Respiratory Physiology of Urban Insects

By

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Abstract

Respiratory physiology of urban insects was studied. Specific urban insect pests studied included silverfish, *Lepisma saccharina* L., firebrats, *Thermobia domestica* (Packard), and bed bugs, *Cimex lectularius* L. All of these species frequently infest human dwellings and are unusual in their ability to survive extended periods of starvation, sometimes more than a year. Closed system respirometry was used to measure the standard metabolic rate (SMR) of silverfish, firebrats, and bed bugs. Closed system respirometry was also used to measure the post-feeding and post-molting metabolic rates of the common bed bug. SMR results indicated distinct differences both within and among species. Post-feeding and post-molting metabolism results indicated a pattern in respiratory decline during starvation possibly unique to bed bugs. All results were interpreted with respect to the life histories of the insects being studied.

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Introduction

Metabolism

All behaviors and physiological functions necessary for survival depend on a source of energy. Metabolism in animals is responsible for generating this energy in a form which is both easily usable and storable (short term). In animals, metabolism is largely driven by the chemical conversion of organic materials (food) into stored energy (ATP). A variety of factors can affect metabolism including temperature, mass, activity, and starvation. Therefore, to truly understand differences among species, a standardized measure must be used to properly compare metabolic rates. In ectothermic species (the focus of this thesis), the measure is termed the standard metabolic rate (SMR). Ectothermic species are those which regulate their internal body temperature primarily through external sources of heat. SMR is defined as the total amount of O_2 consumed over a given time, at a given temperature for post-absorptive ectothermic animals at rest (Moyes and Schulte, 2008). Post-absorptive animals are those which have completely digested a previous meal. Ectothermic animals are those whose body temperature is regulated by the external environment. SMR has been reported in multiple arthropod groups including ants (Lighton, 1988; Vogt and Appel, 1999), beetles (Burges, 1960), cockroaches (Dingha et al., 2009), crickets (Hack, 1997), fleas (Fielden et al., 2001), moths (Schneiderman and Williams, 1953), spiders (Anderson, 1970), termites (Shelton and Appel, 2001), and ticks (Fielden et al.,

1999; Lighton and Fielden, 1995). Metabolic rates often differ between groups, with some showing greater metabolic rates (ants, beetles, spiders) relative to others (ticks) (Lighton and Fielden, 1995). These relationships can be useful in understanding the life history of the species being studied.

Activity is one factor that commonly complicates metabolic measurements. In insects, activity can drastically increase metabolic rate (Bartholomew et al., 1985; Lighton et al., 1987; Lighton and Feener, 1989; Lighton and Duncan, 1995; Snelling et al., 2011). The increase in metabolic rate with activity creates a major problem for those attempting to compare species. Without some form of standardization (animal at rest or a defined speed of movement) it is nearly impossible to make accurate comparisons among species. Older studies, specifically those using microrespirometrs, did not account or correct for movement because the animal was hard to observe inside of the water bath (Scholander, 1942). However, recent studies have begun addressing this issue through observation of individuals during respiratory studies. The ability to observe insects during respirometry studies is due in large part to new technology. Vogt and Appel (1999) used video monitoring during closed system respirometry to identify and exclude all animals which had significant activity, thereby assuring that they were only measuring the true SMR.

Temperature is also a key component to understanding SMR in ectothermic animals. Ectothermic animals are unable to maintain a stable internal body temperature and therefore their metabolic rate is dependent upon the ambient temperature of their environment. The effects of temperature on insect metabolism differ among species and even among different strains or different life stages of the same species (Dingha et al., 2009; Vogt and Appel, 1999). To quantify the effects of temperature on metabolic rates, Q_{10} is often reported. Q_{10} is defined as the

change in metabolic rate for a 10°C change in temperature (Chown and Nicolson, 2004). When the relationship between Log_{10}(MR) and temperature is linear, Q_{10} can be thought of as a constant across that temperature range, whereas when the relationship is nonlinear, Q_{10} must be considered a variable which changes with temperature (Lighton, 1989; Vogt and Appel, 1999). Q_{10} can also be useful for understanding the life history of different species or different life stages within a species. Lower Q_{10} values indicate a greater ability to maintain a steady metabolic rate during changes in ambient temperature. Currently, few studies have explored and compared Q_{10} between different insect taxa, despite the important biological and life history data composed in this measure.

Body mass has a critical role in understanding and modeling metabolic rates. Mass scaling relates metabolic rate (usually in W or μW) to mass, usually in log form. One hypothesis states the metabolic rate should scale with 3/4 body mass across all orders of life ranging from cells to complex organisms (West et al., 1997, 1999; West et al., 2002). This model, known as the 3/4-power law, has been widely excepted since its origin in the 1930s (Kleiber, 1932). However, recent studies have suggested that the 3/4-power law may not be the most accurate description of mass scaling, particularly within species or between species with smaller mass ranges (Chown et al., 2007; Glazier, 2005). Specifically, Chown et al. (2007) present an alternative model termed the cell-size model which predicts metabolic rates to have a scaling exponent between 0.67 (2/3) and 1. This model is largely based on the increase in size of organisms relating to either an increase in the size of their cells or an increase in the number of cells. In this model, a 2/3 mass scaling factor indicates only an increase in cell size, while a mass scaling exponent of 1 indicates only an increase in cell number (Chown et al., 2007). The contrasting opinions and data illustrate the need for further studies into mass scaling.

