	-4 72	-1 40

Appendix 3.4. Tukey-Kramer grouping for CT$_{max}$, corrected for individual live mass, least squares means of seven species of dung beetle retrieved from a disturbed temperate pasture ecosystem at Armidale, NSW. Least squares means with the same letter are not significantly different. Alpha = 0.05.

Species	**Estimate**	**Grouping**	
S. rubrus	52.8644		A
E. fulvus	50.1809		B
			B
E. intermedius	49.7978		B
O. binodis	45.8547		C
			C
O. granulatus	45.3651		C
			C
A. fimetarius	44.2005	D	C
		D	
O. australis	43.0382	D	

CHAPTER FOUR

THE EFFECTS OF TEMPERATURE ON THE PHYSIOLOGY AND NESTING BEHAVIOUR OF A TUNNELLING DUNG BEETLE, *ONTHOPHAGUS BINODIS*, AND ITS IMPLICATIONS FOR THE PROVISION OF ECOSYSTEM FUNCTION.

4.1 – Abstract

Rising temperatures as a result of climate change are expected to influence the distribution and interactions of insects. The nature of these changes will be a result of both the severity of warming and the ability of species' to mitigate its impacts. The capacity to alter behaviour or physiology in response to increased temperatures has been suggested as a potential mitigating mechanism. The dung beetle, *Onthophagus binodis*, forages during the day where it is exposed to highly variable air temperatures; however it feeds and reproduces in dung or below the soil where thermal variation is greatly reduced. We use temperature controlled chambers and temperature data from the field to test the plasticity of several thermal traits under simulated warming regimes of ambient fluctuating temperatures +0°C, +2°C, +3.5°C, and +5°C. Flow-through respirometry was used to measure CT_{max}, metabolic rate, and thermal sensitivity at ten day intervals. Brood ball burial depth, brood ball volume, and brood ball abundance were recorded to identify changes in larval provisioning behaviour. Treatment temperature did not have an effect on CT_{max}, metabolic rate, or thermal sensitivity. Warming had no effect on the depth of brood ball burial, but did increase the number of brood balls produced. Beetles from the +5°C treatment produced significantly larger brood balls; however this difference was only observed when the total number of brood ball produced was low. The response of *O. binodis* to climate change will likely be mediated by changes in behaviour rather than within-generation phenotypic plasticity.

4.2 – Introduction

The response of insect populations to climate change is expected to be largely driven by the associated changes in temperature, therefore an understanding of species thermal biology is crucial (Bale, Masters et al. 2002). The sensitivity of an organism to temperature can be described by plotting its performance across a range of body temperatures (Huey and Kingsolver 1989). The breadth of an organisms thermal performance curve is constrained by the minimum (CT_{min}) and maximum (CT_{max}) body temperature it can tolerate before suffering permanent physiological damage, with optimal performance often occurring closer to CT_{max} (Angilletta Jr, Niewiarowski et al. 2002). With the increasing frequency and severity of extreme temperature events populations will be more frequently exposed to deleterious temperatures (Alexander, Zhang et al. 2006).

Phenotypic plasticity is thought to be advantageous in thermally heterogeneous environments and when colonising novel environments (Yeh and Price 2004), and is expected to be an important mechanism underpinning insects’ response to climate change (Kingsolver, Ragland et al. 2009). Plasticity of thermal tolerance and metabolic rate has been observed in numerous insect species (Chown and Terblanche 2006, Overgaard, Kristensen et al. 2011, Andrew, Hart et al. 2013), however not all species share this capacity (Terblanche, Clusella-Trullas et al. 2010). Inter-species heterogeneity in the plasticity of thermal traits will engender a correspondingly diverse range of responses to increasingly variable temperature regimes, determining changes in community dynamics and ecosystem function (Woods and Harrison 2002, Fordyce 2006, Sgrò, Terblanche et al. 2016). Scarabaeinae beetles display a remarkable degree of plasticity in development, physiology, and behaviour (Valena and Moczek 2012) however little is known concerning their plasticity in response to heat. The unique behaviour and ecological niche inhabited by tunnelling dung beetles makes generalising results from studies on other insect groups difficult.

Acclimatisation (acclimation in a laboratory setting) is a form of phenotypic plasticity that permits individuals to adaptively alter their physiology in response to environmental cues that signal changing conditions, or allow for compensation such that an individual is able to maintain its current functionality when faced with changing environmental conditions (Whitman 2009). Individuals are not infinitely plastic and are limited in the range of environments they can respond to (Moczek, Kijimoto et al. 2014). This process is often reversible, allowing the organism to adapt to short term or seasonal changes in environmental conditions (Bacigalupe, Barria et al. 2017). Acclimation allows an organism to compensate for deleterious conditions, such as via metabolic compensation, allowing it to maintain its current functionality in a changing environment while plasticity of tolerance thresholds allows range expansion. Trans-generational acclimatisation allows parents to adaptively alter their offspring's physiology based on the parents' knowledge of the current environment (Beckers, Anderson et al. 2015).

The vulnerability of insect populations to climate change is therefore dependent on their proximity to CT_{max} and capacity to mitigate the adverse effects of temperature via behavioural or physiological adaptation. The potential for physiological plasticity is limited (Snell-Rood, Van Dyken et al. 2010) and varies between species (Nyamukondiwa, Terblanche et al. 2011) and populations (Sgrò, Overgaard et al. 2010, Kleynhans, Mitchell et al. 2014), with heat tolerance and metabolic rate having limited plasticity in some insects but not others (Terblanche, Clusella-Trullas et al. 2010). In the context of climate change it is expected that populations with a higher degree of plasticity will either be better able to compensate for changing conditions and maintain functionality while persisting in their current environment, or be more able to expand to a new environment with more suitable conditions (Sgrò, Terblanche et al. 2016). The expression of a new phenotype as a result of changing conditions can impact inter and intra-specific interactions altering community structure and function (Pigliucci 2005, Pertoldi, Bijlsma et al. 2007).

Ecosystems cannot be described in terms of the prevailing macroclimatic conditions, rather they must be thought of as a heterogeneous network of microclimates in which abiotic conditions can be highly variable relative to the broader climatic conditions (Andrew, Hill et al. 2013): it is the abiotic conditions of these microclimates that mediate the physiological response of the organisms that inhabit them (Hallett, Coulson et al. 2004). Insects often utilise the wide range of microclimates available to them to regulate body temperature and avoid stressful conditions (Sunday, Bates et al. 2014, Hemmings and Andrew 2016). Behavioural adaptations such as retreat-site selection (Goldsbrough, Hochuli et al. 2004), foraging regime (Cerdá, Retana et al. 1998), increased running speed (Andrew, Hill et al. 2013), orientating body parts to increase heat dissipation (Lactin and Johnson 1996, Cerdá 2001), and the periodic use of microclimates for thermal respite (Wehner and Wehner 2011, Smolka, Baird et al. 2012). The increasing reliance on behavioural thermoregulation as a result of warming may alter species interactions (Angilletta, Wilson et al. 2003) and prove insufficient in buffering from adverse effects under some warming scenarios (Deutsch, Tewksbury et al. 2008). While physiological plasticity is often put forward as the primary mechanism by which populations will deal with climate change behavioural plasticity is an important aspect of a species thermal biology (Basson and Clusella-Trullas 2015). Insects may also adapt behaviourally with temperature preference, feeding rate, temperature thresholds for activity, and locomotor activity being plastic traits (Dillon, Wang et al. 2009, Lachenicht, Clusella-Trullas et al. 2010, Terblanche, Clusella-Trullas et al. 2010).

The Scarabaeinae, known as true dung beetles, are a group of coprophagous scarabs comprised of *ca* 6000 species (Simmons and Ridsdill-Smith 2011). Dung is fundamental in the life-cycle of dung beetles, playing a key role in reproduction and development (Hanski and Cambefort 1991), acting as a microhabitat (Hanski and Koskela 1977), as well as serving as a food source for both larvae and adult beetles (Byrne, Watkins et al. 2013). The majority of Scarabaeinae species are paracoprids, reproducing by tunnelling below a source of dung and provisioning it with masses of dung known as brood balls, a behaviour unique to the group (Scholtz, Davis et al.

2009). Dung beetles are rare among insect in that adult beetles invest significant time and effort in raising their young, being one of a few insects that display bi-parental care (Hunt and Simmons 1998) and one of the only beetles species that provision resources for their young, upon which the young are entirely dependent (Bishop, Griffiths et al. 2000). This relocation of dung and soil during the creation of these nests alters resource distribution and drives a number of ecological processes (Davis, Chown et al. 2000).Temperature induced changes to the number, structure, and depth of brood balls therefore has important implications for the ecology of *O. binodis* such as potential changes to population structure as a result of a change in the frequency of males expressing the “major” morph, with “major” and “minor” morphs being behaviourally distinct, and altered levels of ecosystem function caused by changes in dung burial.

The quality and/or quantity of dung used by adults to create brood balls has implications beyond the immediate survival of the larvae. Onthophagine dung beetles display great morphological plasticity, with males expressing either a “minor” morph, with small horns indistinguishable from that of a female, or a “major” morph where the male has large exaggerated horn like structures (Mattila and Hanski 2014). Unlike most other insects, pupation is triggered by the depletion of the brood ball rather than larval mass (Moczek, Hunt et al. 2002). This development of major and minor males is not determined by genetics but rather the crossing of a critical weight threshold prior to pupation (Shafiei, Moczek et al. 2001), beyond which almost all males emerge as majors (Moczek 1998). Regardless of morph, adults with a higher body mass have preference for warmer microclimatic conditions, with differences of up to 15°C being observed (Shepherd, Prange et al. 2008).

Onthophagus binodis, known as the humpbacked dung beetle, is a small paracoprid that grows up to 13mm in length (Tyndale-Biscoe 1990). Native to South Africa, it was introduced to Australia as part of the Australian Dung Beetle Project (Bornemissza 1976). *Onthophagus binodis* is sexually dimorphic, major males possessing a large pronotal horn with females and minor males develop a small ridge.

The development of a particular morph is associated with a variety of behavioural traits including favouring of direct competition over stealth in reproductive contests (Simmons and Kotiaho 2007), increased reproductive success, the extent to which the male aids the female in larval provisioning (Cook 1988, Cook 1993), and reduced dung residence time (Hunt, Kotiaho et al. 1999). It is bi-voltine with larvae taking ~10 weeks to complete development (Ridsdill-Smith 1993). *Onthophagus binodis* abundances peak from spring to autumn, flying during daylight hours, but they are active during the winter in warmer areas and are therefore considered a high priority for dispersal into areas of Australia where dung beetle activity is low during these times (Doube 2007).

