



Dung beetles' responses to changing environments; implications on ecosystem service delivery

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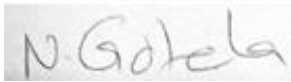
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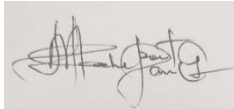
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Dedication

I dedicate this thesis to my children, for having endured my absence during my fieldwork trips, hospitalisations and long hours of work. I love you guys.

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Abstract

Dung beetles are coprophagous insects belonging to the families Scarabaeidae and Geotrupidae. They are a very important insects' group, acting as significant ecosystem engineers through dung burial, decomposition, nutrient cycling, bioturbation and seed dispersal in natural and human managed systems. Despite these benefits, most essential ecosystem services are not economically quantified and as such, their roles as natural capital in natural and human managed systems have largely been neglected. To date, the global climate change and anthropogenic activities such as the widespread use of synthetic pesticides for livestock endo- and ectoparasites, has largely led to loss of or shifts in some the biodiversity and richness of this natural capital. It has been hypothesised that in order to determine a suitable time for activity, insects may monitor environmental variables such as light, temperature and diel events such as sunrise and sunset. However, information on how environmental stress resistance may influence diel activity times and ecosystem services provision remains scant, especially on dung beetles in arid, tropical environments such as Botswana that are more vulnerable to the impacts of climate change and associated anthropogenic activities. This work is therefore aimed at investigating i) the diversity and activity patterns of dung beetle species in Botswana (ii) the effects of temperature (and adaptation mechanisms thereof) as an environmental variable in influencing activity times and (iii) implications on ecological services provision. This study found that Botswana is richly abundant with Scarabaeinae dung beetles belonging to 8 tribes namely, Canthonini, Coprini, Dichotomini, Gymnopleurini, Oniticellini, Onitini, Onthophagini and Carabaeini. Most of the species belong to the Onthophagini tribe, and this tribe comprised the most abundant species. Coprini and Scarabaeini were however the least abundant tribes. Furthermore, the study also found that small-bodied beetles from the Gymnopleurini tribe such as *Allogymnopleurus indicageous*, *Gymnopleurus aenescens* and *Gymnopleurus ignitus* preferred diurnal activity, compared to large-bodied beetles from the Coprini tribe (*Copris elephenor* and *Catharsius calaharis*), and the Scarabaeini tribe (*Scarabaeus zambeianus*, *Scarabaeus goryi*) which preferred crepuscular/nocturnal activity (Chapter 2). Second, an investigation between activity time and temperature tolerance found that day active dung beetle species generally had higher maximum temperatures for activity measured as critical thermal maxima (CT_{max}) than crepuscular and nocturnal species. This implies that heat may significantly play a role in choice of diel activity time in dung beetle species by constraining some species to be active at specific period of the day corresponding to their thermal limitations. However, there was no clear correlation between body mass and CT_{max} suggesting complex associations across species, body mass and heat tolerance that warrants further investigation (Chapter 3). Third, this study revealed that tropical dung beetles' physiological and ecological activities were generally negatively affected by low temperatures. Results showed that low temperature stress may offset dung beetles' ecological services through reduction in dung removal. Dung ball creation between diurnal and nocturnal species interacted with temperature, with diurnal species producing significantly fewer balls at low temperatures, whilst nocturnal beetles were not significantly affected (Chapter 4). Fourth, based on the trade-off theory, plastic responses to variable high (VT-H) and variable low temperatures (VT-L) were investigated in a day active dung beetle, *Allogymnopleurus thalassinus*. Results showed that effects of acclimation were significant for heat tolerance, significantly increasing and reducing CT_{max} values for variable temperature high (VT-H) and variable temperature low (VT-L) respectively. Similarly, effects of acclimation on HKDT were significant, with variable temperature high significantly increasing HKDT, while variable temperature low reduced HKDT. In addition, effects of acclimation on ecological traits showed that beetles acclimated to variable high temperatures were ecologically more efficient in their ecosystem function (dung removal) compared to those acclimated at variable low temperatures. *Allogymnopleurus thalassinus* nevertheless, had low acclimation response ratios, signifying

limited scope for complete plasticity for ULTs tested here. This result supports the 'trade-off' theory, and that observed limited plasticity may unlikely buffer *A. thalassinus* against effects of climate change, and by extension, albeit with caveats to other tropical ecological service providing insect species (Chapter 5). Fifth, functional responses experiments were conducted to help quantify the effects of increasing mean temperatures consequence of climate change and dung beetle species density on dung removal, an essential ecosystem service on three telecoprid species: *Allogymnopleurus indigaceus*, *Scarabaeus zambezianus* and *Khepher prodigiosus* (Chapter 6). Results showed that *K. prodigiosus*, exhibited greatest dung utilisation efficiency overall across dung masses, compared to the other two species. Nevertheless, both *S. zambezianus* and *A. indigaceus* utilization increased with both warming and beetle density, whereas *K. prodigiosus* performance was less temperature and density-dependent. Results on dung ball number differed across species and correlated positively with temperature and densities, with *S. zambezianus* producing significantly most balls overall. These results show that temperature and competition largely have a significant effect on dung beetle species' fitness and thus functional efficiency and ecosystem service delivery. This work provides insights on the survival mechanisms of tropical species against environmental stress and provides a framework for the conservation of these natural capital species that inhabit arid environments under rapidly changing environmental climate. Such studies are critical in serving as early warning systems for predicting the potential effects of climate change on biodiversity and fore warning against threats to the integrity of ecosystems and their essential services. Incorporation of such scientific information in conservation policies may help safeguard this neglected but essential natural capital and consequently ecological services provision in the future.

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Chapter 1

General Introduction and literature review

1.1. Biology and ecology of dung beetles

1.1.1. Life cycle of dung beetles

Dung beetles are insects from the order Coleoptera, families Scarabaeinae and Geotrupidae. The subfamily Scarabaeinae consists of coprophagous beetles which rely on mammalian dung for feeding and nesting (Raine *et al.*, 2018). They are the most studied due to their wide global distribution, the variety of their ecological services to the ecosystem (reviewed in Nichols *et al.*, 2008; Scholtz *et al.*, 2009), cost effectiveness for surveying (Gardener *et al.*, 2008), ease and rapidness of sampling e.g. using pitfall traps, and their sensitivity to anthropogenic activities (Nichols *et al.*, 2007). Dung beetles follow a holometabolous type of development, comprising eggs, larvae, pupa and adult. The life cycle of a dung beetle begins with an individual digging out of the soil to find a fresh dung pat resource. Subsequently, male and female pairs mate and the female lays eggs.

Dung beetles exhibit three types of nesting behaviour: endocoprid, paracoprid and telecoprid (Hanski & Cambefort, 1991). Endocoprids line and lay their eggs directly in the dung pat, and are often referred to as dwellers. These eggs are usually at risk of desiccation as well as predators. Paracoprids on the other hand dig tunnels beneath the dung pad and place their dung balls underneath. While some beetles dig one straight deep tunnel into the ground, others make branched channels where they put one dung ball in each channel. Each tunnel may consist of one or multiple nest balls. As such, this group of dung beetles is often referred to as tunnelers. Telocoprids make nest ball which they then roll away from site and bury it underneath the soil (Hanski & Cambefort, 1991). The ball rolling behavior is suggested to have evolved as a way of avoiding competition from tunnellers and dwellers (Scholtz *et al.*, 2009; Simmons & Ridsdill-Smith, 2011). Thus, this group of dung beetles is also referred to as rollers. A female dung beetle can lay about 10-80 eggs in her lifetime, which usually takes about 1-2 days to hatch into a larva at optimal environmental conditions of temperature and humidity. The larva

then feeds on the dung, develops and grows through the holometabolous cycle. The pupal stage lasts from two weeks to several months depending on the species and prevailing environmental conditions (Vessby, 2001). This is the most variable stage among different dung beetle species. The pupa remains in a dung ball until it is fully matured, thereafter, it will emerge as an adult, relocate to a fresh dung pat, mate and start the life cycle process all over again. The beginning of the rainy season determines the emergence of the adults from the soil for most of the species (Novais *et al.*, 2016).

1.1.2. Ecological significance of dung beetles

Natural systems provide the ecosystem with services ranging from food production to waste removal, called ecosystem services. Dung beetles are the key drivers of these ecosystem services (Soliveres *et al.*, 2016), with a wide range of services beneficial to humans known as ecological services (Fisher *et al.*, 2009). These services include nutrient cycling, pollination, decomposition, parasite control, secondary seed dispersal, soil-bioturbation and related services (reviewed in Nichols *et al.*, 2008; Scholtz *et al.*, 2009; Eggleton, 2020).

Nutrient cycling

Mammalian dung is rich in nutrients such as nitrogen and phosphorus (Voigt *et al.*, 2015), which are essential for plant growth. These nutrients in the dung are transferred into the soil in two ways; by rain as it infiltrates into the dung, and by soil fauna in the form of dung pellets (Yamada *et al.*, 2007). Dung beetles play a major role in the transfer of dung balls into the soil through their dung burial behaviour (Maldonado *et al.*, 2019), and have been reported to increase soil nutrients (Bertone *et al.*, 2015), by incorporating nitrogen, ammonium compounds and phosphorous to the soil (Maldonado *et al.*, 2019).

Parasite control

Gastrointestinal parasite infections are the most common and economically important challenges in livestock production worldwide (Charlier *et al.*, 2009; Rodríguez-Vivas *et al.*, 2017). Helminth eggs are shed from a parasitised host through faeces, where they will hatch and develop until third instar infective larval stage. At third instar, the larva will leave the dung and infest surrounding herbage, where they will be ingested by another grazing ruminant host (Banks *et al.*, 1990; Cable *et al.* 2017; Wang *et al.*, 2020). It has been reported that the successful development, growth and survival of the larvae outside host is dependent on environmental temperature and moisture, therefore anything that alters these parameters can contribute to the management of gastrointestinal infections (Banks *et al.*, 1990; Sands & Wall, 2016; Cable *et al.*, 2017). Dung beetles have been reported to reduce the moisture content of the dung pat (Nichols *et al.*, 2008), thereby making it an unsuitable breeding site for insect pests (e.g. flies) and parasites. In addition, dung beetles also help control flies such as the horn flies (*Haematobia irritans*), an obligate bloodsucking ectoparasite of cattle and face flies (*Musca autumnalis*), which use dung as breeding grounds for their larvae (Oyarzún *et al.*, 2008). By fragmenting dung and burying dung balls under soil, these beetles also reduce the survival of parasite development through desiccation, and thus help in overall parasite control.

Decomposition

Agriculture is the largest anthropogenic sources of greenhouse gases (O'Mara, 2011; Berllaby *et al.*, 2013), with about two-thirds of the emissions from the dairy and beef production (Slade *et al.*, 2016). In 2010, agriculture and associated land use activities were estimated to contribute about 30% of the global anthropogenic emissions (Tubiello *et al.*, 2013), with the main sources of greenhouse gases in agriculture from enteric fermentation and fluxes from manure (Slade *et al.*, 2016). Nevertheless, by constructing nesting ball and burying it under the soil, dung beetles

remove dung deposited on the soil thereby inhibiting ammonia volatilisation (Huerta *et al.*, 2013). Thus, dung beetles contribute to the mitigation of climate change through the reduction of methane (CH₄) emissions by 14.5% and nitrous oxide (N₂O) emissions by 2.0%, resulting in an overall reduction of 7% in CO₂ equivalents (Slade *et al.*, 2016).

Seed dispersal

Large herbivores are the most important drivers of vegetation dynamics in grazed ecosystems (Erschbamer *et al.*, 2003). This is because during grazing, they consume more seeds, cause less damage to the seeds during chewing and rumination processes, and disperses the seeds with the dung over long distances (Kuiters *et al.*, 2010). The dispersed dung attracts dung beetles which through feeding and nesting bury those seeds unintentionally (Nichols *et al.*, 2008). When beetles bury the seeds, they increase the probability of seed germination, as well as protect seeds from predation (Shepherd & Chapman, 1998). This secondary seed dispersal by dung beetles is very advantageous for plants, as some seeds that are initially deposited in places that are not appropriate for seed survival or seedling establishment benefit from relocation (Howe & Smallwood, 1982). Larger beetles were reported to bury larger seeds than smaller dung beetles (Andresen, 2002), probably because larger dung beetles make and roll bigger dung balls than smaller beetles.

Soil bioturbation

Bioturbation is the displacement and mixing of soil particles by animals or plants thereby increasing soil aeration and water porosity (Nichols *et al.*, 2008). Through their tunneling behavior, dung beetles play a major role in bioturbation when they are mixing organic matter with both top- and subsoil from deeper soil horizons to the surface (Mittal, 1993). Furthermore, dung beetles have been reported to improve water infiltration, soil moisture and

aeration in the soil (Bang *et al.*, 2005), by creating pores into the soil as they tunnel down to bury their nest balls. These soil properties are vital for agro-ecosystem functioning.

1.2. Dung beetle diversity and distribution

1.2.1. Diversity

Morphological characteristics have been widely used in the taxonomic classification of dung beetle species (Nel & Scholtz, 1990; Philips *et al.*, 2004; Davis *et al.*, 2008; Tshikae *et al.*, 2008; Davies *et al.*, 2020; Sands *et al.*, 2021, although molecular techniques are now commonly used in the phylogeny studies (Monaghan *et al.*, 2008; Medina *et al.*, 2013; Buse *et al.*, 2018; Mohtasebi *et al.*, 2020). There are reports of inconsistencies in the taxonomic classification of dung beetle species belonging to the Onthophagus and Ontecellini tribes (Philips, 2016). Furthermore, reports of taxonomic revisions (Branco, 2010; Roggero *et al.*, 2015) as well as the discovery of new taxa in dung beetles exist (Krikken, 2009). For example, most of the taxa in the Onthophagini tribe such as the *Proagoderus lansberg*, *Diastellopalpus lansberg*, *Digitophagus balthasar*, *Strandius balthasar*, and *Euonthophagus* which were previously considered subgenera have been recognised as genera (Davis *et al.*, 2008; Davis *et al.*, 2020). However recently, in order to stabilise morphological taxonomy, structures within the genitalia have become popular for both the taxonomic and systemic purposes. Genitalia is reported to provide taxonomically useful characters which can separate organisms at species level in situations where no other morphological characters will suffice (Tarasov & Solodovnikov, 2011).

The current beetle classification separate dung beetles (Scarabaeinae) into rollers and tunnelers, 12 traditionally recognized tribes (Cambefort, 1991) and six bio-geographical regions (Davis *et al.*, 2002; Davies *et al.*, 2020). The 12 “traditional” tribes of the subfamily Scarabaeinae include; Canthonini, Eucraniini, Eurysternini, Gymnopleurini, Scarabaeini, Sisyphini,

Dichotomini, Onitini, Phanaeini, Coprini, Onticellini and Onthophagini (Philips *et al.*, 2004). Despite the global distribution of dung beetles, only four of 12 tribes namely Deltochilini, Dichotomiini, Coprini, and Onthophagini have a global distribution (Tarasov & Génier, 2015), with about half of the known worldwide Scarabaeinae beetles belonging to the Onticellini and Onthophagini tribes (Schoolmeesters *et al.*, 2010). The number of dung beetles (coprophagic Scarabaeoidea) is about 7000 species worldwide (Byk & Piętko, 2018), with about 780 species in Southern Africa (Doubé, 1987; Doubé, 1990; Davis *et al.*, 2020). According to previous studies about 67 species have been identified in Botswana to date (see Tshikae *et al.*, 2008; Sands *et al.*, 2021) and numerous still unknown. Species in Botswana are from 8 of 12 dung beetle tribes namely: Gymnopleurini, Canthonini, Dichotomiini, Coprini, Onthophagini, Scarabaeini, Oniticellini and Sisyphini. Diversity comprises species richness and species abundance (Hamilton, 2005), where species richness refers to the number of species, while species abundance refers to the relative abundances of individuals within each species. Species diversity is most simply measured by counting species (MacArthur, 1965), and may be computed using a wide variety of indices (Morris *et al.*, 2014). Furthermore, there has been no consensus as to which index is more appropriate and informative (Morris *et al.*, 2014), nevertheless the most commonly used indices are the Shannon's diversity index and the Simpson's diversity index (Magurran, 2004; Gorelick, 2006).

The diversity of dung beetles is influenced by environmental variables such as temperature, humidity, and resource availability (Beck *et al.*, 2017; von Hoermann *et al.*, 2020; Silva *et al.*, 2020). Temperature is reported to be a major diversity gradient determinant in most if not all ectotherms (Gebert *et al.*, 2020), due to the fact that it affects various factors of insects such as growth, development, performance, fitness, metabolic activity and survival (Chown & Nicolson, 2004; Angilletta, 2009). It has been reported that insects' richness and abundance increase during period of higher temperature and decreases during periods dominated by low

temperature (Wolda, 1988). In addition, very low temperatures have been associated with decrease in insects' diversity due to the decrease in annual thermal budget required for growth and development (Mittelbach *et al.*, 2007). Rainfall provides moisture which triggers the emergence and activity of most dung beetle species (Hanski & Cambefort, 1991; Novais *et al.*, 2016) and rains also coincides with timing for abundance in food resources for most insects. In addition, dung beetles are sensitive to anthropogenic activities such as overuse of agricultural chemicals (Sand & Wall, 2018; Ambrožová *et al.*, 2020) and intensive land clearing for industrialisation (Noriega *et al.*, 2021). For example, the use of ivermectin to control cattle internal parasites have been reported to results in chemical residues in the dung, affecting both species richness and abundance due to mortality of larvae, immature adults and reduced egg production (Rosales *et al.*, 2011; Ambrožová *et al.*, 2020). Apart from chemical residues, dung beetles were reported to be sensitive to factors such as chemical residues, climate change, habitat loss or modifications, and changing dung resource availability; these factors are dynamic and will continue to vary more so in the future (Nichols *et al.*, 2008). As such, the dung beetle diversity of an area may also be dynamic and continues to change in tandem with changing environments.

1.2.2. Distribution of dung beetles

Communities of dung beetles usually consist of many species feeding on the same resources (Sands *et al.*, 2021). According to the principle of competitive exclusion, two species cannot occupy the same ecological niche, or else one of the species will be excluded (Hardin, 1960). However, it has been documented that dung beetles exhibit both spatial and temporal distribution along several niche partition axis such as dung type preference, seasons, habitat, nesting behavior and activity times (Estrada *et al.*, 1993; Dacke *et al.*, 2003; Filgueiras *et al.*,

2009; Pryke *et al.*, 2013; Niino *et al.*, 2014; Okamoto & Tojo, 2021; Hernández, 2002; Lopes *et al.*, 2011) as a way of resource sharing and co-existence mechanisms.

The spatial distribution of dung beetles occurs due to selection pressures such as; food preference, habitat preference and thermal requirement. Dung beetles have been documented to have preference for dung type; where most species are attracted more to omnivorous mammal dung compared to herbivore and carnivore one (Estrada *et al.*, 1993; Filgueiras *et al.*, 2009). This suggests that the species richness of an area is determined by the type of dung available (see Sands *et al.*, 2021). It has also been reported that, dung beetles show some specialisation regarding vegetation cover and soil type (Barbero *et al.*, 1999; Kunz & Krell, 2011; Silva *et al.*, 2020). Habitat heterogeneity in the different plant formations have been reported as one of the factors that drive dung beetle species guild, species richness and distribution patterns (Silva *et al.*, 2020), such that species composition differs between open habitats and forests (Galante *et al.*, 1991). Species have been reported to prefer certain habitats over others (Pryke *et al.*, 2013; Niino *et al.*, 2014) for example, *Onthophagus* species occur primarily in open habitats (Ebert *et al.*, 2019).

The temporal distribution of dung beetles on the other hand is driven by season, activity time and succession. Seasonal activity in dung beetles is reported to be influenced by rainfall, temperature and life history strategies (Cambefort, 1991; Doube, 1990; Halffter, 1991). These parameters determine the quantity and quality of dung food resources, as well as provide humidity which also triggers adults' emergence and onset of activity (Cambefort, 1991; Doube, 1990). Dung beetles spend most of their life in the dung or in the soil beneath dung pats, and only out to find new dung pats. Different species of dung beetles come out of the soil at different times of the day. Depending on the species, some species are active at night (nocturnal), while others are active during the day (diurnal) and yet others are active at dawn and/or dusk (Caveney *et al.*, 1995). In addition, variations in the length of flight has also been

observed in different species, where some diurnal species fly throughout the day and some nocturnal throughout the night, while others have restricted flight periods lasting only for a few hours (Doube, 1990). Species are also reported to exhibit distinct patterns of successional occurrence (Gittings & Giller, 1998). The maintenance of such daily and successional activity patterns may be a mechanism enabling coexistence of competing species (Feer & Pincebourde, 2005). According to Pessôa *et al.* (2017), species may have evolved different diel activity timing as a co-existence mechanism to avoid intense competition for resources among the different species. Nevertheless, studies on activity times have been limited to the Onthophagini, Coprini, Gymnopleurini and Sisyphini tribes (Doube, 1990; Caveney *et al.*, 1995; Niino *et al.*, 2014), and not exhaustive across all the 12 dung beetle tribes. There are two studies on activity times on local (Botswana) dung beetle communities (see Tshikae *et al.*, 2008; Sands *et al.*, 2020). However, these results are not exhaustive of all species, warranting the need for more comprehensive studies at local scale on local dung beetle guilds.

Animals are adapted morphologically, behaviourally and physiologically to either day or night environmental conditions. However, this notion has not been explored across all organisms. For example, studies report that the retinal structure of a diurnal animal is poorly adapted to low light intensity of the night, as such reducing efficiency of activity and its ability to avoid predators (Jacobs, 1993; Van Schiik & Griffiths, 1996). Furthermore, it is hypothesised that large insects are easily seen by their natural enemies than small ones during the daylight, as such more likely to be active during the night than during the day (Rydell *et al.*, 1996). Indeed, studies on dung beetles show that diurnal dung beetle species are smaller in size than nocturnal species (Hernández, 2002), however this may vary across locations and dung beetle species hence, the need to assess this in dung beetle species.

1.2. Climate change and effects on insects

1.2.1. Ectotherms and thermal stress

Being ectotherms, most insects have body temperatures in constant equilibrium with prevailing ambient environment. As such, their bodily temperatures constantly fluctuate with changing ambient environments (Chown & Nicolson, 2004; Angilletta 2009). Nevertheless, each insect species has an optimal temperature range for development (Ricalde *et al.*, 2012) and activity (Vannier, 1994; Ricalde *et al.*, 2012). It is at this optimum temperature that several physiological functions such as growth, metabolism, and reproduction reach a maximum (Vannier, 1994; Reznik *et al.*, 2009). Thermal effects on insect performance traits within different temperature ranges were summarised using a thermal performance curve (TPC) (Vannier, 1994; Angilletta, 2009; Furlong & Zalucki, 2017). TPCs tend to take a general generic shape, with performance typically increasing proportionally with temperature, reaching maximum at optimum temperature (T_{opt}), beyond which any increase in temperature causes performance decline. In consequence, TPCs describe the effects of temperature on processes that affect insect biological rate processes (Schuttle *et al.*, 2011); although somewhat often varying across taxa, ontogeny, metrics tested and magnitude of climate variability (Deutsch *et al.*, 2008; Clavijo-Baquet *et al.*, 2014; Kingsolver *et al.*, 2013; Vasseur *et al.*, 2014; Thompson *et al.*, 2018).

Temperature is always changing (IPCC, 2007; 2014) therefore organisms need to adapt (Chown & Nicolson 2004; Angilletta 2009). The temperature tolerance of an insect is described by the thermo-biological scale (Vannier, 1994; **Figure 1.1**), which states that beyond the optimum temperature, an increase or decrease in temperature does not result in immediate death of an insect, but first an insect will enter physiological states known as the critical thermal limits (CTLs). CTLs are categorised as critical thermal minima CT_{min} for low temperature and critical thermal maxima CT_{max} for high temperature. Critical thermal maxima (CT_{max}) has been

defined as the temperature at which an organism loses the ability to self-right due to loss of co-ordinated muscle function due to high temperatures, while critical thermal minima (CT_{min}) is the temperature at which an organism loses the ability to self-right due to loss of co-ordinated muscle function due to low temperatures (Gotcha *et al.*, 2018). In both cases, an organism lose the ability to respond to a mild stimuli e.g probing (Nyamukondiwa & Terblanche, 2009). Ecologically, these limits represent significant temperature thresholds that may stop key life defining processes, e.g. flying, walking, and mating or mate finding, oviposition and others (see Chown & Nicolson, 2004). Nevertheless, CTLs are not static, and may change in many terrestrial ectoderms due to plastic changes in phenotypes (Hoffman *et al.*, 2013) and lifestages

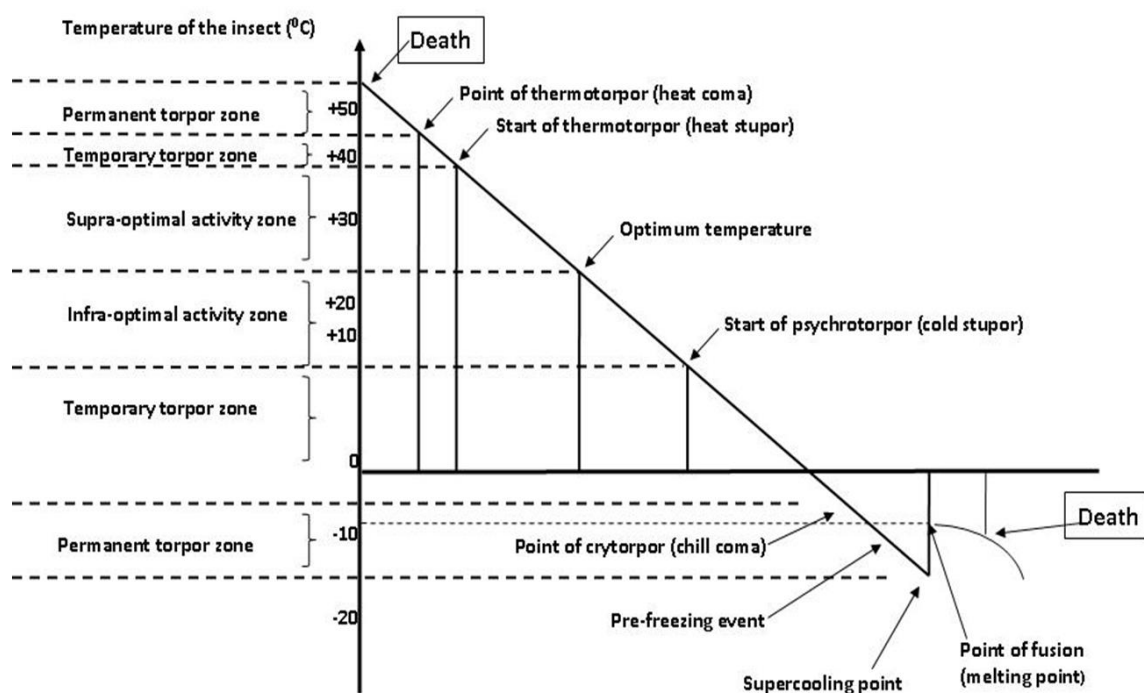


Figure 1.1: The Thermo-biological scale (Vannier, 1994)

Beyond either CT_{max} or CT_{min} , an insect will enter into a coma, known as heat- and cold coma respectively. Finally, beyond either heat or cold coma an insect will enter a stage of irreversible trauma and death (Chown & Nicolson, 2004). Critical thermal maxima (CT_{max}) refers to the

temperature at which an insect lose coordinated muscle function due to high temperatures , while CT_{min} refers to the temperature at which an insect lose coordinated muscle function due to low temperature (Gotcha *et al.*, 2018). All these traits are easily measurable in the laboratory (see Terblanche *et al.*, 2007; Sinclair *et al.*, 2015) and represent significant proxies of thermal tolerance (Castañeda *et al.*, 2005; Hazell *et al.*, 2008; Nyamukondiwa & Terblanche, 2009; Kelty & Lee Jr, 2001; Nyamukondiwa *et al.*, 2013; Sinclair *et al.*, 2015; Chidawanyika *et al.*, 2017; Gotcha *et al.*, 2018) while linking well with biogeographical patterns (Somero, 2005; Lancaster, 2016). CTLs are easy to measure and are often assessed using dynamic protocols e.g., by heating or cooling an organism until it loses its righting response (Terblanche *et al.*, 2007) or using static plunge protocols (see Chown & Nicolson, 2004). When measuring CTLs, insects are subjected to ramping protocols, where the temperature is either being heated or cooled at a specific rate, until either CT_{max} or CT_{min} is reached (Terblanche *et al.*, 2007; Gotcha *et al.*, 2018; Nyamukondiwa *et al.*, 2018).

Heat coma has been used as a measure of heat tolerance whereby an organism is heated in a temperature-controlled column, the time taken for an insect to be knocked down by heat stress is recorded as the heat knockdown time (HKDT) (Huey *et al.*, 1992; Sorensen & Loeschcke, 2001; Weldon *et al.*, 2011; Parkash & Ranga, 2014; Bauerfeind *et al.*, 2014; Gotcha *et al.*, 2018). Chill coma on the other hand has been used to assess cold tolerance by measuring the temperature at which insects go into coma as a consequent of low temperature stress. The time needed to recover (self-right) following chill coma is recorded as the chill coma recovery time (CCRT), and has been used in several studies as a reasonable proxy of cold tolerance in insects (Castañeda *et al.*, 2005; Sinclair *et al.*, 2007; Karl *et al.*, 2014; Scharf *et al.*, 2015).

1.2.2. Effects of thermal stress on insects

Effects of climate change on insects' species diversity and abundance has been well documented. In insects, body temperatures hence physiology is e strongly affected by environmental temperatures (Dillion *et al.*, 2009). Insects being ectotherms, are therefore expected to suffer from both heat (Zhang *et al.*, 2014) and cold (Renault *et al.*, 2002) temperature stressors, potentially reducing survival and reproductive success leading to elevated extinction risks. Studies show that high temperatures as a result of climate change are likely to result in loss of insects' species (Raven *et al.*, 2021), reducing their diversity and distribution. Although dung beetles are vital due to the ecological services they provide, studies on their thermal tolerance to warming remain at infancy (see Nyamukondiwa *et al.*, 2018; Holley & Andrew, 2019) but their evolutionary biology and conservation are well studied (see Scholtz *et al.*, 2009). Nevertheless, the few studies available on thermal tolerance of dung beetles' report that warming is likely to reduce the survival, fitness and performance of dung beetle species (Nyamukondiwa *et al.*, 2018; Holley & Andrew, 2019a; b). Although dung beetles are also experiencing low temperatures in the natural environment, much of the focus on climate change effects on biodiversity have mainly focussed on warming. However, climate change may also come with increasing temperature variability, including cold waves (Harris *et al.*, 2018) Thus, the effects of low temperatures on dung beetle fitness and consequent effects on ecological services delivery is equally vital as high temperature and and thus, also warrants investigation.

1.3. Thermal plasticity

When faced with unfavourable temperatures, an insect either employs behavioural adaptation (through e.g., migration distributional changes) or physiological adaptation; otherwise, it faces extinction (Parmesan *et al.*, 1999; Somero, 2010). Physiological adaptation can either be through phenotypic plasticity or by natural selection (genetic) (Hoffmann & Sgrò, 2011).

Natural selection involves change in the genotype of an organism and, as such, act over a long time (Oms *et al.*, 2017; Svensson *et al.*, 2020). Phenotypic plasticity on the other hand is the environment-dependent expression of alternative phenotypes by the same genotype (Lande, 2009; Chevin *et al.*, 2013; Svensson *et al.*, 2020); thereby allowing organisms to respond quickly to changing environmental conditions (Oms *et al.*, 2017; Macagno *et al.*, 2018). Various behavioural, morphological and physiological plastic phenotypic responses are used by various insects in order to cope with changing temperatures (Lencioni, 2004; Sheikh *et al.*, 2017). Generally, most species develop a combination or several of these survival strategies (Irons *et al.*, 1993) and use them interchangeably or in an overlapping manner to survive stressful environments across the different developmental stages. Effects of plasticity can be reversible and/or irreversible (Hoffmann *et al.*, 2003; Weldon *et al.*, 2011), and can be induced either during development (developmental acclimation), during adulthood (adult acclimation) (Parkash *et al.*, 2014), in one developmental stage and manifest in another- carry-over effects (Stoks & Cordoba-Aguilar, 2012; Salis *et al.*, 2018) or can be passed on across generations- transgenerational effects/plasticity (Sgro *et al.*, 2016; Tougeron *et al.*, 2020).

1.3.1. Behavioural plastic responses against thermal stress

According to Mamantov & Sheldon (2021), behavioural plasticity may mitigate some of the impacts of climate change on dung beetles. Some of the behavioural responses to stressful temperature in insects include shade seeking, hibernating during winter, summer diapause (aestivation) and migration (Sheikh *et al.*, 2017). Behavioural adaptations are usually the preferred first line of defence for most organism because of their associated lower metabolic costs. Despite this, most of the research has been focused on physiological plastic responses against thermal stress (Helmuth & Hofmann, 2001; Andersen *et al.*, 2010; Bellantuono *et al.*, 2012; Chen *et al.*, 2018; González-Tokman *et al.*, 2020). This has created a research lag in

behavioural plastic responses, which are only being studied now (Macagno *et al.*, 2018; Mamantov & Sheldon, 2021). Dung beetles may often use their dung balls as thermal heat sinks as a way of thermoregulation (Smolka *et al.*, 2012).

Insects have been documented to monitor environmental variables in order to determine a time suitable for activity, resulting in the sorting of organisms' activity into day and nighttime activities. However, whether this diel differences in activity times among species is a behavioural plastic response against thermal stress remains at infancy. To my knowledge, there are limited investigations that looked into the effects of evolution of thermal tolerance in dung beetles, i.e. profiling the thermal biology of nocturnal versus diurnal dung beetles (see chapter 3 and 4.) However, there has been few studies on thermal tolerance and activity times in other insects (Jayatilaka *et al.*, 2011; Andrew *et al.*, 2013; Bishop *et al.*, 2017). These studies found that thermal tolerance could be the underlying factor behind differences in activity times in ants. Furthermore, two morphological traits namely body size and colour, which have been shown to have strong links to thermal tolerance, have however been linked to differences in foraging or diel activity times among insects (Pessôa *et al.*, (2017). Results of elytra predominant colouring showed that nocturnal beetles are black, while diurnal species show bright colours such as green, blue, yellow and brown as an adaptation mechanism against predation (Hernandez, 2002). In addition, body size, in combination with light, have been reported to influence vulnerability to predation, thereby acting as a mechanism which separate species activity times (Rydell *et al.*, 1996), where large dung beetles restrict themselves to nocturnal activity, while smaller species are diurnal (Hernández *et al.*, 2011; Guevara & Avilés, 2013). Predators of dung beetles include 409 species of birds, mammals, reptiles and amphibians (Young, 2015), most of which are of diurnal activity pattern.

1.3.2. Physiological plastic responses against thermal stress

If the environment changes in such a way that insects cannot adapt using behavioral or morphological shifts, they may compensate physiologically. The ability of an individual organism to modify its phenotype to increase fitness through physiological, behavioural, or morphological modifications, typically taking place within a single generation, is termed “phenotypic plasticity” (West-Eberhard, 2003; Ghalambor *et al.*, 2007; Whitman & Agrawal, 2009). These physiological plastic responses that increase an organism’s resistance after exposure to some form of non-lethal stress are referred to as acclimation or hardening (West-Eberhard, 2003; Chown & Terblanche, 2007), depending on the time-scale and severity of the treatment (reviewed in Seebacher, 2005; Sgrò *et al.*, 2016). Physiological plastic responses against heat stress include the expression of heat shock proteins (Liu *et al.*, 2017), and against cold stress include carbohydrate cryoprotectants, depression of supercooling points, membrane remodeling, the inhibition of apoptosis, Hsp upregulation and ion homeostasis (Denlinger & Lee, 2010). Although hardening and acclimation are often used interchangeably, these terms are different. Hardening refers to short term (minutes to hours) while acclimation refers to long term (days to weeks) exposure to sub lethal conditions which confers improved survival to an otherwise lethal stress environment (Chown & Nicolson, 2004).

Acclimation can be induced in the laboratory through processes known as rapid cold hardening (RCH) (for low temperatures) and rapid heat hardening (RHH) (for high temperatures) (see Rinehart *et al.*, 2000; Sørensen *et al.*, 2016). According to the beneficial acclimation theory, exposing insects to moderate stress improves their survival to that particular stress later in life, compared to an organism that did not have the opportunity to acclimate (pre-exposure) to that particular environment (Leroi *et al.*, 1994). Studies have shown that insects which have been pre-exposed to sub-lethal heat perform better under more severe stress of the same type (either direction of the temperature scale) than those insects that have not been pre-conditioned (Sorensen & Loeschcke, 2001; Chidawanyika & Terblanche, 2011) or cold (Kelty & Lee, 2001;

Sinclair *et al.*, 2007; Denlinger & Lee, 2010). As such, this form of phenotypic plasticity is adaptive and optimizes fitness under stressful environments.

Due to climate change, cases of extreme events such as heat waves are becoming more frequent and intense (Esperk *et al.*, 2016; Stillman, 2019). Therefore the impact of climate change on ectotherms is predicted to be greater and more heterogeneous than previously thought (Colinet *et al.*, 2015; Kingsolver & Buckley, 2015), especially for subtropical and temperate species (Hoffmann *et al.*, 2013; Kingsolver *et al.*, 2013; Malhi *et al.*, 2020). Temperature and precipitation are the two main climatic factors that strongly impacts on ecosystems by reducing habitat suitability to beneficial species such as dung beetles. For example, in Africa, the past decade has been the warmest on record (Perkins-Kirkpatrick & Lewis, 2020; WMO, 2020) with land bordered areas in the dry tropics of Southern Africa including Botswana, experiencing the warmest temperatures at much faster rates than the global average (Dube, 2003; WMO, 2020). Such rapid increase in temperatures is likely to exceed upper thermal limits (UTLs) for terrestrial organisms endemic to these hot, arid habitats, particularly those in direct contact with hot soil such as dung beetles that provide fundamental ecological functions. Thus, tropical organisms will be more vulnerable to climate change and associated heat stress since their habitat temperatures are close to their upper thermal limits (UTLs) (see Chown & Nicolson, 2004). However, in response to frequent temperature peaks, organisms may evolve higher basal heat tolerance or greater plasticity enabling stronger induced heat tolerance. However, the degree to which phenotypic plasticity will cushion these organisms against fluctuating stressful and extreme temperatures, and how this may shift organismal performance remains largely unknown, especially in less studied areas such as sub-Saharan Africa. Very few, if any studies have been conducted to identify which dung beetle species are more vulnerable and which ones are tolerant (through plasticity or otherwise)) and thus, likely to survive for future conservation planning purposes.