Two additional factors which may strongly impact metabolism are starvation and molting (for arthropods). Many arthropod species, such as ticks, bed bug, reduviids, and thysanurans, have developed lifestyles allowing them the ability to survive extended periods of starvation. Zwicky and Wigglesworth (1956) were able to measure and characterize changes in metabolic rate before, during and after feeding for *Rhodnius prolixus* Stål. Their results indicated a major decline in respiration after molting and a major increase in respiration after feeding (Zwicky and Wigglesworth, 1956). In addition, their results indicated a peak in metabolic rate during molting. Bradley et al. (2003) conducted a similar study on *R. prolixus* which indicated similar trends, although they elected to model CO_2 release in a number of ways (per gram wet weight, per gram dry weight, without gut blood content, etc.). Bradley's results indicated a peak in metabolism during molting. Fielden et al. (1999) conducted a study on the effects of feeding on metabolism on the American dog tick *Dermacentor variabilis* (Say). They found a dramatic increase in tick metabolic rate with feeding, however, they did not model how metabolism changed for an extended period after feeding. Prolonged bouts of starvation are not only common in many species of arthropods, but also in other animals, notably snakes. Snakes generally consume large meals and then do not feed again for weeks to months or sometimes even years (Greene, 1997). Secor and Diamond (1997) modeled changes in metabolic rate of the Burmese python, *Python molurus* (L.) after feeding until metabolic rate returned to a resting stage. The pattern observed by Secor and Diamond for *P. molurus* strongly resembles that found by Zwicky and Wigglesworth (1956) for *R. prolixus*, with a large increase in metabolic rate after feeding and then an exponential or power function decline thereafter. This is of particular interest, because the only similarities between snakes and insects capable of surviving extended periods of starvation are the ectothermic nature of both and the feeding strategies of both. When molting

alone is considered, Defur (1990) showed the blue crab, *Callinectes sapidus* Rathbun, had a peak metabolic rate during ecdysis. From these studies it is clear that both starvation and molting play major roles in insect metabolism, but despite their importance have received limited attention.

Metabolic measurements are capable of determining how fast the animal is producing energy (ATP) and also capable of determining what substrate is being metabolized to generate energy. The respiratory quotient (RQ) is an indirect measure of the substrate being metabolized and is equal to the amount of CO_2 produced divided by the amount of O_2 consumed. Theoretical RQ values range from 1 to 0.7, with values around 1 indicting pure carbohydrate metabolism, values around 0.835 indicating pure protein metabolism and values around 0.7 indicating pure fat metabolism (Livesey and Elia, 1988). However, because these RQ values are theoretical and based on single metabolic studies they often differ significantly from measured values. Therefore, RQ is often used to understand general trends or shifts in metabolism. RQ has been measured and reported for a number of insect taxa, although most of these studies are from the early half of the twentieth century (Fink, 1925; Frew, 1929; Kleinman, 1934). More recently, Vogt and Appel (1999) used RQ to understand diet and metabolic substrate in different castes of the red imported fire ant, *Solenopsis invicta* Buren. Vogt and Appel (1999) provided some evidence indicating RQ may change with temperature in the red imported fire ant. This is supported by some of the older literature. Specifically, (Kleinman, 1934) found temperature to effect RQ in both the grasshopper, *Chortophaga viridifasciata* (De Geer), and larvae of the Japanese beetle, *Popillia japonica* Newman, results which conflicted with previous reports. Despite these findings, RQ is sometimes used as a standard to calculate O_2 consumption based on measured CO_2 production values because of the difficulties associated with making accurate measurement of O_2 (Lighton and Fielden, 1995). Sinclair et al. (2011) provided evidence for the

risk of using RQ to estimate metabolism. Their study demonstrated that a pseudo-change in metabolic rate based on CO_2 measurements was actually only a change in metabolic substrate (RQ) with no actual change in metabolic rate. Therefore, it is important that both components of RQ (O_2 and CO_2) be measured directly and only used for adjustments in insects previously measured and characterized.

Despite the importance and value of metabolic measurements, urban insect (those closely associate with human habitats) metabolic rates have received only limited attention. Measuring and gaining a better understanding of the metabolic rates of a variety of urban insects is important primarily because of the importance of urban insect pests.

Importance of Urban Insect Pests

Insects have been found within human dwellings since the beginning of recorded history. The insects (arthropods) most commonly reported inside human dwellings include ants, beetles, cockroaches, flies, fleas, mites, silverfish, spiders, and termites (Mallis, 2011). These species have the potential to cause a variety of problems for humans including structural damage to property, physical and psychological harm to humans, consumption of human food, and financial distress as a result of management efforts (Mallis, 2011). In addition to these effects, insects within the home place humans at health risks. These risks include potential allergies or allergic reactions, mechanical transmission of bacterial and viral diseases, and fungal or bacterial growth promotion (Mallis, 2011).

Urban insects are ubiquitously found throughout human environments and it would be difficult to find a human dwelling not infested with some urban pest. Despite the location and diversity of human dwellings, they all share one common theme: humans can inhabit them. This

fact provides perfect insight into why insects are found everywhere we are: if we can inhabit and tolerate it, there must be an insect which can also inhabit and tolerate it as well. Interestingly, there are reports stating that unwelcomed cockroaches were found aboard space ships. The benefit for urban insects lies in the relatively consistent environment offered by human homes. Outdoor environmental conditions fluctuate widely and can be rather difficult to withstand, but indoor environments offer favorable temperatures, food supply, and water. In addition, indoor environments offer overwintering and reproductive sites for many species and protection from predation.

Once inside, urban insect can be difficult to manage. Management efforts are routinely compounded by the complex environment being treated. To properly manage urban insects, pest management professionals (PMPs) must have a working knowledge of the pest, and of the building structure and construction type being treated. In addition, when insecticides are necessary they are highly regulated in terms of what insecticides can and cannot be used and some locations cannot be treated at all (Mallis, 2011).

This thesis will focus on three important urban insect species: silverfish, *Lepisma saccharina* L., firebrats, *Thermobia domestica* Packard, and bed bugs, *Cimex lectularius* L. Despite the differences in life history between thysanurans (firebrats and silverfish) and bed bugs, all three species are common pests in the urban environment and can survive extended periods of starvation, making them some of the more difficult urban pests to manage. The biology, behavior, and problems associated with each species are discussed below.

Silverfish and Firebrats

Firebrats, *Thermobia domestica* Packard and Silverfish, *Lepisma saccharina* L., are two common thysanuran pests of the urban environment. Both species are characterized by having three long caudal appendages and a body generally covered in scales (Triplehorn et al., 2005). Their bodies are somewhat elongated and all life stages look similar to each other, except for differences in size and the presence of an ovipositor in adult females (Triplehorn et al., 2005). The presence of an ovipositor also allows for distinction between males and females.