In this study custom-made temperature-controlled chambers and data collected from the field were used to replicate natural daily temperature fluctuations and simulate climate warming scenarios to investigate how increasing temperatures may affect temperate paracoprid dung beetles and the provision of ecosystem functions. The questions addressed were: (1) Are dung beetles currently living close to the limits of their thermal tolerance? (2) Do dung beetles possess the physiological plasticity to increase their thermal tolerance? (3) Are increases in temperature associated with an increase in metabolic rate? (4) Does higher temperature exposure throughout their life history reduce dung beetle fecundity? (5) Do tunnelling female dung beetles attempt to mitigate the impact of temperature on the development of their young by altering brood ball burial depth? (6) Do female dung beetles alter the structure of their brood balls in response to increasing temperature?

4.3 – Methods

4.3.1 – Study animals and site

Onthophagus binodis was chosen for several reason; it is one of the most dominant and abundant species in the region therefore its capacity to respond to increasing temperatures will likely impact the future community structure of the region; as one of the larger diurnal paracoprids in the region it is particularly important from an ecosystem services perspective; Onthophagine dung beetles appear to have highly plastic behaviour and morphologies but their physiological plasticity is unknown. Adult *Onthophagus binodis* were collected from a pasture at the University of New England's Kirby SMART Farm, Armidale, NSW, Australia (30.53°S, 151.62°E; 1079m ASL) between the 15th and 23rd of March 2016. The area has a cool temperate climate with an annual mean temperature range of 7.4°C-19.5°C and 759.6mm of rainfall (Bureau of Meteorology 2016). Cattle producing the dung used here had not been drenched in the past six months. Dung pats showing signs of dung beetle activity were collected and returned to the university where beetles were floated out of the dung. Individuals were sexed using the shape of the pygidium and separated into single sex populations. A 1:1 sex ratio was observed in the Kirby population, and was therefore used in the experiment. Beetles were maintained in 9 L plastic containers, filled with 12cm of sand, which were stored in a glasshouse until the experiment began on the 24th of March 2016. While being maintained beetles were fed using the same dung as in the experiment.

4.3.2 – Dung

All dung used in the experiment was collected on the 22nd of November 2015 from a dairy farm (-30.6, 150.69) at Manilla, NSW, Australia. Fresh dung pats (less than an hour old) that showed no sign of coprophilous activity were collected in 2 L bags which were heat sealed then frozen the day of collection. Cattle from the site were last drenched 4 months prior to collection. Dung was kept in a freezer for up to seven months and thawed at room temperature and homogenised prior to use.

4.3.3 – Chamber design

Eighteen temperature controlled chambers were built for this experiment. The chambers were constructed from PVC pipe (height 50cm, diameter 30cm) with a PVC cap lid. A semicircular clear Perspex window (radius 15cm, diameter 29cm) was installed in the lid to allow the beetles to experience a natural diurnal cycle and light. Three 10mm diameter holes were drilled into the Perspex to allow ventilation and prevent the build-up of condensation (Figure 4.1, Figure 4.2).

A custom built temperature control unit was installed on the PVC half of each lid. These temperature controls units consisted of a Peltier cell (68.09 watts, 40mm x 40mm, Techbrands, Rydalmere, NSW, Australia) with one side attached to a fan assisted heatsink (Pure Rock CPU cooler, 150 watts TPD, BeQuiet!, Glinde, Germany) mounted externally on top of the lid and the other side attached to an internal heatsink (84 x 45 x 50mm aluminium convection heatsink, 9 fins, Techbrands, Rydalmere, Australia) and fan (39.5 cfm 80mm, Sunon, Kaohsiung City, Taiwan). Temperature control units were powered by Switchmode Laboratory Power Supplies (13.8 Volts, 40 Amps, Techbrands, Rydalmere, Australia). A custom made control unit attached to a DS18B20 digital thermometer probe (resolution ± 0.5°C), suspended from the lid, was used to monitor the internal temperature of the chamber. The control unit used a proportional control scheme to automatically switch between heating and cooling, allowing chamber temperatures to remain steady.

Instructions were sent to the chambers via custom built software, created using LabView (National Instruments: http://www.ni.com/newsletter/51141/en/) graphical programming, running on a computer. Chambers were connected in series using RS232 serials ports with the final chamber connecting to a RS485 adaptor which was connected to the computer. The Modbus communications protocol was used to transfer data between the computer and chambers. The software allows the temperature of each chamber to be set using one of three methods; the chamber can be set to a single temperature, chamber temperature can be set to track ambient temperature in real-time using a thermometer probe connected to one of the chambers (RS232 serial port), and chamber temperature can be set using a lookup table with predetermined temperature set for certain times of the day. For this experiment the lookup table method was used with a set temperature for each hour of the day (See below). The program uses linear interpolation between the current set temperature and the next set temperature to allow for a gradual change in temperature from one hour to the next, emulating natural conditions. The control units polled the chamber temperature every 30 seconds. The software allows for a ±X°C offset to be applied to the base temperature of each chamber. This function was used to create the four treatments for this experiment.

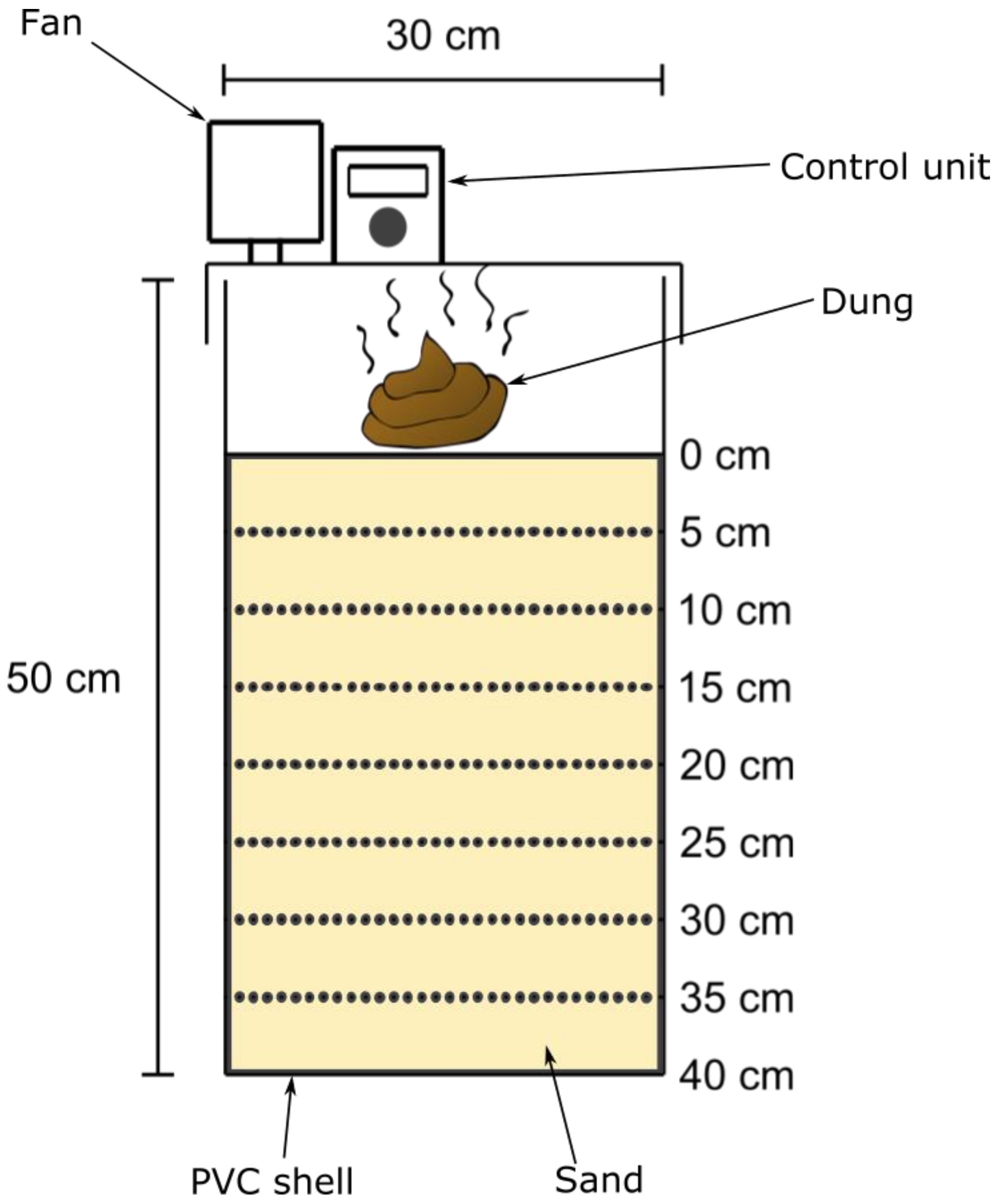


Figure 4.1. Design of the experimental temperature chambers. Chambers were constructed from PVC with a diameter of 30cm and a height of 50cm. Internal temperature could be set using either the control unit on the chamber itself or via the custom-built software. Temperature was regulated using Peltier cells connected to a fan assisted heatsink.