1.4. Changing environments and ecosystem service delivery

The sustainability of ecosystem services under changing environments (climate and anthropogenic changes) depends on the organisms' responses to these changes and how their fitness and /ecosystem service efficacy are altered by these changes (Prather *et al.*, 2012). Indeed, dung beetle ecological service delivery efficiency is reportedly impacted by temperature (Slade & Roslin, 2016; Wu *et al.*, 2021), as well as human activities, e.g., the use of pesticides (Sands & Wall, 2018; Ambrožová *et al.*, 2021). Studies show that ecological services provided by dung beetles are affected by the diversity of beetle functional groups (rollers, tunnelers, dwellers) and species composition of dung beetle assemblages (O'Hea *et al.*, 2010). For example, dung decomposition was reported to vary with dung beetle abundance, high decomposition was recorded when there was high decomposer biomass (O'Hea *et al.*, 2010). In addition, dung beetle functional group diversity was found to increase dung removal, thereby increasing secondary ecological functions (Milotić *et al.*, 2019). The limitation of these studies and many others was that they investigated one variable at a time and ignored trophic interactions. Although trophic interactions are highly context-dependent and mediated by many factors such as temperature, resource density and non-trophic interactions among inter- and intraspecific consumer groups, they highly influence species' net output in ecological service delivery (Cuthbert *et al.*, 2021). Therefore, effects of climate change on ecological services should account for the interactive effects of species, temperature, densities and resource availability. Therefore, there is a need to investigate these multivariate effects (species, food density and temperature stress) on ecological service provided by dung beetles.

1.5. Economic value of ecological services provided by dung beetles

To the best of my knowledge, attempts to quantify the economic benefits of ecosystem services provided by dung beetles have been made by only three studies (see Fincher, 1981; Losey & Vaughan, 2006; Beynon *et al.*, 2015). The economic value of dung beetles in U.S. pasture systems has been estimated to be USD 2 billion annually (Fincher, 1981). The study focused on the services associated with reduced pest fly populations, reduced livestock gastrointestinal parasites, reduced pasture fouling, and increased nitrogen cycling. However another study estimated the value by computing the difference between the costs of services when the beetles are present and when they are absent (Losey & Vaughan, 2006), that is, equating beetle activities to alternate services for generating benefits. They estimated that ecosystem services provided by dung beetles might save the cattle industry USD 380 million per year. In the UK, the contribution of dung beetle activity towards gastrointestinal parasite management was estimated to be £188 million per year in conventional cattle farming systems (Beynon *et al.*, 2015). This study attributed about an additional £6.2 million per year in addition to savings on the anthelmintic drugs. These studies show that although the economic value of dung beetles is not obvious, but the per capita national environmental cost savings from dung beetles are high and economically significant.

1.6. Justification of study

Botswana has over 2.1 million cattle (Botswana-Agricultural sectors, 2017) accounting for 2.6% of Botswana's GDP. In addition, Botswana has a rich diversity of vertebrate wildlife (Winterbach *et al.*, 2014) including an elephant population estimated at ~122 700 (Schlossberg *et al.*, 2019) all of which produces dung daily. If this dung is left unprocessed, it can harbor a huge diversity of ectoparasites for livestock (de León *et al.*, 2020), pollute water bodies thereby encouraging abundance of aquatic parasites (Buxton *et al.*, 2020), increase cost of water

treatment, and increase the release of greenhouse emissions from unprocessed dung into the atmosphere (Openshaw, 2015). In Botswana, dung beetles serve as natural capital responsible for processing cattle and wildlife dung, keeping the environment and waterbodies clean apart from enriching soil nutrition and other ecological services and benefits already explained (as reviewed in Nichols *et al.*, 2008). However, knowledge of specific local dung beetles' diversity in Botswana is limited (Tshikae *et al.*, 2008; 2013) if at all available (except see Scholtz *et al.*, 2009 for a broader regional overview) and has not been explored, although the ranges of biotic and abiotic factors affecting their distribution across the environmental continuum have been reported (Tshikae *et al.*, 2013; Davis *et al.*, 2008). Furthermore, the country is landlocked in the semi-arid sand covered plains of the Kalahari Desert (see Tshikae *et al.*, 2013; Davis *et al.*, 2008), making it a zone of highly variable climate as it is prone to the influence of global El Nino Southern Oscillations phenomenon (Dube, 2003). The seasons are characterised by hot and wet summer, and cold and dry winter periods, and has been subjected to anthropogenic activities such as overuse of pesticides (Machekano *et al.*, 2020) and extensive land clearing for intensive agricultural production, conditions which have been documented to reduce dung beetle species richness and abundance (Slade *et al.*, 2011). Studies have shown that different species contribute unequally to ecological service delivery (Manning & Cutler, 2018) and that dung beetle communities' with functionally diverse species are reported to provide multiple ecosystem services (Manning *et al.*, 2016) compared to monocultures. Therefore, knowledge of dung beetle diversity along with their thermal tolerance and plasticity profiles is essential not only for identifying ecologically efficient species but also separating vulnerable from tolerant species on local guilds for future conservation investments in order to safeguard ecosystem service delivery. Furthermore, it has been reported that substantial costs can be incurred in the restoration of dung beetle ecological services through programs that re-introduce propagule populations (Edwards, 2007). Therefore, policy makers and

conservationists need to appreciate and preserve these natural resources before the populations reach critical IUCN red list levels (Davis *et al.*, 2020) due to climate stress and focus their investment efforts towards their conservation early. This will protect the ecosystems from the consequences of climate change through ecosystem breakdown, or worse still, species extinction.

1.7. Aim and Objectives

The aim of this study was to investigate thermal responses of dung beetles in the context of climate change, local species diversity, ecological evolution of activity/foraging time and ecological service delivery.

Dung beetles are coprophagous insects from the order Coleoptera, families Scarabaeidae and Geotrupidae. They provide various ecological services that are significantly dependant on dung beetle biodiversity (Beynon *et al.*, 2015; Braga *et al.*, 2013; Tixier *et al.*, 2015; Manning & Cutler, 2018), which in turn, is affected by climate change (Wu *et al.*, 2021), or other contributing anthropogenic activities (Sands & Wall, 2018; Ambrožová *et al.*, 2021). Therefore, in line with the aim of the study the first result chapter (**Chapter 2**) investigated the diversity and diel activity timing of dung beetles in Khumaga, (Botswana) (**Objective 1**). Establishing the local diversity inventory of these dung beetles was not only significant for national, regional and scholarly documentation, but also for planning and safeguarding local biodiversity assemblages against anthropogenically changing environments. The specific objective of this chapter, therefore, was to:

- i) To determine the diversity and activity times among a community of dung beetle species.

I hypothesized that Khumaga village has a wide variety of dung beetle species exhibiting different diel activity times owing to abundant dung food resources available in this area because of the livestock-wildlife sympatry.

Behavioural plastic changes form the most immediate compensatory responses insects use to deal with stressful temperatures (Even *et al.*, 2012; Wong & Candolin, 2015). Nevertheless, the role of activity time in buffering dung beetles against thermal stress and climate change has not been explored regardless of its significance in shaping dung beetle biodiversity and reducing competition. Due to the increase in global environmental temperatures and their effects on biodiversity, it is therefore important to investigate how increase in temperature is likely to influence activity times of dung beetle species (**Objective 2**). In the third chapter, I investigated how heat stress may shape diel activity timing in dung beetles. The objective of this chapter was:

- ii) To determine the effects of heat stress on activity timing of sympatric local dung beetle species.

I hypothesized that heat stress tolerance may affect dung beetle diel activity timing may have evolved as a form of thermoregulatory compensation aligned to each species' thermal tolerance levels. For example, those beetles that cannot tolerate heat may be more restricted to nocturnal activities while those that can withstand heat to diurnal activity.

Empirical evidence exists supporting that weather anomalies will increase with climate change, albeit more for heat (Timmermann *et al.*, 1999; Easterling *et al.*, 2000) than cold waves (Wang *et al.*, 2010; Van Der Walt & Fitchett, 2021). Nevertheless, a wide variety of studies have shown that low temperatures also affect species population phenology and fitness (Angilleta, 2009; Ma *et al.*, 2017; Denlinger & Lee, 2010; Govindan *et al.*, 2020), and is a major constraint to organismal function and ecological services through limiting insect activity (Denlinger &

Lee, 2010). Low temperature events remain a critical concern because in future, they are likely to increase in frequency or duration concurrent with increasing mean temperature (Van Der Walt & Fitchett, 2021; Schiermeier, 2021). As such, investigating effects of low temperature stress on dung beetles' essential ecosystem services remains essential as climate change ensues in arid tropical regions (Van Der Walt & Fitchett, 2021). To my knowledge no study to date has explored this, and therefore in the fourth chapter, I investigated the effects of cold stress on activity time and implications on dung beetle ecological service delivery (**Objective 3**). The objective of this chapter was:

- iii) To determine the relationship between low temperature stress tolerance and the activity times of locally existing dung beetle species and the net effect on ecological service delivery.

I hypothesized that low temperature tolerance may also restrict activity timing, for example those beetles with higher cold tolerance may do better under lower night temperatures (nocturnal) and that low temperature stress may offset ecological service delivery.

The concept of phenotypic plasticity is a crucial aspect of species adaptation in the face of climate change (Chevin *et al.*, 2010) under which both the absolute thermal tolerance and thermal plasticity are important aspects of thermal resilience (Stillman, 2003; Somero *et al.*, 2010; Huey *et al.*, 2012). Although organisms may evolve higher basal heat tolerance or greater plasticity enabling stronger induced heat tolerance, this trait is not universal, and may be absent or differs in magnitude of protection offered among species (van Heerwaarden *et al.* 2016). Therefore, in the fifth chapter, I investigated the basal thermal tolerance and plasticity thereof of a diurnal species, *Allogymnopleurus thalassinus* (Klug, 1855) native to and living in the arid tropics (**Objective 4**).

The objective of this chapter was:

- iv) To determine basal thermal tolerance and plasticity thereof of the locally most abundant diurnal species and its ability to maintain ecological service delivery under climate change associated variable heat or cold stress.

I hypothesized that due to its diurnal behavior, the species will have high basal heat tolerance but limited thermal plasticity (see discussions in Gunderson & Stillman, 2015; van Heerwaarden *et al.* 2016; Barley *et al.*, 2021). Results from this chapter will give insights on the vulnerability of diurnal species in the face of global warming and help explain how plasticity may compensate for climate change effects but lack thereof may expose ecologically important species to high risk of extinction. This serves as an early warning system for early investment in conservation measures for target species.

The strength of ecological services provided by many insects is closely tied to their trophic interactions. However, trophic interactions in the tropics are highly context-dependent, being mediated by factors such as temperature, resource density and non-trophic interactions among inter- and intraspecific consumer groups (Cuthbert *et al.*, 2021). Nevertheless, little has been done on how climate change will affect ecological functions in the context of trophic level interaction strengths (but see Slade & Roslin 2016). Therefore, there is a need to investigate the effects of species, food density and temperature stress on ecological service provided by dung beetles. In the sixth chapter, I therefore investigated dung beetle ecological services delivery (utilisation and dung ball creation) at different dung masses across three beetle species, at different intraspecific beetle densities and different acclimation temperature regimes (**Objective 5**). The objective of this chapter was:

- v) To quantify effects of species, temperature, density and resource availability on ecological functions of a native, locally available telecoprid dung beetle species.

I hypothesized that dung beetle ecological service delivery varies interactively with species, temperature, dung availability and beetle density. Knowledge gathered from this chapter will

assist in the identification of functionally efficient dung beetle species under multiple (biotic and abiotic) stress factors, which need to be protected in order to secure their ecological services in the future.

In general, this thesis provides critical information on predicting the effects of climate change on local dung beetle diversity and their potential adaptation. My results provide advisory insights on future conservation and maintenance of dung beetles, their essential ecological services and the integrity of ecosystems as a whole. Application of the scientific findings and recommendations from this study to conservation policies may help protect dung beetles; a neglected but, essential natural capital in developing economies and consequently safeguard ecological services provision in the future.

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Chapter 2

Niche partitioning in dung beetles and the relationship between beetle size and diel activity

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2.1. Introduction

Insects comprise the most diverse and successful group of multicellular organisms on the planet, accounting for ~66% of all animal species (Jankielsohn, 2018). They contribute significantly to vital ecological functions of the ecosystem by fulfilling essential functional roles that benefit humans- termed ecological services (Ouyan *et al.*, 2013). These ecological services include pollination, pest control, decomposition, maintenance of wildlife species, food provision and related (Noriega *et al.*, 2018; reviewed in Nichols *et al.*, 2008). However, because the importance of these services is poorly understood, they are persistently undervalued and are susceptible to degradation (Losey & Vaughan, 2006). Chief amongst the factors contributing to loss of ecological services include anthropogenic habitat degradation (Alvarado *et al.*, 2019), agriculture and use of pesticides (Ambrožová *et al.*, 2020) and climate change (Wu *et al.*, 2021a).

Dung beetles are coprophagous insects from the families Scarabaeidae and Geotrupidae. By feeding on a range of domesticated animals and wildlife dung, they contribute to ecological service delivery of decomposition, nutrient cycling, bioturbation, pest control and secondary seed dispersal (reviewed in Nichols *et al.*, 2008). The value of ecosystem services provided by dung beetles is valued at over US\$380 million annually in the United States (Losey & Vaughan, 2006). This value is to a greater extent also attributable to dung beetle biodiversity (Beynon *et al.*, 2012; Braga *et al.*, 2013; Tixier *et al.*, 2015; Manning & Cutler, 2018). Ecosystems containing higher number of species have a higher chance of containing functionality and likely to contain more efficient ecosystem service provider species than ecosystems with lower species, in what is known as the “sampling effect” (Loy & Brosi, 2022). This is a phenomenon in which resources utilisation increases depending on species identity than proportions of biologically diverse ecosystems (Fridley, 2001). Nevertheless, based on the “complementary

effect”, species use resources differently such that total resource use is higher in a community with higher biodiversity (Loreau & Hector, 2001). There are about 7000 species of dung beetles worldwide, with most of the species found in Africa (Goolsby *et al.*, 2017).

Botswana lies in the southern part of Africa. The country has over 2.1 million cattle population (Botswana-Agricultural sectors, 2017), and a rich diversity of vertebrate wildlife including lions, African wild dogs, cheetahs and brown hyaenas (Winterbach *et al.*, 2014). The country is also a home to the largest global elephant herd, estimated at ~122 700 (Schlossberg *et al.*, 2019). The high number of cattle and elephants, in addition to other animals (both domestic and wild) indicate high dung availability which have been reported to influence both species diversity and richness. This animal diversity and numbers produce enormous dung resources, which should be matched with an equally diverse and abundant coprophagous species. However, despite high animal diversity and numbers, the diversity of dung beetle assemblages potentially feeding on abundant dung resources and cleaning the environment remains limited in Botswana (Tshikae *et al.*, 2018; but see Sands *et al.*, 2021). The Botswana Natural Museum in Gaborone also only has 33 identified dung beetle species specimens (per my observations), a number that may not be a clear reflection of the country’s dung beetle diversity. As such, this national database needs to be updated in order to give a more representative species inventory that provides a guide to conservation efforts. This may help conserve dung beetle diversity and populations to safeguard the maintenance of ecosystem function in anthropogenically shifting habitats (Tixier *et al.*, 2015; Griffiths *et al.*, 2016).

Several factors affecting dung beetle diversity have been discussed elsewhere including ambient and soil temperatures (Larsen *et al.*, 2005; Slade *et al.*, 2007; Morelli *et al.*, 2002). As such, spatial ambient environments may determine the species richness and its maintenance

(Belmaker & Jetz, 2015; Gebert *et al.*, 2020). Dung beetles are also found in defined guilds that are functionally and taxonomically dependent upon the environment (Liberal *et al.*, 2011). For example, the presence of a variety of dung-producing mammals has effects on the relative abundance and diversity of dung beetles' guilds (Cambefort & Walter, 1991). Since, such resources can vary in space and time, resource partitioning and competition between co-occurring species play a major role in structuring dung beetle communities (Hanski, 1991). Insects may exhibit niche partitioning along several ecological axes such as food preference, seasons, habitat, functional groups and activity times (Hanski & Cambefort, 1991; Hill, 1996; Jayatilaka *et al.*, 2011; Whipple & Hoback, 2012; Chao *et al.*, 2013) or competitive exclusion (Hardin, 1960). Thus, diversity studies may also be important in explaining these phenomena. Different factors account for beetle diversity and numbers at community level. For example, empirical studies show greater attractancy of omnivorous mammal- to herbivore-faeces (Estrada *et al.*, 1993; Filgueiras *et al.*, 2009). In addition, seasonal dung beetle activity is influenced by temperature and rainfall which determines the quantity and quality of dung, as well as provides humidity which also triggers adults' emergence and onset of activity (Cambefort, 1991; Doube, 1991; Halffter, 1991). Dung beetles have been shown to exhibit differential preference between open land and forest habits (Pryke *et al.*, 2013; Niino *et al.*, 2014), whereby small sized diurnals species were found to prefer open land habitats (Niino *et al.*, 2014). Nevertheless, these spatial and temporal variations in dung beetle diversity are said to be influenced by thermoregulatory strategies (Verdu *et al.*, 2007). Third, dung beetle species can be niche partitioned into at least three, according to nesting and feeding strategies *vis*; 'rollers' (telecoprids), 'tunnellers' (paracoprids) or 'dwellers' (endocoprids) and seldom 'stealers' (kleptocoprids). This niche differentiation facilitates the co-existence of closely related species with ecologically similar guilds (Whitmore, 1990; Okamoto & Tojo, 2021).

One of the most important and striking niche partitioning strategies among dung beetles is the separation in diel activity periods (Feer & Pincebourde, 2005; Chao *et al.*, 2013; Niino *et al.*, 2014). This mechanism allows several, and seldom up to 100 species to temporally colonise the same dung pat (Bernon, 1981). Several factors such as temperature, light intensity, competition for food, body size, and colour and predator avoidance have also been linked to the differences in diel activity timing across species (Feer & Pincebourde, 2005; Welbergen, 2008; Narendra *et al.*, 2010; Detoni *et al.*, 2015; Pessôa *et al.*, (2017). For example, desert dwelling ectotherms cease activity when temperatures rise to lethal levels during midday (Cole *et al.*, 2010). Similarly, foraging onset in desert ants was significantly influenced by surface temperature (Jayatilaka *et al.*, 2011). Indeed, behavioural responses e.g., changing activity timings are the first responses that insects use to respond to unfavourable environments (Wong & Candolin, 2015). Research on diel resource partitioning within dung beetle assemblages is limited (see Doube, 1991; Caveney *et al.*, 1995; Niino *et al.*, 2014). This study therefore investigated i) dung beetle diversity in Khumaga village, Botswana, ii) niche partition mechanism employed by existing species and iii) the relationship between dung beetle activity and beetle size. Given that the village is at the interface of a human community and national park, it hosts a large diversity of domestic animals and wildlife. We hypothesise that, given the large animal diversity, it should support a rich diversity of coprophagous dung beetles associated with diverse animal dung. This study is therefore significant in that understanding how animal diversity and anthropogenic activities affects the diversity and co-existence mechanisms of dung beetle communities.

2.2. Materials and methods

2.2.1. Study site

Dung beetles were collected in Khumaga village along Boteti riverbank (S20°28.348; E024°30.657). Khumaga village is characterised by small scale pastoralism mainly cattle and goats (Walker *et al.*, 2015) and is at the interface with Makgadikgadi Pans National Park which hosts a wide variety of wildlife. The area is characterised by diverse vegetation, sandy soils and water which forms critical resources for domestic and wild animals, hence promote abundant and diverse dung beetle species. Sampling was done in summer (February 2020), the time for peak populations for most dung beetle species due to conducive weather conditions (temperature and rainfall) as well as food availability. In this area, the rainfall season starts around November and end March, with rainfall amount ranging between 450 and 500 mm (Sands *et al.*, 2021), with most of the rainfall recorded between January and February (Nyamukondiwa *et al.*, 2018). Temperature during the months of January and February, which is the most peak time for most dung beetle species roughly range between 18.5 °C and 46 °C, with an average of 23°C (Nyamukondiwa *et al.*, 2018).

2.2.2. Determination of species activity time

Ten pitfall traps (Hernández, 2002; Braga *et al.*, 2013; Nyamukondiwa *et al.*, 2018), set 50 m apart (Bertone *et al.*, 2005) were put along a transect line along Boteti River and Makgadikgadi Pans National Park. The traps consisted of plastic containers (2 L) buried flush with the ground and covered with fine wire mesh, with some traps covered with 15 mm diameter wire mesh, while some traps were covered with 30 mm internal diameter wire mesh to accommodate capturing of a large array of beetle sizes (< 30mm). Fresh cattle dung (500g) was placed on top of the wire mesh as bait. Although studies show that dung beetles show variable preferences to dung types, cattle dung was used in this study because it was the most abundant and has

been used in several related studies. Beetle collection was done twice a day; before sunrise (0500-0600hrs) and before sun set (1800-1900hrs) during a 24h cycle for a period of 3 days in order to determine the activity time of the species. During collection, beetles from each bucket, were placed in labelled container with a perforated lid. The containers were thereafter taken to camp, where individuals of each species from each container were counted (Bertone *et al.*, 2005). Beetles from each container were sorted by size, only picking medium to large-bodied individuals. For the purpose of this study, I only counted medium to large beetles, which included beetles of body length greater than 5mm. I chose to count only larger beetles because larger sized beetles have been reported to provide higher ecological functions (Kaartinen *et al.*, 2015; Nervo *et al.*, 2014) and said to be most functionally efficient (Larsen *et al.*, 2008; Lahteenmaki *et al.*, 2015, as well as more prone to climate change extinction (Larsen *et al.*, 2008). Although smaller bodied (body length less than 5mm) were not counted for diel activity pattern determination, they were however sampled in order to determine the area's full species composition. During counting, morphological features such as body colour, size and shape were used to sort individuals into species. Species were thereafter given temporary identification numbers, with five individuals representing each species preserved in 70% ethanol in labelled vials (date of collection, place of collection, coordinates, and time of collection), for further identification. These specimens are housed in the Ecophysiological entomology laboratory at the Botswana International University of Science and Technology.

2.2.3. *Species identification*

As previously indicated, morphological features such as body colour, size and shape were used to sort individuals into morphospecies. Samples were further identified in the Ecophysiological entomology laboratory at the Botswana International University of Science and Technology using voucher specimens from Botswana National Museum and Royal Museum

of London Describe the main morphological features used to identify beetles here. To verify the identity of morphologically identified species, molecular techniques were employed. Insects' tissues such as leg for relatively larger individuals and whole specimen for relatively smaller individuals were used. Genomic DNA was extracted from the insect tissue using the ZR Quick-DNA Miniprep Plus Kit™ (Zymo Research, Catalogue No. D4068). The COI target region was amplified using OneTaq® Quick-Load® 2X Master Mix (NEB, Catalogue No. M0486) using COI primers (**Table 2.1**). The mitochondrial cytochrome C oxidase I (COI) was chosen as gene of interest for my DNA barcoding because it is the most common DNA barcoding gene (Wu *et al.*, 2021b). In addition, COI has been defined as the standard DNA barcoding gene in the identification of eukaryotes due to its relative conservatism (Costa *et al.*, 2007). The PCR products were run on a gel and gel extracted with the Zymoclean™ Gel DNA Recovery Kit (Zymo Research, Catalogue No. D4001). The extracted fragments were sequenced in the forward and reverse direction (Nimagen, BrilliantDye™ Terminator Cycle Sequencing Kit V3.1, BRD3-100/1000) and purified (Zymo Research, ZR-96 DNA Sequencing Clean-up Kit™, Catalogue No. D4050). The purified fragments were analysed on the ABI 3500xl Genetic Analyzer (Applied Biosystems, ThermoFisher Scientific, (Massachusetts, United State) for each reaction for every sample.

Table 2.1: Three sets of universal primers that were used which target the COI gene.

Name of Primer	Sequence (5' to 3')	Reference
LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer <i>et al.</i> , 1994
HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	Folmer <i>et al.</i> , 1994
Lep-F	ATTCAACCAATCATAAAGATATTGG	Hebert <i>et al.</i> , 2003
Lep-R	TAAACTTCTGGATGTCCAAAAAATCA	
COI-Pat	CAACATTTATTTTGATTTTTTGG	Simon <i>et al.</i> , 1994
COI-Jerry	TCCAATGCACTAATCTGCCATATTA	

2.2.4. Beetle body size and mass

To investigate the relationship between beetle size and activity time, beetle' body sizes were estimated by using simple linear measurements such as body length (Costa & Gomes-Filho, 2002). Measurements for body length as a proxy for size for each species (n = 20) was determined by measuring the mean distance between the head and abdomen extremities of the beetle using a digital caliper (Hernández, 2002). Dry mass of dung beetle species (n = 20) was determined gravimetrically following methods by Gibbs & Marzkin (2001). Briefly beetles from each species (n = 20) were individually weighed in pre-weighed 30ml ventilated plastic vials using a RADWAG microbalance (model AS 220.R2, Poland) to obtain wet body mass. Thereafter the beetles were dried in an oven (UF160, Memmert, Germany) at 60 °C for 24 h to obtain the dry mass.

2.2.5. Data analyses

. Species identifications were done using gross morphology and molecular techniques as described and are presented in the results section as tables. Further, characterisation of species

nesting behavior was done using a combination of field observations and information from literature. CLC Bio Main Workbench v7.6 was used to analyse the AB1 files generated by the ABI 3500XL Genetic Analyzer. The COI BLAST search on NCBI was performed and results correspond to the similarity between the sequence queried and the biological sequences within the NCBI database. Data was analysed using STATISTICA 13.2 (Statsoft Inc., USA). All residuals were tested for normality using the Shapiro–Wilk test before analysis. All the data met the assumptions of ANOVA; therefore, it was analysed using ANOVA, and Tukey HSD post-hoc test was used to separate statistically significant groups. Both one way and factorial ANOVA were used to investigate the relationships between independent variables i.e species, activity time on the dependent variable i. e. the number of beetles.

2.3. Results

2.3.1. Species composition

Species composition from sampling area revealed 38 dung beetle species from 7 dung beetle tribes namely, Coprini, Gymnopleurini, Oniticellini, Onitini, Onthophagini, Scarabaeini and Sisyphini, with majority of these species belonging to the Onthophagini tribe (**Table 2.2**). Molecular identification matched the morphological identification of only 7 samples with about 94.2 – 99% similarities, identifying those species as; *Onitis alexis*, *Metacatharsius opacus*, *Digionthophagus gazella*, *Onthophagus bicallosus*, *Euoniticellus intermedius* and *Onthophagus signatus* (see **Table 2.2**). In addition, the molecular identification of 17 of the specimens showed different species results from gross morphology expectation (**Table 2.2**), which match similarities ranging from 85.9-98% similarity. An additional 11 species, which were only identified at genus level using voucher specimens, were able to be identified up to species level using BLASTING. Only one specimen, which was identified using voucher

specimens as *Neosisyphus calcaratus*, could not be amplified, hence was unable to be identified by molecular techniques (**Table 2.2**).

Table 2.2: Dung beetle species diversity along the Boteti River, Khumaga, Botswana. Beetle collection was done February 2020 using pitfall traps baited with cattle dung. The beetle samples were identified using voucher specimen from Botswana National Museum and specimens from the Royal museum of London. Molecular verification of species was done using Sanger sequencing and COI insect identification was done by BLAST on NCBI.

Sample ID	Voucher specimen ID	Tribe	Molecular ID	GenBank Accession
*NG1	<i>Onitis alexis</i>	Onitini	<i>Onitis alexis</i> (99%)	KU739467.1
#NG2	<i>Neosisyphus calcuratus</i>	Sisyphini	N/A	N/A
^NG3	<i>Gymnopleurus ignites</i>	Gymnopleurini	<i>Gymnopleurus sericeifrons</i> (95.6%)	MN344054.1
*NG5	<i>Metacatharsius opacus</i>	Coprini	<i>Metacatharsius opacus</i> (94.2%)	AY131864.1
*NG6	<i>Digitonthophagus gazella</i>	Onthophagini	<i>Digitonthophagus gazella</i> (98.8%)	KU739497.1
^NG7	<i>Allogymnopleurus thalassinus</i>	Gymnopleurini	<i>Gymnopleurus virens</i> (94.4%),	AY131900.1
^NG8	<i>Caccobius ferrugineus</i>	Onthophagini	<i>Onthophagus fimetarius</i> (90%)	KU739452.1
*NG10	<i>Onthophagus bicallosus</i>	Onthophagini	<i>Onthophagus bicallosus</i> (98.6%)	KU739472.1
!NG11	<i>Onthophagus species1</i>	Onthophagini	<i>Onthophagus similis</i> (93%),	KU739432.1
!NG12	<i>Onthophagus species2</i>	Onthophagini	<i>Onthophagus cuniculus</i> (86.6%)	KU739451.1
^NG17	<i>Catharsius calaharicus</i>	Coprini	<i>Catharsius molossus</i> (92.5%)	KU739435.1
^NG18	<i>Kheper prodigiosus</i>	Scarabaeini	<i>Kheper subaeneus</i> (93.5%)	AF499756.1
*NG24	<i>Euoniticellus intermedius</i>	Onititellini	<i>Euoniticellus intermedius</i> (96%)	KU739490.1
^NG29	<i>Gymnopleurus aenescens</i>	Gymnopleurini	<i>Allogymnopleurus indigaceus</i> (89%)	MN345504.1
!NG32	<i>Scarabeus species 1</i>	Scarabaeini	<i>Scarabaeus pius</i> (89.21%),	KP752059.1
^NG39	<i>Copris elephenor</i>	Coprini	<i>Copris lunaris</i> (89.9%)	JF889540.1
!NG42	<i>Copris species 1</i>	Coprini	<i>Copris tripartitus</i> (86.1%)	NC_045923.1
^NG44	<i>Onthophagus verticallis</i>	Onthophagini	<i>Onthophagus extensicollis</i> (96.1%)	MW982775.1
^NG45	<i>Onthophagus suffuses</i>	Onthophagini	<i>Onthophagus signatus</i> (98%)	EF188216.1
^NG46	<i>Onthophagus stellio</i>	Onthophagini	<i>Onthophagus vulpes</i> (87.8%)	KU739474.1
*NG47	<i>Onthophagus signatus</i>	Onthophagini	<i>Onthophagus signatus</i> (98.7%)	EF188216.1
#NG48	<i>Onthophagus aeruginosus</i>	Onthophagini	<i>Phalops smaragdinus</i> (89.6%)	KU739495.1
^NG49	<i>Onthophagus quadraticeps</i>	Onthophagini	<i>Onthophagus sp.</i> (87.6%)	KU914961.1
!NG50	<i>Onthophagus species 3</i>	Onthophagini	<i>Onthophagus haematopus</i> (89%),	KU739478.1
!NG53	<i>Gymnopleurus species1</i>	Gymnopleurini	<i>Allogymnopleurus indigaceus</i> (89%)	MN345504.1
^NG54	<i>Allogymnopleurus thalassinus</i>	Gymnopleurini	<i>Allogymnopleurus indigaceus</i> (89%)	MN345504.1
^NG55	<i>Scarabaeus goryi</i>	Scarabaeini	<i>Scarabaeus pius</i> (92%)	KP752057.1
^NG57	<i>Afrostrandius plebejus</i>	Onthophagini	<i>Onthophagus cuniculus</i> (88.3%)	KU739451.1
^NG60	<i>Copris cornifrons</i>	Coprini	<i>Copris doriae</i> (91.7%)	KU739435.1
#NG61	<i>Metacatharsius triglodytes</i>	Coprini	<i>Onthophagus bonasus</i> (87.9%)	KU739459.1
!NG63	<i>Onthophagus species4</i>	Onthophagini	<i>Onthophagus fimetarius</i> (85.9%)	KU739452.1
^NG66	<i>Onthophagus sp. nr.sugillatus</i>	Onthophagini	<i>Onthophagus turbatus</i> (88.9%),	MT677918.1
!NG68	<i>Scarabaeus species 2</i>	Scarabaeini	<i>Scarabaeus pius</i> (89.21%)	KP752059.1
^NG71	<i>Esarabaeus remii</i>	Scarabaeini	<i>Scarabaeus pius</i> (93.3%)	KP752066.1
!NG72	<i>Scarabaeus species 3</i>	Scarabaeini	<i>Scarabaeus isidis</i> (91.8%)	MW983441.1
!NG73	<i>Scarabaeus species 4</i>	Scarabaeini	<i>Scarabaeus pius</i> (89.5%)	KP752065.1
!NG76	<i>Copris species 2</i>	Coprini	<i>Copris lunaris</i> (90%),	JF889540.1
^NG77	<i>Scarabaeus zambezianus</i>	Scarabaeini	<i>Scarabaeus pius</i> (92.5%)	KP752059.1

*Specimens identified positively using both morphology and molecular.

^Specimen identified using morphology but molecular tools yielded same genus but different species.

Specimen identified morphologically but unidentified molecularly.

! Morphologically unidentified specimens but species identified through BLAST.

Specimen only identified using gross morphology.

2.3.2. Dung beetles' activity times

The sorting, identification and counting of only species of beetle length greater than 5mm resulted in 16 dung beetle species characterised as large bodied species (**Table 2.3**). Effects of species on dung beetle numbers were statistically significant ($F_{(15, 64)} = 3.73$, $p < 0.05$), with *S. zambeziensis* recorded as the most abundant large bodied species while *G. ignitus* was the least abundant. Effects of activity time on dung beetle number were also statistically significant ($F_{(1, 94)} = 6.73$, $p < 0.05$), with most beetles preferring nocturnal activity compared to diurnal activity (**Figure 2.1**). Effects of species and activity time on dung beetle numbers were also statistically significant ($F_{(15, 64)} = 3.87$, $p < 0.05$) indicating that activity time was species dependent (**Table 2.3**). Further grouping of species by nesting behavior revealed only two types of behavior namely paracoprid and telocoprid (**Table 2.4**). None of the species in our study exhibited endocoprid behaviour. This is expected as these are mostly the smallest that dwell in dung. Sorting of species by body size (beetle length) resulted 16 of the dung beetle species of body length greater than 5mm length, which were characteristic as large bodied species (**Table 2.5**).

Table 2.3.: Activity time of large-bodied dung beetle species, collected from Khumaga village during the summer of February 2021. Beetles were collected using pitfall traps over a period of 3 days. Activity time for a species was determined as the time period in which majority of the individual were collected. From pitfall traps for 3 days. Diurnal species were those which were collected from pit fall trap in the evening before sunset (1800-1900hrs), while nocturnal species were those species which were collected in the morning before sunrise (0500-0600hrs) (Hernández, 2002).

Species	Diurnal	Nocturnal	Total	Activity time
<i>Scarabaeus zambeianus</i>	4	1877	1881	Nocturnal
<i>Kheper. Prodigiosus</i>	3	50	53	Nocturnal
<i>Scarabaeus goryi</i>	6	329	335	Nocturnal
<i>Copris elephenor</i>	10	490	500	Nocturnal
<i>Digionthophagus gazelle</i>	85	211	296	Nocturnal
<i>Catharsius calaharis</i>	0	46	46	Nocturnal
<i>Metacatharsius opacus</i>	1	13	14	Nocturnal
<i>Copris cornifrons</i>	5	71	76	Nocturnal
<i>Metacatharsius triglodytes</i>	52	188	240	Nocturnal
<i>Escarabeaus remii</i>	1	188	189	Nocturnal
<i>Afrostrandius plebejus</i>	0	49	49	Nocturnal
<i>Onitis alexis Klug</i>	22	200	222	Nocturnal
<i>Gymnopleurus aenescens</i>	8	0	8	Diurnal
<i>Euoniticellus intermedius</i>	103	0	103	Diurnal
<i>Allogymnopleurus indigaceous</i>	25	0	25	Diurnal
<i>Gymnopleurus ignites</i>	6	0	6	Diurnal
Total	331	3712	4043	

Table 2.4: Nesting behaviour of dung beetle species identified in Botswana. Paracoprids dig tunnels and bury brood balls directly under a dung pad, Telocoprids move dung balls for a certain distance away from the original dung pad before burying them in the ground and endocoprids form their nests directly in the dung pad (Gebert *et al.*, 2020).