Thysanurans develop by ametabolous metamorphosis, which is characterized by immatures that look identical to adults, only gradually increasing in size with each molt. Thysanurans have an indeterminate number of instars and continue to molt throughout their lifetime even after reaching sexual maturity (Sweetman, 1938). Development time from egg to adult is dependent on temperature and different for both species (Lindsay, 1940; Sweetman, 1938, 1939). Firebrats generally develop from egg to adult (sexual maturation) in 92-105 d at their preferred temperature (32-37°C) (Sweetman, 1938). Silverfish development generally takes over a year at their preferred temperature (22°C), although reports exist for this time being as short as 3-4 months in tropical climates (Sweetman, 1939). Once sexually mature, both species can live for long periods of time. Firebrats are capable of living for 2-3 years, given the right conditions, although average time is generally shorter (Adams, 1933; Sweetman, 1938). Silverfish live on average between 2-3 years (Sweetman, 1939).

Both thysanuran species commonly feed on carbohydrates and proteins. Their diet is often mixed and in vitro can include oats, oatmeal, animal protein, and (on occasion) each other (Adams, 1933; Sims and Appel, 2012; Sweetman, 1939). Because of their tendency to eat carbohydrates and starches, both species will commonly feed on book pages, book bindings, and

cardboard boxes. These feeding habits can lead to large amounts of damage, if left untreated. The difficulties associated with thysanuran control is likely linked to their ability to feed on such a wide variety of foods (Sims and Appel, 2012). Both species are commonly found in and around their food. In particular, firebrats are generally found at warmer temperatures (32-41°C), with high relative humidity (RH, 76-85%) (Sweetman, 1938). Such locations commonly included boiler rooms, bakeries, and steam tunnels. Silverfish tend to be found in cooler environments with temperatures ranging from 22-27°C and with high relative humidity (> 75%) (Sweetman, 1939). Such locations include attics, basements, and bathrooms.

Firebrats have some unique characteristics unknown in other thysanurans. Beament et al. (1964) showed firebrats were capable of actively absorbing water from the atmosphere down to 45% RH. Noble-Nesbit (1969) was able to provide more information about the critical level (now 43% RH) and found that water loss occurs below this humidity level. Noble-Nesbit (1970, 1975) further determined the tail, and specifically the anus to be the site of active water uptake. This behavior allows for firebrats to avoid desiccation, even in environments with relatively low water content in the air and no free water. This behavior is also likely energy expensive, but quantification of the metabolic cost has not previously been reported.

Bed Bug History

Bed bugs have been a problem since the beginning of recorded history (Usinger, 1966). The origin of association with humans likely arose during the time humans first started inhabiting caves (Usinger, 1966). Bed bugs likely made the switch from their bat hosts to humans during this time. From this point on, bed bugs continued their close association with humans, moving with humans from caves to developed structures (homes). Bed bugs were

present in ancient Egypt (Panagiotakopulu and Buckland, 1999), the middle ages, and up to the middle of the twentieth century (Potter, 2011). Despite a temporary absence from mainstream society in the latter half of the twentieth century, bed bugs have resurged. Throughout their history, bed bugs routinely infested human habitation regardless of socio-economic status (Potter, 2011). However, overcrowding and poor living conditions of impoverished people often foster ideal conditions for bed bugs to prosper.

Bed bugs have been difficult to manage throughout their existence. Historically, humans have tried a variety of chemicals for management, including: mercury chloride, pyrethrum, sulfur (burned), and hydrogen cyanide, although few proved both safe and effective in the long run (Potter, 2011). Effective and affordable control was not realized until around the time of World War II when the organochlorine insecticide DDT became commonly used for urban insect control (Potter, 2011). DDT was highly effective and provided a relatively safe, residual treatment option for bed bugs, until it was banned in 1972. Although DDT was no longer available, development of other syntheci insecticides ruther limited be bug activity from most developed countries until the 1990s (Boase, 2001; Doggett et al., 2004; Hwang et al., 2005).

The resurgence of bed bugs has been both quick and unexpected. Their increased presence has resulted in a large increase in the number of citations and published works concerning bed bugs (Delaunay et al., 2011). Many factors have been implicated in contributing to their resurgence, notably insecticide resistance, increased travel, change in urban insect chemical application process (sprays to baits, etc.), and a general lack of sociatal awareness (Cooper, 2011; Doggett et al., 2004; Romero et al., 2007). Regardless of the reason for the bed bug resurgence, they are back and proving extremely difficult to manage.

Bed Bug Biology

The common bed bug (*C. lectularius*) is a small (5-6 mm) hematophagous insect which usually feeds on human blood (Usinger, 1966). Their bodies are brown (adults) to white (first instars), and can take on a red color, especially after feeding (Usinger, 1966). They are dorso-ventrally flattened and oval in shape, with each life stage looking similar to the last except increasing in size (Usinger, 1966). Their segments also overlap, but are joined by flexible connective tissue, permitting abdominal expansion during feeding thus allowing them to take in large blood meals (Usinger, 1966). Adult males can be differentiated from females by the presence of a reproductive structure known as a paramer that gives their abdomen a more pointed appearance (Usinger, 1966). In addition, adult females usually have scarring on the ventral side of their abdomen from multiple mating events (traumatic insemination) (Usinger, 1966).

Bed bugs develop by hemimetabolous metamorphosis. They have 5 nymphal instars and must feed prior to molting from one instar to the next (Usinger, 1966). Total development time varies depending largely on temperature and access to food. Developmental times (egg to adult) can range from 24 d (30°C) to 128 d (18°C), as long as food is continually available (Usinger, 1966). Relative humidity affects development, with problems in molting arising when relative humidity falls below 20% (Usinger, 1966).

Mating is unique within the Cimicidae and is known as traumatic insemination (Usinger, 1966). Bed bug mating is termed traumatic because the male mounts the female and then injects his sperm directly into her abdominal cavity, bypassing connection with her functional reproductive tract (Reinhardt and Siva-Jothy, 2007; Usinger, 1966). Males use a reproductive organ known as a paramere to inject their sperm. The paramere acts like a hypodermic needle allowing the male to pierce the female's cuticle and inject sperm into her hemolymph (Reinhardt

and Siva-Jothy, 2007; Usinger, 1966). Females have also evolved a counter adaption to this type of mating. The area where males usually pierce to inject sperm is known as the spermalege and it has been modified to better recover after traumatic insemination (Usinger, 1966). After traumatic insemination, sperm is stored in the seminal vesicles until fertilization occurs (Usinger, 1966). Mating is also interesting in bed bugs because it is male controlled. This behavior is indicative of the intersexual conflict hypothesis, which states that males and females are constantly evolving new strategies and defenses to gain control over fertilization (Parker, 2006; Reinhardt and Siva-Jothy, 2007). Once mated, female bed bugs can lay between 200-500 eggs in their lifetime (Cooper, 2011).