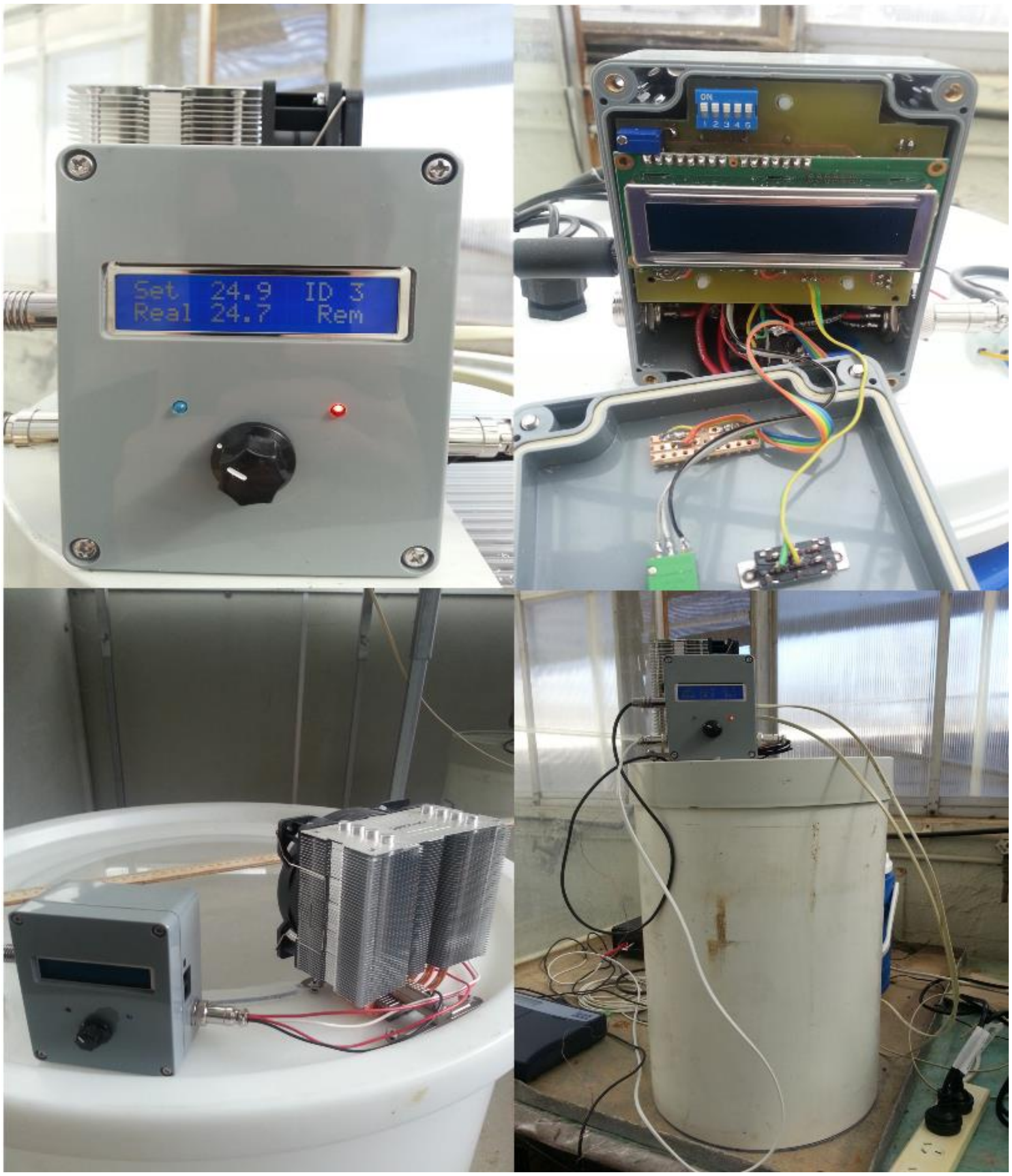


Figure 4.2. Temperature controlled chambers used in the study.

4.3.4 – Experimental design

The base temperature regime was determined by averaging hourly data logged from five Decagon 5TE soil temperature sensors buried 5cm below the soil surface (resolution ± 0.1°C, accuracy ± 1°C) in the area surrounding the pasture where the beetles were collected. These soil temperature sensors were installed and maintained by the University of New England's Precision Agriculture Research Group as part of the SMARTFARM program. The temperature data was collected from the 20th-26th of February 2016. Summer temperatures were chosen as that is when the beetles are most at risk of thermal stress and approaching their CT_{max}. The base thermal regime of each chamber was set to a diurnal cycle using soil temperature from the corresponding time of day. Temperatures ranged from a low of 24.8°C at 06:00 to a high of 29.2°C at 18:00 (Appendix 4.1). The four treatments applied in this study were +0°C (n=5), +2°C (n=5), +3.5°C (n=4), and +5°C (n=4) increases from the base regime. Temperatures inside each chamber were measured using a thermocouple on the soil surface.

The inside of the chambers was marked at 5cm intervals and filled with 40cm of sand. The moisture content of the sand was standardised across all chambers at 4% volumetric soil water content using an MPM160 moisture probe meter (± 0.1 VSW%) as at levels below this *O. binodis* only produce broods in the moist sand directly below the dung (Barkhouse and Ridsdill-Smith 1986). A one litre dung pat was placed in each chamber using a 15.5cm diameter mold followed by forty *O. binodis* (20 male, 20 female). This was done for one replicate of each treatment daily until all chambers were filled (24th March – 27th March). The fifth replicates of the +0°C and +2°C treatments were filled on the 26th and 27th of March respectively. After the beetles had been in a chamber for 10 days it was dug up, the broods removed, and thermolimit respirometry performed on two beetles. The sand from each chamber was sifted and its VSW% was readjusted to 4%, old dung was removed and replaced with fresh. This process was repeated every 10 days for a total of 50 days.

The number of broods at each 5cm depth interval was recorded. If a brood intersected two depth intervals then it was recorded as being in the interval where the majority of the broods mass was observed. Each brood was measured along three axis using a Kincrome digital Vernier calliper (resolution ± 0.01mm, accuracy ± 0.03mm). Broods were then placed in a 5L plastic container, covered in moist sand, then stored in a static temperature controlled cabinet (Thermoline TRH-300-SD) at 25°C and 75% relative humidity. Once the second generation of beetles had emerged they were maintained in the glasshouse as described above. The experiment was then repeated using this second generation of beetles with replicates being initiated from the 12th-15th of July (Appendix 4.2). The second generation of beetles was run for 30 days rather than 50 due to logistical issues. A large number of the broods did not produce adult beetles resulting in an insufficient number to exactly replicate the initial experiment. Twenty beetles were placed in each chamber (10 male, 10 female), with the amount of dung per chamber reduced to 500 ml to account for this. The number of replicates was also reduced; +0°C (n=4), +2°C (n=4), +3.5°C (n=4), +5°C (n=3).

4.3.5 – Thermolimit respirometry

Thermolimit respirometry was conducted using a push mode flow-through system (Andrew, Ghaedi et al. 2016). An air pump (HB-40, Hiblow USA, Saline, MI, USA) was used to flow outside air through a scrubber column containing soda lime to remove CO_2 then a column containing silica gel and Drierite to remove H_2O. The airflow was then split and passed through two mass flow control valves (Side-Trak 840L, Sierra Instruments Inc., Monterey, CA, USA) connected to a mass flow controller (MFC-2, Sable Systems, Las Vegas, NV, USA) which regulated airflow to 200 ml min^{-1}, through two 15 ml glass cuvettes, and into a LiCor 7000 CO_2/H_2O infra-red gas analyser set to reference estimation mode. Reference estimation mode allows gas concentrations in both of the LiCor sampling cells to be measured independently, with the caveat that more frequent calibrations are required. Beetle movement was measured in each channel using separate activity detectors (AD2 Activity Detector, Sable Systems International, Las Vegas, NV, USA). Activity and gas concentration data was recorded every one second.

Each assay consisted of a male and female beetle, from the same temperature controlled chamber, being run simultaneously. A ratio of 1:1 male-to-female was chosen as this sex ratio was observed in the field, allowing for a natural sex ratio to be maintained throughout the experiment. All animals were fasted for a minimum of 12 hours prior to the assay by placing them in mesh cages inside their respective temperature controlled chambers. Assays for each set of replicates were run consecutively in one day. To control for the potential effect of the beetles daily activity cycle on their metabolism the order in which each treatment was assayed was rotated over the four days so that each treatment was tested during all times of the day. A 10 minute baseline was recorded with empty cuvettes before and after each assay.

Animals were subject to a ramping temperature protocol using Grant GP200/R4 programmable waterbaths (resolution ± 0.1°C). The starting temperature was 25°C, this was maintained for five minutes to allow the internal temperature of the cuvettes to reach equilibrium with the surrounding water. After this equilibration phase temperature was increased by 0.25°C min^{-1} (Lighton and Turner 2004, Terblanche, Sinclair et al. 2005), stopping at 55°C. Temperatures inside the cuvettes were recorded every one second using a PicoTech TC-08 datalogger (resolution ± 0.025°C, accuracy ± 0.5°C; Pico Technology Ltd, Cambridge, UK) and a type T copper-constantan thermocouple. Data from the datalogger and LiCor were combined in excel using the timestamps recorded by each program.

4.3.6 – Data extraction

ExpeData data acquisition and analysis software (Emlen and Philips 2006) was used for all data extraction. Prior to data extraction CO_2 release rates were corrected for baseline drift and converted to CO_2 ml h^{-1}. CT_{max}, $\dot{V}CO_2$ mg^{-1} h^{-1}, thermal sensitivity, and intercept were extracted for each beetle. CT_{max} was determined using the inflection point of the absolute difference sum residuals (Lighton and Turner 2004). $\dot{V}CO_2$ mg^{-1} h^{-1} released prior to CT_{max} was calculated by calculating the integral of CO_2 ml h^{-1} vs. hours for CO_2 emitted between the end of the initial five minute equilibrium period and the CT_{max} of each beetle. To determine thermal sensitivity CO_2 ml h^{-1}was log transformed and least squares linear regression performed on log transformed CO_2 ml h^{-1} and cuvette temperature (Terblanche, Sinclair et al. 2005). When metabolic rate does respond to temperature changes via acclimation it often comes in the form of a change in the intercept of the metabolic rate-temperature relationship, rather than changes in the slope (Irlich, M et al. 2009). The slope and intercept of the metabolic rate-temperature relationship were extracted and thermal sensitivity calculated using the equation $10^{(10*slope)}$ (Lake, MacMillan et al. 2013).

4.3.7 – Data analysis

CT_{max}, $\dot{V}CO_2$ ml h^{-1}, thermal sensitivity, and intercept of the MR-Temperature relationship were analysed using generalized linear mixed models. Independent factors were treatment, time interval (nested within generation), generation, and log transformed weight as a covariate. Weight was non-significant for CT_{max} and was therefore removed from the final model. Chamber (nested within generation) was a random variable in all models. Measurement intervals were modelled with time interval (nested within generation). Time interval was modelled as an R-side effect to allow for unequal variances (Boykin, Camp et al. 2010). Data for $\dot{V}CO_2$ ml h^{-1}, thermal sensitivity, and intercept were skewed and non-normal. As is often the case with biological systems (Koch 1966) a lognormal distribution was found to best fit the data, indicating a multiplicative effect of variables (Limpert, Stahel et al. 2001, Limpert and Stahel 2011).