Tribe	Species	Nesting behaviour	Reference
Coprini	<i>Copris elphenor</i>	Paracoprid	Scholtz et al., 2009 Giller & Doube, 1989
	<i>Copris cornifrons</i>	Paracoprid	Scholtz et al., 2009
	<i>Catharsius calaharicus</i>	Paracoprid	Scholtz et al., 2009
	<i>Metacatharsius opacus</i>	Paracoprid	Scholtz et al., 2009
	<i>Metacatharsius triglodytes</i>	Paracoprid	Scholtz et al., 2009
Gymnopleurini	<i>Allogymnopleurus thalassinus</i>	Telocoprid	Holter & Scholtz, 2005
	<i>Allogymnopleurus indigaceus</i>	Telocoprid	Holter & Scholtz, 2005
	<i>Gymnopleurus aenescens</i>	Telocoprid	Holter & Scholtz, 2005
	<i>Gymnopleurus ignitus</i>	Telocoprid	Holter & Scholtz, 2005
Oniticellini	<i>Euoniticellus intermedius</i>	Paracoprid	Dabrowski et al., 2019 Scholtz et al., 2009
Onitini	<i>Onitis alexis</i> Klug		Dabrowski et al., 2019
Scarabaeini	<i>Kheper prodigiosus</i>	Telocoprid	Holter & Scholtz, 2005
	<i>Scarabeus goryi</i>	Telocoprid	Holter & Scholtz, 2005
	<i>Scarabeus zambezianus</i>	Telocoprid	Holter & Scholtz, 2005
	<i>Scarabeus remii</i>	Telocoprid	Holter & Scholtz, 2005
Sisyphini	<i>Neosisyphus calcaratus</i>	Telocoprid	Holter & Scholtz, 2005
Onthophagini	<i>Digitonthophagus gazelle</i>	Paracoprid	Dabrowski et al., 2019
	<i>Onthophagus verticallis</i>	Paracoprid	Scholtz et al., 2009
	<i>Onthophagus quadraticeps</i>	Paracoprid	Scholtz et al., 2009

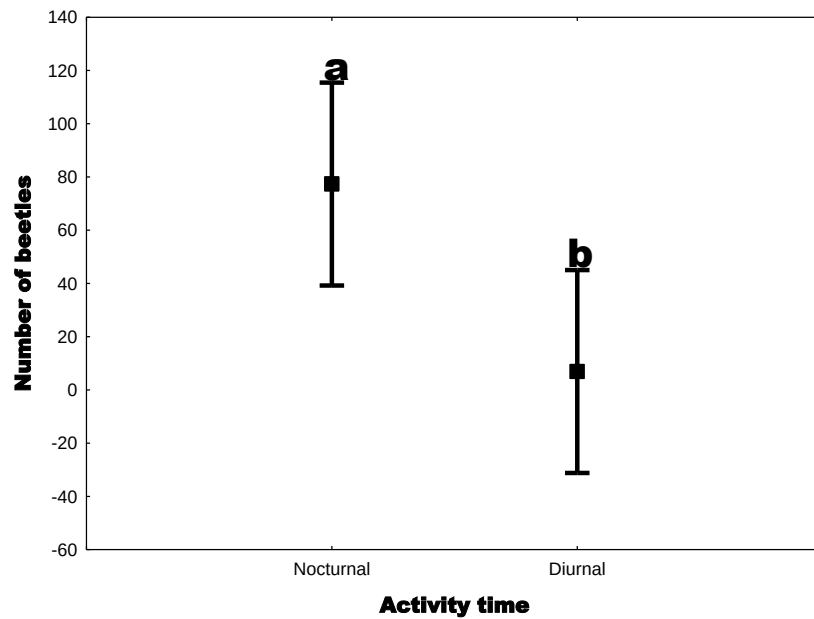


Figure 2.1: Number of large-bodied dung beetle species grouped by activity time (nocturnal or diurnal). Nocturnal active individuals were collected from traps around 0500-0600hrs (before sunrise), while diurnal active individuals were collected around 1800-1900hrs (before sunset). Values represent means and 0.95% confidence limits.

2.3.3. Beetle body length and activity time

Effects of species on beetle size (length) were significant ($p < 0.05$). *Khepher prodigiosus* exhibited the longest length (bigger size) while *E. intermedius*, *O. gazella*, *G. ignitus* and *G. aenescens* all exhibited the shortest length (smaller size). Effects of activity time on beetle body length was also statistically significant ($p < 0.05$). Nocturnal species showed significantly broader body length than diurnal species (**Table 2.5**). Effects of species * activity time were also significant ($p < 0.05$). Nocturnal species (*K. prodigiosus*, *S. goryi*, *S. zambezianus*, *C. elephenor*, *C. calaharis*, *C. cornifrons*, *E. remii*, *O. alexis* and *M. opacus*) showed significantly longer beetle body length than diurnals species (*A. indigaceuous*, *G. ignitus*, *G. aenescens* and *E. intermedius*) (**Table 2.5**).

Table 2.5: Beetle size and activity time in dung beetles. Dung beetle body size was measured as body length represent the distance from anterior margin of head to the posterior edge of the abdomen of each beetle, while beetle dry mass was determined by the gravimetric method. Values for beetle length and mass are represented as mean $\pm$ SEM. Turkey HSD was used to differentiate statistically significant groups, and groups with same letter are not significant from each other.

Species	Activity time	Beetle length (mm)	Beetle dry mass (mg)
<i>Scarabaeus zambezianus</i>	Nocturnal	26.90 $\pm$ 0.313 ^c	550.50 $\pm$ 29.327 ^c
<i>Kheper. Prodigiosus</i>	Nocturnal	39.21 $\pm$ 0.407 ^a	1107.80 $\pm$ 78.950 ^a
<i>Scarabaeus goryi</i>	Nocturnal	34.61 $\pm$ 0.242 ^b	911.80 $\pm$ 49.826 ^b
<i>Copris elephenor</i>	Nocturnal	21.56 $\pm$ 0.181 ^e	416.73 $\pm$ 23.348 ^e
<i>Digionthophagus gazelle</i>	Nocturnal	9.28 $\pm$ 0.165 ^{jk}	37.26 $\pm$ 2.910 ^{jk}
<i>Catharsius calaharis</i>	Nocturnal	25.75 $\pm$ 0.363 ^d	522.50 $\pm$ 21.128 ^d
<i>Metacatharsius opacus</i>	Nocturnal	13.33 $\pm$ 0.484 ^h	65.86 $\pm$ 3.02 ^h
<i>Copris cornifrons</i>	Nocturnal	14.86 $\pm$ 0.045 ^g	153.52 $\pm$ 1.634 ^g
<i>Metacatharsius triglodytes</i>	Nocturnal	11.52 $\pm$ 0.074 ⁱ	40.46 $\pm$ 2.938 ⁱ
<i>Escarabeus remii</i>	Nocturnal	20.61 $\pm$ 0.043 ^e	181.66 $\pm$ 10.019 ^e
<i>Afrostrandius plebejus</i>	Nocturnal	9.96 $\pm$ 0.120 ⁱ	24.18 $\pm$ 1.626 ⁱ
<i>Onitis alexis Klug</i>	Nocturnal	16.05 $\pm$ 0.244 ^f	185.68 $\pm$ 11.00 ^f
<i>Gymnopleurus aenescens</i>	Diurnal	9.35 $\pm$ 0.174 ^{jk}	39.82 $\pm$ 3.895 ^{jk}
<i>Euoniticellus intermedius</i>	Diurnal	8.37 $\pm$ 0.125 ^k	13.77 $\pm$ 0.878 ^k
<i>Allogymnopleurus indigaceus</i>	Diurnal	11.86 $\pm$ 0.137 ⁱ	77.36 $\pm$ 5.059 ⁱ
<i>Gymnopleurus ignites</i>	Diurnal	9.36 $\pm$ 0.099 ^{jk}	32.72 $\pm$ 2.766 ^{jk}

2.4. Discussion

This study revealed 38 dung beetle species from Khumaga village in Botswana. This number represents lower species diversity than the 67 species recorded from Chobe National Park (Tshikae *et al.*, 2008) mainly because most of the tiny species (< 5mm) were not considered in my study. Nevertheless, it should be noted that we used morphology to finally identify species, due to the fact that our results from molecular analysis showed many mismatches between our morphology and GenBank matches. The degree of similarity (%) between morphology and molecular results (BLAST results) may be relatively low because of (i) intraspecific species variation and/or (ii) the sequences for some of these particular species had not been deposited in the GenBank due to limited studies available on this and iii) we realised later that 70%

ethanol concentration, was slightly on the lower side for DNA preservation. This therefore suggests taxonomic complexities in these dung beetle's species that may need to be urgently resolved through further studies. Nevertheless, I strongly feel our morphological identifications are still key in informing species inventory locally, as still to date most studies use morphology to identify dung beetle species (see Nyamukondiwa *et al.*, 2018; Davis *et al.*, 2020; Sands *et al.*, 2021).

Six of the species found in this study namely *S. zambezianus*, *M. opacus*, *M. troglodytes*, *O. plebejus*, *O. quadraticeps*, *O. verticallis* have been recorded in Botswana at Chobe National Park (Tshikae *et al.*, 2008). This result is not surprising as dung beetle assemblages are affected by factors such as temperature, humidity, habitat characteristics and energy resource availability (Beck *et al.*, 2017; von Hoermann *et al.*, 2020; Silva *et al.*, 2020). Dung beetles have been reported to show some specialisation with regard to vegetation cover and soil type (Barbero *et al.*, 1999; Kunz & Krell, 2011; Silva *et al.*, 2020). Although I did not map the soil and vegetation types, the differences in habitat may thus account for differences in species diversity recorded here from that recorded in Chobe national park (see Tshikae *et al.*, 2008). In addition, Khumaga is at the interface of a national park and human dwellings (village) and thus has dung resources from both livestock and wildlife while Chobe national park may lack domestic animal dung resources as such, the differences in dung beetle diversity reported here may be attributable to differences in resources between the two study sites (Tshikae *et al.*, 2008; Beck *et al.*, 2017) but enlightens on dung beetles spatial distribution across heterogeneous microhabitats and overall species richness of natural capital in tropical Africa. Similar differences in dung beetle species diversity with habitat type has also been reported in other countries (see e.g., Ganaswary *et al.*, 2015; Anto & Vinod, 2017).

Furthermore the 38 species I recorded in this study are less than the 52 species which were recorded at the same site by Sands *et al.* (2021). This may be due to the fact that the current

study (i) only used cattle dung for pitfall trapping hence this could have influenced the ‘lower’ species richness observed here, especially that Sands et al. (2021) used four different types of dung, and (ii) small size beetles ($\leq 5\text{mm}$) were not considered. Nevertheless, studies reported no evidence of exclusive preference to any one dung type (Dormont *et al.*, 2004; Tshikae *et al.*, 2008). Similarly, in dung type niche partitioning studies, Sands et al. (2021) documented that most dung beetle species were found on cattle dung, a result consistent with Barbero *et al.* (1999). Furthermore, habitat heterogeneity in the different plant formations have been reported as one of the factors that drives dung beetle species guild, species richness and distribution patterns (Silva *et al.*, 2020) such that species composition differs between open habitats and forests (Galante *et al.*, 1991). As such, dung beetle species exhibit habitat fidelity (see e.g., Pryke *et al.*, 2013; Niino *et al.*, 2014; Ebert *et al.*, 2019). Furthermore, anthropic influences such as fire outbreak and habitat fragmentation have also been reported to influence dung beetle diversity (Davis *et al.*, 2001; Halffter & Arellano, 2002; Silveira *et al.*, 2016).

Classification of species by activity time showed that *S. zambezianus*, *S. goryi*, *K. prodigiosus*, *C. elephenor*, *C. calaharicus*, *M. opacus*, *M. triglodytes*, *C. cornifrons*, *O. gazella*, *O. alexis*, *A. plebejus* and *E. remii*, were nocturnal while only *E. intermedius*, *G. aenescens*, *G. ignitus* and *A. indigaceus* were diurnal (**Table 2.3 & 2.5**). These results are in agreement with other studies which indicated that the nocturnal niche is largely filled by dung beetles of the tribe Coprini (Caveney *et al.*, 1995), while the diurnal niche is usually filled with ball rollers of the tribe Gymnopleurini and Sisyphini. While *O. alexis* is reported here as a nocturnal species, studies show that it is crepuscular, i.e., active during dawn and/or dusk (Houston & McIntyre 1985; Caveney *et al.*, 1995). The current study only investigated nocturnal and daytime active beetles owing to logistical constraints. Future studies should thus also consider sampling during twilight, to disentangle across daytime, night active and crepuscular species. Physiological

responses to diel changes in abiotic conditions may be the main selective pressure resulting in the ecological sorting of diurnal and nocturnal activity patterns among insects (Jayatilaka *et al.*, 2011; Guevara & Avilés, 2013; Bishop *et al.*, 2017). Thus, variations in day and night ecological conditions may drive insects' community patterns such as diversity, abundance and activity.

The differences in activity times among the different dung beetle species observed in this study therefore suggest possible differences in thermal tolerance in these species. This hypothesis however needs to be investigated by determining thermal tolerance limits of diurnal and nocturnal species before drawing up more robust conclusions. Nevertheless my results are in agreement with other studies that have linked insect temperature activity limits to their peak activity times (Bishop *et al.*, 2017; Garcia-Robledo *et al.*, 2018). Furthermore, the results also agree with the hypothesis that temperature constrains animal behaviour by temporarily restricting their activities to more favourable temperature times (McMunn, 2018). The difference in activity times across different species has been linked to interspecific competition (Hanski & Cambefort, 1991). Foraging in the dark, allows species to exploit dung voided at night before it becomes available to dawn and day flying species, thereby contributing to reduced direct competition between day, crepuscular and night species (Caveney *et al.*, 1995). Species that are active at same time of the day may also colonise dung pads at different times due to slight differences in median flight onset times. These subtle shifts in flight onset and duration may result in spatial and temporal partitioning of shared resources, hence allowing for species co-existence (Tilman, 2007). In addition, niche differentiations that arises as a result of difference in activity times also enhance mate recognition and selection (Jacot *et al.*, 2008) and, may also allow for species to forage at near thermal optima (Bishop *et al.*, 2017).

Large bodied species were found to either be paracoprids or telocoprids, with no endocoprids. Endocoprids are usually small beetles ($\leq 5\text{mm}$) body length (Stanbrook *et al.*, 2020). As such, the absence of endocoprids in the current study may have been methodological, since we only picked relatively larger beetles ($\geq 5\text{mm}$). Telocoprids have been reported to be more functionally efficient than both paracoprids and endocoprids (Niino *et al.*, 2014; Bastro-Estrella *et al.*, 2016). Telocoprids were reported to remove a higher proportion (87.46%) while paracoprids only removed smaller portion (12.54%) of the dung (Bastro-Estrella *et al.*, 2016). In a different study, paracoprids were found to remove more dung than endocoprids at both short and long periods (Niino *et al.*, 2014).

Effects of paracoprid beetle size on macronutrient recycling was statistically significant, with large beetles moving more nitrogen, potassium, phosphorus and calcium than the smaller beetles (Stanbrook *et al.*, 2021). Stanbrook *et al.* (2021) defined large beetles ($>25\text{ mm}$), intermediate size ($>15\text{mm}$ to $<25\text{mm}$), and small sized (>5 to $<15\text{ mm}$). In the current study, only four species *vis K. prodigiosus* ($39.21\pm0.407\text{mm}$), *S. goryi* ($34.61\pm0.242\text{mm}$), *S. zambezianus* ($26.90\pm0.313\text{mm}$) and *C. calaharius* ($25.75\pm0.363\text{mm}$) satisfied this definition. Dung beetles contribute unequally to ecological services delivery (Manning & Cutler, 2018). Thus, understanding local species diversity and the efficacy of different species is the first step towards conservation efforts of functionally efficient species. Indeed, mixtures of species are reportedly more efficacious than best functioning monocultures for a particular function through niche complementarity (Milotic *et al.*, 2017). Studies have shown that dung beetle biomass is positively correlated to dung removal efficiency (Niino *et al.*, 2014; Tixier *et al.*, 2015), where body mass heterogeneity (species from each functional group) have been reported to contribute to greater dung removal (Niino *et al.*, 2014).

Furthermore, there was a significant interaction between beetle size and activity timing, with relatively larger dung beetles being active at night, while the relatively smaller ones, were active during the day. This may point to the role of beetle body size in selecting for foraging or activity timing (see Feer & Pincebourde, 2005). These results agree with other studies which found nocturnal insects to be generally larger than diurnal insects (Hernández *et al.*, 2011; Guevara & Avilés, 2013). Larger insects are easier to see than small insects, as such likely to be easily targeted by natural enemies during the day (Berger *et al.*, 2006) than at night. It is also believed that visual predators are responsible for the strict nocturnal activity of many prey animals in a wide variety of taxa (Kronfeld-Schor & Dayan, 2003). Furthermore, larger insects heat up faster and cool slower and as such are prone to overheating should they be active during the day particularly in high tropical temperatures. As such, temperature tolerance also accounts for activity timing in dung beetles (Guevara & Avilés, 2013; Sun *et al.*, 2020).

Furthermore, correlations between beetle body temperature and body mass suggest that larger beetles are better able to maintain their body temperatures than smaller insects despite environmental temperatures (Verdu *et al.*, 2006). These enable large insects to fly over a wide range of temperatures, while limiting smaller insects from flying during the night due to inability to maintain high temperatures required for flight during the cooler night temperatures. Body temperature thresholds needed to initiate flight in insects is usually higher than 30 °C (Advani *et al.*, 2019). As such diurnal activity is often associated with smaller body size (Slade *et al.*, 2007), because night temperatures may be too low and thus energetically costly for flight initiation (Verdu *et al.*, 2006). Furthermore, being small, and having higher surface area to volume ratio, exposes small, bodied beetles to high loss of heat during night foraging. As such, it makes evolutionary sense that bigger beetles may be more restricted to night-time activity

while smaller ones are restricted to daytime activity (the latter also loses heat faster, and thus better adapted to daytime high temperatures).

While reports suggest that insects do exhibit different diel activity pattern (Jayatilaka *et al.*, 2011), Levy *et al.* (2019) showed that daily activity patterns may not be fixed. Changes of activity patterns have been reported in a number of species due to changes in environmental factors such as temperature (Brown *et al.*, 1979). In addition, increase in maximum daily temperature may results in midday temperatures being too hot, thereby resulting in bi-modal activity times among species (see e.g., Bucy & Breed, 2006). Climate change predicts increase in mean temperatures and variability, reduction in vegetation covers and decrease in water availability (Luo *et al.*, 2015; Padrón *et al.*, 2020), as such interactions among these factors can influence temperature variation of microclimates. These changes in microclimate may, thus result in further changes in diel activity patterns. For example, temperature has been reported to affect various factors of an insect body such as survival, performance, fitness and metabolism (Chown & Nicolson, 2004; Angilletta, 2009). In order to avoid the increased metabolic costs of daytime activity; diurnal species may shift to nocturnal activity with lower environmental temperature (Levy *et al.*, 2019). These changes in activity patterns may thus likely affect future intra- and inter-specific relationships in species thereby affecting co-existence. Furthermore, switching of activity patterns may also come with costs, such as asynchrony between activity time and physiological and biochemical processes, and lack of sync between activity timing of dung producers/resources and that of the dung beetles. This asynchrony may directly affect the efficiency of these ecosystem service providers.

In conclusion, this study also showed differences in diel activity timing which could likely be as a result of different species trying to cope with differences in temperatures between day and

night cycle as well as ensuring co-existence among dung beetle species. Future work should investigate the thermal tolerance of diurnal and nocturnal species, as well as investigate how changes in temperature may influence activity timing of local species identified here. These results are important for the conservation of local guilds of dung beetle species diversity and the maintenance of ecosystem service integrity in anthropogenically shifting environments. The results obtained from this study also show that small communities may host diverse dung beetle species that provide essential ecosystem services and that the species diversity may be spatially dependant. This knowledge (of species composition in Khumaga area) is therefore a very important, spatio-temporal dung beetle species inventory data set for the government and associated stakeholders for the development of policies related to land use management and the conservation of ecosystem services and preservation of natural capital. Future studies should improve the current knowledge by also investigating small bodied species for a full complement of dung beetle diversity and abundance across the country to contribute to sustainable management and conservation efforts (Wei *et al.*, 2016). I also recommend that future studies look into how anthropogenic changes in the environment likely affect both species diversity, abundance and efficacy of local dung beetle species (see e.g., Sands & Wall, 2018), and also cover species diversity in agro-ecosystems. In addition, there is need to sample across different seasons and replicate these seasons to get a more comprehensive species inventory of the area studied here. Other studies have shown that warming may affect the behaviour, physiology, growth and survival of insects (Gotcha *et al.*, 2018; Nyamukondiwa *et al.*, 2018; Holley & Andrew, 2019). Thus, future studies should also investigate the effects of climate change on dung beetle physiology and/or fitness and consequent effects on ecosystem service provision.

2.5. References

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Chapter 3

Heat tolerance and activity time in coprophagous beetle species (Coleoptera: Scarabaeidae)

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3.1. Introduction

The rate and magnitude of climate warming are increasing (Meehl & Tebaldi, 2004; Poloczanska *et al.*, 2013); consequently, the structure, function, and provision of many ecological services are often affected (Walther *et al.*, 2002; Ju *et al.*, 2015; Graham *et al.*, 2015). Several reports indicated a higher risk of climate warming to tropical species (Tewksbury *et al.*, 2008), which are physiologically constrained to compensate through phenotypic plasticity (Ghalambor *et al.*, 2006; Stillman, 2003). However, there is increasing evidence that temporal variation in foraging behaviour among generalist species could facilitate adaptation to climate change (Shaffer *et al.*, 2017). Studies revealed that variation in foraging behaviour among species can reduce niche overlap within a population, thereby decreasing competition (Shaffer *et al.*, 2017). Consequently, species niche differentiation can be sustained through utilising different types of foods, such as using only a subset of the resources (Bolnick, 2002; Bell *et al.*, 2009; Navarro *et al.*, 2017), spatially different environments, or temporal species separation (Jayatilaka *et al.*, 2011; Esch *et al.*, 2017).

Dung beetles, most of which belong to subfamily Scarabaeidae, are generalist species that consume mammalian dung. There are approximately 7000 dung beetle species worldwide, with many of the species found in Africa (Goolsby *et al.*, 2017). The diversity of this group stems from differences in body size (Vaz-de-Mello, 2008; Edmonds & Zidek, 2004), shape (Hernández *et al.*, 2011), resource relocation behaviour for feeding, nesting, and diel activity period (Hernández, 2002; Lopes *et al.*, 2011). Three distinct nesting behaviours have been observed in dung beetles (Hanski & Cambefort, 1991), including endocoprids (or dwellers), paracoprids (or tunnellers), and telocoprids (or rollers; Hanski & Cambefort, 1991). By burying dung in the ground, dung beetles contribute to nutrient recycling (Nichols *et al.*, 2008), and act as biological control agents for internal (Sands & Wall, 2016) and external parasites (Bergstrom

et al., 1976). Additionally, dung burial was reported to play a role in reducing carbon dioxide and nitrogen into the atmosphere (Smolka *et al.*, 2012). In the absence of dung decomposers, ~80% of the nitrogen in dung is lost by volatilisation. However, in the presence of dung beetles, nitrogen loss can be reduced to ~5–15% (Gillard, 1967). Moreover, dung beetles create channels in the soil, thereby improving water retention and soil aeration, and reducing soil compaction.

Although the economic value and ecosystem services provided by dung beetles are often ignored in management and policy decisions (Beynon *et al.*, 2015), their benefits in some parts of the world are increasingly becoming appreciated. For example, the economic benefits of dung beetles have been estimated to be ~US\$380 million per year in the United States (Losey & Vaughan, 2006). This estimate includes the reduced use of fertilisers, parasite transmission, and livestock damage due to pest flies. Similarly, in the United Kingdom, the benefits of dung beetles have been estimated to be US\$60 million per year (Beynon *et al.*, 2015). Moreover, it was reported that substantial costs can be incurred when restoring ecological services provided by dung beetles through programs that re-introduce propagule populations (Edwards, 2007). Therefore, understanding how climate change might impact foraging behaviour and ecosystem services is of paramount economic importance for conserving existing biodiversity and associated essential ecosystem functions.

Dung beetle species typically forage at particular times of the diel cycle. Species are characterised as diurnal, crepuscular, or nocturnal (Dacke *et al.*, 2003). Behavioural responses are normally the first responses that insects use to respond to changing environments, owing to their favourable energetic costs (Even *et al.*, 2012; Wong & Candolin, 2015). Indeed, there is evidence that animals can modify their foraging densities in response to biotic and abiotic cues

(Hamer *et al.*, 2009). Recent theoretical and empirical data predicted increasing mean temperatures, and magnitude and frequency of heat waves (Walther *et al.*, 2002; Meehl & Tebaldi, 2004), which have already negatively impacted biodiversity and ecological services (Walther *et al.*, 2002; Ju *et al.*, 2015). However, how this acute heat stress may affect dung beetles and whether these species will behaviourally respond through shifts in activity time is unknown.

This study explored differences in acute heat tolerance across diurnal, crepuscular, and nocturnal dung beetle species by (1) determining critical thermal maxima (CT_{max}), (2) heat knockdown time (HKDT), and (3) the relationship between body size (morphometric) measurements and activity patterns. This information helps elucidate the evolution of current activity times and the ecological principles underlying dung beetle co-existence in nature. The results may also provide insights into possible future abundance of dung beetles and their related ecosystem services in a warming and increasingly divergent climate (Karl *et al.*, 1991). Furthermore, the results may assist in modelling biological diversity, conservation, and the mechanistic basis of temporal foraging activity.

3.2. *Materials and methods*

3.2.1. *Sampling site and trapping methods*

Dung beetles were collected in November 2018 from Khumaga Village (S20.46801; E24.51491; 918 m.a.s.l), Central District, Botswana—a semi-arid region with Kalahari sandy soils. Khumaga has an abundance of wildlife and domestic animals, which supports high coprophagic species diversity (Nyamukondiwa *et al.*, 2018). The typical average annual rainfall is 34.4 mm and average temperature is 23.6°C (Nyamukondiwa *et al.*, 2018; Botswana Meteorological Services, pers comm). In the study area, dung pat temperature was observed to range from 22.1

to 37.2°C (mean, 28.2°C), whereas ambient temperatures ranged from 18.6 to 46.6°C (summer mean, 26.5°C; see detailed climate data trends in Nyamukondiwa *et al.*, 2018).

Based on modified methods (Sands & Wall, 2016; Nyamukondiwa *et al.*, 2018) beetles were captured using pit fall traps, which consisted of plastic containers (2 L) that were buried flush with the ground and covered with fine wire mesh of varying sizes (15- and 30-mm internal diameter) to accommodate a large array of beetle sizes. Fresh cattle dung (500 g) was placed on top of the wire mesh as bait. The traps were covered with overhead plastic plates for protection from rain and direct sunlight (Niino *et al.*, 2014). Beetle collection was done in four periods during a 24-h cycle to establish activity time (diurnal; crepuscular, or nocturnal behaviour) of each species. Specimens were collected at night before sunrise (nocturnal species), 1 h after sunrise and 1 h before darkness (crepuscular species), and daytime (diurnal species) (e.g. Hernández, 2002). Captured beetles were placed in insulated cooler boxes that contained soil and dung for feeding during transportation to the laboratory. In the laboratory, beetles were sorted into morpho-species. Thereafter, five individuals per species were used for species identification using keys from the Royal Museum of London. Our voucher specimens of diurnal (*Gymnopleurus aenescens*, *G. ignitus*, and *Euoniticellus intermedius*), crepuscular (*Onthophagus (Afrostrandius) plebejus*, *Onitis alexis*, and *Onthophagus gazella*), and nocturnal (*Copris elephenor*, *Scarabaeus zambezianus*, and *Metacatharsius opacus*) were kept in the Eco-physiology Laboratory, Department of Biological Sciences and Biotechnology, Botswana International University of Science and Technology. Beetles were kept in climate chambers set at conditions similar to the collection site ($27 \pm 1^\circ\text{C}$, $65\% \pm 2\%$ RH, 12: 12 L: D photoperiod). All experiments were done within 5 days from the day the specimens were collected to minimise stress associated with captivity.

3.2.2. Physiological traits assays

CT_{max} assays

Individual beetles were placed into a double-jacketed chamber with 10 ‘organ pipes’ connected to a programmable bath (Grant GP200-R4, Grant Instruments, UK) (Nyamukondiwa & Terblanche, 2009). A thermocouple (type K, 36 SWG) connected to a digital thermometer (Fluke 54 series II, Fluke Cooperation, China; accuracy: 0.05°C) was inserted into the organ pipe to record chamber temperature. CT_{max} experiments started at an environmentally benign set temperature of 27 °C, from which temperature was increased at a rate of 0.25 °C/min until the beetles reached the upper temperature limit of activity (CT_{max}) (Nyamukondiwa & Terblanche, 2009). This was repeated for n = 20 beetles per species. The experiment was performed on nine species (three diurnal, three crepuscular, and three nocturnal species). In this study, CT_{max} was defined as the temperature at which each individual insect lost coordinated muscle function and consequently lost the ability to respond to mild stimuli (such as gentle prodding). This loss of muscle function coincided with death such that recovery was not possible upon removal from the assay.

HKDT assays

HKDTs were assayed using standardised protocols (Nyamukondiwa & Terblanche, 2009; Gotcha *et al.*, 2018). Ten beetles were individually placed in vials (30 mL) for small beetles (*G. aenescens*, *G. ignitus*, *E. intermedius*, *Onthophagus (Afrostrandius) plebejus*, *Onitis alexis*, and *Othophagus gazella*, and *M. opacus*), and tubes (60 mL) for large beetles (*C. elephenor* and *S. zambeziensis*) and placed in a Memmert climate chamber (HPP 260, Memmert GmbH + Co.KG, Germany). HKDTs (**Table 3.1**) were selected based on preliminary assays of insect upper critical temperatures of activity (for example, Mutamiswa *et al.*, 2018). A video recording camera (HD Covert Network Camera, DS-2CD6412FWD-20, Hikvision Digital Technology Co., Ltd., China) linked to a computer was connected to the climate chamber and

used to monitor knockdown activity. HKDT was defined as the time (in min) at which an individual beetle lost activity following acute heat stress.

Table 3.1: Summary table showing heat knockdown temperatures used in HKDT experiments. These values were obtained following preliminary experimental investigations.

Species	Knock down temperature (°C)
<i>G. aenescens</i>	54
<i>G. ignitus</i>	54
<i>E. intermedius</i>	50
<i>O. (A)plebejus</i>	49
<i>O. gazelle</i>	50
<i>O. alexis</i>	50
<i>C.elephenor</i>	48
<i>S. zambezianus</i>	50
<i>M. opacus</i>	48

Beetle dry mass

Dry mass of each of diurnal (*G. aenescens*), crepuscular (*O. gazelle*), and nocturnal (*C. elephenor*) species was gravimetrically determined following the methods described by Gibbs & Marzkin (2001). Beetles ($n = 20$) for each activity time were individually weighed in pre-weighed 30-mL ventilated plastic vials using a RADWAG microbalance (model AS 220.R2, Poland) to obtain wet body mass. Thereafter, the beetles were dried in an oven (UF160, Memmert, Germany) at 60 °C for 24 h to obtain the dry mass. This dry mass was used to determine the relationship between body size and acute heat tolerance (CT_{max} and HKDT).

3.2.3. Data analyses

Data were analysed using STATISTICA 13.2 (Statsoft Inc., USA) and R 3.3.0 (R Development Core Team, 2016). All residuals were tested for normality using the Shapiro–Wilk test before analysis. CT_{max} data met the assumptions of ANOVA; therefore, it was analysed using one-way ANOVA, and Tukey HSD post-hoc test was used to separate statistically significant groups in STATISTICA. However, HKDT data did not meet the assumptions of ANOVA; thus, they were analysed using a generalised linear model with a Gamma family function in R. Correlation analysis was used to study the relationship between beetle dry mass and heat tolerance (CT_{max} and HKDT).

A phylogeny of the tested dung beetle species was constructed using nine nucleotide sequences of the cytochrome oxidase subunit I gene obtained from the NCBI database (*Scarabaeus zambesianus*; AF499770.1, *Copris lunaris*; JF889540.1, *Euoniticellus intermedius*; MN344203.1, *Metacatharsius opacus*; AY131864.1, *Onitis alexis*; MN343811.1, *Onthophagus gazelle*; EU162450.1, *Onthophagus fimetarius*; AY131925.1, *Gymnopleurus virens*; AY131900.1, *Gymnopleurus humanus*; JN804628.1). Congeneric proxies were used when species samples could not be retrieved owing to contamination or seasonal restraints (that is, for *C. elephenor*, *Onthophagus (A) plebejus*, *G. aenescens*, and *G. ignitus*). The tree was drawn to scale, with branch lengths (above the branches) in the same units as the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.*, 2004) in units of number of base substitutions per site. Codon positions included were 1st+2nd+3rd+Noncoding. All positions that contained gaps and missing data were eliminated. There was a total of 549 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar *et al.*, 2016).

To quantify the influence of phylogenetic signal on CT_{max} and HKDT responses to activity time (diurnal, crepuscular, or nocturnal behaviour), phylogenetic generalised least squares modelling was conducted using means for each species; this enables estimation of λ in each model using maximum likelihood, and tests whether λ significantly differs from 0 (no phylogenetic signal) or 1 (expectation under Brownian motion). Moreover, we compared results from models with λ fixed at 0 and 1 via AIC to further examine whether inclusion of phylogenetic signal influenced the effects of activity time or model fit.

3.3. Results

3.3.1. CT_{max} and activity time

CT_{max} significantly varied across dung beetle species (**Table 3.2**). The diurnally active *G. aenescens* and *G. ignitus* showed the highest CT_{max} , whereas diurnal *E. Intermedius*, crepuscular (*Onitis alexis*, *Onthophagus gazella*,) and nocturnal *S. zambezianus* showed moderate heat tolerance. Crepuscular *Onthophagus plebejus* and nocturnal; *C. elephenor*, and *M. opacus* had the lowest CT_{max} (**Figure 3.1**). The effects of beetle activity time (diurnal, crepuscular, or nocturnal behaviour) on CT_{max} was also significant ($P < 0.05$; **Table 3.2**). The CT_{max} for diurnal species was significantly higher than that for crepuscular ($P < 0.0001$) and nocturnal species ($P < 0.0001$). Nocturnal dung beetles had a significantly lower CT_{max} than both crepuscular ($P < 0.0001$) and diurnal species ($P < 0.0001$). Alternatively, crepuscular species were intermediate between diurnal and nocturnal, with lower CT_{max} than diurnal species ($P < 0.0001$), but higher CT_{max} than nocturnal species $P < 0.0001$). No evidence for phylogenetic signal was found ($\lambda = 0$); λ was not significantly different from 0 ($P > 0.05$), but was significantly different from 1 ($P < 0.05$). Models with λ fixed at 0 also minimised information loss compared with when λ was fixed at 1 ($\Delta AIC = 2.25$), with no overall effect on the significance of activity time for CT_{max} .

Table 3.2: Summary of ANOVA showing the effects of (i) species and (ii) activity time (diurnal, crepuscular and nocturnal) on critical thermal maxima (CT_{max}). Species with same activity time were pooled together to study effects of activity time on CT_{max} .

Effect	<i>SS</i>	<i>DF</i>	<i>MS</i>	<i>F</i>	<i>P</i>
Species	816.1	8	102.0	146.0	< 0.01
Error	119.5	171	0.7		
Activity time	527.1	2,	263.5	112.7	< 0.001
Error	413.8	177	2.3		

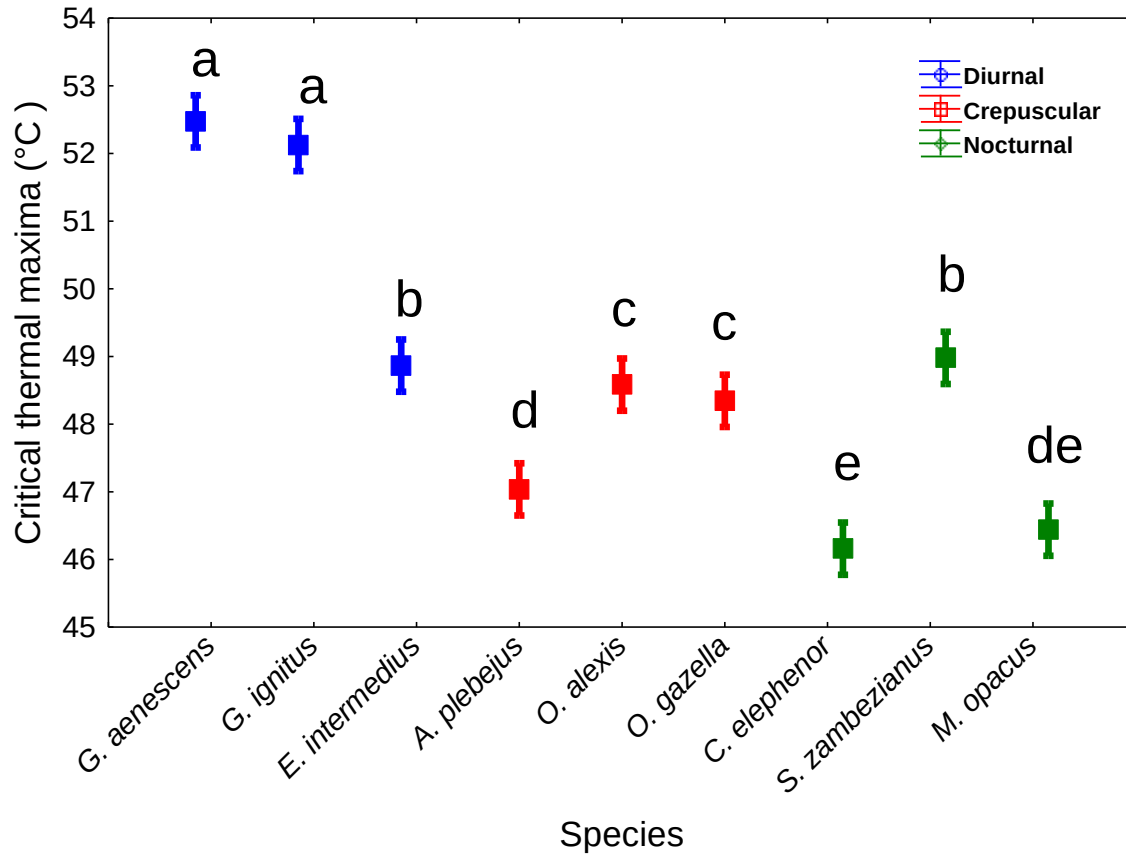


Figure 3.1. Effects of (i) species and (ii) activity time (diurnal, crepuscular, and nocturnal) on the critical thermal maxima (CT_{max}) of dung beetle species. Diurnal species include *Gymnopleurus aenescens*, *Gymnopleurus ignitus*, and *Euoniticellus intermedius*; crepuscular species include *Onthophagus (Afrostrandius) plebejus*, *Onitis alexis*, and *Onthophagus gazella*; and nocturnal species include *Copris elephenor*, *Scarabaeus zambezius*, and *Metacatharsius opacus*. Values represent means ± SE (95% CLs). Tukey HSD post-hoc test was used to separate statistically heterogeneous groups at $P = 0.05$. Means with the same letter are not statistically different.