Feeding is a complicated process for bed bugs and involves host location, undetected feeding, and food digestion. Host location involves a combination of heat, CO_2, and kairomones from the host (Reinhardt and Siva-Jothy, 2007; Usinger, 1966). Some researchers have speculated on the importance of each host cue, but it is still unknown specifically what role each signal plays in host location. Bed bugs are also attracted to a variety of hosts, not only humans (Reinhardt and Siva-Jothy, 2007; Usinger, 1966). These hosts include rabbits, chicken, mice, and bats (Usinger, 1966). After finding a host, feeding then proceeds. To feed, the bed bug first secures itself to its host with its forelegs and then inserts its stylet into the host (Usinger, 1966). Engorgement can take as little as 3 minutes (first instars) and as long as 10-15 minutes (adults) (Usinger, 1966). After feeding, the same host cues that attract bed bugs repel them (Aboul-Nasr and Erakey, 1968). This is why bed bugs are never found living on their hosts, but rather in areas close to their host, except in cases of sever infestation. After ingesting a meal several times their body weight and returning to their harborage, bed bugs begin condensing and digesting their blood meal (Usinger, 1966). Digestion occurs with aid from microorganisms housed in

structures known as mycetomes (Reinhardt and Siva-Jothy, 2007; Usinger, 1966). These gut symbionts likely assist in obtaining vital nutrients from blood (Reinhardt and Siva-Jothy, 2007). Feeding is particularly important in bed bugs because of the close relationship between feeding and all aspects of bed bug life, not necessarily seen in other organisms. The frequency of feeding is highly dependent on temperature. Bed bugs have been known to feed as often as every three days (27°C), but generally at room temperature (23°C) bed bugs feed weekly (Usinger, 1966).

While not feeding, bed bugs tend to be found in cracks and crevices surrounding the location of their host. In apartments, houses, and hotel rooms bed bugs are commonly found around the bed. Areas bed bugs are commonly located include the mattress seam, box spring, bed frame, and headboard (Cooper, 2011). As bed bug populations grown, their locations become harder to pinpoint as they begin to disperse and move further away from the bed (Cooper, 2011). In this situation, bed bugs can be found behind picture frames, in electrical fixtures and furniture. Bed bugs are also commonly found in furniture which is used frequently, including chairs and couches (Cooper, 2011). Due to their dorso-ventrally flattened body, bed bugs have the ability to squeeze into small cracks and crevices and can be very difficult to see or identify. In addition, their eggs are usually laid in their harborage areas making identification and removal of eggs extremely difficult. While not feeding, bed bugs also tend to be found in aggregations that include all life stages. Bed bugs aggregation is still poorly understood, however we do know some of the factors responsible for this behavior. Levinson and Bar Ilan (1971) identified bed bug attraction to previously occupied paper disks. More specifically, Olson et al. (2009) found bed bugs to be attracted to feces from other bed bugs, although this behavior faded with increased starvation. The airborne component of the bed bug aggregation

pheromone was determined to be a blend of ten chemicals, all important in attracting bed bugs (Siljander et al., 2008). The antennae have an important role in aggregation (Olson et al., 2009), although it is still unclear the relative importance of tactile and airborne pheromone recognition. Bed bug aggregation may be due to a number of factors. Benoit et al. (2007) suggests that aggregation is potentially a mechanism for water conservation, possibly do to a humidified boundary layer of water. In addition, aggregation could also be beneficial for mate location because those living in a group are the most likely to be able to locate a mate. Also, aggregation sites indicate locations where development was successful.

Bed bugs are capable of withstanding a wide range of environmental conditions despite the general stability of their "natural" environment, the human home. One of the major problems associated with bed bug control is their ability to withstand extended periods of starvation (Usinger, 1966). Typical survival times during starvation vary greatly depending on temperature (Usinger, 1966). Survival times for adults have been reported up to 425 d post feeding (10°C) and as little as 29 d post feeding (37°C) (Usinger, 1966). Survival time during starvation also varied between strains, with insecticide resistant strains being less capable of surviving starvation than susceptible strains (Polanco et al., 2011). In addition to surviving extended periods of starvation, bed bugs are also able to withstand extremely dry environments and show very low rates of water loss (Benoit et al., 2007). Benoit et al. (2007) report 50 % of adult females capable of surviving for over 16 days at 0% relative humidity without access to food or water. Furthermore, Benoit et al. (2007) reported a low net transpiration rate (0.2%/h) and a high desiccation tolerance (30-40% loss in body water). Therefore, in the average human home bed bugs would rarely face any challenge associated with water loss. Bed bugs are not know to have any special modification to survive extreme temperatures, either hot or cold (Benoit et al., 2009).

Benoit et al. (2009) determined that neither water loss nor 2 week exposure to 4°C modified cold tolerance, although exposure to 0°C did improve survival at -16°C. In addition, Benoit et al. (2009) were able to identify heat shock proteins present in bed bug hemolymph, however these proteins failed to change bed bug heat tolerance.