To assess the effect of temperature on brood burial depth the cumulative total broods buried at each depth were calculated for each time interval. From this the average burial depth per time interval was calculated for each replicate $\bar{x}_i = \Sigma n_{i,d} \times d / \Sigma N_{i,d}$. Replicates that did not produce any broods were removed prior to analysis as a data entry of "0cm" would suggest that brood balls had been created but left unburied. A generalised linear model was fit using a lognormal distribution with average burial depth (per 10 days) as the dependent variable, temperature, time interval(nested within generation), and generation as independent variables and log transformed brood abundance (per 10 days) as a covariate. Time interval was modelled as an R-side effect to allow for unequal variances.

To determine the effect of temperature on brood ball production a generalised linear model was fit using a negative binomial distribution. Prior to analysis the number of brood balls produced per chamber per measurement interval was collapsed to give a single value for each chamber. Treatment, generation, and treatment*generation were independent factors in the model (Boykin, Camp et al. 2010).

Prior to analysis x,y, z axis brood ball measurements were used to calculate volume, assuming an ellipsoid shape (Kishi 2014). Ellipsoid volume was modelled with a generalised linear mixed model using a lognormal distribution with an autoregressive covariance structure used to model the effect of measurement interval. Independent factors in the model were treatment, measurement interval, generation nested within time interval, log transformed brood abundance was a covariate with the interaction term abundance*treatment included to allow for unequal slopes of the covariate across treatment groups. The depth at which each brood was found was added as a random factor. On day 40 chamber 12 (+3.5°C) produced extremely small brood balls, this change was not reflected in other replicates nor in brood ball measurements from this chamber on days 30 and 50, therefore brood ball data for this chamber on day 40 was omitted prior to analysis. Measurement interval was included in the model as an R-side effect to allow for unequal variances (Boykin, Camp et al. 2010, SAS Institute Inc. 2013).

All post hoc analysis was performed using the least squares means and the Tukey-Kramer adjustment used to adjust for multiplicity. For all analysis denominator degrees of freedom were calculated using the Kenward-Roger approximation (Kenward and Roger 2009). Both the Satterthwaite and Kenward-Roger methods are appropriate for analysis of unbalanced designs using mixed models, however the Kenward-Roger method provides a more conservative test than Satterthwaite by reducing the bias of the variance-covariance matrix (Spilke, Piepho et al. 2005). When a model specifies an unequal covariance structure, as is the case for the measurement interval factor in our models, use of the Kenward-Roger adjustment may result in non-integer denominator degrees of freedom (Spilke, Piepho et al. 2005, Stroup 2015). All statistical analyses were carried out using the GLIMMIX procedure in SAS® 9.4 software (SAS Institute Inc. 2013).

4.4 – Results

4.4.1 – *CT_{max}*

There was a significant effect of measurement interval on CT_{max} ($F_{6,\ 70.01} = 20.93$, $p < 0.001$) with the CT_{max} of both generations declining over the course of the experiment. CT_{max} during the first 10 days was similar for both generations ($t = -0.68$, $p = 0.99$). After this the CT_{max} of beetles from the second generation declined faster than in the field generation, dropping 1.99°C ± 0.35 between 10-20 days compared to the 0.70°C ± 0.34 of generation one. This relatively rapid drop in CT_{max} is reflected in the significant effect of generation ($F_{1,\ 70.16} = 10.34$, $p = 0.002$) with the second generation having an overall CT_{max} 0.61°C lower than beetles collected from the field. It took 50 days for the CT_{max} of generation one to get as low as the CT_{max} of the second generation on day 30 ($t = -0.52$, $p = 0.99$). No significant difference in CT_{max} was detected between treatments ($F_{3,\ 218.6} = 2.48$, $p = 0.062$) (Table 4.1).

4.4.2 – Metabolic rate and thermal sensitivity

Warming treatments had no effect on the metabolic rate of *O. binodis*. $\dot{V}CO_2$ ml h^{-1} ($F_{3,\ 33.37} = 1.59$, $p = 0.209$), thermal sensitivity ($F_{3,\ 36.51} = 0.63$, $p = 0.602$), or intercept ($F_{3,\ 27.32} = 1.36$, $p = 0.277$) were all unaffected by warming. Weight was a significant predictor of metabolic rate ($F_{1,\ 101.9} = 229.53$, $p < 0.001$). The second generation had a lower metabolic rate than the field generation ($F_{1,\ 26.29} = 12.00$, $p < 0.002$) which was primarily a function of the field generations increased metabolic rate on days 20 and 30 ($F_{6,\ 76.59} = 4.02$, $p < 0.002$)(Figure 4.3). The thermal sensitivity of metabolic rate to changes in temperature was unaffected by time ($F_{6,\ 56.45} = 0.69$, $p = 0.657$), generation ($F_{1,\ 35.4} = 0.03$, $p = 0.872$), or weight ($F_{1,\ 79.48} = 1.21$, $p = 0.275$). The intercept of the MR-T relationship increased over time, peaking on days 20 and 30 for generation two and days 40 and 50 for the field caught generation, with a significant effect of beetle weight ($F_{1,\ 45.49} = 4.26$, $p = 0.045$)(Figure 4.4), however, these differences were non-significant after correcting for multiple comparisons (Appendix 4.3).

4.4.3 – Brood balls

There was a significant interaction effect between treatment temperature and generation on mean brood abundance ($F_{3, 20 54} = 3.60$, $p = 0.031$). There was no effect of treatment temperature on the total number of broods produced by the field caught generation over the 50 days they spent in the warming chambers. Warming did significantly increase the total broods produced by the second generation, with the +2°C ($t = -3.37$, $p = 0.044$) and +5°C ($t = -3.50$, $p = 0.037$) treatments resulting in significantly more broods than the +0°C control. Brood production was increased at +3.5°C compared to the control, however this difference was non-significant ($t = -1.18$, $p = 0.878$). The first generation produced significantly more broods than the second generation across the +2°C ($t = 2.83$, $p = 0.009$), +3.5°C ($t = 4.37$, $p < 0.001$) and +0°C ($t = 5.39$, $p < 0.001$) controls, with both generations producing a similar number of brood balls under +5°C warming treatment ($t = 2.02$, $p = 0.055$) (Figure 4.5).

There was no effect of warming treatment on mean burial depth. All warming treatments buried broods at shallower depths than the control but differences were insignificant (Appendix 4.4). The field generation buried their broods deeper than the second generation ($F_{1, 89.06} = 97.46$, $p < 0.001$) with both generations altering their burial depth over time ($F_{7, 45.39} = 15.14$, $p < 0.001$). Beetles from the first generation maintained a stable burial depth for the initial 30 days of the experiment after which beetles began burying broods at shallower depths. Between days 30 and 40 brood burial decreased significantly ($t = 5.07$, $p < 0.001$), burial depth continued to decrease with a further significant decrease between days 40 and 50 ($t = 3.54$, $p = 0.029$). Inversely, the second generation saw a significant increase in burial depth between days 10 and 40 ($t= 3.39$, $p = 0.027$). Brood ball burial depth was independent of log transformed brood abundance across all generations and treatments ($F_{1, 85.23} = 2.15$, $p = 0.146$).

There was a significant interaction effect of treatment temperature and brood abundance on individual brood ball volume ($F_{3, 4277} = 3.1$, $p = 0.025$) therefore the least-squares means were calculated at five levels of log transformed brood abundance; 0.7, 1.0, 2.0, 3.0, 4.0, and 4.5 with the first and last values being the highest and lowest recorded abundances respectively (Figure 4.6). When the number of broods produced was low brood ball volume increased with treatment temperature, with significant differences between the +0°C and +5°C treatments at log abundances 0.7, 1.0, and 2.0. At log abundance 3.0 and 4.5 the volume of +5°C broods had declined and differences were no longer significant. Across all treatments abundance of brood balls was negatively correlated with volume ($F_{1, 4278} = 5.57$, $p = 0.0183$). The second generation produced smaller broods than the field generation ($F_{1, 4279} = 35.03$, $p < 0.001$).

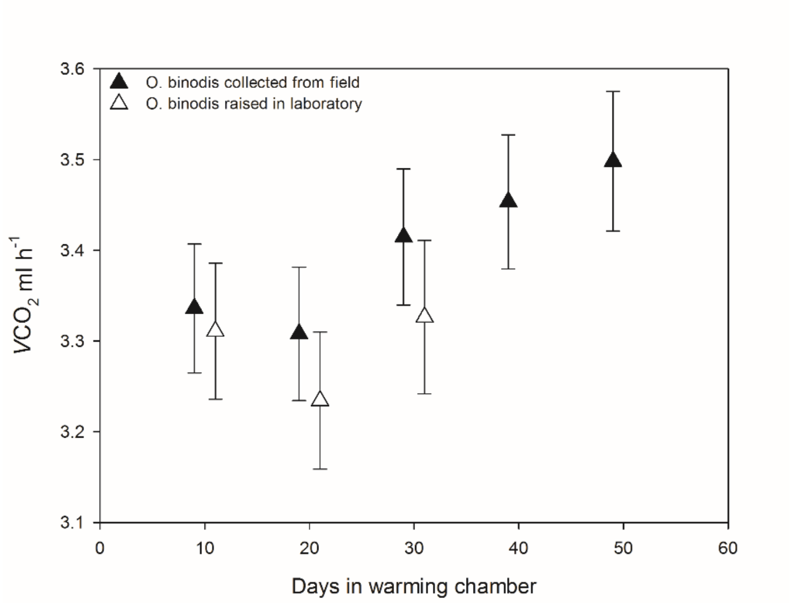


Figure 4.3. $\dot{V}CO_2$ ml h-^{1} of *Onthophagus binodis* from Armidale, Australia. Adult beetles were collected from the field and placed in temperature controlled chambers for 50 days (closed triangles). Brood balls produced by these beetles were collected and maintained at 25°C until beetles emerged. This lab reared generation of beetles was then placed in the warming chambers for a total of 30 days (open triangles). $\dot{V}CO_2$ ml h-^{1} was measured every ten days. Values are least-squares means adjusted for multiplicity and covariates with 95% confidence intervals.