3.3.2. HKDT and activity times

HKDT showed significant differences across species (**Table 3. 3**). The longest survival time at lethal high temperature was recorded for *Onitis alexis* (67.00 min), *E. intermedius* (57.82 min), and *S. zambezius* (44.87 min), whereas the shortest survival at lethal temperatures was

recorded in *G. aenescens* and *G. ignitus* (**Figure 3. 2**). The effects of activity time on the HKDT of dung beetles was also statistically significant ($p < 0.05$; **Table 3.3**). HKDT among dung beetle species was significantly longer ($P < 0.05$) in crepuscular > nocturnal > diurnal species. This indicates that, when subjected to acute heat stress, crepuscular species are more likely to survive for long periods than both diurnal and nocturnal species. Despite diurnal species having significantly higher CT_{max} , they were more susceptible to acute heat stress compared with the other two groups. Consistent with CT_{max} results, phylogenetic signal was not found for the effect of activity time on HKDT ($\lambda = 0$), with λ significantly different from 1 ($P < 0.05$) but not from 0 ($P > 0.05$). In turn, models with λ fixed at 0 minimised information loss compared with λ fixed at 1 ($\Delta AIC = 2.60$), and phylogenetic signal was not detected when evaluating the effect of activity time on HKDT.

Table 3.3: Summary results of generalized linear model assessing the effects of (i) species and (ii) activity time on the Heat Knockdown Time (HKDT).

Effect	<i>Df</i>	<i>SS</i>	<i>MS</i>	<i>F-value</i>	<i>P</i> (<i>>F</i>)
Species	8	51827	6478	131.6	< 0.001
residuals	174	8567	49		
Activity time	2	2507	1253.5	3.898	< 0.05
Residuals	180	57886	321.6		

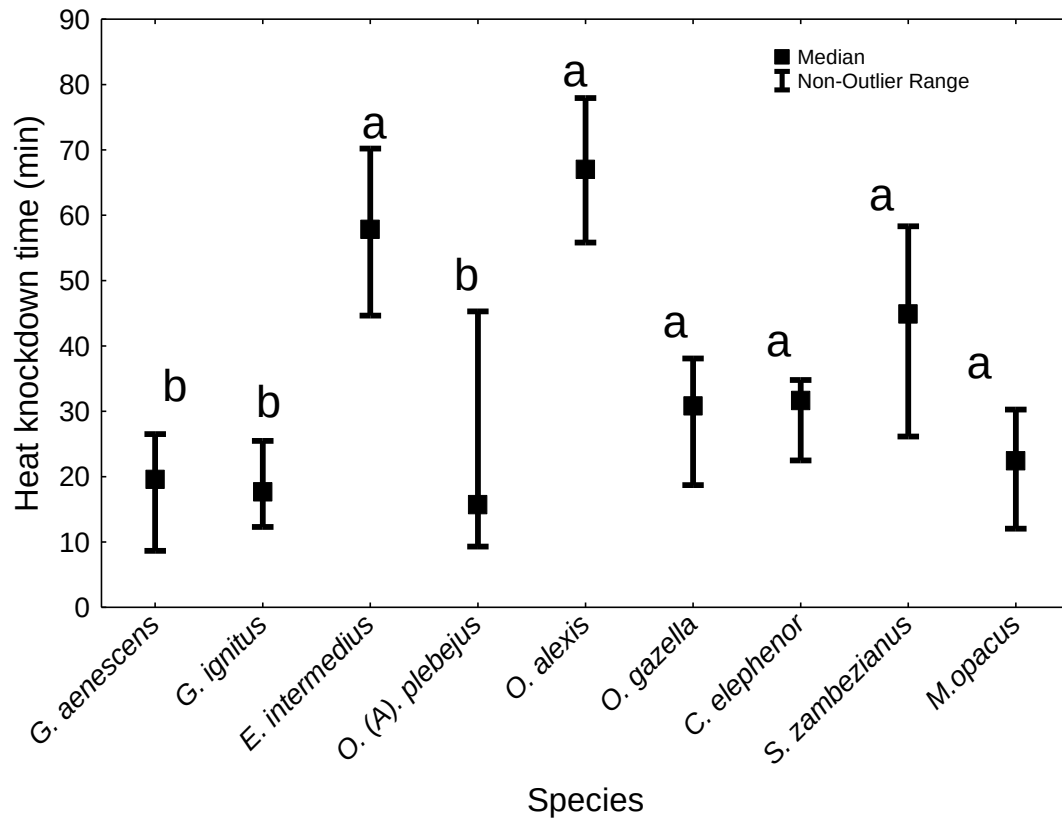


Figure 3.2: Effects of species on heat knockdown time of dung beetle species. Diurnal species include *Gymnopleurus aenescens*, *Gymnopleurus ignitus* and *Euoniticellus intermedius*; crepuscular species include *Onthophagus (Afrostrandius) plebejus*, *Onitis alexis*, and *Onthophagus gazella*; and nocturnal species include *Copris elephenor*, *Scarabaeus zambezius*, and *Metacatharsius opacus*. Statistical significance was analysed through generalised linear model. Values represent median ± 1.0 non-outlier range coefficient.

3.3.3. CT_{max} / HKDT and beetle mass

There was no significant ($P > 0.05$) correlation between CT_{max} and beetle body mass (see **Table 3.4**). Although the strength of the correlation was weak ($r < 0.40$), the direction of the relationship varied across species (**Table 3.4**). The relationship between CT_{max} and beetle body mass was negative for *G. aenescens*, but positive for *Onthophagus gazella* and *C. elephenor* (see **Table 3.4**; **Figure 3.3**). The negligible negative relationship between body size and CT_{max}

in *G. aenescens* may indicate higher acute tolerance for smaller compared with larger beetles. However, the overall results indicate that, within species, CT_{max} was not influenced by body mass.

There was no significant ($P > 0.05$) correlation between HKDT and beetle dry mass (Table 3.4). The strength of this correlation was weak ($r < 0.4$). However, unlike in CT_{max}, these correlations were positive across all species (Table 3.4; Figure 3.4); this indicates no association between HKDT and beetle body mass.

Table 3.4. The relationship between traits of high temperature tolerance and beetle dry mass. Analysis was done using correlation analysis in STATISTICA. r represents the coefficient of correlation.

Traits of high temperature tolerance	R	P
Critical thermal maxima (CT _{max}) vs dry mass		
Species		
<i>G. aenescens</i>	-0.3739	0.1044
<i>O. gazelle</i>	0.3232	0.1645
<i>C. elephenor</i>	-0.0017	0.9942
Heat knockdown time (HKDT) vs dry mass		
Species		
<i>G. aenescens</i>	0.0686	0.7738
<i>O. gazelle</i>	0.2145	0.3639
<i>C. elephenor</i>	0.0819	0.7313

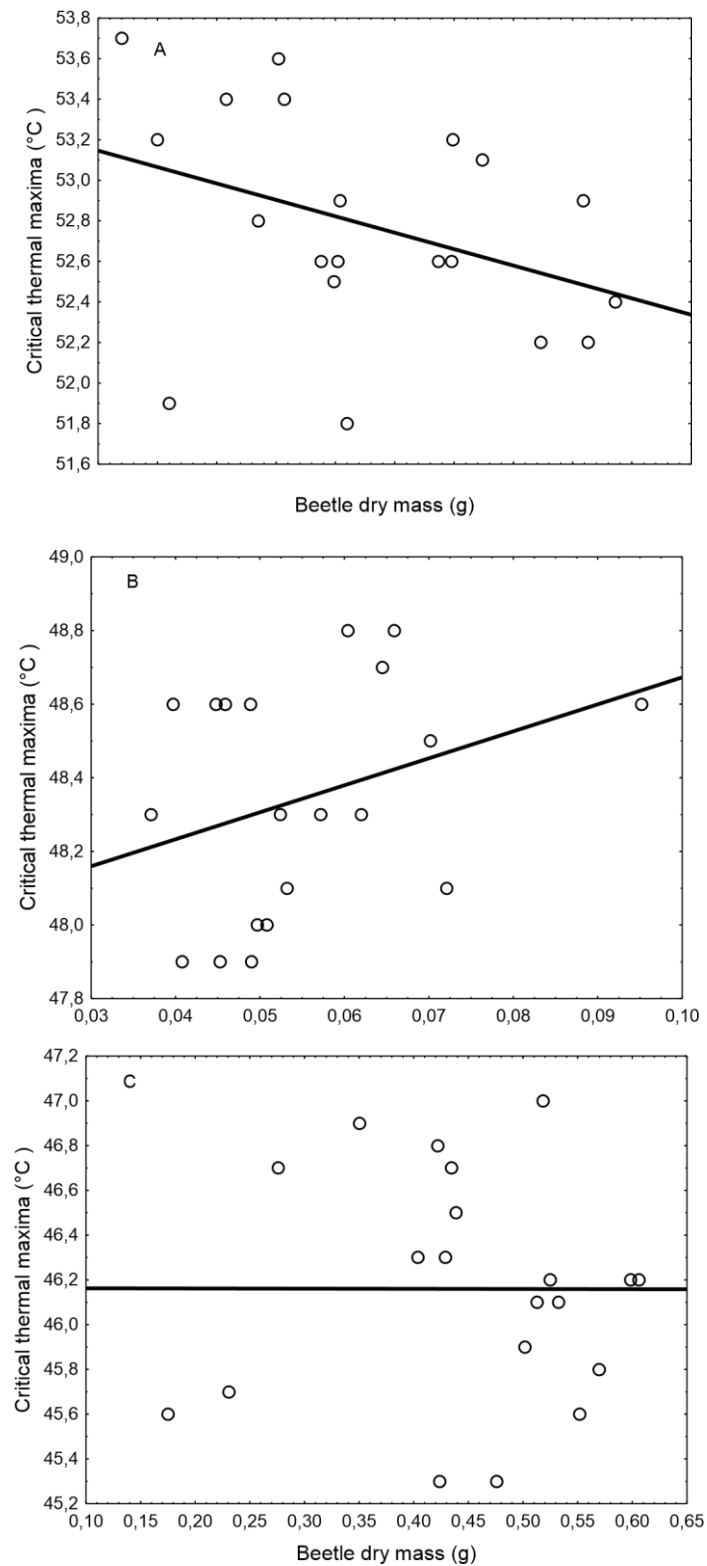


Figure 3.3. The relationship between CT_{max} and beetle dry mass in diurnal *Gymnopleurus aenescens* (A), crepuscular *Othophagus gazella* (B) and nocturnal *Coprís elephenor* (C). Statistical significance was determined at $p < 0.005$. r represents the coefficient of correlation.

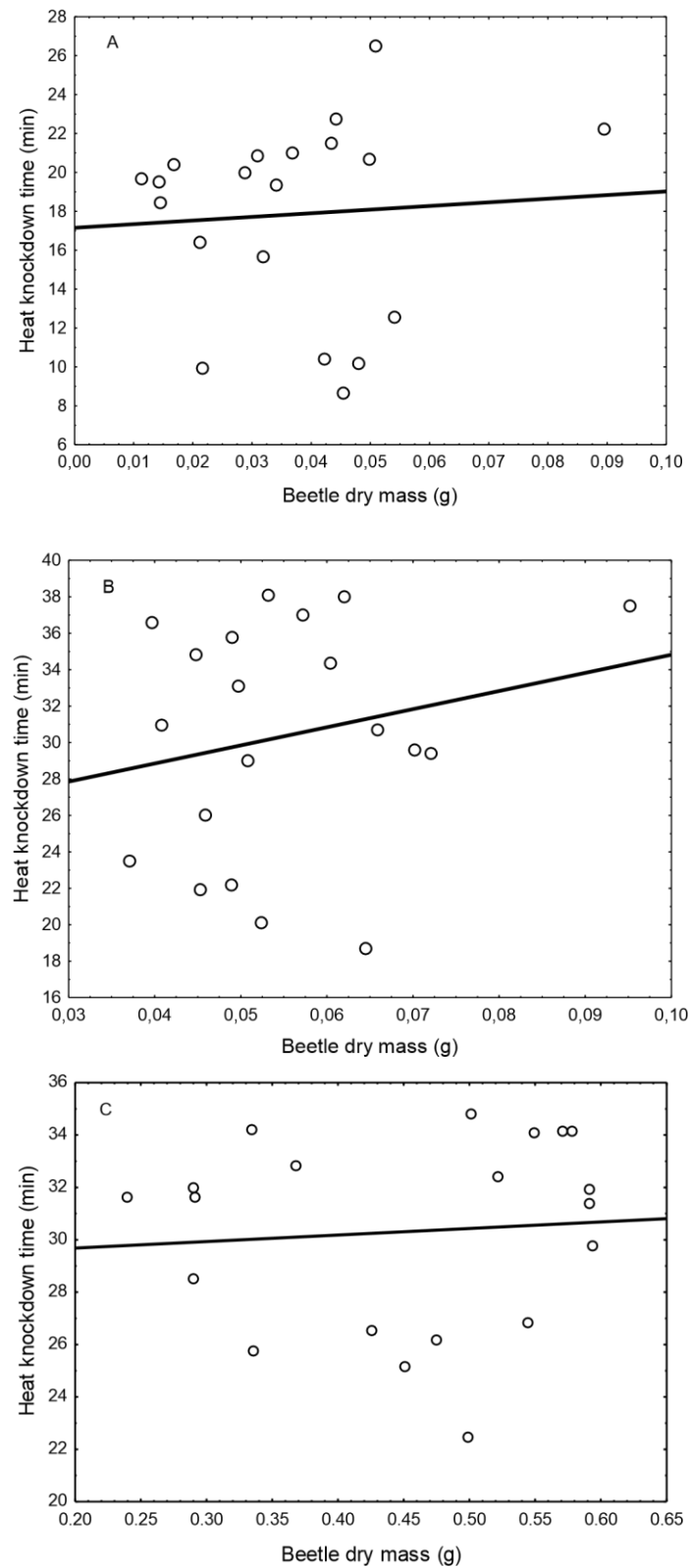


Figure 3.4; The relationship between CT_{max} and beetle body mass in diurnal *Gymnopleurus aenescens* (A), crepuscular *Othophagus gazella* (B) and nocturnal *Copris elephenor* (C). Statistical significance was determined at $p < 0.005$. r represents the coefficient of correlation.

3.4. Discussion

The results showed marked differences in CT_{max} across dung beetle species. This is consistent with study which showed that CT_{max} varied within and across insect species (Agosta *et al.*, 2018).). The differences in CT_{max} between species may be due to the distinct micro-thermal environments that are experienced by individual species (Katzenberger *et al.*, 2018), which are seldom associated with their temporal foraging diurnal cycle timing. Other studies have reported that the CT_{max} of different species and populations differ with geographic range (Esch *et al.*, 2017; Garcia-Robledo *et al.*, 2018); therefore, that the differences in heat tolerance across the dung beetle species could be due to differences in thermal micro-habitat use among species with the same activity time (Baudier *et al.*, 2015; Nyamukondiwa *et al.*, 2018; Katzenberger *et al.*, 2018). I also found no significant evidence for phylogenetic constraint on CT_{max} , which demonstrates that heat tolerance and activity timing may not be linked to dung beetle relatedness.

As I predicted, when dung beetle species were grouped by activity time, diurnal species had significantly higher CT_{max} . This result indicated that diurnal species may have evolved inherent heat tolerance that enables them to be active during the day when temperatures are sub-optimally higher for other species. To my knowledge, this is the first report to evaluate the role of CT_{max} on activity times in dung beetles. Consistent with my results, temperature was shown to influence foraging time in ants (Hurlburt *et al.*, 2008), and the flight and daily activities of Geotrupinae dung beetle species (Gallego *et al.*, 2018). Additionally, species with higher CT_{max} were reported to have higher forage densities at higher temperatures than species with lower CT_{max} (Stuble *et al.*, 2013). However, species abundance at certain time periods and consequently their foraging behaviour may also be a trade-off between thermal tolerance and ecological dominance (Cerdá *et al.*, 1998). Consequently, animals are rarely active at critical

thermal limits (McConnachie *et al.*, 2007), although they may be forced to forage at these limits because of predation pressure or competition (Jayatilaka *et al.*, 2011). Nevertheless, high temperature tolerance does not significantly vary across latitudinal ectotherms (Deutsch *et al.*, 2008); previous reports revealed that tropical species may be living near their upper thermal limits to activity (Tewksbury *et al.*, 2008; Deutsch *et al.*, 2008). Additionally, other studies also reported that temperate species may also be threatened by climate warming (Andrew *et al.*, 2013; Johansson *et al.*, 2020). For the tropical dung beetles reported here, activity close to their upper thermal limits means heat tolerance is a significant trait. Increased heat waves associated with climate change (Meehl & Tebaldi, 2004) affect (1) species directly through temperature-associated mortality and abundance (Chown & Nicolson, 2004; Holley & Andrew, 2019a) and (2) the consequent ecosystem services after the loss of the dung beetles or their activity (Kinzner *et al.*, 2019; Holley & Andrew, 2019b). Nevertheless, organisms can still maintain ecological services through temporary shifts in activity times, and changes in morphology, physiology, and phenotypes (Chown & Nicolson, 2004; Walther *et al.*, 2002).

Apart from evolving higher heat tolerance, high temperature tolerance also depends on environmental history that may confer an acclimatory response (Nyamukondiwa & Terblanche, 2010). Thus, another possible reason for higher CT_{max} values in the diurnal species compared with the crepuscular and nocturnal species could be their prior environments. The diurnal species may occupy a warmer thermal niche than the crepuscular and nocturnal species (Esch *et al.*, 2017), which indicates higher heat acclimation for the diurnal species. Studies on several insect taxa have reported beneficial acclimatory effects to low and high temperatures (Sinclair *et al.*, 2003; Fischer *et al.*, 2010; Nyamukondiwa *et al.*, 2010; Nyamukondiwa *et al.*, 2011; Weldon *et al.*, 2011; Chidawanyika & Terblanche, 2011; Bubliy *et al.*, 2012; Gotcha *et*

al., 2018). Thus, the diurnal species reported here may benefit from occupying a warmer thermal niche (see discussions of beneficial acclimation in Leori *et al.*, 1994)

Nocturnal species had the lowest CT_{max} compared with both crepuscular and diurnal species (**Figure 3.1**). My results are consistent with those of other studies which showed that ant species that are active during the night have lower CT_{max} than those that are active during the day (Jayatilaka *et al.*, 2011; Esch *et al.*, 2017; Garcia-Robledo *et al.*, 2018). Furthermore, species with lower CT_{max} have been found to be less active during high temperatures (Stuble *et al.*, 2013), which indicates that their low CT_{max} could restrict them from being active at night when temperatures are cooler. This is because resistance to environmental stress is affected by a wide range of factors, of which the thermal environment is the most important (Fischer *et al.*, 2010; Andersen *et al.*, 2010). Thus, the environmental temperature to which an organism is exposed can either modulate or decrease resistance to thermal extremes (Bauerfeind *et al.*, 2014). Additionally, studies have reported that cold acclimation may impair or have no effect on heat tolerance (Karl *et al.*, 2014; Scharf *et al.*, 2015; Gotcha *et al.*, 2018); thus, organisms that have been pre-exposed to a cold environment are likely to have lower CT_{max} . This result indicates that mechanisms for cold and heat tolerance may be decoupled. As average mean temperatures and variability are increasing, night temperatures are also becoming increasingly warmer (Meehl & Tebaldi, 2004; Ju *et al.*, 2015). Warming night temperatures may also directly affect nocturnal beetle activities and consequently affect their ecological roles.

Similar to CT_{max} , HKDT was also observed to be species-specific; that is, there were HKDT differences across species (**Table 3.3; Figure 3.2**). HKDT differences could be due to differences in species' inherent basal heat tolerance, which is the trait in question. Nevertheless, these HKDT results indicate that, under acute heat stress, species might reduce their activity time to avoid acute heat stress environments. This change in activity may have negative impacts

on ecosystem services provided by these species. However, to cope with immediate acute heat stress, organisms may compensate by shifting phenology (Parmesan *et al.*, 2003), geography (Chown & Nicolson, 2004), or activity time (Tewksburg *et al.*, 2008; Walther *et al.*, 2002). Congruent with the CT_{max} results, we also found no evidence of phylogenetic constraint on HKDT, which indicates that acute high temperature tolerance and activity timing may not be linked to dung beetle relatedness.

When species were pooled together by activity time, HKDT was significantly longer in crepuscular > nocturnal > diurnal species, which indicated that crepuscular beetles had higher survival under acute heat stress. Additionally, crepuscular species probably had higher heat tolerance (HKDT) owing to their small–medium body size compared to nocturnal species. Smaller insects generally take longer to warm up compared with larger insects owing to their higher surface area-to-volume ratio (Amore *et al.*, 2017). Contrastingly, diurnal species exhibited the shortest survival under high heat stress. The reasons for this anomaly are unknown but may be related to species and traits that depend on heat tolerance.

The correlations between body mass and heat tolerance (CT_{max} and HKDT) were nevertheless non-significant. This indicated that body mass was not affect heat tolerance, at least for the metrics tested here. Furthermore, the different directions of the relationships between body mass and heat tolerance across the species tested point to a complex relationship between the two traits that warrants further investigation. Nevertheless, a negligible negative relationship between day-active *G. aenescens* body mass and CT_{max} may indicate higher heat tolerance (CT_{max}) in smaller beetles. Smaller-bodied species warm up slower and, dissipate heat faster than larger species upon overheating. Thus, there may be a higher cost of foraging at night for smaller species because of excessive radiant heat loss. Conversely, larger body size facilitates

faster warming during night temperatures, which facilitates flight and activity at lower temperatures. Moreover, once larger species warm up, they take longer to dissipate the heat owing to thermal inertia. There may also be a higher cost of activity during the day for larger species owing to faster heat gain and a higher probability of overheating due to slower radiant heat loss.

In conclusion, my results show that day-active dung beetle species generally had higher maximum temperatures for activity (measured as CT_{max}) than crepuscular and nocturnal species. This indicates that heat may play a significant role in choice of activity time in dung beetle species by constraining some species to be active during specific periods of the day. Thus, selection for heat tolerance may be a key trait in the evolution of activity time in dung beetles that were analysed in this study. In the face of increasing acute heat stress with climate change, and as days and nights get hotter; it is likely that diurnal species pushing their activity time closer to crepuscular or fully nocturnal as a way of avoiding the heat of the day. Moreover, warmer nights may constrain the inherent nocturnal fitness and essential ecological roles of nocturnal beetles. These probable shifts in foraging patterns across species that use the same resources and habitats are likely to result in disruption of the balance of ecological niche differentiation based on distinctive temporal activity. This may increase competition, thereby affecting species co-existence and general fitness, and contribute to the decline of some species. Studies have shown that the benefits provided by dung beetles depend on species richness and abundance in a given habitat. Therefore, anything that affects species diversity, abundance, and spatio-temporal distribution may likely reduce the ecological services provided by this group of insects. My results also show no clear correlation between body mass and CT_{max} , which indicates complex associations across species, body mass, and heat tolerance that warrant further investigation. Furthermore, I also showed no significant phylogenetic effects on

foraging activity and heat tolerance traits. However, my conclusions are based on a restricted number of species that were sampled from the same region. It has been documented that ecological service provisions under climate change are very context- and species-dependent (Drieu & Rusch, 2017). Thus, more studies are needed that incorporate a greater number of divergent species that are sampled from different geographic regions to account for the impact of heat tolerance and phylogeny on foraging activity and ecological services under shifting climates.

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Chapter 4

Effects of low temperature tolerance on activity and ecosystem service delivery in coprophagous beetle species (Coleoptera: Scarabaeidae)

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4.1. Introduction

Anthropogenic climate change has increased stressful weather events, increasing the frequency, duration and magnitude of extreme temperatures (Meehl & Tebaldi, 2004; Wernberg *et al.*, 2013). Empirical evidence exists that these anomalies will increase with climate change, albeit more for heat waves (Timmermann *et al.*, 1999; Easterling *et al.*, 2000) than cold waves (but see Wang *et al.*, 2010; Van Der Walt & Fitchett, 2021). Several empirical studies have, nevertheless, shown that low temperatures can affect species population phenology and fitness (Angilleta, 2009; Ma *et al.*, 2017; Denlinger & Lee, 2010; Govindan *et al.*, 2020), and is a major constraint to organismal function and ecological services through limiting insect activity (Denlinger & Lee, 2010). Given the historical, current and projected climate change trajectories associated with extreme events (see LTAS, 2013; Jury, 2013; Kodra *et al.*, 2011; Peings *et al.*, 2013; Harnik *et al.*, 2016; Kim & Lee, 2019), although biased towards increases in mean temperatures, low temperature events remain a critical concern because they are likely to increase in frequency or duration concurrent with increasing mean temperature (see e.g Van Der Walt & Fitchett, 2021.). As such, investigating effects of low temperature stress on essential ecosystem services remains essential as climate change ensues in arid tropical regions (Van Der Walt & Fitchett, 2021).

Insects faced with low temperature stress have evolved a number of mechanisms to maintain key fitness activities under sub-optimal temperatures (Lee, 2010). These mechanisms can be behavioral, morphological and/or physiological strategies (Lencioni, 2004; Sheikh *et al.*, 2017). Some of the behavioral strategies that can be used by insects to survive low temperatures include hibernation (Lee, 2003), migration, and seeking warm shelter. Furthermore, some insect species can restrict foraging to specific times of the day to avoid sub-optimal temperatures (Jayatilaka *et al.*, 2011). Physiologically, insects use three low temperature

survival strategies, namely: chill susceptibility, freeze avoidance and freeze tolerance (Bale, 1993). Chill susceptible insects succumb to non-freezing low temperature stress (Bale, 1993, 1996; Andersen *et al.*, 2015). Freeze-avoiding insects cannot tolerate the formation of ice within their bodily fluids and, as such, depress the temperature at which their bodily fluids will freeze, a phenomenon called supercooling (Bale, 1991; Andersen *et al.*, 2015; Sheikh *et al.*, 2017). Freeze tolerant insects tolerate low temperature through intracellular ice formation with much of the water converted to extracellular ice (Denlinger & Lee, 2010; Danks, 2004). Regardless of compensatory modifications, when insects are exposed to low temperature environments, this can result in reduced activity periods and slowed physiological processes (Muir *et al.*, 2014) thereby reducing activity, development and reproduction (Lee *et al.*, 2010). Consequences of low temperatures on dung beetles in southern Africa, and particularly in Botswana, may be dire, since distributions are largely dependent on livestock and wildlife (Wallgren *et al.*, 2009). The removal of dung resources for these animals, more so under stressful climate environments, is critical in maintaining ecosystem integrity. While climate change and its effects on the environment are increasingly becoming apparent (Walther *et al.*, 2009; IPCC, 2014), most empirical studies have solely focused on insect physiological responses to high temperature (see Marshall & Sinclair, 2012; MacMillan *et al.*, 2016; Overgaard & MacMillan, 2017; Lubawy *et al.*, 2019; Johansson *et al.*, 2020). The impacts of these low temperature events, nevertheless, also threaten ecological service provision. As such, coupling the investigation of effects of low temperature on physiological traits and subsequent ecological services is crucial for explaining the fate of ecological structures and functions under climate change (Chown & Nicolson, 2004; Angilletta, 2009; Chevin *et al.*, 2010).

Numerous ecologically relevant traits have been used to measure the low temperature tolerance of chill susceptible tropical species (see Chown & Nicolson, 2004). Amongst these, the critical

thermal minima (CT_{min}) (Nyamukondiwa & Terblanche, 2009; Hazell & Bale, 2011; Kelty & Lee, 2001; Kellermann *et al.*, 2012; Gotcha *et al.*, 2018) and chill coma recovery time (CCRT) (MacDonald *et al.*, 2004; Sinclair *et al.*, 2007; Weldon *et al.*, 2011; Karl *et al.*, 2014; Findsen *et al.*, 2014; Scharf *et al.*, 2015; Andersen *et al.*, 2015) have been used in empirical studies as reasonable proxies of low temperature tolerance. CT_{min} is the lowest temperature allowing activity whereas CCRT refers to the time taken to recover following chill coma. Ecologically, at temperatures below CT_{min} , organisms lose key activity traits that potentially determine their fitness such as movement, feeding, mating, flying and others. Similarly, a prolonged CCRT is a fitness disadvantage, since the organism under chill coma will be immobile and thus vulnerable to other biotic and abiotic stress factors including predation. Chill coma temperature involves cooling an organism from a starting temperature until the loss of physiological righting response. Chill-coma recovery time is therefore the time it takes an individual insect to self-right following re-exposure to benign temperatures and is widely documented in literature (David *et al.*, 1998; Gibert *et al.*, 2001; Nyamukondiwa & Terblanche, 2009; MacMillan & Sinclair, 2011a; Gotcha *et al.*, 2018; Nyamukondiwa *et al.*, 2018). Similarly, critical thermal limits have also received a lot of attention, owing to their ecological relevance and ability to provide quick indications of organismal temperature activity ranges (Vannier, 1994; Chown & Nicolson, 2004; Terblanche *et al.*, 2007). However, there have been debates around critical thermal limits and methodological contexts (see e.g. Terblanche *et al.*, 2007) and that the ramping rates significantly impact these traits. While most studies have used ramping rates between 0.25 °C /min and 0.5 °C/min, the choice of ramping rates is usually a compromise between ecological soundness and achieving maximum throughput (Kleynhans *et al.*, 2014; MacMillan & Sinclair 2011b; Chown & Nicolson, 2004; Terblanche *et al.*, 2006).

Coprophagic (dung) beetle species are coleopterans that use dung as food and juvenile refuge. By feeding and removing mammalian dung from the ecosystem, they provide an invaluable ecological service that is often ignored in management and policy decisions (Beynon *et al.*, 2015). They are partially responsible for cleaning the environment, nutrient cycling, bioturbation, plant growth enhancement, secondary seed dispersal, soil aeration and parasite control (Nichols *et al.*, 2008). Environmental warming has been associated with changes in dung beetle species distributions (Erasmus *et al.*, 2002; Larsen, 2012), populations (Wu & Sun, 2012) and reduction in egg and larval sizes (Wu & Sun, 2012). Furthermore, climate warming may reduce dung decomposition (Wu *et al.*, 2011), dung burial and reproduction (Holley & Andrew, 2019b). Despite this evidence, little is known on the low temperature tolerance for tropical dung beetles, and the likely fate of ecological services they provide under the increasing stressful low temperatures associated with climate change. To accurately predict the survival, and thus efficient provision of ecological services for tropical species, a comprehensive database of current and projected low temperature data needs to be matched with accurate index of individual species' low temperature tolerance and potential service delivery performance under such conditions (Bale, 1991, 1996).

Different dung beetle species occupy distinct temporal niches and have different activity patterns during the day and throughout different seasons (Jayatilaka *et al.*, 2011; Gotcha *et al.*, 2020). The maintenance of such seasonal and daily activity patterns allows them to exploit a diverse range of food resources, avoid natural enemies and reduce competition (Jayatilaka *et al.*, 2011). However, whether low thermal tolerances have been linked to foraging activity profiles remains understudied (but see Gotcha *et al.*, 2020). More specifically, the role of low temperature tolerance in the evolution of diurnal, crepuscular or nocturnal activity patterns remains unknown. Although behavioural modifications are normally the first and immediate

responses that insects use to respond to changing stressful environments (Even *et al.*, 2012), much work has rather focussed on impacts of physiological effects on the population dynamics and biogeography of insects (Deutsch *et al.*, 2008; Gotcha *et al.*, 2018, Nyamukondiwa *et al.*, 2018). To my knowledge, little work has documented how stressful low temperatures can affect dung beetle behaviours, particularly dung removal efficiency and choice of foraging activity times. Moreover, no studies have looked at the ecological cost of surviving low temperature environments, through e.g., loss of ecological service provision (but see Holley & Andrew, 2019a; b). To partly address the void on tropical insect responses to low temperature, here, I used dung beetle species of three different foraging times (i.e., nocturnal, diurnal and crepuscular) combining both physiology and ecology to study (i) low temperature tolerance across six dung beetle species, two of each diurnal, crepuscular and nocturnal, and (ii) the effects of low temperature tolerance on ecological services, using dung removal efficiency as proxy of ecological services.

4.2. Materials and Methods

4.2.1. Sampling site and trapping methods

Dung beetles were collected in February 2020 from Khumaga Village (S20.46801; E24. 51491; 918 m.a.s.l), Central District, Botswana. This location has abundant wildlife and domestic animals that support high coprophagic species diversity (Nyamukondiwa *et al.*, 2018). Khumaga has an average annual rainfall of 34.4mm, most of it occurring in January and February of the summer season and an average temperature of 26.5 °C. Beetles were captured using pit fall traps, based on methods by Sands & Wall (2016) and Nyamukondiwa *et al.*, (2018). Briefly, a total of 30 pit fall traps (fifteen traps for each mesh size) were used. The traps consisted of plastic containers (2 L) buried flush with the ground and covered with fine wire mesh of varying sizes (15 mm and 30 mm internal diameter) to accommodate capturing of a

large array of beetle sizes (< 30mm). Fresh cattle dung (500g) was placed on top of the wire mesh as bait. The traps were covered from above with plastic plates to protect from rain and direct sunlight (Niino *et al.*, 2014). Beetle collection was done in four periods during a 24 h cycle to establish the activity time (diurnal; crepuscular and nocturnal behaviour) of each species. Specimens were collected at night up to before sunrise (nocturnal species), an hour after sunrise and an hour before darkness (crepuscular species), and daytime between sunrise and sunset (diurnal species). Species were classified as diurnal or crepuscular or nocturnal if the majority of its individuals were collected in one of the specified periods (Hernández, 2002) and following published literature (see Blume, 1984; Caveney *et al.*, 1995; de Oca & Halffter, 1995; Nyamukondiwa *et al.*, 2018; Pokhrel *et al.*, 2020). Beetles were placed in insulated cooler boxes containing moist soil and dung for feeding during transportation to the laboratory. The specimens were kept in climate chambers set at conditions similar to the site of collection (28 ± 1 °C, 65 ± 2 % RH, 12:12 LD photoperiod) and provided with fresh dung for food *ad libitum*. All experiments were done within 5 days from the day the specimens were collected to minimise stress associated with laboratory captivity.

4.2.2. Identification of dung beetle species

Dung beetles were identified using both gross morphology and molecular techniques. In the laboratory, beetles were sorted into morpho-species and five individuals per species were used in the identification of species using specimens from the Natural History Museum (London, United Kingdom) and morphological features (Nyamukondiwa *et al.*, 2018). My voucher specimens of diurnal (*Allogymnopleurus indigaceus*, *Euoniticellus intermedius*), crepuscular (*Onitus alexis*, *Onthophagus gazella*) and nocturnal (*Copris elephenor*, *Scarabaeus zambezianus*) were kept in the Eco-physiology Laboratory, Department of Biological Sciences and Biotechnology, Botswana International University of Science and Technology. Briefly,

two individuals of each species were used for mitochondrial DNA extraction and analysis using either a leg or whole organism for large and small beetles, respectively (Monaghan *et al.*, 2007). The DNA was extracted using a commercial kit (Zymo-research Quick-DNA Tissue/Insect Miniprep Kit (Zymo Research, USA); extraction procedures were conducted as per the manufacturer's instructions. After extraction of the total genomic DNA, 2 µl of the DNA was used for PCR. For this procedure, the barcoding primers LCO1490 and HCO2198 that amplify the insect mitochondrial cytochrome oxidase 1 gene (COI) were used. For each of the samples, 2 µl of each of the primers along with the 2 µl of insect DNA was added to 12.5 µl of the NEB Q5[®] High-Fidelity 2X Master Mix that was previously added with 6.5 µl of Nuclease free water, to a total of 25 µl in 0.2 ml PCR tubes. The PCR conditions used were as follows: 98 °C for 3 min (initial denaturation); 98 °C for 30 sec (denaturation); 47 °C for 30 sec (annealing), and; 72 °C for 45 sec (extension). Thirty-two cycles were used for PCR amplification and the final extension was at 72 °C for 5 min. Results were confirmed in an Agarose gel electrophoresis. The successful samples were sent for Sanger sequencing at a commercial sequencing facility (Inqaba Biotec Pty Ltd, South Africa).

4.2.3. Physiological traits

Two physiological traits were measured, critical thermal minima (CT_{min}) and chill coma recovery time (CCRT). These metrics (CT_{min} and CCRT) are considered standardised and are the most useful proxies of cold tolerance, linking well with insect species' distributions (Sinclair *et al.*, 2015; Andersen *et al.*, 2015). For CT_{min} , individual beetles were placed into a double jacketed chamber with ten 'organ pipes' connected to a programmable water bath (Grant GP200-R4, Grant Instruments, UK) (see Nyamukondiwa & Terblanche, 2009; Sinclair *et al.*, 2015; Mutamiswa *et al.*, 2018; Nyamukondiwa *et al.*, 2018). This was repeated twice for each experiment to yield $n = 20$ beetles per species. A thermocouple (type K, 36 SWG)

connected to a digital thermometer (Fluke 54 series II, Fluke Cooperation, China; accuracy: 0.05 °C) was inserted into the control organ pipe to record the chamber temperature. Experiments started at an environmentally benign set temperature of 28 °C, from which temperature was ramped down at a rate of 0.25 °C /min until the beetles reached the lower temperature limit of activity (CT_{min}) (Nyamukondiwa & Terblanche, 2009; Gotcha *et al.*, 2018). In this study, CT_{min} was defined as the temperature at which each individual insect lost co-ordinated muscle function, consequently losing the ability to respond to mild stimulus (e.g. gentle prodding).