Bed Bug Medical Importance and Management Difficulties

Bed bugs can inflict severe damage on their hosts, especially when populations are allowed to persist over time. The damage comes in a variety of forms, but bites are the most notable and resemble those inflicted by mosquitoes that result in an itchy red persistent welt (Goddard and deShazo, 2009; Usinger, 1966). The severity of this reaction varies among individuals; ranging from no reaction to experiences of extreme itching and large welts or even lesions (Fletcher et al., 2002; Goddard and deShazo, 2009; Usinger, 1966). Reactions to bed bug bites often change over time. For a majority of people, the first bite of a bed bug often leads to a delayed reaction (several days) or no reaction at all (Reinhardt et al., 2009). However, with increased exposure the latency period between bite and reaction slowly disappears with the two events occurring almost simultaneously (Reinhardt et al., 2009). It is also worth noting that when bed bug populations become large enough they have been reported to cause anemia in some patients (Pritchard and Hwang, 2009). Bed bug bites can be particularly hard to diagnose because they generally appear similar to other causes, such as hives or other insect bites (Scarupa and Economides, 2006). Without a positive identification of the bed bug, bites are nearly impossible to identify as being caused by bed bugs. In addition to the physical effects bed bugs can have on their host, perhaps the most alarming effects come through psychological damage. Bed bugs can commonly leave their victims emotionally affected far past the time of bed bug

removal. These effects are hard to quantify, but it is easy to understand because there is nothing like the feeling of being a prisoner in your own home, a place generally reserved for tranquility and rest. Bed bugs can be initial causative agents of Ekbom (restless legs) syndrome (Ekbom, 1960; Hinkle, 2010). Their presence often results in a delusional parasitosis that can persist long after proper management of a bed bug infestation. Despite all of the problems bed bugs can cause, they have not been found capable of transmitting any diseases (Goddard and deShazo, 2009). Infections associated with bed bugs have been reported to generally come from secondary infections from scratching or re-irritating bites (Cooper, 2011).

Goals and Objectives

The goal of this thesis is to better understand the effects of abiotic and biotic factors on metabolism of urban insect pests. This thesis takes a basic approach at better understanding standard metabolic rates while simultaneously offering insight into problematic and often understudied species. The importance and relevance of metabolism in a basic sence is discussed throughout while simultaneously gaining insight into the biology of urban pests species which could prove important to future control efforts. These goals are specifically addressed in thysanurans and bed bugs in the following three chapters.

In chapter II, the objective was to determine the effects of temperature, mass, and life stage on the metabolisms of two thysanuran species: the common silverfish and the firebrat. To investigate these effects, closed system respirometry was used and both O_2 consumption and CO_2 production were measured across a range of temperatures and masses of both species. Metabolic rates were compared and modeled for the effects of each variable. In addition, the results were

interpreted with the life history of each species and used to better understand metabolic mass scaling in insects.

In chapter III, the objective was to determine the effects of starvation and molting on the metabolic rate of the common bed bug. Closed system respirometry was used to measure both O_2 consumption and CO_2 production to determine metabolic rate and respiratory quotient. The metabolic rates of each life stage of the common bed bug were measured for over 800 h of starvation to determine how metabolic rate changes with starvation. The results were compared among life stages and with other animals which can undergo similar prolonged periods of starvation.

In chapter IV, the objective was to determine the effects of temperature, mass, and life stage on the metabolic rate of the common bed bug. Closed system respirometry was used to measure both O_2 consumption and CO_2 production across a range of temperatures and masses for all life stages. Metabolic rates were compared between life stages and modeled with temperature and mass. In addition, metabolic rates were also compared with ticks and other arthropods (ants, beetles, spiders, thysanurans). The results are interpreted with the life history of the common bed bug and also used to better understand metabolic mass scaling in insects.

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Chapter 2

Standard metabolic rates of *Lepisma saccharina* and *Thermobia domestica*: effects of temperature and mass

Abstract

Silverfish, *Lepisma saccharina* L., and firebrats, *Thermobia domestica* (Packard), are two common thysanuran pests in the urban environment. Both species can survive for extended periods without feeding, suggesting that they have some metabolic modifications compared with other insects which cannot tolerate extended starvation. To investigate potential metabolic modifications and to compare silverfish and firebrats, we measured the standard metabolic rate of both species at five temperatures (10, 20, 25, 30, 40°C) across a range of body masses using closed system respirometry. Temperature had a stronger effect on firebrat mass specific $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}) than on silverfish mass specific $\dot{V}_{O_2}$ for adults (> 0.00700 g: firebrat Q_{10} = 2.32, silverfish Q_{10} = 2.07) and immatures (< 0.00700 g: firebrat Q_{10} = 2.86, silverfish Q_{10} = 2.57). In addition, temperature had a stronger effect on the mass specific $\dot{V}_{O_2}$ of immatures than adults for both firebrats and silverfish. Respiratory quotients showed complex relationships with temperature from 10-40°C, indicating a change in metabolic substrate with temperature. These results are interpreted with respect to the life histories and environment of both species. Finally, metabolic rates are compared with those of ticks and other arthropods.

Introduction

Metabolic rates have been reported for a vast number of arthropod taxa including ants (Lighton, 1988; Vogt and Appel, 1999), beetles (Burges, 1960), cockroaches (Dingha et al., 2009), crickets (Hack, 1997), fleas (Fielden et al., 2001), moths (Schneiderman and Williams, 1953), spiders (Anderson, 1970), termites (Shelton and Appel, 2001), and ticks (Fielden et al., 1999; Lighton and Fielden, 1995). In many cases, metabolic rates can be used to gain insight into an organisms' life history or adaptation to a particular environment. Lighton and Fielden (1995) used metabolic rates and mass scaling to better understand the ability of ticks to survive extended periods of starvation in comparison to other arthropods. Vogt and Appel (1999) used metabolic rates to gain a better insight into differences in diets between fire ant castes (*Solenopsis invicta* Buren). In addition, scaling of metabolic rates with mass is currently an area of debate. The 3/4-power law has been the accepted model of mass scaling for years, but it has recently undergone scrutiny with other models predicting mass scaling coefficient ranging from 0.67 to 1 (Chown et al., 2007; Glazier, 2005; Kozlowski et al., 2003; West et al., 1997; West et al., 2002). Despite the large number of taxa which have been measured and the uncertainty about the 3/4-power law, two primitive insect species whose respiratory physiologies have received almost no attention are firebrats, *Thermobia domestica* (Packard), and silverfish, *Lepisma saccharina* L.

Firebrats and silverfish are two common thysanuran pest species of the urban environment. Both species consume proteins and starchy materials and have the potential to cause extensive damage if left untreated (Adams, 1933; Lindsay, 1940; Meek, 2011; Sweetman, 1938, 1939). In addition, both species are capable of surviving for greater than two years under the right conditions (Adams, 1933; Lindsay, 1940; Meek, 2011; Sweetman, 1938, 1939).