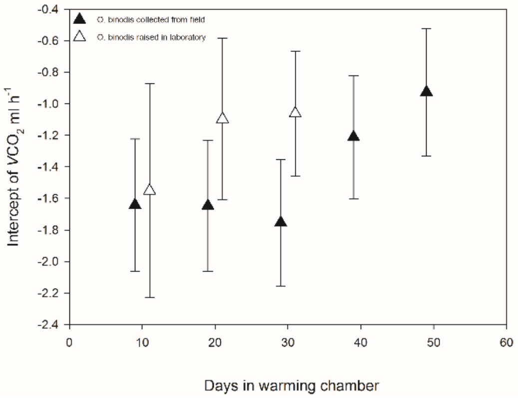


Figure 4.4. Log-transformed intercept of the regression line of log-transformed CO_2 vs. temperature for *Onthophagus binodis*. Adult beetles were collected from the field and placed in temperature controlled chambers for 50 days (closed triangles). Brood balls produced by these beetles were collected and maintained at 25°C until beetles emerged. This lab reared generation of beetles was then placed in the warming chambers for a total of 30 days (open triangles). CO_2 was measured every 10 days. Values are least-squares means adjusted for multiplicity and covariates with 95% confidence intervals.

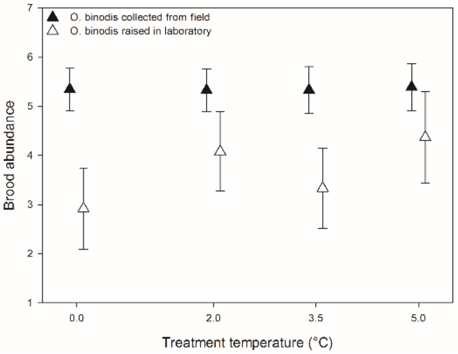


Figure 4.5. Effects of treatment temperature on the number of brood balls produced by adult *Onthophagus binodis*. Adult beetles were collected from the field and placed in temperature controlled chambers for 50 days (closed triangles). Brood balls produced by these beetles were collected and maintained at 25°C until beetles emerged. This lab reared generation of beetles was then placed in the warming chambers for a total of 30 days (open triangles). All values are least squares means with 95% confidence intervals.

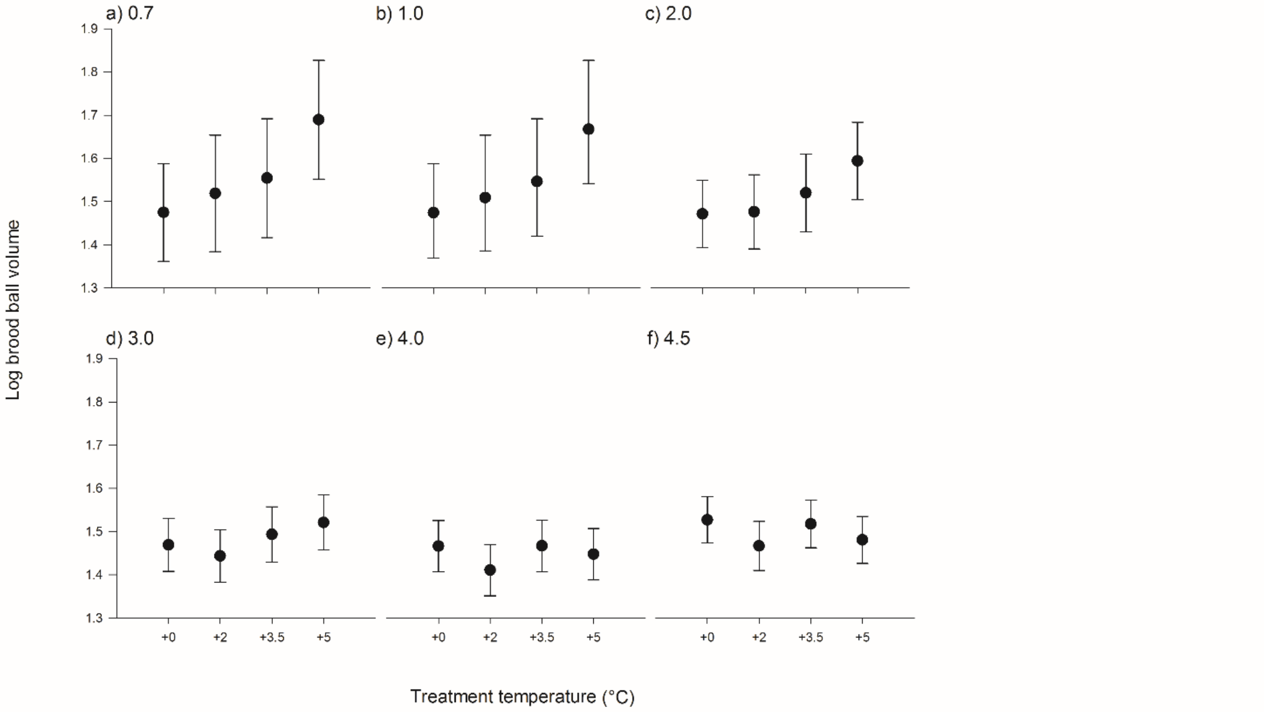


Figure 4.6. Log-transformed brood ball volume of *Onthophagus binodis* at six levels of log-transformed brood ball abundance a) 0.7 b) 1.0, c), 2.0, d) 3.0, e) 4.0, f) 4.5. 0.7 and 4.5 correspond to the minimum and maximum number of brood produced respectively. Values are least-squares means adjusted for multiplicity and covariates with 95% confidence intervals

Table 4.1. Mean ± SD values for CT_{max}, $\dot{V}CO_2$ ml h^{-1}, thermal sensitivity, intercept, brood ball production, brood ball burial depth, and brood ball volume, for each of the four treatment temperatures; control +0°C, +2°C, +3.5°C ,and +5°C.

	Treatment			
	+0°C	**+2°C**	**+3.5°C**	**+5°C**
CT_{max} (°C)[1]	46.43±1.68	45.93±1.89	45.94±1.67	45.77±1.48
$\dot{V}CO_2$ ml h^{-1}(2)	32.28±8.84	28.02±8.93	30.34±10.01	30.63±11.35
Thermal Sensitivity[3]	2.51±0.63	2.43±0.67	2.34±0.62	2.44±1.08
Intercept[4]	-0.13±0.41	-0.13±0.45	-0.03±0.42	-0.02±0.63
Brood ball production[5]	44.92±27.96	39.28±20.42	41.86±23.08	45.13±23.8
Brood ball burial depth (cm)[6]	9.12±7.02	9.43±5.34	9.99±5.83	9.09±5.37
Brood ball volume (cm^3)[7]	4.61±1.17	4.32±1.18	4.62±1.34	4.47±1.18

[1] CT_{max} is the temperature at which spiracular activity ceases,

[2] $\dot{V}CO_2$ ml h^{-1} is the volume of CO_2 ml^{-1} released prior to reaching CT_{max},

[3] Thermal Sensitivity is the sensitivity of the rate of CO_2 ml^{-h} to changes in temperature from 25°C to CT_{min} or CT_{max}.

[4] The intercept of the regression line for $\dot{V}CO_2$ ml h^{-1}

[5] Number of brood balls produced per chamber per 10 days

[6] Depth at which brood ball were buried below the dung

[7] The cylindrical volume of brood balls calculated using x, y, z measurements and assuming an ellipsoid shape

All values are the untransformed mean ± SD.

4.5 – Discussion

In this study we examined the plasticity of *O. binodis'* thermal biology and nesting behaviour under four warming regimes in order to determine the effects of increased temperatures as a result of climate change. Despite producing several thousand broods an insufficient number of adult beetles emerged to fully replicate the study, therefore study of the second generation took place with only 20 beetles per chamber. A factor that may have played a role in the low number of 2nd generation beetles emerging is the use of dung from dairy cattle whose diet had been supplemented by grains. *Onthophagus binodis* larvae from broods constructed from pasture fed cattle dung have greater survivability and greater mass than those raised on grain fed dung (Dadour and Cook 1996). Furthermore, a greater percentage of broods buried by *O. binodis* using grain fed dung did not contain egg chambers (Dadour and Cook 1996). This confounds our results as brood production cannot be directly extrapolated to fecundity in this case. We considered the use of this dung preferable to that of pasture fed cattle as it ensured that all dung was of a similar nutritional content, being collected on the same day, and we could be sure that the dung was free from chemical contaminants. There was difficulty finding pasture fed cattle where a sufficient volume of dung could be collected over such a short time and where the cattle had not been drenched. It is unknown whether chamberless "broods" are food balls or incomplete/failed broods, even if many of the brood balls produced were food balls rather than true brood balls, changes in brood abundance, burial depth, and volume have implications for the provision of ecosystem function.

4.5.1 – Thermal physiology

The mean CT_{max} of *O. binodis* in this study was 46.06°C ±1.67, similar to the CT_{max} reported for insects in general, which display minor geographic variation and tends to be similar regardless of taxonomic group (Addo-Bediako, Chown et al. 2000). Despite thermal ecology playing a large role in structuring dung beetle communities, with species utilising thermal niches to minimise competition, few species have had their thermal tolerances directly assessed. Four species of tropical dung beetle

Dichotomius annae (39.3°C), *D. satanas* (37.5°C), *D. colonicus* (41.3°C), and *Phanaeus quadridens* (42.1°C) have slightly lower CT_{max} than that of *O. binodis* or the temperate *Canthon imitator* (42.8°C) (Sheldon and Tewksbury 2014). Species from similar temperate climates, such as *Jekelius hernandezi* and *J. punctatolineatus* from the warm-temperate south-eastern Iberian peninsula (38°61'N, -0°48'W), had CT_{max} of 44.69°C and 47.19°C respectively (Gallego, Verdú et al. 2016), while three endocoprid Aphodiine species from the United Kingdom (54°42'N, 2°29' W) had CT_{max} ranging from 41.2°C-44.4°C (Birkett, Blackburn et al. 2018). The elevational range of dung beetle species is related to their thermal tolerances; species with broader thermal windows occupy larger elevational ranges (Chown and Gaston 1999), however, it appears that for some species this increase in range is not strictly due to thermal conditions placing greater constraints on less tolerant species as the CT_{max} of *O. binodis* far exceeds the highest recorded air temperature in the Armidale region (37.1°C) (Bureau of Meteorology 2017).

Onthophagus binodis displayed no plasticity of thermal biology when subjected to temperature increases. The lack of plasticity of *O. binodis'* thermal biology may therefore be a result of its lifestyle as it experiences a highly variable thermal environment foraging on the wing, experiencing air temperatures, but spends significant time feeding and reproducing in the dung and underground where temperatures are more stable. This behaviour allows the beetle to feed and thermoregulate simultaneously, invoking a lower evolutionary cost than physiological acclimation (Snell-Rood, Van Dyken et al. 2010).