For CCRT, beetles ($n = 20$ beetles per species) were tested following methods by Weldon *et al.*, (2011). Beetles were individually placed in screw-cap plastic vials with two 1 mm diameter holes pierced through the caps for ventilation. The vials were then placed into a large zip-lock bag which was then plunged into a water bath set at 0 °C for 1 hour. These assay conditions are sufficient to induce chill coma (see Weldon *et al.*, 2011). Following chill coma, the plastic vials were then transferred to a climate chamber set at $28 \pm 1^\circ\text{C}$ (average temperature at site of beetle collection) and CCRT was recorded through observations from the camera (HD Covert Network Camera, DS-2CD6412FWD-20, Hikvision Digital Technology Co., Ltd, China). CCRT was defined as the time (minutes) required for each beetle to stand on its legs (self-righting), without any interference or stimulation from the observer, following chill coma (as in David *et al.*, 1998; Gotcha *et al.*, 2018). Environmental microclimate temperatures were recorded using thermocron iButtons (Dallas Semiconductors, Model DS1920) [0.5 °C accuracy; 30-min sampling frequency] across ten sites in Botswana for two years (see Machekano *et al.*, 2019). Temperatures eliciting chill coma and those stopping activity (CT_{min}) were frequently recorded across all ten sites.

4.2.4. Ecological traits assays

Two ecological traits, *vis* dung ball number and size, representing proxies of ecological services, were measured. To investigate how low temperature tolerance may influence dung removal efficiency, dung ball number and size were measured at three temperatures (28 °C (benign temperatures), 23 °C ($\Delta T = -5$ °C) and 18 °C ($\Delta T = -10$ °C)) in two selected species: *A. indigaceus* (diurnal species) and *S. zambezianus* (nocturnal species), because these were the most abundant species in the trapping area. Dung rolling behaviour in these species was identified through observation. Soil was placed into three replicate plastic containers (length 50cm, width 30cm and depth 15cm), and five *S. zambezianus* dung beetles of mixed sex were placed in each container. The containers were covered with a screen mesh (2mm diameter) to simultaneously allow aeration and prevent the beetles from escaping. These replications were then placed in a climate chamber set at 28 °C and 12h/12h light: dark ratio, simulating 12 hours of daylight and 12 hours of darkness. These temperature and photoperiod combinations represent the average temperatures prevailing in the natural environment during summer (see Nyamukondiwa *et al.*, 2018) and thus served as a control condition. Beetles were given 30 minutes to acclimatise to the new environment after which 200g of freshly homogenised cattle dung was placed at the centre of each container. After 12 h of night cycle (for *S. zambezianus*), all dung balls were counted and the diameter for each ball measured using an electronic digital calliper (E- base measuring tools Co., model SV-03-150, Size 6in/150mm, Pert Industries, Johannesburg, South Africa). The dung ball horizontal and vertical diameters were measured for the same ball, from which average dung ball diameter was calculated for each ball. The procedure was repeated for *A. indigaceus*, as well as at 23 °C and 18 °C with new individuals for both species. For *A. indigaceus*, dung ball production was measured during the 12 h day cycle in keeping with its natural diurnal activity times.

4.2.5. Statistical analyses

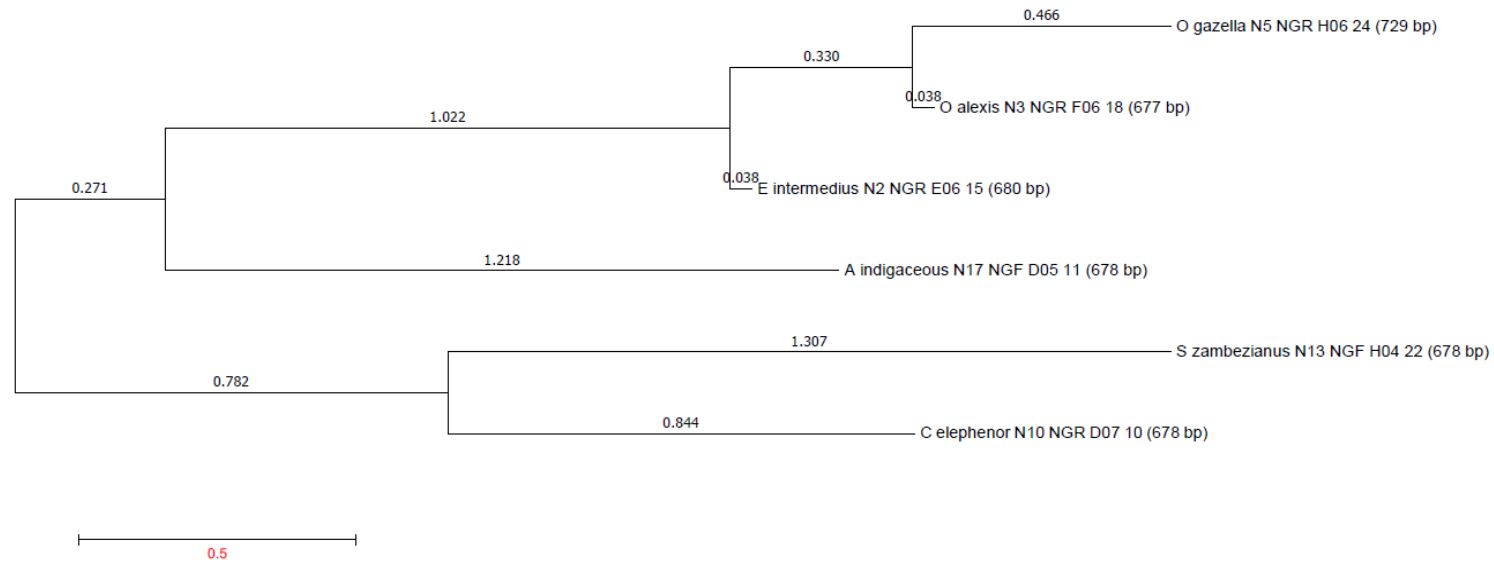
Data were analysed using STATISTICA 13.2 (Statsoft Inc., Tulsa, Oklahoma) and R version 3.3.0) (R Development Core Team, 2016). Data for CT_{min} and CCRT were analysed using separate generalised linear model (GLMs) with Gamma links as functions of species (6 levels) and activity time (3 levels). Effects of temperature (3 levels) and species (2 levels) on dung ball size and number were investigated through separate factorial ANOVAs, as normality assumptions were satisfied (Shapiro-Wilks's test, $p > 0.05$). The interactions between these variables in each model were included. In all models, Tukey comparisons were used post hoc to examine significant effects.

Further, a phylogeny of the six dung beetle species was constructed using nucleotide sequences of the cytochrome oxidase subunit I gene. The tree was drawn to scale, with branch lengths (above the branches) in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.*, 2004) and are in the units of the number of base substitutions per site. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There was a total of 549 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar *et al.*, 2016).

The tree (**Figure 4.1.**) was then used to investigate phylogenetic constraints on low temperature tolerance (CT_{min} and CCRT). Phylogenetic generalised least squares (PGLS) models were used to determine any influence of phylogenetic signal on CT_{min} and CCRT responses to activity times. Mean species-specific response values were first combined with the phylogeny among the respective species to account for relatedness. Then, PGLS models were fit for each response to obtain maximum likelihood estimates and p -values for λ (0-1), with values of 0

corresponding to no phylogenetic signal, and 1 to expectations under Brownian motion. The model used maximum likelihood estimation to simultaneously examine statistically whether phylogenetic signal differed from 0 or 1 for each response variable. However, I acknowledge that the low number of species included in the present study reduces the potential to detect significant phylogenetic effects. The mean daily minimum microclimate temperature data was plotted using SigmaPlot® (IBM Statistics, USA)

1



2

3 **Figure 4.1:** A phylogenetic tree of the tested dung beetle species constructed using nine nucleotide sequences of the cytochrome oxidase subunit I gene. The tree was drawn to
 4 scale, with branch lengths (above the branches) in the same units as the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed
 5 using the Maximum Composite Likelihood method (Tamura *et al.*, 2004) in units of number of base substitutions per site. Codon positions included were
 6 1st+2nd+3rd+Noncoding. All positions that contained gaps and missing data were eliminated. There were a total of 549 positions in the final dataset. Evolutionary analyses
 7 were conducted in MEGA7 (Kumar *et al.*, 2016).

4.3. Results

4.3.1. CT_{min} and activity time

CT_{min} values differed significantly among the six species (**Table 4.1**), with nocturnal *C. elephenor* exhibiting the lowest critical thermal minimum (greatest cold tolerance), which was significantly different to all species ($p < 0.001$) except *S. zambezianus*, the other nocturnal species ($p > 0.05$; **Figure 4.2**). In turn, *S. zambezianus* exhibited significantly lower CT_{min} than the remaining four species (all $p < 0.001$). CT_{min} between crepuscular species *O. gazella* and *O. alexis* did not significantly differ ($p > 0.05$), whilst diurnal *E. intermedius* was significantly higher than *O. gazella* ($p < 0.01$) but not *O. alexis* ($p > 0.05$; **Figure 4.2**). Last, diurnal *A. indigaceus* CT_{min} was significantly higher than all other species (all $p < 0.001$). Activity time also significantly (**Table 4.1**) influenced CT_{min} across species with diurnal species exhibiting significantly highest CT_{min} values (i.e., lowest cold tolerance; both $p < 0.001$) and crepuscular species significantly less cold tolerant than nocturnal ones overall ($p < 0.001$; **Figure 4.2**).

Table 4.1: Summary results of effects of (i) species and (ii) activity time on the CT_{min} and CCRT. P-values in bold font represent statistical significance.

Trait	Effect	Df	F-value	<i>p</i>
CT_{min}	<i>Species</i>	5	330.56	< 0.001
	residuals	114		
	<i>Activity time</i>	3	248.01	< 0.001
	residuals	117		
CCRT	<i>Species</i>	5	157.25	< 0.001
	residuals	114		
	<i>Activity time</i>	3	21.56	< 0.001
	residuals	117		

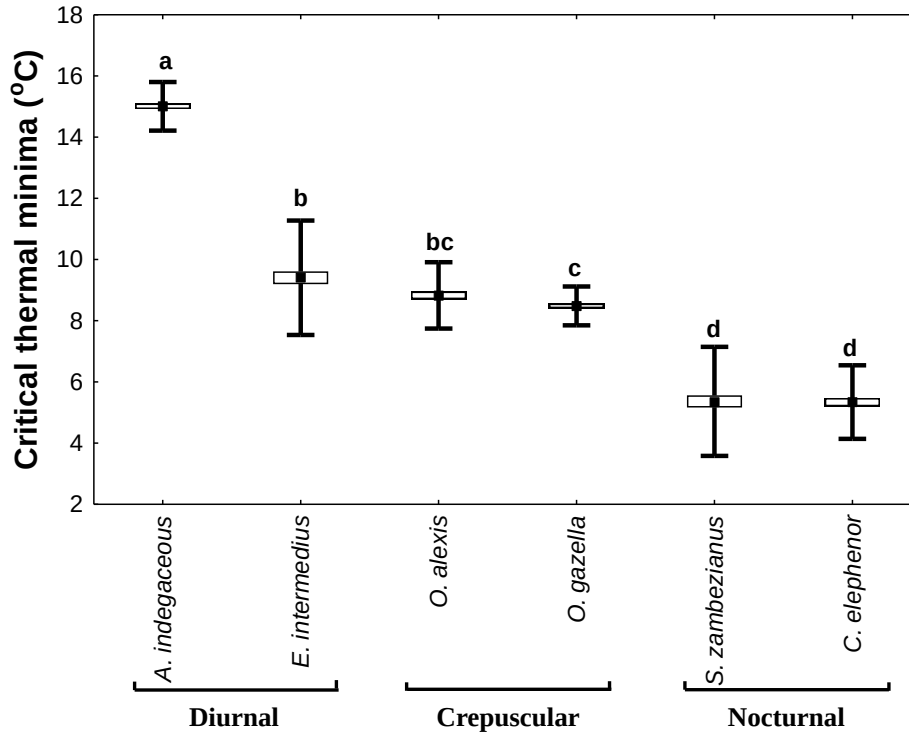


Figure 4.2. Critical thermal minima (CT_{min}) of diurnal, crepuscular and nocturnal dung beetle species. Values and boxes represent means $\pm$ 95% confidence limits and interquartile ranges, respectively. Values with the same letter are not statistically different.

4.3.2. Chill coma recovery time and activity time

CCRT values were also significantly different among species, with crepuscular *O. gazella* exhibiting significantly lowest CCRT ($p < 0.001$; **Table 4.1**), except in comparison to diurnal *E. intermedius* ($p > 0.05$; Figure 4.3). In turn, diurnal *E. intermedius* had significantly lower CCRT than the remaining four species (all $p < 0.001$). In addition, CCRT of diurnal *A. indigaceus* was significantly lower than nocturnal *C. elephenor* and crepuscular *O. alexis* (both $p < 0.001$), but not nocturnal *S. zambeziensis* ($p > 0.05$). Last, for *O. alexis* and *C. elephenor*, CCRT did not differ significantly ($p > 0.05$), but both were higher than the other species (all $p < 0.001$; **Figure 4.3**). Activity time also significantly (**Table 4.1**) affected CCRT, whereby diurnal species had significantly lowest CCRT (both $p < 0.001$), while nocturnal and

crepuscular differences were not statistically different ($p > 0.05$; **Figure 4.3**). No significant phylogenetic signal was observed ($\lambda = 0$), with λ significantly different from 1 (i.e. expectation under Brownian motion; $p < 0.01$) but not 0 (i.e. no signal; $p > 0.05$) for both $CT_{\min}$ and CCRT. It should however be noted that the low number of species considered here reduces the capacity to detect phylogenetic signal.

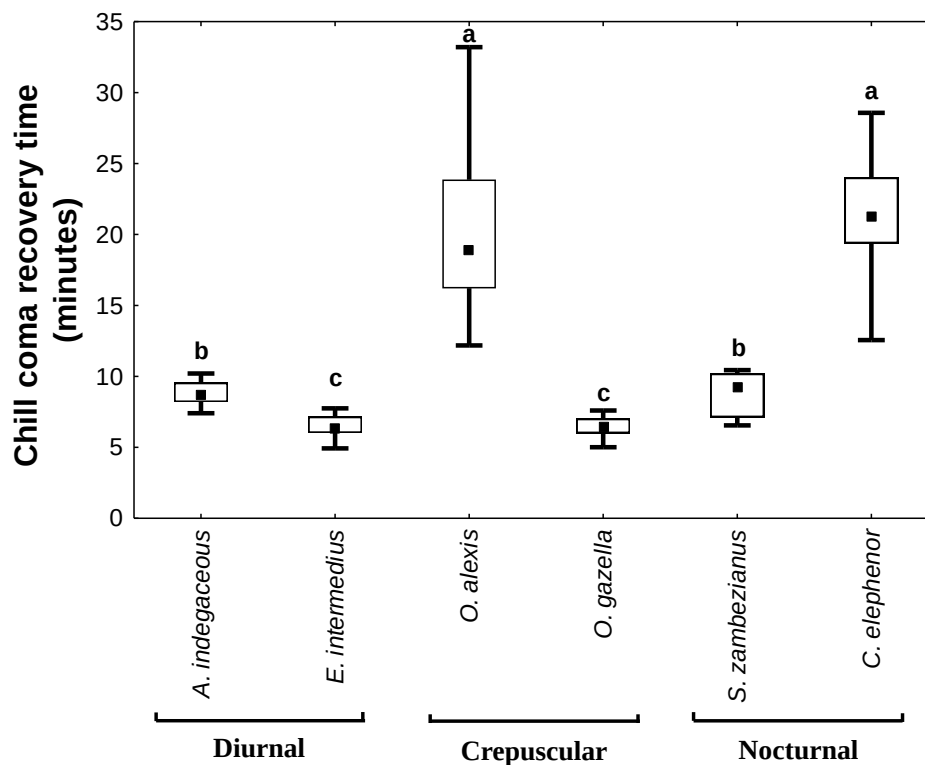


Figure 4.3 Chill coma recovery time (CCRT) of diurnal, crepuscular and nocturnal dung beetle species. Values and boxes represent medians $\pm$ 95% confidence limits and interquartile ranges, respectively. Values with the same letter are not statistically different.

The mean daily minimum temperature recorded from ten sites over 24 months showed that minimum temperature ranged between -0.33°C to 22.01°C . Furthermore, there was a prolonged persistence of these low temperatures over the time the microclimate temperature records were taken (see **Figure 4.4**). Approximately 57% (403 of 705) of values were below

15.1 °C, the CT_{min} for the most vulnerable species (*A. indigaceus*), while only 18% (125 of 705) of values were below of 5.3°C, the CT_{min} for the most resilient species (*C. elephenor*). In addition, about 34% (238 of 705) of values were below the average CT_{min} (8.7°C) for all species in this study. Similarly, temperatures eliciting chill coma (0°C) were also recorded (see **Figure 4.4**).

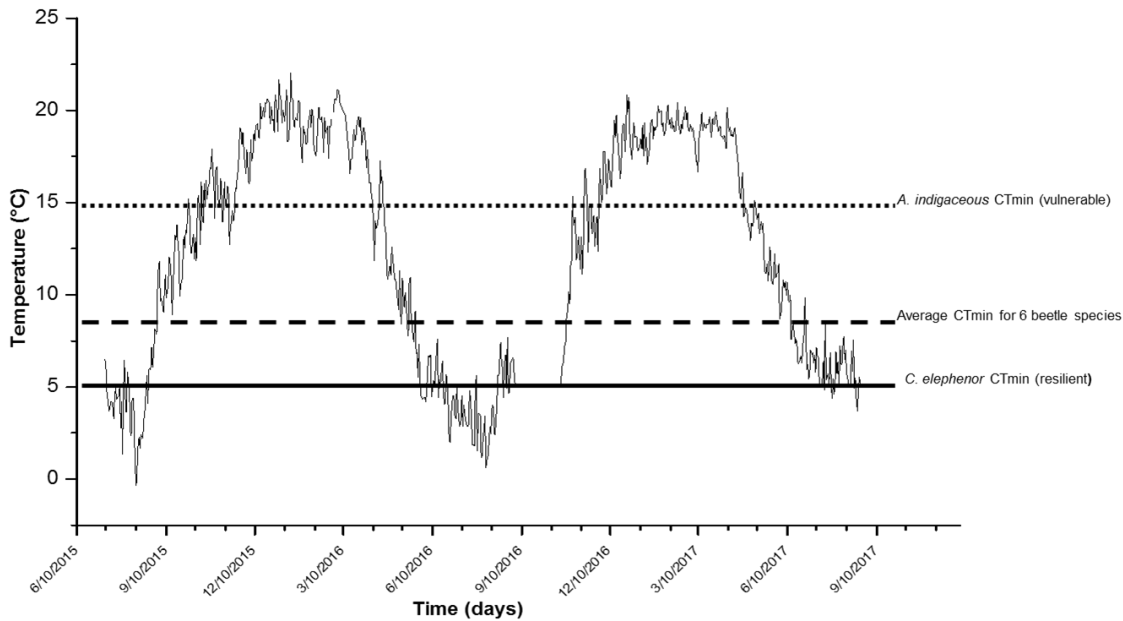


Figure 4.4: The mean daily minimum temperatures from ten ecological sites around Botswana for a period of 24 months prior to the study (July 2015 – August 2017). The horizontal lines represent how low environmental temperature records went below the species with the highest CT_{min} (most vulnerable species, *A. indigaceus*), the average CT_{min} for all the beetle species in the study and the species with the lowest CT_{min} (the most resilient species, *C. elephenor*).

4.3.3. Ecological traits

The effects of species on dung ball numbers were not significant overall (**Table 4.2**). Effects of temperature on dung ball numbers was however significantly different among species (as indicated by a significant interaction term (**Table 4.2**). *Allogymnopleurus indigaceus* produced significantly fewer ($p < 0.05$) balls at 18 °C than at 28 °C (see **Figure 4.5A**),

indicating low temperatures are likely to reduce the number of dung balls produced for this diurnally active species. Conversely, the nocturnal *S. zambeziensis* dung ball production was not significantly different across temperatures (all $p > 0.05$). Contrastingly, the effect of species on dung ball diameter was only significant overall (**Table 4.2**). Nocturnally active *S. zambeziensis* produced significantly bigger balls than day active *A. indigaceus* (**Figure 4.5B**). Effects of temperature on dung ball diameter were, however, not significant (**Table 4.2**), while interaction effect between temperature and species on dung ball size were also not statistically significant (**Table 4.2**).

Table 4.2: Effects of species and temperature and their interactions on dung ball number and dung ball diameter. p -values in bold font represent statistical significance.

Trait	Effect	Df	F-value	P
Dung ball number	Species	1	0.46	0.51
	Residuals	12		
	Temperature	2	20.0	0.0015
	Residuals	12		
	Species *Temperature	2	4.91	0.027
	Residuals	12		
Average dung ball diameter	Species	1	244.3	0.001
	Residuals	98		
	Temperature	2	0.49	0.60
	Residuals	98		
	Species *Temperature	2	0.064	0.94
	Residuals	98		

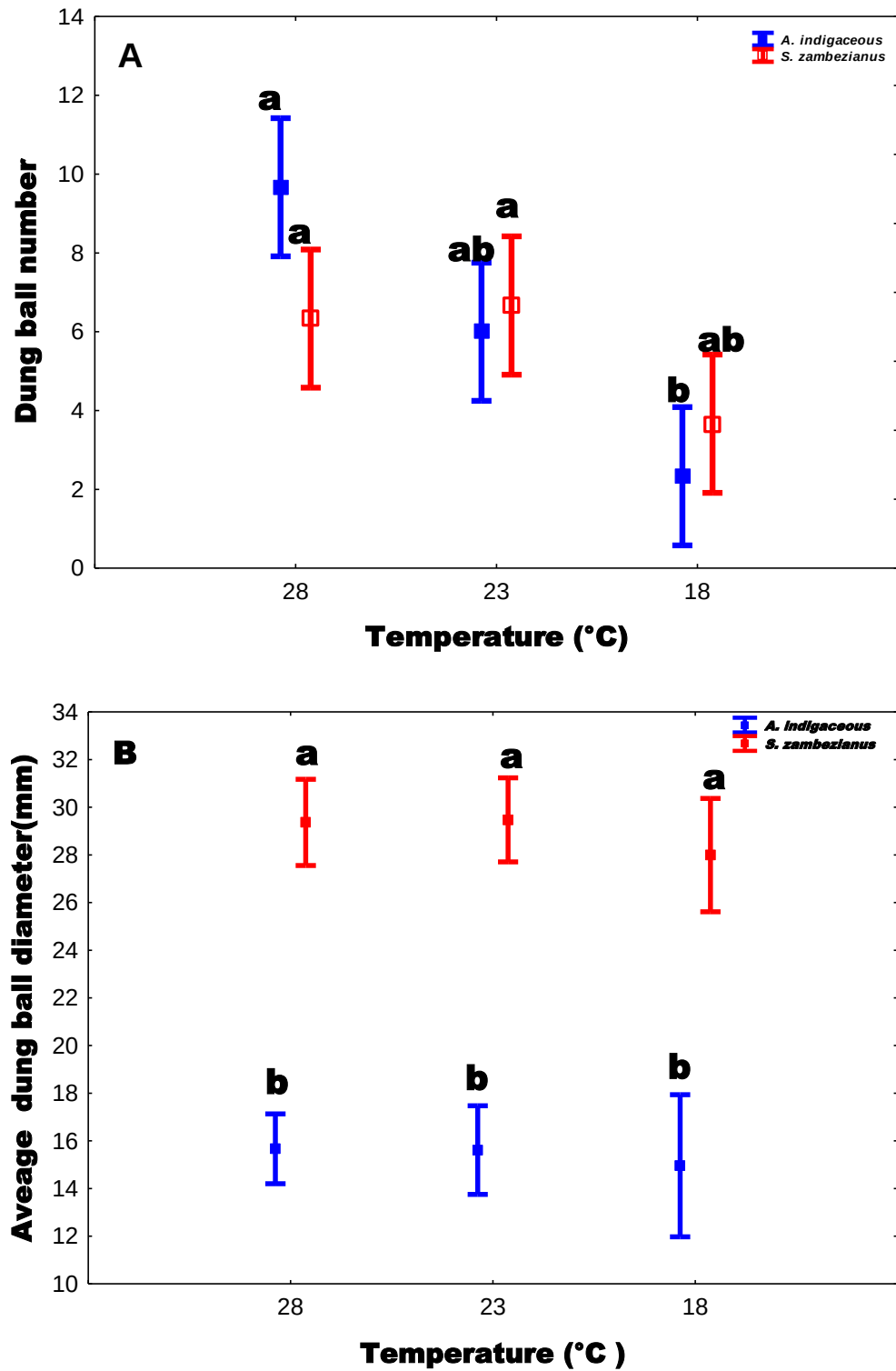


Figure 4.5: Effects of low temperature on (A) dung ball number and (B) dung ball diameter in a nocturnal (*S. zambezianus*) and diurnal (*A. indigaceus*) dung beetle species. Values represent means $\pm$ 95% confidence limits. Means with the same letter are not statistically different.

4.4. Discussion

The results showed nocturnal species had a lower minimum temperature for physiological activity (CT_{min}) than both crepuscular and diurnal species. This aligns with the thermal adaptation hypothesis (Bishop *et al.*, 2017; Garcia-Robledo *et al.*, 2018) and confirms insects temperature activity limits are closely linked to their peak activity times. The results are also in keeping with our hypothesis that temperature constrains animal behaviour by temporarily restricting their activities to more favourable temperature times (McMunn, 2018). Temperature plays an essential role in insect physical activities such as movement. Thus, foraging activity may be a function of the link between temperature and species physiological tolerance (Bishop *et al.*, 2017; Gotcha *et al.*, 2020). In the current study, nocturnal *S. zambeianus* and *C. elephenor* likely evolved this temporal foraging behaviour owing to their superior low temperature tolerance observed here. Conversely, diurnally active *A. indigaceus* and *E. intermedius* and crepuscular *O. alexis* and *O. gazella* likely evolved these day and dawn/dusk foraging behaviours owing to their relatively constrained low temperature tolerance. In addition, no phylogenetic signal was recorded for both CT_{min} and CCRT, suggesting no significant link between low temperature tolerance and relationships across dung beetles tested, albeit the few species tested may limit potential for detecting such. Furthermore, whilst species effects on dung ball numbers were not apparent in isolation, average dung ball size differed with species, suggesting ecosystem service delivery may vary with species. The present study also found evidence for emergent effects between activity time and temperature, with numbers of dung balls reduced by decreasing temperature for diurnal species only. In the absence of any physiological adjustments, for example through phenotypic plasticity (Sgro *et al.*, 2016), this result shows that low stressful temperatures associated with climate variability may impair ecosystem services rendered by dung beetle species through reduction in quantity of dung removed.

Species-specific differences in CT_{min} were recorded. *Euoniticellus intermedius* showed a lower CT_{min} than *A. indigaceus* symbolising differences in physiological strategies in surviving cold tolerance between species of the same foraging patterns (Lombardero *et al.*, 2000). Higher cold hardiness for nocturnal species resonates well with their activity timing and the thermal adaptation hypothesis. A number of factors have been documented to affect thermal tolerance in insects, including body size (see Burton & Eium, 2020; Gotcha *et al.*, 2021). Indeed, nocturnal *S. zambeziensis* and *C. elephenor* generally have larger body size compared to both diurnal and crepuscular species (see Gotcha *et al.*, 2020). It thus, follows that larger bodied insects are more likely to be nocturnal while smaller bodied are day active (Slade *et al.*, 2007; Chown & Klok, 2011). This result corroborates with the notion that body size affects key physiological fitness traits (Calder, 1984). Based on the thermal inertia hypothesis, larger nocturnal species warm faster than smaller diurnal ones, while small bodied species warm up slower (Verdu *et al.*, 2006). In related studies on aquatic invertebrates, the constant evolution of smaller body size has been caused by failure of larger bodied organisms to tolerate high temperature in warming aquatic environments (Burton & Eium, 2020). It is thus, plausible that low temperature tolerance by larger bodied nocturnal species may have contributed to the evolution of nocturnal activity behaviour in our study species.

Chill coma recovery time was shorter in diurnal species than both crepuscular and nocturnal species, indicating higher cold resistance for diurnal relative to crepuscular and nocturnal species. These results are, however, inconsistent with CT_{min} observations, suggesting mechanisms for resistance between these two cold tolerance traits may be decoupled (Sinclair *et al.*, 2015; MacMillan 2019). Chill coma recovery time measures the time taken for an insect to recover from chill coma inducing temperature, while CT_{min} is a measure of the lowest

temperature to activity and occurring prior to the onset of chill coma (David *et al.*, 1998). Mechanisms enhancing cold tolerance comprise carbohydrate cryoprotectants (Duman, 2002; Kostal *et al.*, 2011), depression of supercooling points, membrane remodelling, the inhibition of apoptosis and *Hsp* up-regulation (Denlinger & Lee, 2010). However, re-establishment of ion homeostasis (MacMillan *et al.*, 2012), restoration of signal transmission and whole organisms' oxygen supply restoration (MacMillan & Sinclair, 2011a; Overgaard & Macmillan, 2017; Macmillan, 2019) are some of the reported mechanisms accounting for improved CCRT in invertebrates. Previous studies have shown that traits of CT_{min} and CCRT depend on environmental history (Gilbert & Huey, 2001; Hoffmann *et al.*, 2005) and that their relationship is curvilinear (Ransberry *et al.*, 2011). Thus, mechanisms accounting for phenotypic adjustment of CT_{min} and CCRT appear decoupled. I speculate here, that differences in phenotypic compensation, although not measured here, may account for the differences in CT_{min} and CCRT results recorded. Similarly, results showed no phylogenetic constraints to both CT_{min} and CCRT, indicating that cold tolerance and dung beetle relatedness are also decoupled. Nevertheless, this assertion comes with caveats owing to the limited number of species in our phylogenetic analysis context.

While most biological conservation efforts have been skewed to the protection of habitats or specific threatened species (Braby, 2018), few empirical studies have looked at the cost of climate change to natural capital, for example insects and their ecosystem services provision. However, predicting the fate of insects as natural capital may help safeguard their essential ecosystem services and help maintain the integrity of ecosystems. Stressful low ambient temperature can limit an insect's performance (Sinclair *et al.*, 2015) through reduction in metabolism, activity and key fitness, as well as other ecological traits. My assessment of ecological traits showed that lower temperatures reduced dung removal frequency, but not size;

a proxy of natural ecosystem services supplied by dung beetles. Dung ball production was significantly reduced under lower temperatures in *A. indigaceus*. However, for *S. zambezianus*, there was no significant difference between the control (28 °C) and lower temperature, i.e. 23 °C or 18 °C treatments with respect to dung ball number. These results also suggest that stressful low temperatures generally reduce foraging rates for tropical coprophagous beetle species studied here; and that these responses are species-specific. Previous studies have shown that animals can adjust their behaviours such as selecting temporally optimal foraging activity to buffer impacts of unfavourable environments (Hamer *et al.*, 2009; Bale & Hayward, 2010). Furthermore, energy trade-offs can also explain reduction in species foraging at stressful temperatures. For example, keeping warm may come at the cost of increased starvation risk while organisms often trade off low temperature stress for reduced foraging. It thus implies that, to ensure survival, low temperature intolerant species tend to opt to starve but keep warm, while cold tolerant species are able to forage and keep cool (Bishop *et al.*, 2017). As such, it is not surprising that foraging rates (number of balls produced) of diurnal *A. indigaceus* (higher CT_{min}) were significantly affected by low temperature stress, while foraging rates of nocturnal *S. zambezianus* (low CT_{min}) were not. These results agree with Bishop *et al.* (2017) who showed that the activity of lower CT_{min} mountain ants was not constrained by stressful low temperature whereas the activity of those with higher CT_{min} was affected. Nevertheless, although increases in low temperature extremes may expand temporal niches for cold adapted nocturnal versus diurnal species, the net ecological effects of these low temperature stresses may depend on overall temperature variability and consequences for ecological service delivery across beetle species with different foraging behaviours.

Dung ball size (ball diameter) was not affected by lower temperature environments in both *A. indigaceus* and *S. zambezianus*. However, nocturnal *S. zambezianus* had significantly larger

average ball diameters than diurnal *A. indigaceus* across all test temperatures, indicating dung ball size is species dependent. The relatively larger body size of *S. zambezianus* compared to *A. indigaceus* could account for the bigger balls reported in the current study. Similar studies have shown a positive correlation between beetle size and dung ball size (Hanski & Cambefort, 1991; Halffter & Matthews, 1966; Tomkins *et al.*, 1999) and dung ball differences within species (Halffter & Matthews, 1966). A within-species comparative study also showed larger individual *Kheper nigroaeneus* produced significantly larger dung balls than smaller beetles (Tomkins *et al.*, 1999). However, size of dung ball is also dependent on its function. For examples, dung balls made for use as a food resource have been found to be significantly smaller than those meant for reproduction (Hanski & Cambefort, 1991). For the current study, dung balls were examined without accounting for ecological purpose e.g. source of food, breeding chambers (Hanski & Cambefort, 1991), nuptial female gifts and related purposes (Heinrich & Bartholomew, 1979). As such, further investigations may need to incorporate the purpose of the dung ball as a factor.

In conclusion, my results show that tropical nocturnal *S. zambezianus* and *C. elephenor* had lower activity temperatures (lower CT_{min}) than crepuscular and diurnal species. This may mean that nocturnal insects may have evolved enhanced cold tolerance commensurate with their night-time activity behaviour. Second, my CCRT results were less apparent, and trends were equivocal and inconsistent with CT_{min} results. This may point to decoupled resistance mechanisms accounting for traits of CT_{min} and CCRT. Third, my results showed that low temperature stress may offset dung beetles' ecological services through reduction in dung removal, but effects are species-specific. These results are critical in predicting the effects of climate change on biodiversity and the maintenance of the integrity of ecosystems. Specifically, these results will help explain responses of dung beetle species to low temperature

stress and determining how environmental stress may affect essential ecological service provision in future. Future studies should nevertheless investigate the role of plasticity and mechanisms in facilitating physiological thermal tolerance. In addition, future studies should thus consider incorporating more beetle species to increase the probability of detecting possible phylogenetic signals. Furthermore, it would be interesting to quantify functional responses (i.e. feeding rates under different densities) of dung beetles towards different amounts of dung (masses) as temperature changes among species.

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Chapter 5

Limited thermal plasticity may constrain ecosystem function in a basally heat tolerant tropical telecoprid dung beetle, *Allogymnopleurus thalassinus* (Klug, 1855)

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5.1. Introduction

Climate change is expected to increase global mean temperature by 1.5-4.5°C by the end of the century if mitigation measures fail. In Africa, the past decade has been the warmest on record at $1.78 \pm 0.24^\circ\text{C}$ (IPCC, 2007; 2014; Ju *et al.*, 2015; Easterling *et al.*, 2020; WMO, 2021) with land bordered areas in the dry tropics of Southern Africa experiencing the warmest temperatures at much faster rates than the global average (WMO, 2021; Dube, 2003). Such rapid increase in temperatures is likely to exceed upper thermal limits (UTLs) for over 40% terrestrial organisms endemic to the region, particularly those providing fundamental ecological functions (Deutsch *et al.*, 2008). Indeed, the evidence for this is manifesting globally (Perkins-Kirkpatrick & Lewis, 2020) e.g., very recently (June 2021), a record shattering 49.5°C heatwave was recorded in Canada (NOAA, 2021), a scenario that is spatially consistent in other continents (see e.g., UK Met Office, 2021; Kendon *et al.*, 2020; Head *et al.*, 2014; Nangombe *et al.*, 2018; Gergis *et al.*, 2020). These record-breaking high temperatures are linked to climate change and threaten the survival of insect species responsible for sustaining key ecological functions.

Reports show that, the effects of global warming on ecologically significant arthropods are increasingly becoming apparent across various facets of environmental ecosystems, particularly in the arid tropics (IPCC, 2014). Insect ecologists are thus concerned about the risks of species survival, risks of extinction and associated loss of ecosystem functions. For example, reports suggest that in some parts, the foraging frequency of dung beetles has reduced by 31% in the last quarter of the 20th century due to climate change associated heat stress (Carpaneto *et al.*, 2007). Empirical evidence suggests that the magnitude of heat stress is increasing, threatening the survival of ecologically important insect species (IPCC, 2014; Easterling *et al.*, 2020; IPCC, 2007; Carpaneto *et al.*, 2007). For example, recent trends show

increases in extreme high and low temperatures are more emphasized in the arid tropics (Easterling *et al.*, 2020; IPCC, 2007) likely increasing thermal injury through additive stress, reducing survival, abundance and distribution of dung beetles (Walther *et al.*, 2002; Chown & Nicolson, 2004; Huey & Kearney, 2020; Jørgensen *et al.*, 2021). This has created geographical sub-optimal thermal heterogeneity for tropical insects (Chown & Nicolson, 2004; Buyantuyev & Wu, 2010; Aalto *et al.*, 2017) particularly those in hot, arid habitats, likely affecting their organismal function and the efficiency of the whole ecosystem functions. For example, Holley and Andrew (2019) as well as Giannini *et al.* (2012) showed that heat stress impacts ecological functions in different species. For example, in *Onthophagus hectate*, sub-optimal high temperatures reduced both brood ball sizes and depth of burial, likely exposing the next generation immatures to high mortalities due to desiccation and starvation (Wu & Sun, 2012; Mamantov & Sheldon, 2020).