Firebrats and silverfish are frequently prone to bouts of forced starvation, which they are able to endure. Lindsay (1940) reported that one silverfish survived for almost a year without food. Both species undergo ametabolous development with an indeterminate number of instars; both species continue to molt even after reaching sexual maturity (Adams, 1933; Lindsay, 1940; Meek, 2011; Sweetman, 1938, 1939).

Despite these similarities, there are several distinct and important differences between the species. Firebrats usually inhabit warm to hot areas (32-41°C), with high relative humidity (76-85%) (Sweetman, 1938). Such environments can include boiler rooms, steam tunnels or bakeries. Silverfish tend to inhabit cooler environments (22-27°C) with high relative humidity (75-97%) (Sweetman, 1939). Due to their preference for cooler environments, silverfish tend to be found in environments closer in proximity to humans, such as basements, bathrooms, and attics. Within their optimal temperature range, firebrats can complete their lifecycle (egg to adult) in about 2-4 months (Sweetman, 1938), whereas silverfish generally require over a year to complete their lifecycle (Lindsay, 1940).

Currently, thysanuran respiratory physiology and specifically thysanuran standard metabolic rates (SMR), respiratory quotients (RQ) and Q_{10}s have received almost no attention. Standard metabolic rate is defined as the total amount of O_2 consumed or CO_2 excreted, over a given time, at a given temperature for post-absorptive ectothermic animals at rest (Moyes and Schulte, 2008). Respiratory quotients (RQ) is defined as the total amount of CO_2 excreted divided by the total amount of O_2 consumed and can be used as an indicator of what substrate is being metabolized. Specifically, theoretical RQ values range from 1 (pure carbohydrate metabolism) to 0.835 (pure protein metabolism) to 0.7 (pure fat metabolism) (Livesey and Elia, 1988). However, these values are best understood in a relative context because actual values

often differ from theoretical values. Q_{10} is defined as the change in metabolic rate for a 10°C change in temperature (Chown and Nicolson, 2004). Thysanuran metabolic rates have only been reported in the literature once by Edwards and Nutting (1950), who measured the metabolic rate of firebrats across a range of temperatures. However, no information was provided on the size of the animals tested or if they measured and corrected for activity, which significantly affects measurement of SMRs (Bartholomew et al., 1985; Lighton and Duncan, 1995; Lighton and Feener, 1989).

Due to the limited information on thysanuran respiratory physiology, and differences in the temperature preferences of firebrats and silverfish, it would be useful to compare the effects of temperature and mass on the metabolic rates of both species. In addition, information on these primitive insects could prove useful in gaining a better understanding of mass scaling and the ongoing debate regarding the 3/4-power law. In the present study, we measured both O_2 consumption and CO_2 production across a range of temperatures and masses for both species, accounting for movement. In addition, we calculated RQs and Q_{10}s for both species across the same range of temperatures. Finally, we compared the results for Thysanura as a group to values reported for ticks and other arthropods (Lighton and Fielden, 1995).

Materials and Methods

Experimental Animals

Separate firebrat and silverfish colonies were maintained at Auburn University, Auburn, Alabama, U.S.A. The firebrat colonies were started in 1985 and have been maintained continuously thereafter. Firebrats were maintained in 48.6 L plastic coolers (54x30x30cm; The Coleman Company, Golden, CO, USA) at 31±1°C in rolls of corrugated cardboard harborage.

Temperature was maintained within each cooler with a 100W incandescent light bulb surrounded by a 3 L clay pot (Wal-Mart Stores, Inc., Bentonville, Arkansas, USA). Coolers were provisioned with five 70 ml glass water jars covered with lids and fashioned with water wick (Absorbal Inc., Wheat Ridge, CO, USA). Firebrats were provided dry oatmeal (The Quaker Oats Company, Chicago, IL, USA) and pieces of dry Purina® dog chow (Nestle Purina Pet Care, St. Louis, MO, USA).

Silverfish colonies were initiated in 2010 and maintained in 19.0 L plastic containers (40x28x17cm; VWR International, Radnor, PA, USA) at 24±1°C. Containers were filled with shredded white copy paper (various types) and cardboard for harborage. Containers were provisioned with two 70 ml glass water jars covered with lids and fashioned with water wick. Silverfish were provided dry oatmeal and pieces of dry Purina® dog chow.

Prior to testing, groups of silverfish and firebrats were removed from their colonies and placed into 50 ml glass beakers (one for each species), provisioned with wetted water wick, and covered with Parafilm® (American National Can, Chicago, IL, USA). Individuals were counted before and after isolation to ensure that they did not consume one-another. This procedure ensured that all animals were post-absorptive before testing. These containers were maintained in identical conditions to the original colonies and animals were isolated for a minimum of 24 h before testing.

Respirometry Equipment

After both species were isolated for 24h, individuals were weighed to the nearest 0.00001g on a Mettler-Toledo AX205 digital balance (Mettler-Toledo GmbH, Greifensee, Switzerland), and placed into 3 ml respirometry chambers fashioned from 3 ml plastic syringes

(Becton, Dickinson and Company, Rutherford, NJ, USA). Six 1.4 mm diameter holes were drilled in each syringe barrel past the last gradation where the plunger enters the barrel. After animals were placed into individual respirometry chambers, the syringe plunger was inserted to close the syringe but leave the drilled holes open and animals were given at least 1 h to acclimate. After animals were acclimated, respiratory chambers containing animals were placed on a manifold for a minimum of 6 minutes, with dry, CO_2 free air flowing into the manifold at a rate of 230 ml min^{-1}. This procedure purged the respirometry chambers of all ambient CO_2 and H_2O. After purging, a 26 gauge intradermal bevel needle (Becton, Dickinson and Company, Rutherford, NJ, USA) was attached to the respirometry chamber and the volume was adjusted (depending on the life stage and temperature) by pushing the plunger past the drilled holes. Each respirometry chamber was sealed by attaching a rubber stopper (size 000) to the needle. Each syringe was placed into an incubator at one of five temperatures (10, 20, 25, 30, 40°C) in a completely randomized design. A minimum of 10 replicates were used for each temperature, with one replicate being equal to one respirometry chamber. Most sizes were incubated between 2-7 h, depending on mass. However, 10°C proved difficult to get results for some of the smaller sizes (< 0.00700g) and therefore they were tested between 18-50 h at 10°C. The incubator was illuminated with 15 W white fluorescent light and animals were monitored for activity using a SONY® DCR-SX85 video camera (Sony Corporation, Minato, Tokyo, Japan). Video recordings (MPEGs) were reviewed using Windows Media Player Classic version 6.4.9.1 (© 2002-2009). Playback was done manually, but an average play rate of 1 min per sec was used and allowed for adequate measurements of total distance traveled. After incubation was complete, an air sample (0.5-1.0 ml) from each respirometry chamber was injected into the respirometry system (described below) which measured the concentration of O_2 and CO_2.