Low temperature tolerances often display greater plasticity than upper thermal tolerances (Chown and Terblanche 2006, Sgrò, Overgaard et al. 2010, Tattersall, Sinclair et al. 2012). Crickets acclimated at 33°C took longer to recover from a chill coma than those at 25°C or 29°C, however there was no difference in heat knockdown time. Metabolic rate increased when acclimated at 25°C compared to 29°C or 33°C, as did its sensitivity to temperature (Lachenicht, Clusella-Trullas et al. 2010). Plastic responses of metabolic rate to low temperatures, rather than high, have also been observed in species of *Glossina* (Chown and Terblanche 2006, Irlich, M et al. 2009). The acclimation temperatures used in our study may therefore have been too high to provoke a plastic response in *O. binodis*; alternatively there may have been an unmeasured effect on the beetle's physiology at low temperatures. In chapter three we provided evidence that maximum lowest temperature, rather than highest temperature, is a better indicator of dung beetle community composition and distribution, as environmental temperatures rarely approach CT_{max}. The breadth of a species' thermal performance curve was the best indicator of the distribution of European diving beetle species, with acclimatory capacity unrelated to range size (Sheldon and Tewksbury 2014).

The South African dung beetle *Scarabaeus spretus* displays a similar increase in metabolic rate when acclimated at low temperatures, although this appears to be a mechanism to conserve water in xeric environments rather than to provide a competitive advantage (Stevens, Jackson et al. 2010). *Scarabaeus galenus* and *S. westwoodi* were both shown to have reduced metabolic rates when acclimated to winter temperatures, however the metabolic rate temperature relationship remained unchanged (Davis, Chown et al. 2000). The thermal sensitivity of *O. binodis* was similar to that reported by Davis, Chown et al. (2000) who observed Q10s between 2.14-2.84 for six species of *Scarabaeus*. *Scarabaeus* are telecoprids, creating balls of dung and rolling them away from the source, rather than burying them in the soil below the dung like *O. binodis.* Whilst moving its brood ball it is directly exposed to the elements and is at a higher risk of thermal stress and water loss than tunnelling species.

The CT_{max} and metabolic rate of *O. binodis* changed significantly with time, decreasing and increasing respectively. In insects, age can influence thermal tolerance in several ways: A linear decline in CT_{max} with age; a non-linear decline; or no effect (Bowler and Terblanche 2008). Metabolic rate-temperature relationship was found to be highly invariant in the Tsetse fly *Glossina morsitans morsitans* across a range of acclimation temperatures and ages (Terblanche, Sinclair et al. 2005). Metabolic rate also increased due to age in these flies.

4.5.2 – Brood Balls

Nest building and brood ball provisioning behaviour in tunnelling dung beetles is highly plastic with beetles responding to multiple factors. *Onthophagus taurus* has been observed to alter the size of its brood balls in response to soil moisture, producing heavier brood balls in drier soils (Hunt and Simmons 2004). Along with *O. taurus*, *O. atripennis* (Kishi and Nishida 2006) produces lighter brood balls when supplied with dung of higher nutritional quality (Moczek 1999, Hunt and Simmons 2004). The amount of nutrition supplied to the larvae before pupation is the primary determinant of morph, as male beetles only expresses the major horned phenotype upon crossing a critical weight threshold whilst a larvae. The weight threshold at which a male develops a horn is itself a plastic trait (Snell-Rood, Van Dyken et al. 2010). *Euoniticellus intermedius* alter their nesting behaviour by creating dense clumps of brood balls when soil moisture is low (Barkhouse and Ridsdill-Smith 1986). Many aspects of nesting and brood production have rapidly diverged between recently isolated populations. We found the brood balls produced had a mean length of 30.4mm, a mean y diameter of 17.52mm and a mean z diameter of 15.91mm. These results are similar to those reported for a population of *O. binodis* from Western Australia by Smith, Hall et al. (1982) who measured a mean length of 37mm and diameter of 17mm.

The generation of *O. binodis* caught from the field did not alter the number of brood balls they produced in response to warming, however beetles from the lab reared second generation did produce more brood balls. This may be an artefact of the small sample size rather than a biological effect or the shortened duration of the experiment for the second generation. It has been suggested that the frequency of contact between adult *O. binodis* may invoke a density response allowing them to determine the level of competition prior to nesting as adult density (Smith, Hall et al. 1982). As the number of beetles per chamber was halved for the second generation contact between individuals was less likely, the significant effect of temperature on the second generation may indicate that increased brood production as a result of warming is mediated by the density of adult beetles within the chamber. The amount of dung available to mating pairs for brood construction, synonymous with beetle density in this case, was likely the limiting factor in brood production for the field generation. Even when brood production was at its highest there was ample room in the chambers for additional broods and we observed individual brood burial depths of 35cm so beetles were capable of utilising this free space, therefore space was not the limiting factor. As the number of beetles per pat increases competition for dung becomes more intense, reducing brood ball production. When supplied with 1-litre of cattle dung brood ball production of *O. binodis* peaks at densities of 20-30 beetles (Smith, Hall et al. 1982). When running the experiment with the second generation beetle density was halved from 40 to 20 individuals. Despite the reduced number of beetles in each chamber second generation beetles under the +5°C treatment produced almost as many broods as the first generation. While beetles from the +5°C treatment created slightly larger broods most of the time, when the number of broods produced by the second generation was at its peak beetles from this treatment produced among the smallest broods, with +0°C and +3.5°C treatments producing the largest. This pattern mirrors that of brood production for the second generation. As the number of broods being constructed increases the size of individual brood balls decreases. If this effect is due to an increased proportion of incomplete brood balls than this itself is a result of increased temperature as it is unlikely to be a response to increased competition within chambers as adult density has been found to have no effect on the number of incomplete broods or ovipositing (Smith, Hall et al. 1982). That the number of broods produced was not a significant indicator of burial depth suggests that burial depth is due to beetle density rather than a result of lack of space for brood burial.

Burial depth is similarly labile, with Western Australian populations burying brood ball at a shallower depth than populations from the Italy and Indiana. Alongside this change was a parallel divergence of tibial shape indicating that this is not simply a plastic response to environmental conditions but is representative of extremely rapid adaptive evolution (~100 generations), driven by developmental plasticity (Macagno, Moczek et al. 2016). When desiccation rates are high dung beetles are more likely to parasitise the brood balls of conspecifics, utilising them as a food source. Females are also more likely to parasitise the broods of other females as the availability of above-ground dung decreases (Beckers, Anderson et al. 2015). When beetle density, and therefore competition, is high beetles bury broods deeper to reduce the risk of them being encountered as brood balls represent a costly investment in terms of time and energy, as a result of this provisioning dung beetles have among the lowest fecundities of all insects (Simmons and Ridsdill-Smith 2011).

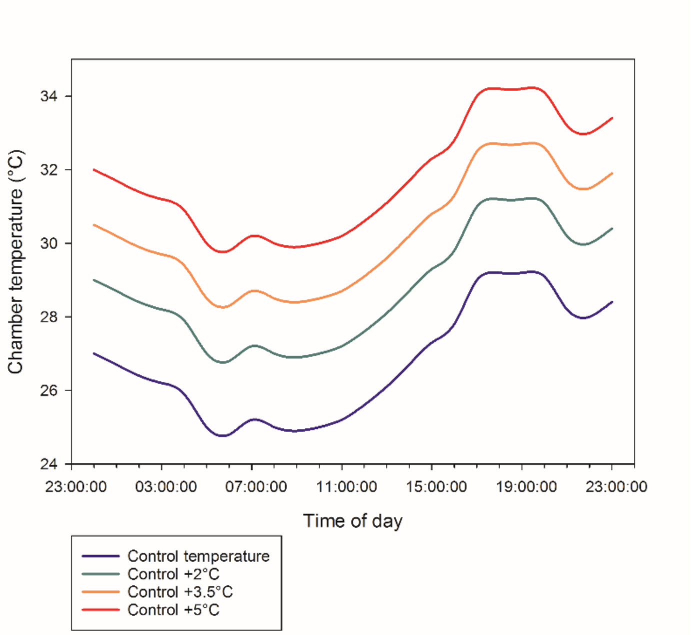
Temperature is known to have an effect on the production of brood and food balls in *O. australis*. The beetle does not produce brood balls below 15°C but dung is still buried in the form of food balls while brood ball production at 20°C and 25°C is identical but females at 25°C produced twice the number of food balls. Individuals at 30°C did not produce broods and died within five weeks (Tyndale-Biscoe and Walker 1992). From this we can infer the *O. binodis* is better suited to warmer environments than the native *O. australis* as the minimum temperature experienced in the +5°C treatment was 29.8°C. At this offset the field generation survived for over 50 days with no effect of temperature on brood production, whereas in the second generation brood ball production was highest at +5°C. This preference for warmer climates is also reflected in the thermal tolerances of the two species' with *O. binodis* being more tolerant of high temperature than *O. australis* but less tolerant of cold temperatures. It may be that increases in temperature of as little as 2°C increase the temporal breadth of *O. binodis'* optimal thermal window, increasing the number of productive hours available to the beetle, without changes to its underlying thermal physiology.