Heat tolerance is a physiological trait of ecological significance and may determine the fate of tropical organisms in the face of climate change. Insects are more susceptible to snaps of extreme temperature events (Clusella-Trullas *et al.*, 2011; Ma *et al.*, 2014). Tropical organisms are especially vulnerable to heat stress because they live in habitats with temperatures close to their UTLs and often lack the capacity to compensate adaptively through phenotypic plasticity (Gunderson & Stillman, 2015; van Heerwaaeden *et al.*, 2016; Nyamukondiwa *et al.*, 2011) partly due high investment in high basal heat tolerance or genetic constrains (Blackbun *et al.*, 2014; Bowler & Terblanche, 2008; Barley *et al.*, 2021). Thus, it follows that variation in UTLs is also lower than that of lower thermal limits (LTLs) even across space and species [reviewed in Chown & Nicolson, 2004]. While UTLs for insects are generally ~40°C with little variation (Bowler & Terblanche, 2008), tropical organisms should thus compensate adaptively in order to maintain functionality under climate change (Sgrò *et al.*, 2016). Unless organisms

compensate in situ to heat stress through behaviour e.g., migration to less hostile environments (Pincebourde & Woods, 2020; Woods *et al.*, 2021), phenotypic plasticity becomes an essential requisite for survival. Therefore, phenotypic plasticity is thus, a critical primary factor in buffering species against the negative effects of heat stress on insect survival through increased magnitudes of thermal safety margins (Gunderson & Stillman, 2015; Gunderson *et al.*, 2017) and may help improve fitness under suboptimal conditions. While climate change continues to push tropical insects closer to UTLs (Dube, 2003; Gunderson & Stillman, 2015), those organisms capable of compensating adaptively may emerge winners of climate change (Gunderson *et al.*, 2017). Thus, evidence exists for a strong selection for either high basal heat tolerance and/or plasticity thereof with climate change (Esperk *et al.*, 2016), depending on species. However, data on specific mechanisms for heat tolerance in individual species of ecological importance is lacking particularly in sub-Saharan dry tropics despite the region's high vulnerability to warming (WMO, 2021; Dube, 2003).

The extent to which phenotypic plasticity buffers climate change effects has been a subject for huge debate (Deutsch *et al.*, 2008; Gunderson & Stillman, 2015; Nyamukondiwa *et al.*, 2011; Calosi *et al.*, 2008; van Heerwaarden & Kellermann, 2020), and moreover, its effects on the maintenance of essential ecosystem function is unclear (Malhi *et al.*, 2020). Theories explaining variation in plasticity and basal thermal stress resistance have been equivocal (Barley *et al.*, 2021). For example, the 'trade-off' hypothesis predicts that higher basal thermal tolerance may come at a cost of phenotypic plasticity (Gunderson & Stillman, 2015; Stillman, 2019). This suggests that the scope for tropical organisms to physiologically compensate for UTLs, e.g., critical thermal maxima (CT_{max}) is constrained (Deutsch *et al.*, 2008; van Heerwaarden *et al.*, 2016; Tewksbury *et al.*, 2008). Furthermore, the evolutionary potential of UTLs is also limited (Blackburn *et al.*, 2014), and acclimation response ratios for CT_{max} are

inherently low (van Heerwaarden *et al.*, 2016; Kelley, 2014), likely impacting tropical organisms under high climate change stress. Contrastingly, empirical support for the trade-off theory has been equivocal (Sgrò *et al.*, 2016). For example, while some species trade-off plasticity for high basal heat tolerance (Gunderson & Stillman, 2015; van Heerwaarden *et al.*, 2016), some organisms with high basal temperature tolerance are reportedly more plastic too (Nyamukondiwa *et al.*, 2011; Calosi *et al.*, 2008; Mitchell *et al.*, 2011). This raises significant ecological questions on the fate of individual species success under climate change and warrants more studies to unravel exact relationships between basal stress tolerance and phenotypic plasticity. Tropical diurnal species are constantly exposed to heat stress in their arid, hot habitats during foraging. By constantly being exposed to soil heat, dung beetles should theoretically adapt to heat and presumably have inherent high basal resistance to heat stress. However, it remains unknown which species trade off plasticity for basal high temperature tolerance, and how that is likely to subsequently impact its essential ecosystem function, e.g., dung removal. Nevertheless, previous reports suggest global warming poses a threat to these species by likely reducing their field fitness and ecological function apart from increasing their risk of extinction (Wu & Sun, 2012; Easterling *et al.*, 2020; van Heerwaarden & Kellermann, 2020; Allen *et al.*, 2016).

Dung beetles are coprophagic species that use dung during feeding and nesting (Edwards & Aschenborn, 1987). Through their coprophagy, they concomitantly contribute to other ecological functions such as nutrient cycling, secondary seed dispersal, reducing parasites and the loss of N₂ due to ammonia volatilization (Bertone *et al.*, 2006; Yamada *et al.*, 2007; Yoshihara & Sato, 2015; Manning *et al.*, 2016; Milotic *et al.*, 2019). They also contribute to reduction in greenhouse gas emissions and facilitate microbial activity through bioturbation (Slade *et al.*, 2016; Penttilä *et al.*, 2006). Thus, dung removal is a valuable economic

contribution to functional efficiency used as a proxy for ecological function provision by dung beetle species (Holley & Andrew, 2019; Mamantov & Sheldon, 2020; Spector, 2006). This makes dung beetles a critical resource (natural capital) globally. The coprophagic teleocoprid, *Allogymnopleurus thalassinus* is a significant ecological function contributor in southern Africa and throughout the continent (although it is widely distributed in other hot arid environments (GBIF, 2020 (online database). Its local abundance makes it a significant component of local biodiversity assemblages, community structure and natural capital (Janssens, 1940; Doube, 2014). Given the current and projected increase in mean temperatures of the savannah land mass with climate change (IPCC, 2007; 2014), it remains unknown how this important species may survive heat stress and whether or not it can remodel its thermal phenotypes through plasticity (see e.g., Calosi *et al.*, 2008; Stillman, 2019). Although several studies have assessed physiological responses of insects to climate change before (see Deutsch *et al.*, 2008; Chown & Nicolson, 2004), only a few have considered the combined effects of physiological and ecological impacts of thermal stress (Gotcha *et al.*, 2021). Similarly, while the effects of temperature on dung beetle functional responses are documented (Wu & Sun, 2012; Slade & Roslin, 2016; Mamantov & Sheldon, 2020; Grittings *et al.*, 1994; Rosenlew & Roslin, 2008; Gotcha *et al.*, 2021), to our knowledge, no studies have simultaneously assessed acclimation effects on both physiological and ecological responses dung beetle species in sub-Saharan Africa (see e.g., Mitchell & Hoffmann, 2010; Terblanche *et al.*, 2010). Furthermore, if indeed *A. thalassinus* is vulnerable to heat stress, the fate of its ecological function associated with heat stress is largely unknown. Building on latitudinal hypothesis (Janzen, 1967) and the findings by van Heerwaarden *et al.* (2016), Gunderson and Stillman (2015) and van Heerwaarden and Kellermann (2020), we hypothesise that (i) *A. thalassinus* may have high basal heat tolerance as a day active forager and that (ii) if present, this high basal heat tolerance is likely traded-off with phenotypic plasticity (see also Gunderson & Stillman, 2015; Somero,

2010; Overgaard *et al.*, 2011). Here I used ecologically relevant physiological- (CT_{max} and heat knockdown time [HKDT]) and ecological-traits as proxies for performance under climate change (Allen *et al.*, 2016; Terblanche *et al.*, 2011) using dynamic protocols (Mitchell & Hoffmann, 2010). The results will provide a profound understanding of species-specific physiological acclimation capacity and associated ecological implications for ecologically important species is essential to predict the fate of ecosystem function under climate change (Giménez Gómez *et al.*, 2020) and provide a framework for the conservation of such species in order to preserve their benefits to the environment and society.

5.2. Materials and Methods

5.2.1. Study site and sampling

Study beetles were collected from Khumaga Village (S20.46801; E24. 51491; 918 m.a.s.l), Central District, Botswana, in February 2020. The summer season represents the peak activity time of most dung beetles. Khumaga village is characterised by small scale pastoralism (mainly cattle and goats) and is at the interface with a protected area (Wildlife Park), Makgadikgadi Pans National Park, which hosts several wild large ruminants, and non-ruminants (DWNP, 2012). This rich animal diversity provides diverse and overlapping dung resources that promote abundant and diverse beetle communities. The beetles were captured using pit fall traps consisting of mini-plastic buckets (~2L) buried flush with the ground and covered with fine wire mesh of 15 mm internal diameter (modified from Nyamukondiwa *et al.*, 2018; Gotcha *et al.*, 2020; Braga *et al.*, 2013; Niino *et al.*, 2014). About 350g of fresh cattle dung was placed on top of the wire mesh as bait. The traps were covered with overhead shading to protect from rain and direct sunlight (Niino *et al.*, 2014). Traps were set at 0600 h every morning and captured beetles were collected from about 1000 h till 1800 h for 6 consecutive days. Collected beetles were placed in insulated cooler boxes with perforated lids containing moist soil and

dung for feeding during transportation to the Eco-physiology Laboratory, Department of Biological Sciences and Biotechnology, Botswana International University of Science and Technology, in Botswana. In the laboratory, beetles were identified using gross morphology (Beetles of Africa, 2021) and Voucher specimens were deposited at the Botswana National Museum. Beetles were kept in a climate chamber set at conditions like those at site of collection ($28\pm1^{\circ}\text{C}$, $65\pm10\%$ RH, 14:12 LD photoperiod) (Nyamukondiwa *et al.*, 2018) prior to experimentation. All acclimations and/or experiments were done within 7 days (as in Mathur & Schmidt, 2017) of specimen collection to minimise confounding effects of laboratory captivity.

5.2.2. Acclimation treatments

Beetles were acclimated using a combination dynamic (fluctuating temperature) protocol in climate chambers (HPP 260, Memmert GmbH + Co.KG, Germany) at $65 \pm 10\%$ relative humidity (RH) under 14L:10D photoperiod. This dynamic acclimation protocol is ecologically sound and may give more reliable estimates of species responses to variability typical of environmental climate change (Chidawanyika *et al.*, 2017; Moretti *et al.*, 2017). For variable high temperature acclimation (VT-H) for CT_{max} and HKDT, 150 beetles were exposed to a climate chamber where temperature was ramped up at $0.5^{\circ}\text{C}/\text{min}$ from a benign temperature of 28°C to 45°C , allowed to remain at 45°C for a duration of 2 h before being ramped down back to 28°C , remain constant for 2 h (at 28°C) before ramping up again in continuous repeated daily cycles (**Figure 5.1**). Similarly, for variable low temperature acclimation (VT-L) for CT_{max} and HKDT, 150 beetles were exposed to a climate chamber where temperature was ramped down at $0.5^{\circ}\text{C}/\text{min}$ from a benign of 28°C (ambient) to 16°C , and then held at 16°C (2 h) before being ramped up back to 28°C , held there for 2 h before ramping back down in repeated daily cycles (see **Figure 5.1**). Acclimation at both temperature extremes may improve both high and

low temperature tolerance (Hoffmann *et al.*, 2003; El-Saadi *et al.*, 2020] owing to potential overlap in heat and cold stress resistance mechanisms. Control beetles were maintained at a constant 28°C. Relative humidity and photoperiod were maintained at 65 ± 10% (RH) and 14D: 10L respectively for all treatments (see also Nyamukondiwa *et al.*, 2018).

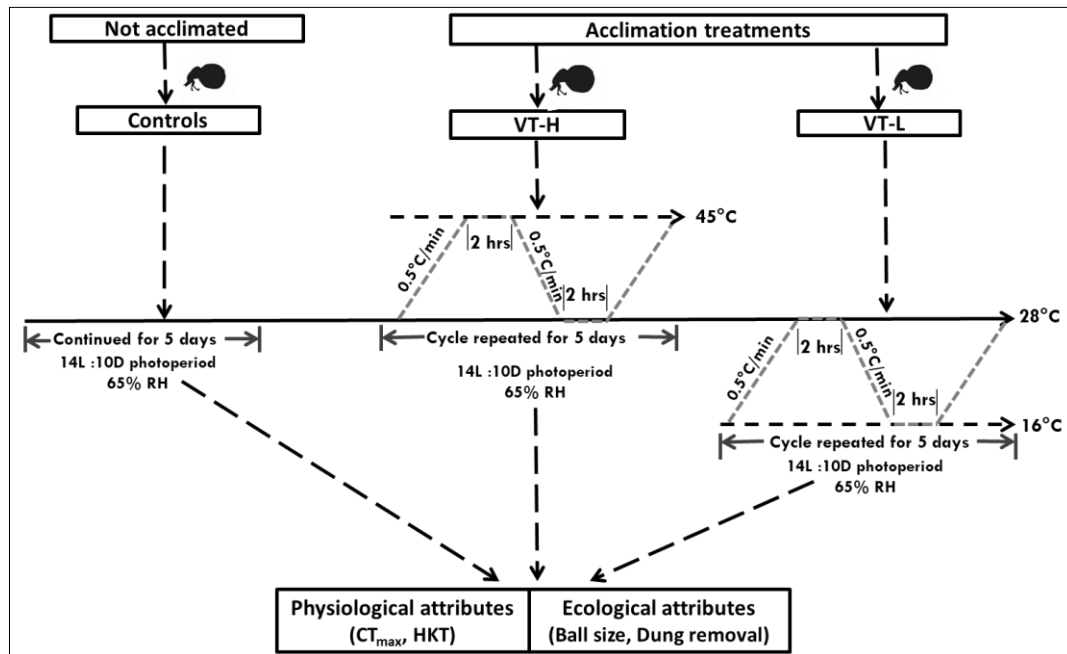


Figure 5.1. Schematic representation of variable temperature acclimation treatments at a ramping rate of 0.5°C/min from a benign temperature of 28-45°C (VT-H) and to 28-16°C (VT-L) at 65 ±10 % RH and 14D:10L photoperiod. Control beetles were maintained at a constant 28°C, 65 ±10 % RH and 14D: 10L photoperiod.

5.2.3. Physiological traits assays

To investigate the effects of plasticity on physiological fitness, I measured physiological functional traits *vis* CT_{max} and HKDT in adults following standardised protocols from (Nyamukondiwa *et al.*, 2018). Critical thermal maximum is a good indicator for an organism ability to survive extreme events, and as such a good measure of resistance mechanism under

extreme heat exposures (Morley *et al.*, 2019). A series of insulated double-jacketed chambers ('organ pipes') was connected to a programmable water bath (Lauda Eco Gold, Lauda DR.R. Wobser GMBH and Co. KG, Germany) filled with 1:1 water: propylene glycol to allow for sub-zero temperatures at the same time regulating the flow of liquid around the chambers. Ten mixed sex adult beetles were counted and individually placed randomly into the organ pipes. In the organ pipes, beetles were allowed to first equilibrate for 10 minutes at 28 °C (benign temperature), before ramping waterbath temperature up at a benign rate of 0.25 °C/min (see Terblanche *et al.*, 2011) until the CT_{max} for each beetle was recorded. Thermal ramping rates may affect adaptive capacity for UTLs (Mitchell & Hoffmann, 2010; Terblanche *et al.*, 2011). Thus, the process was repeated using a faster ramping rate of 0.5 °C/min with a fresh set of beetles. A thermocouple (type K 36 SWG) connected to a digital thermometer (53/54IIB, Fluke Cooperation, USA) was inserted into a control chamber to measure beetle temperature. The body temperature of each individual beetle was assumed to be in equilibrium with the organ pipe temperature as in similar work (see Nyamukondiwa *et al.*, 2018). Each beetle was discarded after recording and for each ramping rate, the process was repeated three times with fresh beetles each time to yield sample size of n=30 (30 replications) for each treatment. In this study, CT_{max} was defined as the temperature at which each individual beetle lost coordinated muscle function, consequently losing the ability to respond to mild stimuli like prodding with thermally inert camel-hair brush (e.g., Nyamukondiwa & Terblanche, 2010; Nyamukondiwa *et al.*, 2018).

For HKDT, ten mixed sex beetles were individually placed in numbered 30 ml polypropylene vials and placed in a climate chamber set at 53 ± 0.5°C (65 ± 10% RH) connected to a camera (HD Covert Network Camera, DS-2CD6412FWD-20, Hikvision Digital Technology Co., Ltd, China) linked to a computer from where observations were recorded (in minutes). The process

was repeated at $55 \pm 0.5^{\circ}\text{C}$ ($65 \pm 10\%$ RH) and each experiment was run three times with fresh beetles each time to yield a sample size of $n=30$ (30 replications) for each acclimation treatment and each HKDT temperature. The HKTs were selected following both preliminary assays and previous studies (Gotcha *et al.*, 2020). Beetles were discarded after each recording. HKT was defined as the time (in minutes) at which each individual beetle lost coordinated activity due to acute heat stress (see Nyamukondiwa *et al.*, 2018; Weldon *et al.*, 2011). All treatments and replicates were all randomised across the different experimental blocks.

5.2.4. Ecological functions assays

To investigate ecological effects of acclimation, I assessed the effects of treatments on two essential ecosystem functions, dung ball size and dung removal. Ball sizes were measured for each of the three treatments following modifications of methods by (Sullivan *et al.*, 2016; Nervo *et al.*, 2014). Fifty mixed sex beetles from each treatment were provided with 500g of manually homogenised fresh cattle dung (Slade *et al.*, 2016) in plastic containers of 4.09 L total volume with effective soil depth of 6 cm. The experiment was replicated 3 times for each of the treatments (VT-H, VT-L and controls). Experimental containers were placed in a climate chamber (HPP 260, Memmert GmbH + Co.KG, Germany) set at 28°C and $65 \pm 10\%$ RH under 14L:10D. After 24 h, 50 completely formed balls (Sullivan *et al.*, 2016) were randomly picked from each container and ball sizes (diameter) were recorded. Ball diameter was measured using an electronic digital Vernier calliper (E-base Measuring Tools Co., model: SV-03-150, size 6in/150mm, Pert Industries, Johannesburg, South Africa). Since most balls were more spheroidal in shape, both the longest and shortest diameters of each ball were measured. Diameter of each ball was thus calculated as the average of the longest and the shortest diameters.

Dung removal experiments were conducted following modified protocols (Slade *et al.*, 2007; Nervo *et al.*, 2014; Slade *et al.*, 2016). Following acclimation treatments, 50 mixed sex beetles were exposed to 200g homogenised dung pats (RADWAG1 Wagi Elektroczne, Model AS220. R2, Poland) in plastic containers of $27 \times 17.8 \times 8.5$ cm (4.09 L volume) with effective soil depth of 6 cm. A thin film of clean multipurpose wiping paper was placed beneath each dung pat to avoid soil sticking to the dung. The experiment was replicated 3 times for each of the treatments. Experimental containers were placed in a climate chamber (HPP 260, Memmert GmbH + Co.KG, Germany) set at 28°C and $65 \pm 10\%$ RH under 14L:10D photoperiod. After 24 h, residual dung that was not balled or buried was weighed and recorded. Water loss was accounted for by using a parallel control experiment with only 200g dung pats but no beetles (Braga *et al.*, 2013).

5.2.5. Data analysis

All the statistical analyses were performed in R version R4.0.2. (R Core Team, 2020). Models were built based on how treatments (acclimation and ramping rates) affected traits of CT_{max} (°C), HKDT (minutes), ball sizes (mm) and dung removal (%). A total of 180 observations were used to assess the effects of the 3 acclimation treatments (Control, VT-H and VT-L) and ramping rate (0.25 and 0.50°C/min) on CT_{max} .

A sample of 180 observations was also considered for HKDT model at three different acclimation treatments (Control, VT-H and VT-L) with 2 different heat knockdown temperature levels (53°C and 55°C) within each treatment respectively. A balanced 90 observations for each of the temperature levels was used treating the data at 53°C and 55°C separately for analysis. A preliminary two-way ANOVA on both CT_{max} and HKDT models were run and showed that the residuals were not normally distributed for HKDT. Thus, a rank

based non-parametric approach was adopted to assess the effects of acclimation on HKDT under the different acclimation treatments, and heat knockdown temperatures. The aligned rank transformation (ART) for nonparametric factorial analyses using only ANOVA procedures was used (Wobbrock *et al.*, 2011). A multifactor contrasts procedure by (Elkin *et al.*, 2021) was implemented to distinguish significant differences on the different factors and levels for the ART method. In order to determine how ball size and dung removal were influenced by the different acclimation treatment levels, one-way analysis of variance (ANOVA) was used. The models were both appropriate as both Shapiro Wilk's test for normality and Levene's test for equal variance assumptions on residuals were satisfied. Shapiro Wilk's p-values were (0.85, 0.34) whilst Levene's test p-values were (0.17, 0.93) for the ball diameter and dung model, respectively.

The responses of *A. thalassinus* to acclimation, termed acclimation response ratio (ARR) was calculated for CT_{max} using the formula:

$$ARR = \frac{\Delta CT_{max}}{\Delta Acclimation}$$

Where,

ΔCT_{max} = recorded change in (CT_{max}) (°C), and

$\Delta Acclimation$ = Difference between holding and acclimation temperature (°C)

following methods by (Kelley, 2014; Qari, 2014; Azra *et al.*, 2020). This was compared to ARR from literature to interpret how the magnitude of plasticity of thermal tolerance may likely buffer *A. thalassinus* under changing climates. Acclimation Response Ratio of 1 shows a positive 1°C shift in CT_{max} for each 1°C acclimation temperature investment suggesting positive plasticity while an ARR of close to zero indicates lack of plasticity and ARR=0.5 indicates little effects of acclimation on CT_{max} plasticity (Morley *et al.*, 2019).

5.3. Results

5.3.1. Basal heat tolerance

The CT_{max} of *A. thalassinus* was higher than 50°C for all treatments and controls (**Figure 5.2; Table 5.1**). The CT_{max} of non-acclimated *A. thalassinus* beetles was $51.32 \pm 0.68^\circ\text{C}$ and $51.73 \pm 1.81^\circ\text{C}$, at ramping rate of 0.25° and $0.5^\circ\text{C}/\text{min}$ respectively. These CT_{max} values indicate extreme heat tolerance and are comparable with other related diurnal beetle species in the same tribe (**Table 5.1**). Similarly, basal HKDT was long, registering 130.79 ± 41.17 and 55.05 ± 2.12 minutes at 53 and 55°C respectively. These HKDT values are also longer than related diurnal species in the same tribe (**Table 5.1**).

Table 5.1: The basal CT_{max} ($^\circ\text{C}$) and HKDT (minutes) for *Allogymnopleurus thalassinus* compared to other diurnal telecoprid species in the same tribe occurring in the same hot and arid environment.

Tribe	Species	CT_{max} ($^\circ\text{C}$) ($\pm\text{SEM}$)	HKDT (minutes) ($\pm\text{SE}$)	Reference
Gymnopleurini	<i>Allogymnopleurus thalassinus</i>	51.3 ± 0.68	130.8 ± 41.12	Current study
Gymnopleurini	<i>Gymnopleurus aenescens</i>	52.5 ± 0.15	19.6 ± 1.12	Gotcha <i>et al.</i> , 2020
Gymnopleurini	<i>Gymnopleurus ignitus</i>	52.1 ± 0.23	17.7 ± 1.04	Gotcha <i>et al.</i> , 2020
Oniticellini	<i>Euoniticellus intermedius</i>	48.9 ± 0.14	57.8 ± 1.62	Gotcha <i>et al.</i> , 2020

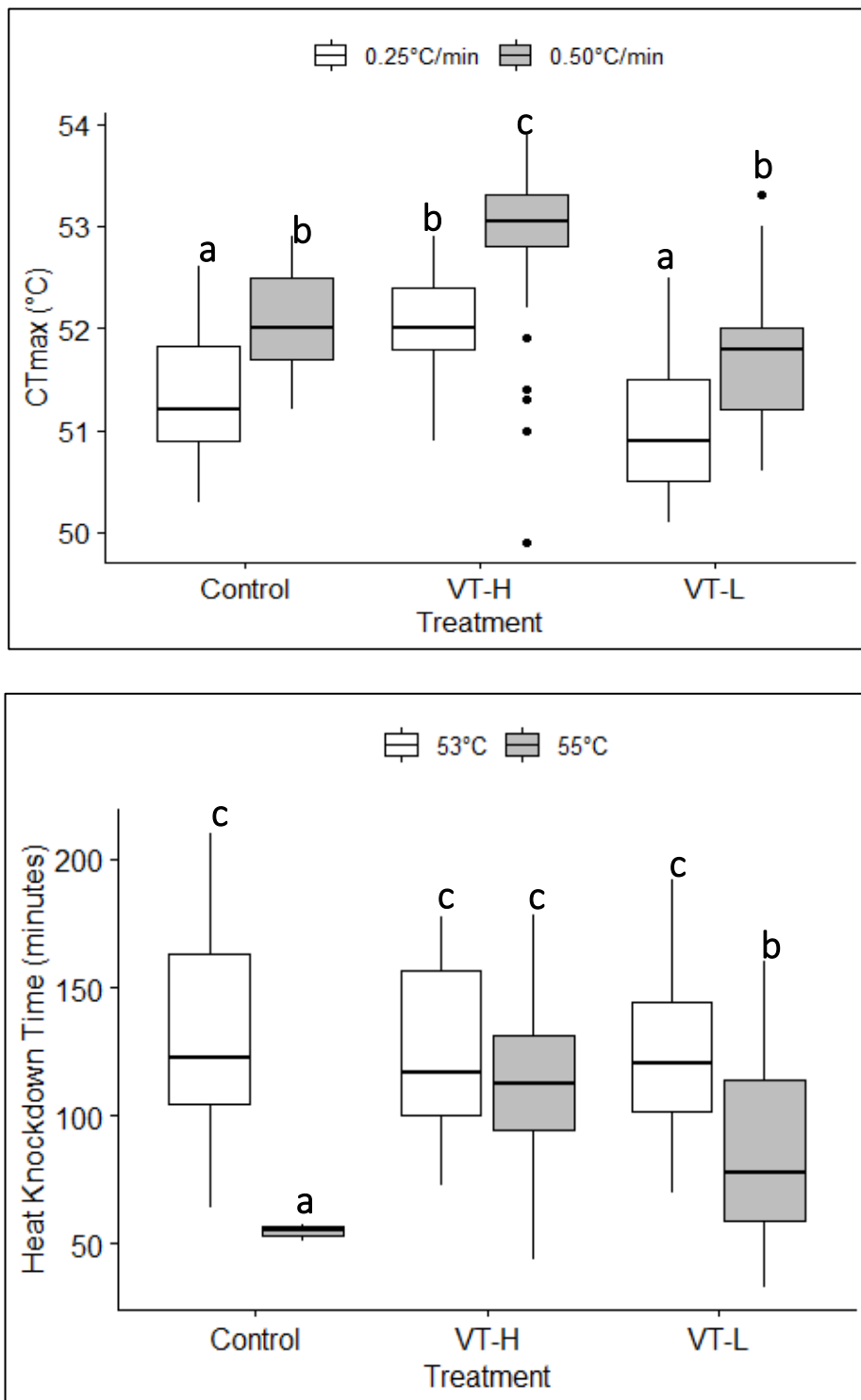


Figure 5.2: The effect of ramping rate and variable temperature acclimation on critical thermal maxima (CT_{max}) and Heat knockdown time (HKDT) at 53°C and 55°C. Each point represents mean $\pm$ SEM and median $\pm$ SE.

5.3.2. Effect of acclimation on heat tolerance

CT_{max} was significantly affected by both acclimation treatments ($p < 0.001$) and ramping rate ($p < 0.001$) although combined interactions of acclimation and ramping rate were not statistically significant ($p = 0.924$) (**Table 5.2**). Variable high temperature acclimation (VT-H) significantly improved CT_{max} compared to control ($p < 0.001$) at all the two ramping rates while variable low temperature (VT- L) on the other hand significantly reduced CT_{max} ($p = 0.0274$) compared to the controls (**Figure 5.2**). Across all acclimation treatments, the 0.5°C/min ramping rate resulted in significantly higher CT_{max} values than the 0.25°C/min one (**Figure 5.2**).

Table 5.2: The effect of acclimation treatment and ramping rate on CT_{max}.

<i>Parameter</i>	<i>DF</i>	<i>SS</i>	<i>SM</i>	<i>F</i>	<i>Pr(<F)</i>
<i>Treatment</i>	2	32.76	16.38	36.906	< 0.001
<i>Ramping rate</i>	1	24.58	24.58	55.389	< 0.001
<i>Treatment × Ramping rate</i>	2	0.07	0.035	0.079	0.924
<i>Residuals</i>		171	75.89		0.444

DF = degrees of freedom, SS= sum of squares, SM= mean sum of squares, F=F ratio, Pr (<F) = p-value associated with the F-statistic.

HKDT was significantly affected by both acclimation treatments and knockdown temperature ($p < 0.05$), with combined interactions of both acclimation treatments and knockdown temperature also being statistically significant ($p < 0.05$) (**Table 5.3**). The HKDT for control adult beetles was 130.79±41.17 and 55.05±2.11 minutes for knockdown temperature of 53 and 55°C respectively. HKDT was consistently significantly higher across all treatments for the 53°C than 55°C heat knockdown temperature (**Table 5.3**). Variable temperature high and low acclimation treatments improved HKDT for the 55°C heat knockdown temperature but not 53°C. Therefore, there were no treatment effects for HKDT for the 53°C heat knockdown

temperature. Interactions between acclimation treatment $\times$ heat knockdown temperature showed that, at 53°C the HKDT of both acclimation treatments (VT-H & VT-L) were not statistically different from the control group, while at 55°C, both variable temperature high and low acclimation treatments significantly increased HKDT (**Figure 5.2**).

Table 5.3: The effect of acclimation treatment and ramping rate on HKDT

<i>Parameter</i>	<i>F</i>	<i>DF</i>	<i>DF-Res</i>	<i>Pr(<F)</i>
<i>Treatment</i>	10.415	2	145	< 0.001
<i>HKT</i>	94.211	1	145	< 0.001
<i>Treatment $\times$ HKT</i>	13.242	2	145	< 0.001

DF = degrees of freedom, DF-Res = degrees of freedom residuals, F = F ratio, Pr (<F) = p-value associated with the F-statistic.

5.3.3 Effects of acclimation on ecological functions

Acclimation treatments significantly affected the mean ball diameter made by *A. thalassinus* ($F_{(3, 147)} = 4376.8$, $p < 0.001$). Variable temperature high (VT-H) acclimated beetles made significantly wider balls (16.19 ± 1.30 mm; $p < 0.001$), than both the non-acclimated beetles (14.35 ± 1.31 mm; $p < 0.001$) and variable temperature low (VT-L) treatments (13.58 ± 1.14 mm; $p < 0.00001$) (**Table 5.4; Figure 5.3**). Similarly, acclimation treatments had significant effects on the proportion of dung removed ($F_{(3, 6)} = 554.32$, $p < 0.001$). The proportion of dung removed followed the same trend; where variable temperature high acclimated beetles, removed a significantly higher proportion of dung compared to controls (**Table 5.4; Figure 5.3**). Furthermore, variable low temperature acclimation came at an ecosystem function cost, significantly reducing dung removal compared to controls (**Table 5.4; Figure 5.3**).

Table 5.4: Parameter estimations of variable high (VT-H) and variable low (VT-L) acclimation treatments on ball diameter and dung removal efficiency. Statistical significance was determined at $p < 0.05$.

Trait	Treatmen t	Estimate (mean)	Standard Error	t-value	Pr(> t)
<i>Ball diameter</i>	Control	14.3463	0.2229	64.37	< 0.001
	VT-H	16.1863	0.2229	72.63	< 0.001
	VT-L	13.5778	0.2229	60.92	< 0.001
<i>Dung removal</i>	Control	25.753	1.261	20.42	< 0.001
	VT-H	40.323	1.261	31.98	< 0.001
	VT-L	18.853	1.261	14.95	< 0.001

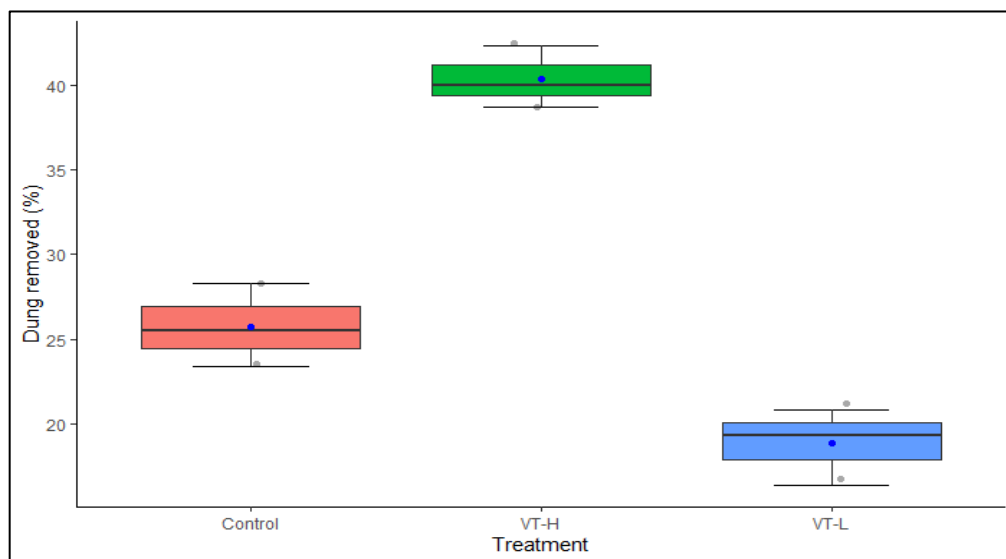
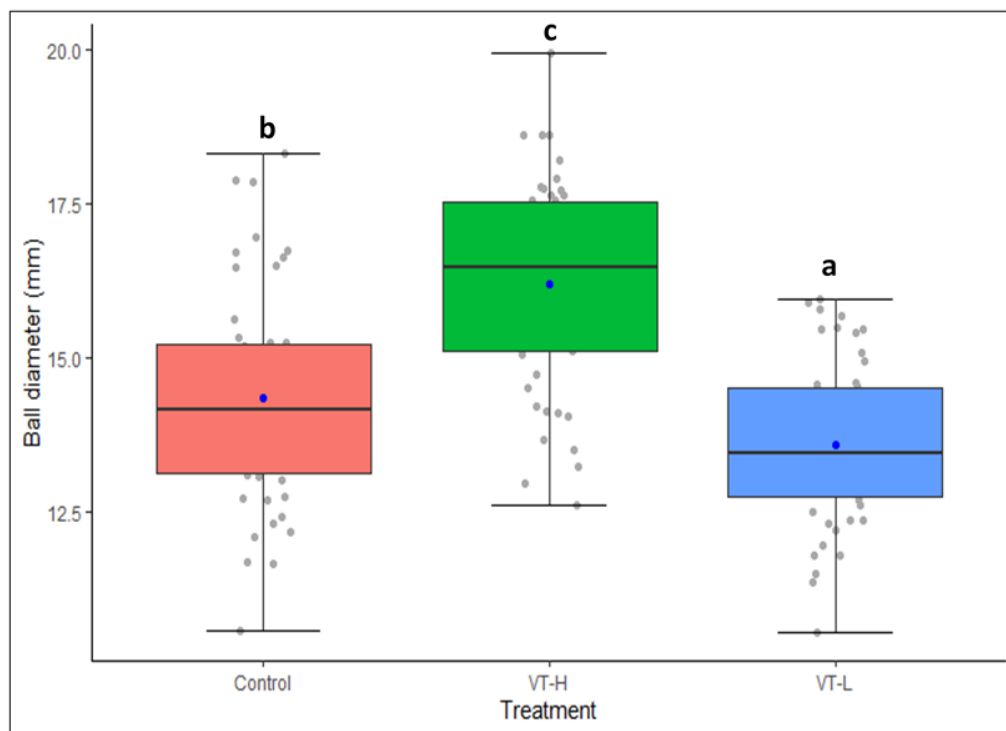


Figure 5.3: The effect of acclimation treatments on ball diameter and dung removal efficiency. Each point represents mean $\pm$ SEM.

5.3.4. Acclimation response ratio (ARR) of CT_{max}

The acclimation response ratio (ARR) of CT_{max} at benign ramping rate ($0.25^{\circ}\text{C}/\text{min}$) for *A. thalassinus* following variable temperature high and variable temperature low acclimation treatments was $0.0284^{\circ}\text{C}/^{\circ}\text{C}$ and $-0.02556^{\circ}\text{C}/^{\circ}\text{C}$ respectively, indicating ($\sim 2.84\%$ compensation capacity and $\sim -2.56\%$ fitness cost respectively). However, when the CT_{max} ramping rate was increased to $0.5^{\circ}\text{C}/\text{min}$, the CT_{max} ARR for variable temperature high acclimation treatment increased to $\sim 0.06^{\circ}\text{C}/^{\circ}\text{C}$, (i.e. 6% compensation capacity) while it decreased for variable temperature low ($-0.026^{\circ}\text{C}/^{\circ}\text{C}$) (-2.6% fitness cost). Compared to results of ARR values of other insects' taxa at a chosen ramping rate of $0.25^{\circ}\text{C}/\text{min}$, the CT_{max} ARR values for *A. thalassinus* was low except compared to *Scarabaeus zambeziensis* and *Copris elephenor* (**Table 5.5**). The list may not be purely exhaustive but represents a significant number of studies found in literature at the time of publication. Also note that CT_{max} values and consequently ARRs may vary depending on CT_{max} methodological context e.g. starting temperature (Jørgensen *et al.*, 2021).

Table 5.5: The critical thermal maxima (CT_{max}) acclimation response ratios (ARR) for *A. thalassinus* compared to other different Orders and their specific species obtained from literature. All the acclamatory response ratio values were calculated only for CT_{max} at ramping rate of 0.25°C/min.