Following testing, each animal was re-weighed to the nearest 0.00001 g. It is important to note that for each group that was tested we also included at least one control respirometry chamber (syringe). The control respirometry chamber contained no animals, but underwent the same procedures as the syringes containing animals. After injecting air samples from the control syringes, we were able to adjust the experimental syringes for residual air which leaked in during the experiment. Oxygen showed no change in the control syringes (no O_2 leakage) and CO_2 leakage into the control syringes typically accounted for less than 2% of the total measured CO_2 in the experimental syringes.

The respirometry system used to measure O_2 and CO_2 was plumbed as follows: prior to entering the injection port (for injection of air samples from respirometry chambers), ambient air was forced through a FT-IR Whatman purge-gas generator (Whatman Inc., Haverhill, MA, USA) which scrubbed the air of both CO_2 and H_2O. Next, the CO_2- and H_2O-free air was forced into a 100 l mixing tank and then into an open manifold, where it equalized to atmospheric pressure. This dry, CO_2-free air was pulled through the respirometry system past an injection port, where air samples were injected from each respirometry chamber. This air was drawn through a Li-Cor 6251 CO_2 analyzer (LI-COR Inc., Lincoln, NE, USA), a Drierite®-Ascarite®-Drierite® (W.A. Hammond Drierite Company LTD., Xenia, OH, USA; Thmoas Scientific, Swedesboro, NJ, USA) tube to remove all CO_2 before O_2 analysis, a Sable Systems Oxzilla II O_2 analyzer (Sable Systems, Henderson, NV, USA), and a Sable Systems mass flow system MFS2 (Sable Systems, Henderson, NV, USA), which pulled air through the system at 100 ml min^{-1} at STP. This system was properly calibrated (zeroed and spanned) regularly. To span we used 100 ppm Certified CO_2 (Airgas South, Theodore, AL, USA) and to zero we used 0 ppm CO_2 generated by passing air through the FT-IR Whatman purge-gas generator and a Drierite®-Ascarite®-Drierite® tube.

Data were recorded and analyzed using Datacan V acquisition and analysis software (Sable Systems, Henderson, NV, USA). The exact incubation period (from the removal of the respirometry chamber from the manifold to when the air sample was injected) was recorded for each animal. Additional information on this type of closed system respirometry can be found in Lighton (1991) and Vogt and Appel (1999).

Statistical Analysis

A t-test (PROC TTEST, SAS-Institute, 1985) was used to compare active versus inactive individuals and determine what level of activity was acceptable. Analysis of covariance (PROC GLM, SAS-Institute, 1985) was used to assess the effects of age (main effect; adults and immatures) and temperature (covariate) on respiratory rates of both silverfish and firebrats. Analysis of covariance was also used to assess the effects of species group (main effect; Thysanura, ticks, other arthropods) and mass (covariate) on metabolism. Multiple regression (PROC GLM, SAS-Institute, 1985) was used to model how variables including temperature and mass affect metabolic rate ($\dot{V}_{O_2}$) and RQ. Analysis of variance (PROC GLM, SAS-Institute, 1985) was used to compare mean RQ and $\dot{V}_{O_2}$ between temperatures and groups. An LSD test was used to determine individual differences among the means. Significance for all tests was determined at $p < 0.05$ (SAS-Institute, 1985). $\dot{V}_{O_2}$ is reported as both ml h^{-1} and ml g^{-1} h^{-1} (mass specific), as specified in the text. Q_{10}s are also reported and discussed. All means are reported with standard errors (±SE).

Results

Activity

Active individuals were identified and removed from the study if they moved >80 mm h^{-1} within respirometry chambers. Mass specific $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}) was compared using a t-test between animals which were defined as showing no movement (0-10 mm h^{-1}) and those which were defined as showing limited movement (11-80 mm h^{-1}). The no movement group ranged from 0-10 mm h^{-1} because very few individuals did not move (0 mm h^{-1}) for the duration of the experiment. However, this group represents individuals which did not even move the length of the syringe per hour and can therefore be considered as not moving. The t-test revealed no significant differences between groups (no movement, limited movement) for firebrats ($p = 0.2522$) and silverfish ($p = 0.1739$) and therefore, all animals moving <80 mm h^{-1} were included in the study. Overall, we discarded 121 firebrat samples (30% of all tested) and 24 silverfish samples (7% of all tested) due to activity (movement) which was determined to significantly enhance $\dot{V}_{O_2}$ measurements.

Division of Adults and Immatures

The effects of age and temperature on mass specific $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}) were assessed for both firebrats and silverfish. Two age groups were established based on our data and biological information of both species: adults (> 0.00700 g) and immatures (< 0.00700 g) (Table 1). Analysis of covariance (age as main effect, temperature as covariate) revealed age, temperature, and the interaction variable (age*temperature) to have a significant effect on mass specific metabolic rate for both firebrats ($F_{3,282} = 1535.0$, $p < 0.0001$) and silverfish ($F_{3,334} = 965.4$, $p < 0.0001$). Further analysis revealed the interaction between age and temperature to be significant

for both firebrats ($F_{1,282} = 53.96$, $p < 0.0001$) and silverfish ($F_{1,334} = 46.12$, $p < 0.0001$), indicating that adults and immatures of both species have mass specific metabolic rates which scale differently with temperature. This is illustrated in Fig. 1, as adult $\dot{V}_{O_2}$ is less affected by temperature than immature $\dot{V}_{O_2}$. Consequently, both adults and immatures were modeled independently.