4.5.3 – Conclusions

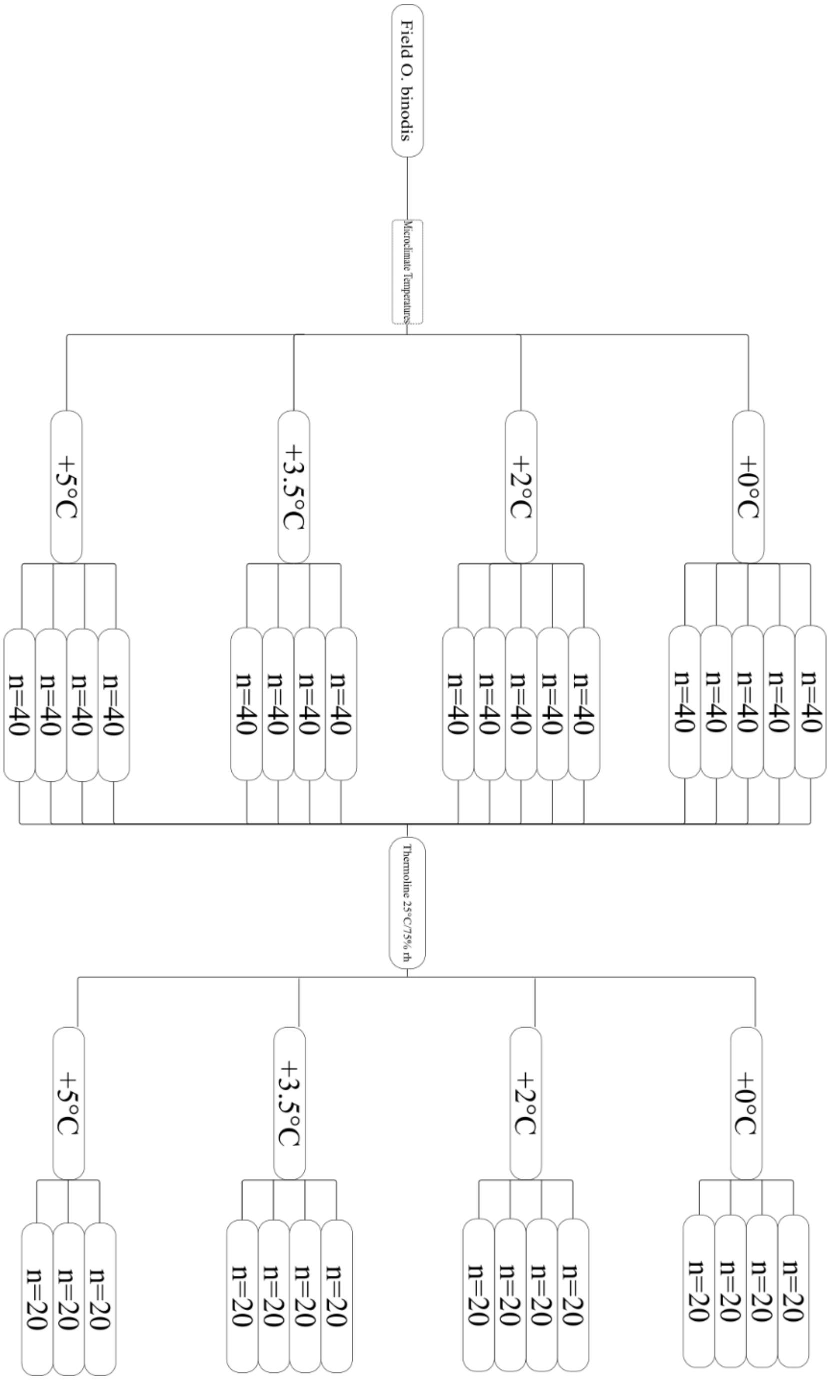
We did not detect a significant effect of warming on the CT_{max}, metabolic rate or its sensitivity to temperature indicating that *O. binodis* does not have a plastic thermal physiology, however their high CT_{max} relative to their environment and their ability to utilise behavioural thermoregulation means that beetles in temperate environments are unlikely to directly influence population of *O. binodis*. Populations in warmer areas may be more affected as they approach temperatures where beetles are physiologically undamaged but thermally stressed, thereby altering inter-species interactions. Changes to nesting behaviour may have implications for the provision of ecosystem function; however more research is required into the ecosystem services provided by dung beetles in Australia as little is known concerning their effect on soil nutrients, plant growth, and seed dispersal. While we detected few effects of increased temperature on *O. binodis,* and indeed it may be the case that there were no direct effects, we cannot be sure that global warming will not affect them in other ways. Due to our experimental design beetles were contained within a chamber for the entire duration of the study. In the field warming may alter emigration and dispersal rates that we could not measure. There are also implications for competition with other species and the effects of warming on community structure. Dung beetle community structure is heavily influenced by temperature, with species utilising thermal partitioning to avoid competition (Krell, Krell-Westerwalbesloh et al. 2003). In temperate climates such as that of Armidale, where thermal tolerances exceed temperatures, the effects of climate change on *O. binodis* will be indirect as changes in minimum temperatures and extreme events alter its foraging window, potentially bringing it into competition with other species.

Phenotypic plasticity is suggested to favour highly variable environments as it reduces variance in offspring fitness from one generation to the next (Snell-Rood, Van Dyken et al. 2010). Paracoprids have among the most complex and unique reproductive behaviour and life-history of any insect group. The phenotypic plasticity of a number of dung beetle traits has been explored while the potential effects of developmental plasticity largely overlooked. It likely that *O. binodis* will adapt to increased temperatures via behavioural rather than physiological plasticity and there is evidence that developmental plasticity plays an important role in the ability of populations to adapt to new environments. This emphasises the need to incorporate behavioural adaptation and plasticity when considering relative vulnerability to climate change and how these changes will affect community interactions.

4.6 – Appendix



Appendix 4.1. Thermal regime of the four treatments applied to *O. binodis*. Temperature regimes are based on soil temperature data gathered from the site of collection. Treatments are the baseline regime +0°C, +2°C, +3.5°C, and +5°C.



Appendix 4.2. Experimental design. *Onthophagus binodis* were collected from UNE SMARTFARM, Kirby, near Armidale. Soil temperature data from the farm was used to construct a thermal regime similar to what the beetle experience in the wild with the addition of temperature offset to simulate climate change.

Appendix 4.3. Differences of least squares means for the intercept of $\dot{V}CO_2$ ml h^{-1} at 10 day intervals among a field caught generation, and lab raised generation, of *Onthophagus binodis*. Significance levels are shown pre and post-adjustment for multiple comparisons Tukey Kramer method

generation	**Day**	**_generation**	**_Day**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Adj P**	**Alpha**	**Lower**	**Upper**
lab	10	lab	20	-0 4543	0 4024	28 92	-1 13	0 2681	0 9448	0 05	-1 2774	0 3687
lab	10	lab	30	-0 4899	0 3741	28 37	-1 31	0 2009	0 8878	0 05	-1 2558	0 2760
lab	10	field	10	0 09106	0 3910	28 74	0 23	0 8175	1 0000	0 05	-0 7089	0 8911
lab	10	field	20	0 09671	0 3893	27 75	0 25	0 8056	1 0000	0 05	-0 7010	0 8944
lab	10	field	30	0 2028	0 3865	28 39	0 52	0 6038	0 9994	0 05	-0 5883	0 9940
lab	10	field	50	-0 3395	0 3822	27 33	-0 89	0 3820	0 9847	0 05	-1 1232	0 4441
lab	10	field	40	-0 6246	0 3848	27 8	-1 62	0 1159	0 7331	0 05	-1 4131	0 1640
lab	20	lab	30	-0 03553	0 2983	24 63	-0 12	0 9062	1 0000	0 05	-0 6504	0 5794
lab	20	field	10	0 5454	0 3189	24 48	1 71	0 0999	0 6811	0 05	-0 1121	1 2029
lab	20	field	20	0 5511	0 3233	25 21	1 70	0 1006	0 6846	0 05	-0 1146	1 2167
lab	20	field	30	0 6572	0 3137	24 12	2 10	0 0468	0 4453	0 05	0 009950	1 3044
lab	20	field	50	0 1148	0 3155	25 21	0 36	0 7190	0 9999	0 05	-0 5348	0 7644
lab	20	field	40	-0 1702	0 3174	25 27	-0 54	0 5965	0 9993	0 05	-0 8236	0 4832
lab	30	field	10	0 5809	0 2773	23 44	2 09	0 0472	0 4461	0 05	0 007871	1 1540
lab	30	field	20	0 5866	0 2781	23 83	2 11	0 0456	0 4372	0 05	0 01247	1 1607
lab	30	field	30	0 6927	0 2715	23	2 55	0 0179	0 2241	0 05	0 1310	1 2544
lab	30	field	50	0 1503	0 2687	22 93	0 56	0 5813	0 9991	0 05	-0 4056	0 7063
lab	30	field	40	-0 1347	0 2755	24 32	-0 49	0 6293	0 9996	0 05	-0 7029	0 4335
field	10	field	20	0 005651	0 2717	23 55	0 02	0 9836	1 0000	0 05	-0 5557	0 5670
field	10	field	30	0 1118	0 2666	23 15	0 42	0 6788	0 9999	0 05	-0 4394	0 6630
field	10	field	50	-0 4306	0 2662	23 62	-1 62	0 1191	0 7359	0 05	-0 9805	0 1193
field	10	field	40	-0 7156	0 2730	24 91	-2 62	0 0147	0 1950	0 05	-1 2779	-0 1533
field	20	field	30	0 1061	0 2668	23 45	0 40	0 6944	0 9999	0 05	-0 4452	0 6575
field	20	field	50	-0 4363	0 2590	22 39	-1 68	0 1060	0 6966	0 05	-0 9729	0 1004
field	20	field	40	-0 7213	0 2658	22 71	-2 71	0 0125	0 1691	0 05	-1 2716	-0 1710
field	30	field	50	-0 5424	0 2520	22 06	-2 15	0 0426	0 4150	0 05	-1 0649	-0 01981
field	30	field	40	-0 8274	0 2581	22 52	-3 21	0 0040	0 0650	0 05	-1 3620	-0 2928
field	50	field	40	-0 2850	0 2586	23 04	-1 10	0 2817	0 9498	0 05	-0 8198	0 2498

Appendix 4.4. Differences of least squares means of brood ball burial depth of *Onthophagus binodis* subjected to one of four warming treatments; +0°C control, +2°C, +3.5°C, and +5°C. Significance levels are shown pre and post-adjustment for multiple comparisons Tukey Kramer method

treatment	**_treatment**	**Estimate**	**Standard Error**	**DF**	**t Value**	**Pr > |t|**	**Adj P**	**Alpha**	**Lower**	**Upper**	**Adj Lower**	**Adj Upper**
+0	+2	0 08273	0 03194	43 63	2 59	0 0130	0 0603	0 05	0 01835	0 1471	-0 00257	0 1680
+0	+3 5	0 05538	0 03310	40 94	1 67	0 1019	0 3507	0 05	-0 01147	0 1222	-0 03325	0 1440
+0	+5	0 08763	0 03346	42 76	2 62	0 0122	0 0567	0 05	0 02014	0 1551	-0 00181	0 1771
+2	+3 5	-0 02735	0 03195	43 09	-0 86	0 3966	0 8271	0 05	-0 09178	0 03707	-0 1127	0 05801
+2	+5	0 004898	0 03062	40 31	0 16	0 8737	0 9985	0 05	-0 05698	0 06678	-0 07716	0 08695
+3 5	+5	0 03225	0 03456	46 97	0 93	0 3555	0 7872	0 05	-0 03728	0 1018	-0 05980	0 1243

CHAPTER FIVE

GENERAL DISCUSSION

Despite the pivotal role temperature has played in shaping the diversity and structure of dung beetle communities their thermal physiology is still poorly understood. As seen in chapter two the thermal biology and ecology of dung beetles in Africa, Mexico, and Europe has received some attention however little work has been conducted on Australian dung beetles. On a global scale physiological data for the group is lacking but there is a particular dearth of information on important thermal traits such as thermal tolerances and metabolic rate. Data on these traits is critical if we are to understand the response of dung beetles to climate change. The work presented in chapter three and four of this thesis are the first steps to filling many of these knowledge gaps. In chapter three we conducted a comprehensive comparison of the thermal physiology of seven species of dung beetle, a substantial addition to the current number of species for which similar data is available (Table 3.3). This is also among the first papers to use respirometry to examine the physiology of an entire community and the first to provide such data on Australia's native species. Using thermolimit respirometry in this way allowed for a close examination of physiological responses under thermal conditions relevant to those experienced in the field and provided physiological evidence for hypotheses put forward in the literature, primarily that endocoprids are better adapted to heterogeneous environments as a result of their uncompetitive reproductive strategy, and that telecoprids prefer warmer habitats to boost their metabolic rate and increase performance (Krell, Krell-Westerwalbesloh et al. 2003).