Order	Species	CT_{max} (°C/°C)	ARR	Reference
	<i>Allogymnopleurus thalassinus</i>	0.0284		Current study
	<i>Metacatharsius opacus</i>	0.085		Nyamukondiwa <i>et al.</i> , 2018
Coleoptera	<i>Scarabaeus zambeziensis</i>	-0.030		Nyamukondiwa <i>et al.</i> , 2018
	<i>Copris elephenor</i>	-0.420		Nyamukondiwa <i>et al.</i> , 2018
	<i>Linepithema humile</i>	0.20		Jumbam <i>et al.</i> , 2008
	<i>Castanophlebia calida</i>	0.19		Dallas & Rivers-Moore, 2012
Hymenoptera	<i>Lestagella penicillata</i>	0.16		Dallas & Rivers-Moore, 2012
Ephemeroptera	<i>Drosophila melanogaster</i>	0.043		Gallego <i>et al.</i> , 2018
	<i>Portunus pelagicus</i>	0.280		Qari, 2014
Diptera	<i>Scylla olivacea</i>	0.495		Azra <i>et al.</i> , 2020
Decapoda	<i>Thalameta crenata</i>	0.360		Azra <i>et al.</i> , 2020

5.4. Discussion

The results show that the arid environment inhabiting dung beetle *A. thalassinus* has high basal heat tolerance as exhibited by CT_{max} values $>50^{\circ}\text{C}$ and long HKDT values (>2 hours), consistent with other arid/desert habitat insect species that exhibit these striking basal heat tolerance traits (Lutterschmidt & Hutchison, 1997; Tewksbury *et al.*, 2008; Huey & Kearney, 2020; van Heerwaarden & Kellermann, 2020; Kafer *et al.*, 2020). Second, variable temperature high acclimation improved CT_{max} for all ramping rates, while variable temperature low acclimation generally reduced heat tolerance (CT_{max}). This may show plastic responses to CT_{max} at high temperature acclimation. Similarly, heat knockdown temperature influenced HKDT, suggesting that the magnitude of temperature stress may affect insect fitness in a

warming climate (van Heerwaarden & Kellermann, 2020). Similar, to CT_{max} , effects of acclimation on HKDT were significant, with variable temperature high and low temperature acclimations both increasing HKDT than controls at 55°C while no acclimation treatment effects were recorded for 53°C. The improved CT_{max} at higher ramping rate agrees with the notion that thermal stress and injury is highly additive (Jorgensen *et al.*, 2021). Thirdly, high temperature acclimation increased net ecological function through significantly higher dung removal and wider ball diameters than low temperature acclimation. Fourth, a qualitative comparison of ARR to like tropical insect species showed that phenotypic plasticity of heat tolerance in *A. thalassinus* adults was low, indicating that plasticity is constrained (Gunderson & Stillman, 2015; Gunderson *et al.*, 2017). When *A. thalassinus* ARR was compared to the general mean ARR for most terrestrial arthropods (0.12-0.16 (°C/°C)) (Gunderson & Stillman, 2015) its ARR was ~5 times lower, indicating considerably constrained high temperature plasticity compared to other insect species. Thus, this work supports the trade-off theory (Somero, 2010; Gunderson & Stillman, 2015; van Heerwaarden *et al.*, 2016; van Heerwaarden & Kellermann, 2020), and that selection for high basal heat tolerance in *A. thalassinus* may have come at a cost of plasticity. This therefore affirms the notion that although having high basal heat tolerance than temperate species, tropical species may be more vulnerable to future global warming because they lack complete plastic responses to heat stress, i.e., phenotypic plasticity is ecologically insufficient (van Heerwaarden *et al.*, 2016; Woods *et al.*, 2021). Acclimation effects were significantly positive on ecosystem function only following high temperature acclimation and would likely plateau. We therefore summarise that although plasticity is present in *A. thalassinus*, it may be insufficient to buffer this species against projected increase in in global warming, moreso in its arid, tropical habitat of southern Africa. Thus, *A. thalassinus*' field fitness and efficiency in contributing to ecological functions in the future may also be constrained under increasing heat stress with climate change.

Critical thermal maximum is a popular ecologically relevant index of heat tolerance measurement (Huey & Kearney, 2020) that is used by many ecologists to measure the capacity of organisms to survive extreme heat and thus as proxy for estimating climate change risks in many species (Terblanche *et al.*, 2011; Nyamukondiwa *et al.*, 2018; Gallego *et al.*, 2018; van Heerwaarden & Kellermann, 2020). My results showed that regardless of acclimation and ramping rates, the basal CT_{max} for *A. thalassinus* was high (> 50°C), indicating that *A. thalassinus* is thermophilic (survives extreme temperatures of > 41°C) (Chown & Nicolson, 2004). This could be attributed to its tropical origins (Janssens, 1940;; Doube, 2014) and day foraging activities (as observed in the field)) in tropical environments where environmental temperatures are normally high during daytime. Dung beetle species from the tribe Gymnopleurini, where *A. thalassinus* belong, are reportedly diurnally active (Caveney *et al.*, 1995). Similarly, in seed bugs, Käfer *et al.* (2020) showed high correlation between CT_{max} with environmental annual mean temperature and mean maximum temperature of warmest months in Austria. In separate studies, the desert ant, *Cataglyphis bombycin* had an extremely high heat tolerance (CT_{max} = 53.6°C) (Gehring & Wehner, 1995; Chown & Nicolson, 2004), partly attributable to its high habitat temperature (desert) environment. Similar reports on dung beetles *Gymnopleurus aenescens* and *Gymnopleurus ignitus* from the same habitats (Khumaga, Botswana) reported CT_{max} values ranging 52-53°C (Gotcha *et al.*, 2020), further confirming how environment (Kafer *et al.*, 2020) may shape basal heat tolerance and adaptation thereof in insects.

HKDT for *A. thalassius* was long (>2 hours), at 53°C HKT, again attesting its high heat tolerance apart from high CT_{max}. This result is in tandem with Gotcha *et al.* (2020), who showed high HKDT in related diurnal dung beetles *Gymnopleurus aenescens* and *Gymnopleurus*

ignitus. Similar observations were observed by Nyamukondiwa et al. (2018), using dynamic acclimation protocols for nocturnally active dung beetle *Scarabaeus zambesianus*. This high basal HKDT (and CT_{max}) in *A. thalassius* could have evolved as an adaptation to daytime activity (foraging during peak heat stress) in stressful arid environments (Bishop *et al.*, 2017; Kafer *et al.*, 2020; Gotcha *et al.*, 2020) where it is abundant (; GBIF, 2020). Although diurnal species are highly heat tolerant physiologically, telecoprids were observed to particularly employ behavioural plasticity mechanisms such as utilising microhabitats (see details in Pincebourde & Woods, 2020; Woods *et al.*, 2021) or using the moist dung balls as thermal refuge (heat sinks) to cool their bodies during rolling (Smolka *et al.*, 2012). Although it is not clear under what environmental temperature this thermal respite behaviour would be initiated, in the midday foraging desert ant, *Ocymyrmex robustior*, thermal respite behaviour was shown to increase when soil temperatures reach 51°C, coinciding with *A. thalassius* CT_{max} . Thus, extreme basal heat tolerance for *A. thalassius* reported, here may be attributable to its tropical origins (; GBIF, 2020) and the latitudinal hypothesis (Janzen, 1967; Kafer *et al.*, 2020) and may form part of its main survival strategy against warming climates.

My results showed that variable temperature high (VT-H) acclimation improved CT_{max} , while variable temperature low (VT-L) acclimation generally reduced CT_{max} . This result suggests plastic responses for this species for CT_{max} following dynamic high temperature acclimation. These results are consistent with results from other studies which show that heat acclimation improves CT_{max} , while on the contrary; low temperature acclimation may not improve heat tolerance (Chown & Nicolson, 2004). Similar to what was observed in other studies, CT_{max} values increased with increase in ramping rate, while slower ramping rates reduced this trait potentially owing to cumulative stress effects (Chown & Nicolson, 2004; Jørgensen *et al.*, 2021). The effects of ramping rates, test temperatures and duration of acclimation are

reportedly complex to disentangle (Chown & Nicolson, 2004; Allen *et al.*, 2016). Indeed, CT_{max} varies with methodological context e.g., starting temperature and ramping rates (Terblanche *et al.*, 2007; Chown *et al.*, 2009). Thus, the higher plasticity (CT_{max}) and survival consequences for higher ramping rates (for CT_{max}) may be attributable to the effect of reduced timing (and stress thereof) at faster heating rates (0.5 °C/minute) relative to slower one (0.25°C/minute). This is in keeping with recent models that assume heat stress and consequent injury is a function of temperature severity and that it is additive (Jørgensen *et al.*, 2021).

Acclimation to high temperature often improves low temperature traits and vice versa owing to shared physiological response mechanisms e.g., Hsps (Hoffmann *et al.*, 2003). However, phenotypic plasticity may also be maladaptive and traded-off with other life history traits (Pelster & Burggren, 2018). In related studies, Kristensen *et al.* (2016) showed that acclimation to low temperature negatively affected heat tolerance in *Drosophila melanogaster*. This suggests a possible trade-off between heat and cold tolerance, as such, represents an additional constraint for this species when facing changing environments (i.e. acute high and low temperature events) in nature. This also affirms the notion that the relationship between heat and cold shock responses is highly asymmetrical e.g., heat acclimation ‘always’ improves low temperature survival while the reverse is not always true (see discussions in Chown & Nicolson, 2004). Similarly, in *Nezara viridula*, CT_{max} showed more plastic responses post heat acclimation than CT_{min} , showing that CTLs may be typically decoupled (Chanthy *et al.*, 2012). In wolf spiders, acclimation also did not modify thermal breadth showing that plasticity may not cushion these species from high temperature stress (Anthony *et al.*, 2021). Thus, the role of short- to medium-term plasticity in the adaptation to variable climatic environments remains largely contested (Nyamukondiwa *et al.*, 2011).

One of the more intriguing aspects of my data is the implications of variable temperature acclimation on *A. thalassius* functional responses (dung removal efficiency). High temperature acclimated beetles made significantly bigger balls and removed a significantly higher proportion of dung compared to both control and low temperature acclimated beetles. In my view, this translates to relatively higher ecological functions at high temperature acclimation compared to the control and low temperature acclimated beetles. In a similar study, Mamantov & Sheldon (2020) showed that *Onthophagus taurus* increased ball size and depth of dung burial following high temperature acclimation, signifying that ecological responses were linked to temperature acclimation. During acclimation, higher temperatures increase metabolic enzyme activity (Hoffmann & Somero, 1995) that likely plateaus at peak (yet unknown) temperature or duration.

The results from this study showed that low temperature acclimation had negative effects on dung beetle ecosystem function, manifesting as significantly smaller dung balls, and lower dung mass removal. Acclimation responses to low temperature may be highly species dependant (Gotcha *et al.*, 2021); as such, we speculate, with caveats that *A. thalassius* may not be adapted to low but high temperature stress owing to its warm tropical origin and diurnal activity patterns (Caveney *et al.*, 1995)). In a similar study, Wu & Sun (2012), showed that a 2.3°C increase in temperature delayed female oviposition maturity and egg hatching by 4.1 and 7.2 days respectively and egg and larval size by 22.1 and 33.4% respectively in *Aphodius erractus*. This signifies that high temperature acclimation affects beetle performance. The current study only tested within-generation adult acclimation responses; thus, future studies should aim to investigate the effects of temperature variability across generations and testing more diverse life history traits. In addition, I could not account for the cost of mounting plasticity and the role of behavioural adaptation (the Borget Effect) (Dallas & Rivers-Moore,

2012; Gunderson & Stillman, 2015; van Heerwaarden & Kellermann, 2020; Mamantov & Sheldon, 2020). Thus, future work may need to test acclimation across ontogeny and investigate the role of carry-over and/or transgenerational plasticity in *A. thalassius*. To avoid competition, dung rollers are also known to abandon their dung balls if they cannot migrate far enough from the dung pat source. As such, future experiments may consider increasing experimental arena sizes, considering mesocosm- or field-approaches to better explain the effects of temperature variability on ecological services. Similarly, future studies may also incorporate dung beetles from diverse locations to better understand the role of local adaptation in buffering climate change effects.

Although critical thermal limits (e.g., CT_{max}) have received considerable attention under climate change, the fate of ecosystem functioning under climate change have been limited (but see Munang *et al.*, 2013; Holley & Andrew, 2019; Gotcha *et al.*, 2021). This work thus, provides novel data on how a diurnally active tropical dung beetle species may be adapted to the predicted global warming and how the ecological service delivery of this species may be affected by heat stress under climate change. This is evidence to argue that future models for species survival under climate change should account for potential losses in ecosystem function and/services. The results showed that (i) *A. thalassius* has extreme basal heat tolerance that presumably helps the species forage diurnally in heat stressing tropical environments; (ii) acclimation to variable high temperature improves *A. thalassius* heat tolerance indicating thermal plasticity, while simultaneously improving ecosystem function (dung removal), (iii) low temperature acclimation constrained *A. thalassius* ecosystem function (dung removal), and (iv) *A. thalassius* has low plasticity as exhibited by the low ARR, as such phenotypic plasticity may unlikely cushion physiological fitness and survival of this species and indeed ecosystem functioning in the face of heat stress associated with climate change. This study

thus contributes empirical evidence to literature supporting the phenotypic plasticity versus basal tolerance ‘trade-off’ theory and contributes to the growing recognition of the need to make practical decisions for ecosystem management to enable continued provision of ecological functions under a range of future human-mediated environmental conditions in sub-Saharan Africa and similar environments.

5.5. References

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Chapter 6

Density-dependent ecosystem service delivery under shifting temperatures by dung beetles.

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6.1. Introduction

Global climate change represents one of the biggest societal challenges of the 20th century (Sala *et al.*, 2000; Thomas *et al.*, 2004; Jactel *et al.*, 2021), and its impacts are increasing across all ecosystems (see Deutsch *et al.*, 2008; Hoegh-Guldberg & Bruno, 2010; Hughes, 2000). Tropical ecosystems, in particular, may be disproportionately affected due to drastically shifting temperature and precipitation patterns (Engelbrecht *et al.*, 2015). Both low and high temperature stresses have been known to affect insects' survival, ecological performance (Sinclair *et al.*, 2015; Holley & Andrew 2019a), ecosystem multifunctionality (Antiqueira *et al.*, 2017), male reproductive sterility (Parratt *et al.*, 2021), growth rates, feeding, movement and metabolism (Ramachandran *et al.*, 2020). Environmental changes, such as warming, have been associated with changes in species distributions (Erasmus *et al.*, 2002; Larsen, 2012), populations' fitness (Wu & Sun, 2012), maintenance of the integrity of multifunctional landscapes (Antiqueira *et al.*, 2017), species diversity and abundance (Chown & Nicolson 2004; Deutsch *et al.*, 2008). Indeed, empirical evidence suggest insects' biodiversity is rapidly declining (Kleijn *et al.*, 2011; Oliver *et al.*, 2015; Raven & Wagner 2021) due to climate change, agricultural intensification, rampant use of pesticides, biological invasions and potential synergistic effects among these factors (Deutsch *et al.*, 2008; Harvey *et al.*, 2020; Jactel *et al.*, 2021; Ricciardi *et al.*, 2021). In the absence of compensatory mechanisms to survive climate change, reports suggest a likely decrease in biological diversity (Carpaneto *et al.*, 2007; Deutsch *et al.*, 2008). Tropical insect species may be the most at risk of extinction from global warming because they are living close to their upper thermal limits and are thus highly sensitive to heat stress (Stillman *et al.*, 2003; Gunderson & Stillman 2015; van Heerwaarden *et al.*, 2016; but see Nyamukondiwa *et al.*, 2011). Therefore, recent increases in mean temperatures and the frequency and severity of heat stress (see Easterling 2000; Meehl

and Tebaldi 2004; IPCC, 2007; 2014; Stillman 2019; Harvey *et al.*, 2020) represent an important challenge to arthropods' fitness and ecological roles.

Coprophagous (dung) beetle species are insects from the order Coleoptera. They use dung as food and nesting resources (Raine *et al.*, 2018). By collecting dung for these uses, they concomitantly contribute to multiple ecosystem services, such as, nutrient cycling, soil bioturbation, pest control, secondary seed dispersal, pollination, and dung decomposition (Nichols *et al.*, 2008; Beynon *et al.*, 2012; Braga *et al.*, 2013; Griffiths *et al.*, 2016; Doube 2018). Tunneller and roller dung beetle species physically transfer nutrient-rich mammalian dung (organic matter) from the soil surface to lower soil layers. By burying dung under the soil, dung beetles reduce the emission of greenhouse gases into the atmosphere (Slade *et al.*, 2016a; Piccini *et al.*, 2017)), as well as increase soil nutrient richness (Yamada *et al.*, 2007), thereby contributing to soils' ecological services. Furthermore, by burying seed-containing dung below the soil surface, dung beetles contribute towards secondary seed dispersal (Shepherd & Chapman, 1998), whereby dung beetles relocate seeds away from predators and parent plants and reduce density-dependent seed mortality (Shepherd & Chapman, 1998). Dung beetles also serve as complementary environmental cleaners, through reduction of fluxes of greenhouse gasses from extensive cattle farming areas, particularly in the tropics where advanced greenhouse gases emission mitigation measures are lacking (Slade *et al.*, 2016). By cleaning the environment, dung beetles thus contribute significantly to the maintenance of ecosystem function in multifunctional landscapes (Santos-Heredia *et al.*, 2018).

Despite the ecological benefits provided by dung beetles, their roles in multifunctional landscapes may be affected by climate change. For example, previous works have shown that ecosystem contribution by dung beetles may be affected by thermal stress (Wu *et al.*, 2011;

Holley & Andrew, 2019b; Gotcha *et al.*, 2020; 2021). Warming has been reported to reduce dung burial in *Sisyphus rubrus* (Holley & Andrew, 2019b), thereby being likely to affect nutrient cycling, and pest control and soil fertilisation. In another study, the effects of temperature regime shifts were intertwined with the foraging ecology of individual species, whereby low temperatures reduced dung ball production in diurnal species (Gotcha *et al.*, 2021). Dung removal has been reported to increase with dung beetle functional group richness (Slade *et al.*, 2007), due to the notion that individual species use resources in various ways, and as such, resource use increases more in communities with higher biodiversity.

The effects of thermal stress, species abundance, functional group richness and species diversity do not, however, operate in isolation and may interact non-additively. For many beneficial species, the strength of their ecosystem service provision is closely tied to their trophic interactions. However, trophic interactions in the tropics are highly context-dependent, being mediated by factors such as temperature, resource density and non-trophic interactions among inter- and intraspecific consumer groups (Cuthbert *et al.*, 2021). For example, studies measuring consumer functional responses (resource use as a function of resource density) have found feeding rates to peak at intermediate temperature levels (Englund *et al.*, 2011; Uiterwaal & DeLong, 2020). Furthermore, consumer mass, relative to that of their resources, has been shown to mediate interaction strengths (Cuthbert *et al.*, 2020), and interactions between multiple consumers can result in non-trophic effects through emergent antagonisms or synergisms, which lessen and heighten consumption rates, respectively (Sentis & Boukal, 2018). While most studies to date have modelled changes in species biogeography with climate change (Deutsch *et al.*, 2008), little has been done on how climate change will affect ecosystem service delivery in the context of trophic interaction strengths (but see Slade & Roslin, 2016). While several reports suggest loss of thermal fitness and biological diversity with climate

change (Deutsch *et al.*, 2008; Stillman, 2019; Harvey *et al.*, 2020; Jactel *et al.*, 2021), none have looked at the responses of coprophagous species under varying temperature stress and species density scenarios under differing resource densities (see Buxton *et al.*, 2020a; b for predatory biocontrol agents). Knowledge on the effect of heat stress on functional ecosystem service efficiencies by these coprophagous species may help shape conservation policy of these and other species in the face of climate change. Here, I investigated dung beetle ecosystem service delivery (utilisation and dung ball creation) among three beetle species, at different intraspecific beetle densities, three starting dung mass levels and three temperature regimes. I hypothesised that dung beetle ecological service delivery varies interactively with species context, operational temperature, dung availability and beetle density (owing to effects of intraspecific competition).

6.2. *Materials and Methods*

6.2.1. *Study site and sampling*

Dung beetles were collected from Khumaga village (S 20°28.348 E 024°30.657), in Botswana during February - March 2021 using pitfall traps (Braga *et al.*, 2013; Nyamukondiwa *et al.*, 2018; Gotcha *et al.*, 2020). This seasonality represents the peak activity time for most dung beetle species in the study region (Sands & Wall, 2017; Nyamukondiwa *et al.*, 2018). The area is characterised by annual rainfall between November and March, ranging between 450 and 500 mm (Sands *et al.*, 2021), with most of the rainfall recorded between January and February (Nyamukondiwa *et al.*, 2018). Khumaga has sandy soils with an average temperature of 23°C, with minimum and maximum temperatures of 18.5 °C and 46 °C, respectively, during the months of January and February (Nyamukondiwa *et al.*, 2018). The village is characterised by small scale pastoralism (mainly cattle and goats) and is at the interface with a protected area, Makgadikgadi Pans National Park, which hosts other large wild ruminants (e.g., giraffes,

gemsboks, impala), non-ruminants (e.g., elephants), carnivores (e.g., lions) and omnivores (e.g., monkeys, baboons) (DWNP 2012). This rich animal diversity provides varying and overlapping dung resources that promote abundant and diverse dung beetle species.

Plastic containers (2L) were buried flush with the ground and covered with fine wire mesh of varying sizes (15mm and 30mm internal diameter) to accommodate dung beetles of varying masses (Sands & Wall, 2017; Nyamukondiwa *et al.*, 2018). Fresh cattle dung (200g) was placed on top of the wire mesh as bait. Although dung beetles are often generalists in the type of dung they use, Sands *et al.* (2021) reported that beetles from the study site mostly prefer cattle dung. Furthermore, cattle dung was the most abundant resource available to beetles at the study site and is by far the most widely used in previous research (Sands & Wall, 2017; Nyamukondiwa *et al.*, 2018; Gotcha *et al.*, 2020). Moreover, our survey results and previous work (see e.g. Errouissi, *et al.*, 2004; Wipple & Hoback, 2012; reviewed in Raine & Slade, 2019; Sands *et al.*, 2021) showed that dung beetle resource use overlap was highest for cattle dung. The traps were covered with overhead plastic plates to protect from rain and direct sunlight (as in Niino *et al.*, 2014; Gotcha *et al.*, 2020). Beetles were collected from traps and fresh dung replaced every 12hrs, after the collection of nocturnal species in the morning i.e. before sunrise (6am), and before sunset (7pm) for diurnal species. From the trap catches, only telecoprid beetles of the species *Allogymnopleurus indigaceus*, *Scarabaeus zambezianus* and *Khepher prodigiosus* were selected, and were placed into insulated containers (100cm length × 35cm width × 35cm depth) containing 20cm soil depth.

The beetle species *A. indigaceus* and *S. zambezianus* represent the most abundant diurnal and nocturnal species, respectively, in this area (Gotcha *et al.*, unpublished data). Nevertheless, nocturnal *K. prodigiosus*, with the largest body size, was incorporated to test effects of body

size on ecosystem services by creating a reasonable body size gradient (i.e. small [*A. indigaceus*], intermediate [*S. zambeziensis*] and large [*K. prodigiosus*]). Measurements for body length for each species ($n = 20$) were made by distance between the head and abdomen extremity, following methods by Hernández (2002). The mean lengths $\pm$ SEM for *A. indigaceus*, *S. zambeziensis* and *K. prodigiosus* were 11.84 ± 0.14 , 26.90 ± 0.31 and 39.21 ± 0.41 mm, respectively. Collected beetles were kept as mixed species of about 200 beetles per container (100cm length $\times$ 35cm width $\times$ 35cm depth), where fresh dung was placed inside for *ad-libitum* feeding before transportation to Botswana International University of Science and Technology in Palapye (Eco-physiological Entomology Research Laboratory). In the laboratory, monocultures of these three species were created and kept in a rearing room set at $28 \pm 1^\circ\text{C}$, 12D:12L photoperiod and $65 \pm 5\%$ RH. These conditions were chosen based on prevailing ambient conditions in the natural beetle collection area (Nyamukondiwa *et al.*, 2018). Beetles were fed daily with cattle dung *ad libitum* in their rearing containers (50cm $\times$ 25cm $\times$ 15cm). These containers housed 100, 50 and 25 individuals of *A. indigenus*, *S. zambeziensis* and *K. prodigiosus* respectively, to account for beetle body size.

Fresh, recently dropped cattle dung was collected from trapping sites and stored in cooler boxes to be used for experimentation. This was done to avoid confounding effects of collecting dung from ‘non-native’ sites or ‘old’ dung that could potentially be infested by other corprophagous species, e.g. beetles and flies. In the laboratory, this dung was weighed and placed into zip lock bags in batches of 100g, 200g and 400g, then refrigerated until use. Before use, the dung was homogenised and then put in a climate chamber (HPP 260; Memmert GmbH + Co.KG, Germany) set at 28°C for 24 h to equilibrate to experimental control temperature (28°C).

6.2.2. Experimental design

Acclimation

To acclimatise beetles to test environments and standardise feeding, 30 beetles of each species were removed from rearing containers and placed in experimental plastic containers (30cm length × 18 cm width × 10cm depth) in groups of 5 individuals per container. Each container had soil of about 2cm depth to provide a optimal medium walking surface for beetles. The beetles were then fed with 500g of dung for 12h in the experimental containers. Thereafter food was removed, and the beetles allowed to stay without food for another 12h, in order to standardise feeding status before treatments (see **Figure 6.1**). The acclimation duration (24h) was chosen primarily to offer the organisms time to experience both the day and night cycle (photoperiod) before the beginning of the treatment. Furthermore, although this acclimation duration was higher than studies on Coleoptera acclimations, e.g., 6h (Allen *et al.*, 2012), 5-15 h (Lü & Liu, 2017), 3 h (Chen *et al.*, 2018), the duration was chosen as a balance between reducing the severity of high temperatures on mortality (see Jørgensen *et al.*, 2021), as well as avoiding prolonged low temperatures effects on individual mobility. This ‘acclimation × time’ interaction is sufficient to elicit acclimation responses (see Weldon *et al.*, 2011).

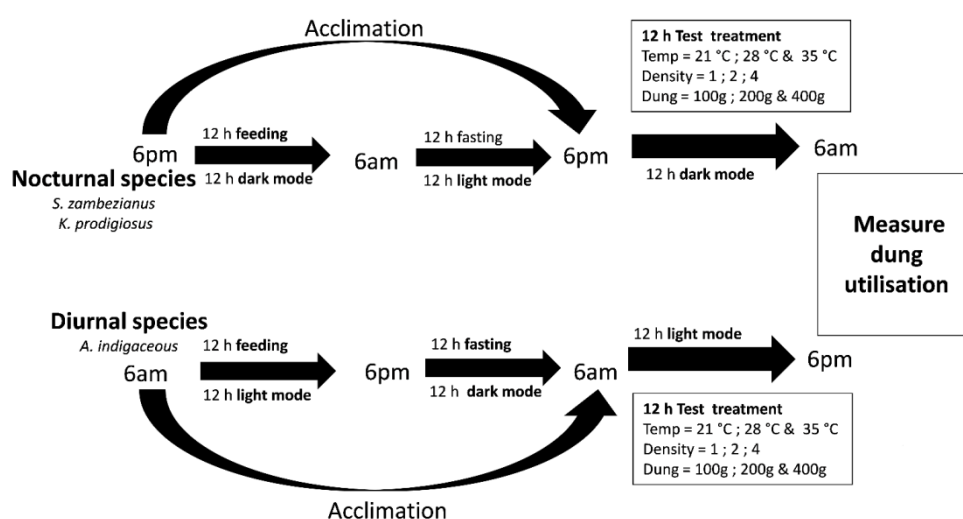


Figure 6.1: Experimental design flow chart: nocturnal and diurnal species were fed for 12h followed by 12h fasting to standardise feeding experience. Thereafter, species were provided

with known dung mass for 12h followed by determination of dung mass utilisation. Dung utilisation was measured in three telecoprid dung beetle species (*A. indigaceus*, *S. zambezianus* and *K. prodigiosus*) across three beetle densities (1, 2 and 4), different starting dung masses (100g, 200g and 400g) and three test temperatures (21 °C, 28 °C and 35 °C). However, *K. prodigiosus* was not tested at 400g due to low field availability.

Treatments

To compare functional efficiency of dung beetles, the dung removal ability of three unsexed telecoprid dung beetle species (*S. zambezianus*, *K. prodigiosus* and *A. indigaceus*) was measured across three beetle densities (1, 2 and 4), three different dung masses (100g, 200g and 400g) and three temperatures (21°C, 28 °C and 35 °C), in a ‘3 (species) × 3 (beetle densities) × 3 (dung masses) × 3 (test temperatures)’ design, i.e., full-factorial experiment. However *K. prodigiosus* was not tested at the highest dung mass (400g) due to limited field availability of individuals. The total numbers of individuals field collected and subsequently used for each species were 500 for each *A. indigaceus* and *S. zambezianus* and 250 for *K. prodigiosus*. The beetle starting densities were based on our field observations and information from literature (Errouissi *et al.*, 2004). Our mean field catches per trap for each 12h sampling period for *K. prodigiosus* and *A. indigaceus* were 1.7 and 1.6, respectively. Thus, based on this, and to circumvent the effects of intraspecific interactions, we chose to start with a beetle density of one. Although *S. zambezianus* was the most abundant species (62.6 beetles per trap per 12h), we had to standardise the number of beetles per species during the experiment to avoid confounds. Dung pat sizes were chosen based on laboratory experimental feasibility and literature (Errouissi *et al.*, 2004). Similar studies by Errouissi *et al.*, (2004) used pat sizes between 30 – 750g; a range overlapping with our methodology. Furthermore, dung pat sizes used here were practical to work with under confined, controlled laboratory conditions (smaller dung mass <100g were likely to be a limitation for the larger species [*K. prodigiosus*], while the larger dung masses >400g would be overabundant for the smaller species [*A. indigaceus*]).

Similarly, the three test temperatures (21 °C, 28 °C and 35 °C) were chosen based on prevailing ambient conditions in the natural habitats, based on empirical data (Nyamukondiwa *et al.*, 2018) and projections of climate change (Engelbrech *et al.*, 2015). Temperature variability associated with climate change is likely to be in the ranges ± 5 °C at RCP 8.5 in southern Africa, should mitigation measures fail (see Engelbrech *et al.*, 2015). Therefore, we wanted to assess effects of temperature beyond the predicted changes, but within fluctuations experienced at the sampling locations.

To begin the treatments, plastic containers (30cm length $\times$ 18cm width $\times$ 10cm depth) were filled with sterilised soil as explained before (2cm depth). Insect screen mesh (100 μ m; 12cm length $\times$ 12cm width) was placed on top of the soil in each container. Pre-weighed dung at one of the three masses was placed on top of the insect screen mesh, and pre-weighed individual beetles (at one of the three species and densities) were added into the containers. The containers were also covered with insect screen mesh (100 μ m) to prevent insects from escaping. Thereafter, the containers were placed in climate chambers set at treatment temperatures (i.e. 21 °C, 28 °C and 35 °C) and 12 L:12 D photoperiod (see **Figure 6.1**). These comprised 72 treatments and 9 controls, replicated five times, resulting in 360 treatments and 45 controls. All controls were run in the absence of dung beetles at each of the test temperatures and dung masses to quantify dung water loss under the same conditions post-experiment. Treatments were structured through time such that acclimation of the nocturnal species was started during the night cycle, while acclimation of diurnal species was started during the day time (see Fig. 1), and replicates were randomised across the different climate chamber compartments. Experimental beetles were thus allowed to feed on the dung for 12h during the day (diurnal species; *A. indigaceus*), and during the night (nocturnal species; *S. zambezianus* and *K. prodigiosus*). To quantify dung utilisation post 12h, the remaining dung on the cover screen

mesh was weighed and utilized dung was calculated by subtracting remaining dung from the initial dung mass (Piccini *et al.*, 2017). Furthermore the number of dung balls produced per replicate was also determined. The two metrics measured (dung mass utilised and number of dung balls made over a 12h time period) were used as reasonable proxies of essential ecosystem services under natural conditions. The two metrics were chosen because dung mass utilisation covers both dung eaten by beetles and dung that was rolled away as balls for food/reproductive investment, while the number of dung balls made may only accounts for reproductive investments. Sex was not considered as a factor since both males and females make and roll dung balls for feeding and brooding (Favila & Diaz, 1996; Sato, 1998; Sullivan *et al.*, 2016; but see Emlen, 1997; Gregory *et al.*, 2015). Furthermore, similar work showed no intra- and inter-specific variations in dung burial rates in two roller beetles between sexes (Carvalho *et al.*, 2018). In addition, no literature explicitly supports or refutes the effect of sex on dung ball numbers or overall removal efficiency; although effects of sex on dung ball size are widely recorded (Tomkins *et al.*, 1999; Sullivan *et al.*, 2016).

6.2.3. Statistical Analysis

To account for water losses due to evaporation, aside from removal by dung beetles, utilisation was qualified using the mean percentage water loss from beetle-free controls at each temperature and dung mass group (i.e., mass utilised minus mean mass lost from evaporation). Dung mass utilisation (i.e., qualified initial mass minus final mass) was analysed using generalized linear models assuming a Gamma error distribution and log link, with species, temperature and dung beetle density included as fully factorial explanatory variables. Dung beetle mass was also included as a single term, with an aggregate mass summed in the multiple dung beetle treatments (i.e., total biomass of dung beetles in each individual replicate). Species was included as a categorical term, whereas temperature, dung beetle density and dung beetle

mass were each continuous term. Separate models were fit at each starting dung mass (i.e. 100g, 200g and 400g), because one species (*K. prodigiosus*) was not tested at the highest dung mass due to limited field availability of individuals.

Similarly, numbers of dung balls created were analysed using generalised linear models assuming a Poisson error distribution and log link as a function of the same explanatory variables above (species, temperature, dung beetle density and dung beetle mass), separately per starting dung mass. All models were checked for overdispersion and zero inflation by comparing fitted residuals to simulations with expected null values (Hartig, 2020). Multicollinearity was assessed considering a threshold variance inflation factor of 5 (Fox & Weisberg, 2019). Analysis of deviance was used to compute F-test for Gamma generalised linear models, and likelihood ratio tests were used for Poisson generalised linear models, with Type III sums of squares in the presence of an interaction term, and Type II sums of squares in the absence of such a term (Fox & Weisberg, 2019). Non-significant terms were removed stepwise in each model, such that the final model included only significant coefficients or underlying terms for significant interactions (Crawley, 2007). Post-hoc comparisons were computed using Tukey adjustments (Lenth, 2020) and statistical significance was considered at $p < 0.05$. All statistical analyses were performed in R v4.0.2 (R Core Development Team, 2020).

6.3. Results

6.3.1. Dung utilization

At the lowest dung mass (100g), nocturnal *K. prodigiosus* (the largest species) exhibited highest utilisation rates (mean $\pm$ SD: 84.71 $\pm$ 9.071g), followed by nocturnal *S. zambeianus* (intermediate size) (54.05 $\pm$ 32.299g) and diurnal *A. indigaceus* (smallest size) (11.76 $\pm$ 6.135g). Significantly higher utilisation rates were exhibited as temperature and beetle density increased

(**Table 6.1a**). Utilisation rates were significantly affected by species in interaction with temperature (**Figure 6.2a**). Utilisation rates of *A. indigaceus* and *S. zambeziensis* increased with temperature, but those for *K. prodigiosus* were contrastingly reduced with warming. Utilisation was significantly more positively temperature-dependent in *A. indigaceus* compared to *K. prodigiosus* ($p < 0.001$), while the other species' temperature trends were statistically similar (both $p > 0.05$).

At the intermediate dung mass (200g), Beetle density and species, but not temperature, had significant effects on dung removal, singularly (**Table 6.1b**). *Kheper prodigiosus* again exhibited highest utilisation rates ($167.56 \pm 24.337g$), followed by *S. zambeziensis* ($75.46 \pm 58.850g$) and *A. indigaceus* ($14.66 \pm 15.970g$). However, significant two-way interactions indicated differences among species according to both temperature and beetle density (**Figure 6.2b**). As with 100g supplies, utilisation rates of *S. zambeziensis* increased with temperature, while *K. prodigiosus* exhibited greatest utilisation at the lowest temperature; *A. indigaceus* utilisation rates peaked at the intermediate temperature. Here, *S. zambeziensis* was significantly more positively responsive to temperature than the other two species (both $p < 0.05$), which were in turn statistically similar ($p > 0.05$). Further, *K. prodigiosus* was significantly least responsive to increasing conspecific densities (both $p < 0.01$), while *A. indigaceus* and *S. zambeziensis* utilisation increased to a similar extent at higher densities ($p > 0.05$); however, *K. prodigiosus* almost depleted a large proportion of the limited available dung across beetle densities, potentially dampening utilisation increases (**Figure 6.2b**).

At the highest dung mass (400g), *S. zambeziensis* again utilised more ($80.44g \pm 69.826g$) than *A. indigaceus* ($9.92 \pm 9.431g$). Dung utilisation was significantly different between species and increased with temperature, beetle density and beetle mass (**Table 6.1c**; **Figure 6.2c**). There were no significant interaction terms. *Allogymnopleurus indigaceus* overall dung utilization peaked at the 200g treatment and was lowest at 400g. In contrast, *S. zambeziensis* and *K.*

prodigiosus feeding rates always increased with dung supply (i.e. positively density-dependent).

Table 6.1: Coefficients from analysis of deviance on Gamma generalized linear models considering dung mass utilization across three species, temperatures, beetle densities and beetle masses at (a) 100g, (b) 200g, and (c) 400g dung. Statistical significance was determined at $p < 0.05$, and significant terms are emboldened.

Dung mass	Term	Df	F-value	p-value
(a)100g	Species	2	37.785	<0.001
	Temperature	1	19.935	<0.001
	Density	1	31.366	<0.001
	Mass	1	0.163	0.688
	Species x Temperature	24	9.199	<0.001
	Species x Density	24	1.410	0.248
	Temperature x Density	14	1.488	0.225
	Species x Temperature x Density	2	0.847	0.431
(b)200g	Species	2	19.115	<0.001
	Temperature	1	3.220	0.075
	Density	1	41.614	<0.001
	Mass	1	0.308	0.580
	Species x Temperature	24	4.870	0.009
	Species x Density	24	6.971	0.001
	Temperature x Density	14	0.294	0.589
	Species x Temperature x Density	2	1.425	0.245
(c)400g	Species	1	16.858	<0.001
	Temperature	1	8.591	0.004
	Density	1	5.365	0.023
	Mass	1	4.780	0.032
	Species x Temperature	1	0.409	0.524
	Species x Density	1	0.023	0.881
	Temperature x Density	14	2.417	0.124
	Species x Temperature x Density	14	0.258	0.613

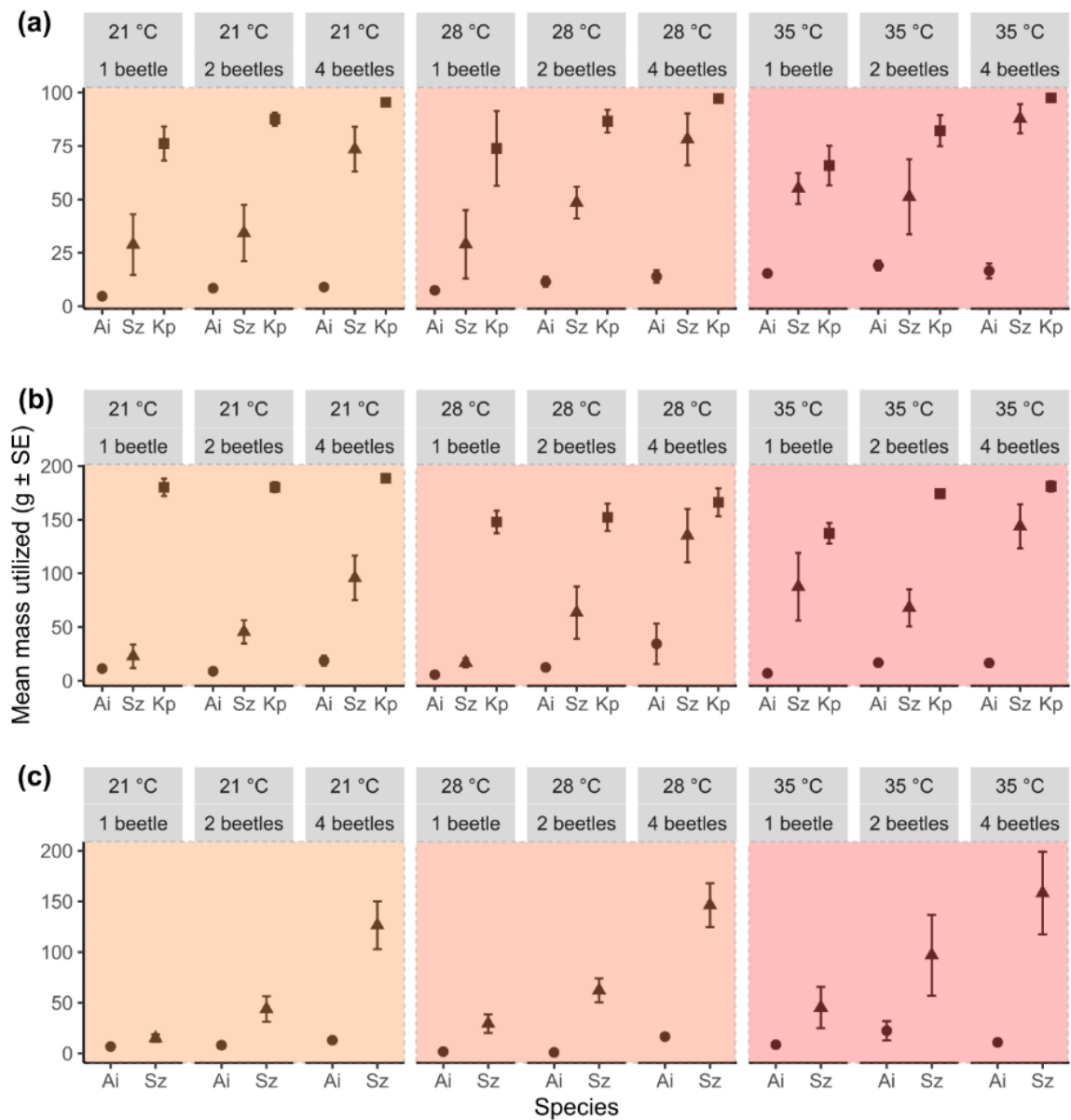


Figure 6.2: Mean dung mass utilization at (a) 100 g, (b) 200g and (c) 400g starting mass among *A. indigaceus* (Ai), *S. zambeziensis* (Sz) and *K. prodigiosus* (Kp) among different temperature treatments and beetle densities. Error bars indicate standard error (SE).

6.3.2. Dung ball production

At the lowest dung mass supply (100g), *S. zambeziensis* produced the most dung balls on average (3.98 ± 2.904) followed by *A. indigaceus* (2.56 ± 1.984) and *K. prodigiosus*

(1.71 ± 0.815). Dung ball creation differed significantly among species and increased significantly according to beetle density, but not temperature (**Table 6.2a; Figure 6.3a**). Here, *S. zambezianus* produced significantly more balls than the other species (both $p < 0.001$), while *A. indigaceus* produced significantly more balls than *K. prodigiosus* ($p < 0.05$). There were no significant interaction terms.

At the intermediate dung mass (200g), *S. zambezianus* again produced the most balls on average (3.56 ± 2.889) followed by *A. indigaceus* (2.78 ± 2.504) and *K. prodigiosus* (2.20 ± 0.944). Dung ball creation differed significantly among species and temperature according to density, owing to a significant two-way interaction (**Table 6.2b; Figure 6.3b**). Here, *S. zambezianus* produced significantly more balls than *K. prodigiosus* ($p < 0.001$), with all other species comparisons statistically similar (both $p > 0.05$). Whereas ball production peaked at the highest temperature in the single and double dung beetle groups, dung ball production related negatively with temperature at the highest beetle density, resulting in significant differences in temperature-dependence as beetle density increased (all $p < 0.05$).

At the highest starting dung mass (400g), dung ball creation by *S. zambezianus* was significantly higher (3.87 ± 3.552) compared to *A. indigaceus* (2.20 ± 1.714). Dung ball creation differed significantly between these species and related significantly positively to beetle density and temperature (**Table 6.2c; Figure 6.3c**). There were no significant interaction terms.

Table 6.2: Coefficients from analysis of deviance on Poisson generalized linear models considering dung ball creation across three species, temperatures, beetle densities and beetle masses at (a) 100g, (b) 200g and (c) 400g of dung. Statistical significance was determined at $p < 0.05$, and significant terms are emboldened.

Dung mass	Term	Df	Chi-square	p-value
<i>(a)100g</i>	Species	2	41.713	<0.001
	Temperature	1	0.024	0.877
	Density	1	61.028	<0.001
	Mass	1	0.006	0.937
	Species x Temperature	24	3.357	0.187
	Species x Density	24	1.696	0.428
	Temperature x Density	14	0.027	0.869
	Species x Temperature x Density	2	1.141	0.565
<i>(b)200g</i>	Species	2	14.609	<0.001
	Temperature	1	4.811	0.028
	Density	1	16.837	<0.001
	Mass	1	0.050	0.824
	Species x Temperature	24	1.800	0.407
	Species x Density	24	1.790	0.409
	Temperature x Density	14	5.826	0.016
	Species x Temperature x Density	4	0.780	0.677
<i>(c)400g</i>	Species	1	20.872	<0.001
	Temperature	1	4.013	0.045
	Density	1	110.365	<0.001
	Mass	1	1.740	0.187
	Species x Temperature	1	0.815	0.367
	Species x Density	1	0.097	0.755
	Temperature x Density	14	0.174	0.677
	Species x Temperature x Density	14	0.505	0.477

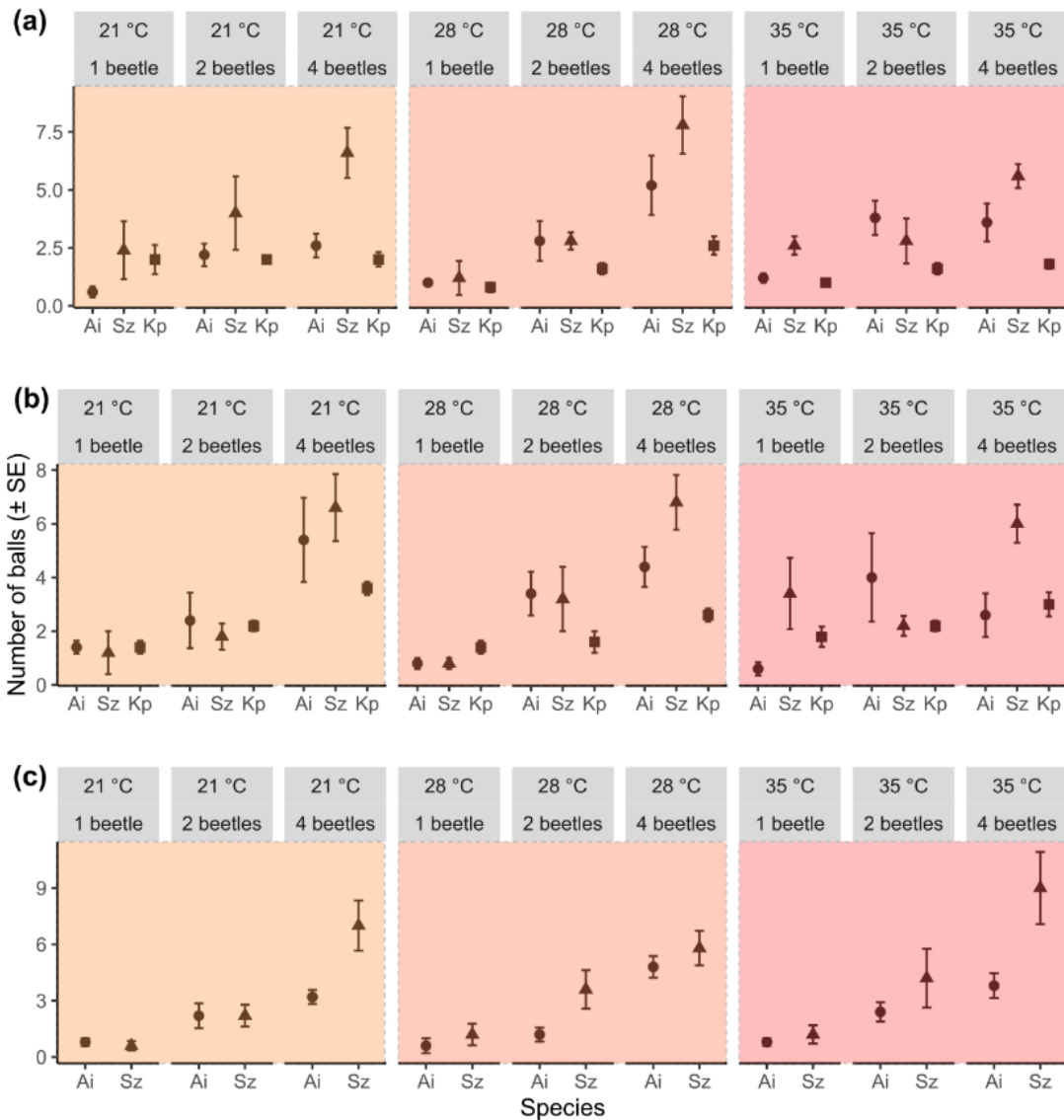


Figure 6.3: Mean dung ball numbers at (a) 100 g, (b) 200g and (c) 400g starting dung masses among *A. indigaceus* (Ai), *S. zambeziensis* (Sz) and *K. prodigiosus* (Kp) at different temperature treatments and beetle densities. Error bars indicate standard error (SE).

6.4. Discussion

This is the first study quantifying the interactive effects of species, temperature, and consumer density on the essential ecosystem function of dung removal from telecoprid dung beetles across multiple starting dung masses. The results showed that dung utilisation rates were firstly species-dependent, with the largest species, *K. prodigiosus*, exhibiting consistently highest dung utilisation rates compared to both intermediately-sized *S. zambeziensis* and smallest-sized *A. indigaceus* across dung masses supplied. Secondly, the results showed statistically higher

utilisation rates as both temperature and density increased at both lowest and highest dung masses. Utilisation rates were further statistically affected by species in interaction with temperature, whereby *K. prodigiosus* exhibited greatest utilisation rates at lowest temperatures, while utilisation rates for *A. indigaceus* peaked at intermediate or high temperatures, and *S. zambezianus* consistently peaked at the highest temperature. Interactions of species with density were, however, not statistically significant except at the intermediate dung mass, with *K. prodigiosus* the least beetle density-dependent. Similar to utilisation, numbers of dung balls produced were species-, temperature- and density-dependent. Generally, *S. zambezianus* produced significantly most balls, and the number of balls increased as dung beetle density and temperature increased. This general increase in dung utilisation may also suggest a lack of interaction, such as interference, between intraspecific dung beetle species studied here.

The results agree with other studies that showed a positive correlation between beetle size and overall mass removed (Tomkins *et al.*, 1999; Gotcha *et al.*, 2020). Similarly, related studies have found large-bodied species to be more functionally efficient at dung removal than small-bodied species (Kaartinen *et al.*, 2013; Nervo *et al.*, 2014; Sullivan *et al.*, 2016; Gotcha *et al.*, 2021). In other comparative studies, results showed that bigger beetles had higher dung mass utilisation than smaller ones both within (Tomkins *et al.*, 1999) and across species (Gotcha *et al.*, 2021). Larger-bodied individual species, e.g., *S. zambezianus*, made significantly bigger dung balls compared to smaller individual species, e.g. *A. indigaceus* (Gotcha *et al.*, 2021). These results therefore suggest that larger *K. prodigiosus* and *S. zambezianus* may be more efficient ecosystem service providers (measured by dung removal) than relatively small *A. indigaceus*; although emergent species differences were found as temperature and beetle density changed.

I also recorded significantly higher dung utilisation rates as temperatures increased across species, and particularly in the case of *S. zambezianus*. The smallest, diurnal *A. indigaceus* typically utilised the least dung across temperatures compared to nocturnal *K. prodigiosus* and *S. zambezianus*. In addition, utilisation rates of *A. indigaceus* and *S. zambezianus* tended to increase with temperature, but those for *K. prodigiosus* were contrastingly reduced with warming. The results therefore suggest that the ecological functional efficiency of bigger-bodied, *K. prodigiosus* at low temperatures may be result of thermal inertia and the Bergmann's Rule, which assumes that larger bodied animals do better under lower temperatures but contrastingly worse at high temperatures; the latter owing to the reduced ability to dissipate heat relative to smaller-bodied species (Zamora-Camacho *et al.*, 2014). These results suggest that both body size and thermal sensitivity (foraging/activity time, see details in Gotcha *et al.*, 2020; 2021) may account for the differences in dung utilisation reported here. The performance of large-bodied *K. prodigiosus* may not be surprising as large body size has been known to enhance many aspects of an organism's performance and dominance (see Bonner, 2006; Zamora-Camacho *et al.*, 2014). Large body size is also associated with higher metabolic costs, and higher vulnerability to biotic pressure, e.g., predation. Dung rolling in telecoprids is also energetically costly (Krell-Westerwalbesloh *et al.*, 2004), and thus an intermediate insect body size has been regarded as being evolutionarily optimal (Roff, 2002). This may partly account for why intermediately-sized nocturnal *S. zambezianus* had high ecosystem service delivery at high temperature relative to the smallest (*A. indigaceus*) and largest (*K. prodigiosus*) species. Indeed, thermal sensitivity varies with species; body size as well as environmental history (see e.g. Nyamukondiwa & Terblanche, 2009; Verble-Pearson *et al.*, 2015; MacLean *et al.*, 2019; Gotcha *et al.*, 2020, 2021). Nevertheless, related studies have also shown that desiccation stress (which is strongly linked to high temperature) (Gibbs *et al.*, 2003; Addo-Bediako *et al.*, 2001), can be compensated for by having a larger body size. Larger body size is associated with larger

water reserves and a reduction in water loss rates, owing to a relatively low surface to volume ratio. However, desiccation tolerance also appears to be species-specific (see Nervo *et al.*, 2021).

The highest heat stress conferred (35°C) did not preclude dung utilisation in any of the tested species. These results are not surprising because an effect of thermal stress on an organism's performance depends on temperature intensity, duration (Chown & Terblanche, 2006) and environmental history (Nyamukondiwa & Terblanche, 2010). Acute, chronic and diurnal changes in sub-optimal heat stress may improve thermal tolerance through phenotypic plasticity (acclimation) (Angilletta, 2009; Sgrò *et al.*, 2016) thus allowing arthropods to compensate adaptively to stressful environments (Whitman & Ananthakrishnan, 2009; Sgrò *et al.*, 2016; Oliveira *et al.*, 2020). As such, rapid acclimation to test temperatures may partly account for the maintenance of ecosystem services at the highest temperature (35°C) tested here. Although phenotypic plasticity has been reported to improve performance through e.g., acclimation (Hoffmann *et al.*, 2003; Chown & Nicolson, 2004; Zerebecki & Sorte, 2011; Sgrò *et al.*, 2016), its capacity to cushion organisms against climate change effects has produced equivocal results (see Calosi *et al.*, 2008; Nyamukondiwa *et al.*, 2011; Gunderson & Stillman, 2015; van Heerwaarden *et al.*, 2016; Machekano *et al.*, 2021). Deleterious effects of acclimation have also been reported, e.g., through heat stress mortality, reduced growth rate, survival (Chown & Terblanche, 2006) and reduced male fertility (Parratt *et al.*, 2021). Therefore, future work could also explore effects of long-term acclimation through more chronic high temperatures on ecosystem service delivery. This result is nevertheless congruent with the expected performance of insects, as ectotherms, which respond positively with temperature (see e.g., Angilletta, 2009; Schulte *et al.*, 2011; Colinet *et al.*, 2015; Furlong & Zalucki, 2017; Tüzün & Stoks, 2018). Indeed, different performance-related metrics, e.g.,

feeding rates, locomotion, growth rates and metabolism, are linked with temperature (Ramachandran *et al.*, 2020). Thus, the correlation between increase in ecosystem service provision (dung utilisation) in several species and increase in temperature reported here may be due to associated species' increase in metabolic rates. However, across study systems, feeding rates of consumers have been shown to peak unimodally at intermediate temperatures (Englund *et al.*, 2011; Uiterwaal & DeLong, 2020), and thus higher temperatures than those employed here could eventually cause a reduction in utilization efficiency.

Beetle density also had significant effects across dung masses, with greater quantities of dung being utilised as beetles increased in density. This result is not surprising as species' abundance and dung biomass are both critical determinants of optimal ecosystem functioning (Tixier *et al.*, 2015). Thus, increased utilisation (dung removal) would be expected with greater beetle density and dung biomass in a positively density-dependant relationship. However, in other trophic systems (e.g., predator-prey), interactions between multiple consumers have been shown to be non-additive, resulting in emergent antagonisms and synergisms which respectively reduce or increase *per capita* feeding rates (Wasserman *et al.* 2016). Here, although there was an increase in dung utilisation as density increased, our results showed that smaller-bodied beetles utilised less dung than large-bodied species at all beetle densities tested. Utilisation rates also tended to increase with dung mass supplied in *S. zambeziensis* and *K. prodigiosus*, while *A. indigacoeus* peaked at the intermediate supply, indicating that an asymptotic feeding rate was not yet reached for the two larger species. My result nevertheless suggests that ecosystem service delivery is likely a function of both *per capita* dung removal and beetle density. Thus, even if smaller-sized beetles utilise less dung *per capita*, they may exhibit higher abundances which compensate at the population-level, relative to larger-bodied species. Field observations support this assertion, in that smaller sized beetles were more

abundant (*S. zambezianus*) than larger ones (*K. prodigiosus*). While the support for species abundance reported here may be anecdotal, species abundance may also be a function of net species reproduction, which largely depends on body size (see Kingsolver & Huey, 2008). Generation time is more prolonged and fecundity lower for large bodied relative to smaller insects (Roff, 2002). Indirectly, longer developmental time may also reduce population abundance through its prolonged direct effects on mortality, e.g., through biotic stress and predation or parasitism (Blanckenhorn, 2000). This may negatively affect population dynamics for large bodied species, e.g., *K. prodigiosus*, and may likely account for their low numbers in natural ecosystems relative to smaller- and medium-bodied ones (*A. indigaceus* and *S. zambezianus* respectively). Future studies should thus investigate the relative abundance of these species and their likely contribution to ecosystem service delivery.

I also found that multiple intraspecific dung beetles tended to interact additively in terms of dung removal, with utilisation and ball creation rising with numbers of conspecifics, and thus more abundant species in ecosystems likely exhibit greater efficiency. Yet, this effect was most pronounced considering *S. zambezianus* and *A. indigaceus*, with the largest species the least positively density-dependent. Notably, however, as dung was not replaced as it was utilised in this study, capacities to detect interactions among consumers are artificially constrained at low resource supplies, particularly for larger beetles which consumed a high proportion of the available dung individually (i.e. *K. prodigiosus*). Regardless, this result is in keeping with the notion that dung beetle species contribute unequally to dung removal. Future studies should thus examine the functional efficiency of multi-species versus species-specific assemblages (see Manning & Cutler, 2018). Incorporation of these species-specific traits in models examining dung beetle ecosystem service delivery may help refine conservation planning under shifting climate and multifunctional environments.

My results for dung ball creation showed that ball production was additionally significantly affected by species at all dung masses. Intermediate-sized *S. zambeziensis* produced significantly more dung balls followed by smallest sized *A. indigaceus*, while the largest *K. prodigiosus* produced the fewest dung balls. Increased number of dung balls produced by *A. indigaceus* and *S. zambeziensis* relative to *K. prodigiosus* may be related to dung ball size and dung availability. Experimental observations have shown that larger beetles make larger dung balls than smaller beetles, making dung resources limiting for bigger-bodied beetles (Tomkins *et al.*, 1999; Sullivan *et al.*, 2016; Gotcha *et al.*, 2021). Future studies on ecosystem services may thus need to increase dung resource availability (i.e., relatively higher dung masses). Efficiency of intermediately-sized *S. zambeziensis* in dung ball number creation as a proxy of ecosystem service delivery may also be linked to the beetle's optimal size and accompanying evolutionary fitness consequences thereof (see Roff, 2002). I also speculate here with caveats that higher number of dung balls for *S. zambeziensis* may partly be due to its higher sensitivity to higher temperatures relative to *A. indigaceus*. Telecoprid dung beetles also use dung balls for thermoregulation via heat sinks (see Smolka *et al.*, 2012). By climbing on the moist dung ball, these beetles often use dung balls as mobile refuge onto which they climb to cool down while rolling dung across the hot ambient environments (Smolka *et al.*, 2012). This therefore suggests that the heat sensitive *S. zambeziensis* individuals may have made more balls as a thermoregulatory compensation at higher temperatures. The sensitivity of *S. zambeziensis* to heat stress is further supported by its nocturnal activity patterns, and relatively reduced upper critical temperatures to activity compared to diurnal species (see Gotcha *et al.*, 2020).

As expected, dung ball production significantly increased with beetle density, again indicating a lack of interference effects among conspecifics. Optimal ecosystem functioning for dung

beetles has been found to largely depend on biomass, species identity, species body size and nesting strategies (Doube, 1990; Piccini *et al.*, 2020). Manning and Cutler (2018) reported that ecosystem functioning was lower at reduced species density and abundance than at higher species richness. However, related studies also suggest that dung beetle ecosystem service delivery and ecosystem functioning require a balance among beetle abundance, beetle biomass and presence of dung beetle species of certain desirable attributes (Braga *et al.*, 2013). Furthermore, the effects of temperature on dung ball production were often statistically significant here, suggesting that across the species tested, number of dung balls created was positively dependent on prevailing temperature. The lack of interference effects amongst conspecifics is significant for optimising ecological diversity and ecosystem function. Future studies should thus look at the interaction effects of temperature, density and heterospecific combinations on ecosystem service delivery in these and other taxa. Enhanced ecosystem service delivery from heterospecific dung beetles will be potentially ideal for optimising ecosystem function in climate change-stressed multifunctional landscapes.

As mean temperatures and climate extremes become severe, fitness of organisms and maintenance of essential ecosystem services remain central themes to ecology. As such, accounting for the effects of species, resource density and temperature stress on ecosystem services delivery should also be critical for multifunctional ecosystems in the face of global change. A reduction of essential ecosystem services, e.g., pollination, organic matter decomposition and collapse of food webs with climate change, has already been widely reported in the literature (Anderson, 1991; Allison *et al.*, 2013; Settele *et al.*, 2016). My results therefore provide evidence for the effects of temperature, species, resource supply and dung beetle density on ecosystem service delivery. Specifically, I have shown that dung beetles contribute unequally to ecosystem service provision, but that effects are density- and

temperature-dependent. I further showed emergent effects for mass utilisation as well as positive effects of warming on dung removal, suggesting the role of climate on species functional efficiency. The results show that larger-bodied species may persist with ecological service delivery under cooler environments, whereas smaller bodied species may be more functionally efficient in moderately higher temperature environments. Thus, it is critical to continue monitoring species-dependent ecosystem service provision in the face of environmental change, and to optimise biodiversity-ecosystem-function. Future studies should consider long-term monitoring of dung beetle species diversity, as well as their ecosystem services and other factors that may reduce their functional efficiency, either singly or synergistically, e.g., insecticides, changing habitats and/or pollution. Some previous research has shown that dung beetle sex is an important driver for tunnel depth (Gregory *et al.*, 2015) and that dung burial behaviour may change between sexes (Emlen, 1997). Thus, future work should also consider the effects of sex on metrics measured here. Similarly, previous work has also shown that dung beetles can use dung for thermoregulation, through using dung moisture as heat sinks (Smolka *et al.*, 2012). Thus, future studies may also investigate how differential dung masses (resource availability) may affect insect behavioural thermoregulation and consequent ecological services. This information is significant for informing sustainable natural capital utilisation and maintenance of ecosystem services.

In conclusion ecosystem service delivery, specifically dung removal, is linked to the interactions across individual species, temperature, resource availability and dung beetle densities. This study has shown that dung removal is positively correlated with beetle body size, with the largest species utilising more dung than the intermediate and relatively small species. Furthermore, beetle size influenced individual species' sensitivity to heat stress, where the intermediate body size outperformed both the larger and smaller species as operational

temperatures increased. The results further showed that larger bodied species may persist with ecological service delivery under cooler environments, whereas smaller bodied species may be more functionally efficient in moderately higher temperature environments. Nevertheless, dung removal increased with beetle densities across temperatures and dung biomass, showing that ecosystem function under changing environmental temperatures require a balance between beetle densities, resource availability and beetle species of certain desirable traits. These results highlight the importance of considering effects of climate change, dung beetle diversity and resource availability on dung removal ecosystem service delivery. Given that thermal tolerance is variable among dung beetle species (Nyamukondiwa *et al.*, 2018), and that dung beetles' species and abundance varies spatio-temporally (Sands *et al.*, 2021), it is therefore important to conserve dung beetle diversity in order to maximise their benefits to the environment and society.

6.5. References

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Chapter 7
General Discussion

This study was aimed at first identifying the diverse range of dung beetles in Khumaga area and then investigate their behavioral and physiological adaptation mechanism against thermal stress, and how these mechanisms can influence species survival and ecosystem service delivery under the context of current and projected climate change and associated anthropogenic activities. As a result of projected global warming associated with climate change, animal species are likely to be exposed to novel and in most cases, challenging environmental conditions, especially stressful temperatures. Results from this thesis show that dung beetles exhibit niche partitioning across time, where some species prefer diurnal activity, while other species prefer crepuscular / nocturnal activity. I further showed that activity times are linked to species thermal tolerance, where species with lower heat tolerance preferred nocturnal activity, while species with high heat tolerance preferred diurnal activity. Similarly, I showed that cold tolerance also had a role in activity time, such that cold tolerant species preferred nocturnal activity, while species with low cold tolerance preferred diurnal activity. The ability of species to adapt through phenotypic plasticity was explored using one of the identified diurnal dung beetle species, *Allogymnopleurus thalassinus*, and the results showed that although diurnal species show high basal heat tolerance, they are less plastic, agreeing with other studies which reported that species with high basal heat tolerant have limited thermal plasticity (Gunderson & Stillman, 2015; Gunderson *et al.*, 2017; Morley *et al.*, 2019).

My results showed that Botswana is endowed with a high diversity of dung beetle species but as an arid, tropical landbordered country, it is highly susceptible to the impacts of climate change, which may affect the survival of these dung beetle species, populations and by extension, their ecological services. Studies have reported that ecosystem services provided by dung beetles are positively correlated with dung beetle diversity (Manning & Cutler, 2018). Furthermore, dung beetle species may be active/forage at different times of the day, presumably

to reduce predation, competition and to avoid stressful temperatures (Feer & Pincebourde, 2005; Pessôa *et al.*, 2017). With the imminent increase in temperature stress such as increased cold and heat waves, as well as increase in temperature variability due to climate change (Davies-Reddy & Vincent, 2017), it is unknown how this group of insects will tolerate these temperature stress. In addition, it is unknown how these stressors will affect their fitness and consequent ecosystem services. I also found that 8/16 large bodied species preferred the crepuscular/nocturnal activity compared to the diurnal activity. Because in Khumaga daily average temperatures are usually higher during the day than the night (see **Figures 3.3; 3.3; 4.2; 4.3**; Bishop *et al.*, 2017; Nyamukondiwa *et al.*, 2018), I therefore concluded that temperature may be one of the reasons for differential activity times suggesting possible evolution of differences in thermal tolerance between nocturnal and diurnal species recorded in my study. In addition, it was also reported that more ecological activity occurs during the night than during the daytime (Andresen, 2002) due to the abundance of beetles during the night, therefore knowledge of activity time in local guilds is critical since more nocturnal species may mean more ecological service provision in terms of ecological services. Still on diversity and activity times, I found that Khumaga area consist of 38 dung beetle species from 7 tribes with majority of the species belonging to the Onthophagini tribes (see Chapter 2). It is in this chapter where I provided new knowledge on the locally available dung beetle species that had not been documented in Botswana before, this is a critical knowledge gap that this study has filled in addition to limited studies on local dung beetles guilds to date (see Tshikae *et al.*, 2008; Sands *et al.*, 2021). In addition, for the first time I categorised local dung beetle species by their activity times as well as highlighting the need to investigate the possibility of daily activity times as an evolutionary adaptation to thermal stress (Chapter 3 & 4) under local climate.

I also found that diurnal species had significantly higher CT_{max} compared to nocturnal species (Chapter 3; Gotcha *et al.*, 2020), suggesting that heat tolerance was likely responsible for restricting dung beetle species to specific foraging / activity diel periods, a trend which likely evolved as adaptation to thermal stress (Bishop *et al.*, 2017). In addition, consistent with results from Chapter 3, nocturnal species had lower minimum temperature for physiological activity (CT_{min}) than both crepuscular and diurnal species (Chapter 4; Gotcha *et al.*, 2020), providing further support that cold tolerance might also have a role in the activity time of species. The results from these two chapters (Chapter 3 & 4) indicated that diurnal species may have evolved inherent heat tolerance that enables them to survive the high temperatures of the day, while nocturnal species have evolved inherent cold tolerances to allow them to survive the low temperature of the night. Other studies have also shown that temperature has an influence on the activity/foraging times of dung beetles (Gallego *et al.*, 2018) and ants (Hurlburt *et al.*, 2008; Bishop *et al.*, 2017). Furthermore, species with higher CT_{max} have been reported to have higher forage densities at higher temperatures than species with lower CT_{max} (Stuble *et al.*, 2013). These results provide knowledge on the possibility of activity times as evolutionary mechanisms for surviving thermal stress in dung beetles (see Gotcha *et al.*, 2020; 2021b). This therefore suggest that activity times also contribute to niche partitioning and low competition thereby enhancing ecosystem service success. However, in nature, many other biotic and abiotic factors play a role in niche partitioning and minimising competition to enhance survival. Diel activity timing for insects is nevertheless not fixed (see Levy *et al.*, 2019) as such may change with projected changing environmental temperatures. Such changes in diel activity may offset ecological balances, since e.g., some beetles always track diel activity of their associated vertebrate dung producers. Change in dung beetle diel activity may, thus, affect their foraging efficacy and hence provision of ecosystem services. Indeed, daily activity times in dung beetles serves as a mechanism of species co-existence and possible mechanism of adaptation to thermal

stress (Gotcha *et al.*, 2020; 2021b). Nevertheless, results showed that morphological traits such as body size play a role in activity times. I found that body size had a positive effect on activity time, such that the crepuscular/nocturnal active beetles exhibited significantly broader beetle size compared to the diurnal ones which exhibited a narrower body size range.

Nocturnal species generally had larger body size than diurnal species, in agreement with other previous studies (Hernández *et al.*, 2011). This finding points to the complex relationship between temperature, morphological traits and activity times, which suggest that species may not always change their activity as soon as temperature changes. Behind this background, species might therefore find themselves having to adapt to changing environments within their specific activity times or else face fitness consequences, population decline and potential extinction. Possible physiological mechanisms against the on-going global warming include phenotypic plasticity and genetic adaptation (Hoffmann & Sgrò, 2011). However genetic adaptation takes a long time since it involves change in the genetic make-up of an organism (Svensson *et al.*, 2020). I therefore investigated phenotypic plasticity of a local diurnal dung beetle *A. thalassinus* to assess whether it is high basal heat or plasticity that plays the major role as an adaptation mechanism that is used by diurnal dung beetles against current and projected global warming (Chapter 5). I found that in order to adapt to its specific diurnal activity time, *A. thalassinus* exhibited high basal heat tolerance; CT_{max} higher than 50°C and took a prolonged time to be knocked down by extreme heat stress; HKDT (130.8 ± 41.12 minutes) at 53°C. This HKDT is significantly longer than that reported for other dung beetle species (see Gotcha *et al.*, 2020), in addition, the high CT_{max} ($> 50^{\circ}C$) means *A. thalassinus* has extreme basal heat tolerance (see Chown & Nicolson, 2004), congruent with arid/desert habitat insect species that exhibit extreme basal heat tolerance. Furthermore, I found that acclimation to variable heat or cold to induce plasticity for both CT_{max} and HKDT, were

effective but to a limited extent. Thus, this species exhibited very low acclamatory response ratios (ARR) signifying limited scope for complete plasticity required to buffer it against stressful environmental temperatures associated with climate change in its arid habitats. These results support the 'trade-off' theory, which predicts that higher basal thermal tolerance may come at a cost of phenotypic plasticity (Gunderson & Stillman, 2015; van Heerwaarden *et al.*, 2016; Barley *et al.*, 2021), therefore for such species, plasticity might not be enough to provide species protection against global warming (reviewed in Barley *et al.*, 2021) thus, they rely on high basal heat tolerance. Heat sensitive species may experience increasingly precautionary trade-offs between investing in thermoregulation versus other key processes as the climate warms (Manson *et al.*, 2017). Thus, in an attempt to adapt to unfavourable temperatures, such species might increase resource investment (time and energy) in thermoregulation, as such reduce the resources available for other key processes such as growth, reproduction and survival (Kearney & Porter, 2004), as such ecosystem service provision might be affected by the ongoing climate change.

The large diversity of dung beetle guilds recorded in this study indicates that beetle competition in Khumaga is likely high, therefore trophic interactions, niche partitioning and resource utilisation efficiency (with and without competition) may need to be teased apart. Literature showed that the strength of ecosystem service provision for most insects is closely tied to their trophic interactions (Englund *et al.*, 2011; Uiterwaal & DeLong, 2020). To help understand this, functional responses experiments quantified the effects of increasing mean temperatures and dung beetle species density and resource availability on dung removal, an essential ecosystem service in three telecoprid species available in Khumaga: *Allogymnopleurus indigaceus*, *Scarabaeus zambeziensis* and *Kheper prodigiosus* (see Chapter 6. Gotcha *et al.*, 2021a). The results showed that *K. prodigiosus*, exhibited greatest dung utilisation efficiency

overall across dung masses, compared to the other two species. Nevertheless, both *S. zambezianus* and *A. indigaceus* utilization increased with both warming and beetle density, whereas *K. prodigiosus* performance was less temperature and density-dependent. Results on dung ball number differed across species and correlated positively with temperature and densities, with *S. zambezianus* producing significantly most dung balls overall. These results show that temperature and competition largely have a significant effect on dung beetle species' fitness and thus functional efficiency and ecosystem service delivery may be affected by projected climate change.

In conclusion, heat stress tolerance may affect dung beetle activity timing and that evolution of temporal activity time may have come as a form of thermoregulatory compensation. Similarly, low temperature tolerance may also restrict activity timing, for example those beetles with higher cold tolerance may do better under lower night temperatures (nocturnal) and that low temperature stress may offset ecological service delivery. The results from my study have shown that dung beetles exhibit niche partition across activity times as mechanisms for co-existence as well as behavioural adaptation mechanisms against thermal stress. Although organisms are reported to adapt to quickly changing environments through phenotypic plasticity (Oms *et al.*, 2017), results from this and other studies showed that phenotypic plasticity is not universal and the degree of plasticity varies among taxa and species, hence some species might be vulnerable to global warming while others are not. In line with these studies, diurnal species such as *A. thalassinus*, thus, inherently have high basal heat tolerance but limited thermal plasticity (see discussions in Gunderson & Stillman, 2015; van Heerwaarden *et al.* 2016; Barley *et al.*, 2021). These results provided insights on the vulnerability of tropical diurnal species living in arid, tropical habitats in the face of global warming and the potential risk of early IUCN red list categorisation of these species (Davis *et*

al., 2020) if not actual extinction. The results help explain how plasticity may compensate for climate change effects in some but not all species. However, a limited or lack of plasticity may expose ecologically important species to high risk of red list category or possible extinction. Functional response results indicated that dung utilisation and removal was highly resource and density-dependent (but dependant on temperature and beetle size), thereby suggesting that species may compensate through temperature tolerance, numbers, body size and resource availability. The results from this study can be used to predict the possible effects of climate change on dung beetle biodiversity and potential consequences on ecosystem service delivery efficiency.

I therefore recommend that in response to the projected global warming, conservationists and policy makers alike, should use results obtained from this study in early warning systems in order to safeguard dung beetle species diversity and abundance in Botswana. This will protect and save the available, precious essential natural capital and its associated ecological services provision in the future. Efforts should be made to determine dung beetle species diversity and abundance across other areas of Botswana not reported here, to provide a complete, national inventory of available natural capital. Policy makers regionally, should incorporate findings from this study in crafting harmonised policies that encourage early investment in dung beetle conservation to improve environmental protection for current and future ecosystem health.

7.1. References

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