There is generally considered to be a trade-off between maximum performance and the breadth of a thermal performance curve, with thermal generalists being capable of moderate performance over a wide range of temperature while thermal specialists maximise their performance during narrow thermal windows (Verberk, Bartolini et al. 2016). We found evidence for the evolution of both strategies in a cool-temperate dung beetle community. *Aphodius fimetarius,* and potentially *O. granulatus*, had metabolic rates that were both relatively high and insensitive to thermal variation at both high and low temperatures, indicative of a thermal generalist. Thermal generalism is beneficial in thermally heterogeneous environments, where temperatures can change rapidly. We found evidence for thermal specialisation in two species of *Euoniticellus* and the telecoprid *Sisyphus rubrus*. These species were sensitive to low temperatures, but also had low metabolic rates, indicating a sharp drop in performance as the temperature drops. Alternatively at high temperature they displayed high metabolic rates that were sensitive to temperature, indicating a sudden increase in performance as temperatures increase. Given the effects of temperature on the activity patterns, habitat associations (Krell, Krell-Westerwalbesloh et al. 2003, Krell-Westerwalbesloh, Krell et al. 2004, Verdú, Arellano et al. 2007) and ball rolling speed (Bartholomew and Heinrich 1978) of telecoprids we would expect them to have a physiology suited towards warmer climates, our observations match this. *Sisyphus rubrus* displayed the highest CT_{max} and metabolic rate of the seven species.

There was little evidence of thermal niche partitioning in paracoprids within genera but there appears to be partitioning among genera. Species of *Onthophagus* displayed similar thermal traits at low temperatures but at high temperatures *O. granulatus* had a significantly higher metabolic rate with a lower slope than *O. binodis* and *O. australis*, suggesting it may be a thermal generalist whose spatial and temporal distribution is more constrained by the presence of more dominant species than temperature per se. *Euoniticellus intermedius* and *E. fulvus* also displayed remarkably similar thermal physiologies, performing worse than all other species at low temperatures, and having similar performance to *S. rubrus* at high temperatures. This begs the question as to what drives the difference in distribution of these two species. A potential answer could be an acclimation response to warm temperatures that affects metabolic water loss as dung beetles are limited by water availability (Davis, Chown et al. 2000). This may be part of a suite of adaptations to xeric environments, along with behavioural plasticity in nest structure.

Consistent with the literature we found that minimum temperature is likely the most important thermal metric for dung beetle community structure at the local scale (Lumaret and Jay-Robert 2002). Most species studied had high thermal tolerances, therefore while increases in maximum temperature may prove advantageous to *Euoniticellus* and *Sisyphus*, warm adapted species, increases in minimum temperature has the potential to influence all species. Should temperatures rise then *Aphodius*'s foraging window will narrow, and environmental temperatures may rise such that *Euoniticellus* species are active and able to compete with *Onthophagus* species that perform better in the cold, having CT_{min} 3°C -4°C lower. This may result in native species being pushed further into closed habitats as temperature become suitable for more dominant species, bringing them into competition with other native species, of which little is known, and threaten ecosystem services they may provide (Derhé, Murphy et al. 2016). Knowledge of the ecosystems services provided by dung beetles in Australia is also an important area that needs further research, particularly the services provided by native species. Secondary seed dispersal is potential function that native beetles could supply that introduced beetles would not fill. Marsupial dung is hard and pelletised, unattractive to introduced dung beetles, however seeds have been found in the dung of possums (Rouco and Norbury 2013) and native cassowaries (Stocker and Irvine 1983). Given the threat posed to native beetles by climate change, and Australia's reliance on introduced dung beetles, a decision might have to be made on the value and reality of conserving Australia's native fauna.

While we found no evidence for physiological plasticity in *O. binodis* in chapter four, the acclimation temperatures I used only covered a +5°C range, a much narrower range than used in most acclimation studies, however these represent a realistic degree of warming. While climatic variability is also a concern, acclimation responses generally take place over the course of days rather than hours, and would therefore require an extended period of temperatures above the 5°C increase we tested. While *O. binodis* did not display an acclimation response to short term (~2 months) increases in temperature I only tested individuals from a single population.

Onthophagine beetles appear to be evolutionarily labile (Macagno, Moczek et al. 2016) and high inter-population variability has been observed in the thermal response of other dung beetle species (Gallego, Verdú et al. 2017). It may be the case that *Onthophagus binodis* and similar species may respond to climate change via long-term evolutionary processes rather than plasticity. A comparative analysis of *O. binodis* populations across the globe would prove extremely illuminating and would aid in describing the role of temperature as an evolutionary pressure. An important benefit of the experimental design used in chapter 4 was that it allowed me to test the effect of climate change on the level of ecosystem function, as many of the ecosystems functions provided by dung beetles are a result of their burial of dung. Examining the effect of climate change on the provision of ecosystem function

Thermolimit respirometry was a key technique utilised in chapters three and four of this thesis. Thermolimit respirometry allowed for the simultaneous measurement of multiple thermal traits which are relevant to both ecologists and physiologists. Thermolimit respirometry differs from traditional respirometry in that the metabolic rate of the individual is measured while it is undergoing increasing thermal stress. This gradual change in temperature more closely replicates ambient temperature changes experienced by beetles in the field. This allows us to see the metabolic response of an individual in real-time as well as providing data on thermal tolerances. The most notable caveat is that the individual tested dies, therefore repeated measurements cannot be performed on the same individual somewhat limiting experimental design. A further disadvantage of thermolimit respirometry is that it is far harder to control for increases in metabolic rate as a result of insect activity. In traditional respirometry individuals can be placed at a static temperature and data recorded for as long as required. Metabolic data from periods of inactivity can then be extracted to provide information on the standard metabolic rate at that temperature. Should the animal display excessive activity during thermolimit respirometry then the metabolic data for that individual will be inflated. This means that individuals displaying excessive activity must be removed from any analysis of metabolic rate or thermal sensitivity. A silver-lining to this is that the thermal tolerance data from these individuals is still usable.

Between an organisms upper and lower thermal tolerances there are a range of temperatures that are stressful and lower performance, but are not lethal. Thermolimit respirometry can provide insight into the sub-lethal effects of temperature on performance. By looking for changes in the respiration pattern of the individual the temperature at which it begins to display a stress response can be pinpointed. Utilising this technique on a dung beetle community would provide valuable information whether the structure of communities or the daily activity patterns observed in the field are the result of interspecies differences in these stress points.

To put the research conducted here into practice the next logical step is to incorporate the physiological data gathered here into mechanistic models, both to verify the accuracy and validity of such models, and to better understand how both biotic and abiotic factors influence the ecology of dung beetles. The obvious candidate species is *Onthophagus binodis* as we have the most complete picture of its thermal biology. Unfortunately we were unable to gather data on its plastic response to low temperatures due to logistical constraints. The dung beetle *Scarabaeous spretus* displayed a plastic response by increasing its metabolic rate when acclimated to 15°C compared to 25°C (Terblanche, Clusella-Trullas et al. 2010). *Onthophagus binodis* distribution is primarily constrained by rainfall, competition from other dung beetles, and minimum environmental temperatures, as they have a CT_{max} significantly higher than the maximum temperature in their environment. Should they display a similar acclimation response to low temperatures then warming due to climate change may result in increased activity at places and times that were historically unsuitable, however Terblanche, Clusella-Trullas et al. (2010) think it unlikely that South African beetles, such as *O. binodis*, should display such response as it appears to have evolved as a mechanism to conserve water in hot environments rather than to increase performance at low temperatures.

Numerous factors influence the behaviour and ecology of dung beetles and teasing out the effects of temperature on communities will require extensive field and laboratory work, focusing on multi-species responses, and studying temperature-dependent species interactions (Slade, Kirwan et al. 2017). The custom temperature chambers from chapter three provide the ideal opportunity for such a study. Briefly, ambient temperature and activity of dung beetle species could be surveyed throughout the day to determine their thermal window (Giménez Gómez, Lomáscolo et al. 2017). Species could then be placed in chambers set to span a range of temperatures based on either programmed temperature regimes or dynamic temperature regimes based off of real-time temperature data. Dung burial and emigration rates would make good measures of performance, with respirometry providing a complete picture of their thermal biology at each acclimation temperature. A confounding factor in such a study would be the effect of density mediated effects of temperature as suggested by differences in brood ball production between the first and second generation of *O. binodis* in chapter three.

In conclusion we have demonstrated the first use of thermal limit respirometry to measure the thermal traits of a dung beetle assemblage composed of the three primary functional groups present in dung beetles as well as the acclimatory capacity of an ecologically important and widespread paracoprid. Consistent with past literature the species studied here displayed a variety of physiological responses to temperature indicative of thermal niche partitioning seen in other communities. While increases in mean and maximum temperature will favour thermal specialists such as *E. fulvus*, *E. intermedius*, and *S. rubrus* changes in minimum environmental temperatures will effect spatio-temporal patterns of activity in the majority of species, altering interactions and community dynamics. This must be taken into account when determining the response of species to climate change as well as density and community mediated responses to such changes.

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