The largest adult males and females (> 0.02500 g) were also compared for both species. Analysis of covariance (sex as main effect, temperature as covariate) revealed no significant interaction between sex and temperature for both firebrats ($F_{1,65} = 3.63$, $p = 0.0611$) and silverfish ($F_{1,90} = 3.09$, $p = 0.0823$), indicating that male and female $\dot{V}_{O_2}$ scaled similarly with temperature. Therefore, all adults were combined and modeled together.

Adult Metabolic Rates

Multiple regression analysis yielded the following equation relating adult firebrat $\dot{V}_{O_2}$ (ml h^{-1}) to temperature (°C) and mass (g):

$$\text{Log}_{10}\dot{V}_{O_2} = -1.964(\pm 0.064) + 0.037(\pm 0.001)\ \text{Temperature} - 0.793(\pm 0.036)\ \text{Log}_{10}\text{Mass}$$

($F_{2,147} = 1556.1$, $p < 0.0001$, $r^2 = 0.9549$). Coefficients for temperature and Log_{10}Mass were both highly significant ($p < 0.0001$). In comparison, silverfish $\dot{V}_{O_2}$ (ml h^{-1}) showed the following relationship to temperature (C) and mass (g):

$$\text{Log}_{10}\dot{V}_{O_2} = -1.651(\pm 0.057) + 0.032(\pm 0.001)\ \text{Temperature}$$

$$- 0.899(\pm 0.032)\ \mathrm{Log}_{10}\mathrm{Mass}$$

($F_{2,193} = 1308.4$, $p < 0.0001$, $r^2 = 0.9313$). Coefficients for temperature and Log_{10}Mass were highly significant ($p < 0.0001$ for both). The interaction term (Temperature*Log_{10}Mass) was not included in either model because it only contributed a 0.2% (firebrats) to 0.3% (silverfish) increase in r^2. We also wanted to understand the effects of either temperature or mass alone. Therefore, we adjusted all adult $\dot{V}_{O_2}$ to 25°C. After adjusting for temperature, the following equation was produced relating adult firebrat adjusted-$\dot{V}_{O_2}$ to mass (g):

$$\mathrm{Log}_{10}\dot{V}_{O_2\,@\,25^{\circ}C} = -1.052(\pm 0.062) + 0.793(\pm 0.036)\ \mathrm{Log}_{10}\mathrm{Mass}$$

($F_{1,148} = 479.6$, $p < 0.0001$, $r^2 = 0.7642$) (Fig. 2). Adult silverfish adjusted-$\dot{V}_{O_2}$ had the following relationship with mass (g):

$$\mathrm{Log}_{10}\dot{V}_{O_2\,@\,25^{\circ}C} = -0.864\ (\pm 0.055) + 0.899\ (\pm 0.032)\ \mathrm{Log}_{10}\mathrm{Mass}$$

($F_{1,194} = 772.9$, $p < 0.0001$, $r^2 = 0.7994$) (Fig. 2). Analysis of covariance (species as main effect, mass as covariate) reveled a significant interaction between species and mass, indicating that firebrat and silverfish $\dot{V}_{O_2}$ scale differently with mass ($F_{1,342} = 4.57$, $p = 0.0332$). Mass can also be related to metabolic rate in μW, where 1 W is equal to 1 J s^{-1}, and 1 ml of O_2 is equal to 20.1 J (Lighton and Wehner, 1993). Because some studies have reported metabolic rates only in terms of μW, we have also reported the power equation relating adult firebrat metabolic rate (μW) to mass (g):

$$MR\ [\mu W] = 433.5(\pm 84.8)\ Mass^{0.753(\pm 0.053)}$$

($F_{1,148}$ =290.3, $p < 0.0001$, $r^2 = 0.6623$). In adult silverfish, the following equation related metabolic rate (μW) to mass (g):

$$MR\ [\mu W] = 808.6(\pm 125.5)\ Mass^{0.909(\pm 0.043)}$$

($F_{1,194} = 648.9$, $p < 0.0001$, $r^2 = 0.7699$).

In addition to looking at the effects of mass only, we also calculated the effects of temperature on mass specific $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}) for adult firebrats:

$$Log_{10}\dot{V}_{O_2} = -1.614(\pm 0.019) + 0.037(\pm 0.001)\ Temperature$$

($F_{1,148} = 2244.9$, $p < 0.0001$, $r^2 = 0.9381$) (Fig. 3). Adult silverfish $\dot{V}_{O_2}$ showed the following relationship with temperature:

$$Log_{10}\dot{V}_{O_2} = -1.482(\pm 0.020) + 0.032(\pm 0.001)\ Temperature$$

($F_{1,194} = 1834.2$, $p < 0.0001$, $r^2 = 0.9043$) (Fig. 3). Analysis of covariance (species as main effect, temperature as covariate) revealed a significant interaction between species and temperature, indicating that mass specific $\dot{V}_{O_2}$ scales differently with between both species ($F_{1,342} = 21.97$, $p < 0.0001$).

Adult Q_{10} and Respiratory Quotient

Mean Q_{10} was calculated by multiplying the slope of the equation relating mass specific $\dot{V}_{O_2}$ by 10 and then taking the antilogarithm of the product (Lighton, 1989). This resulted in mean Q_{10} values of 2.32(±0.04) for adult firebrats and 2.07(±0.04) for adult silverfish. Because the linear relationship between mass specific $\dot{V}_{O_2}$ and temperature fits the data well for both species ($r^2 > 0.90$, Fig. 3), average Q_{10} can be assumed to be a constant across the measured temperature range.

Mean adult firebrat respiratory quotient (RQ) was plotted against temperature for both species (Fig. 4). Analysis of variance found RQ to be significantly different across the measured temperature range for both firebrats ($F_{4,145} = 14.3$, $p < 0.0001$) and silverfish ($F_{4,191} = 9.6$, $p < 0.0001$). The results of the LSD test were identical for firebrats and silverfish. The LSD test found RQ at 10°C to be significantly lower than all other temperatures. It also found RQ at 30°C to be significantly higher than all other temperatures. RQ at 20, 25, and 40°C were found to be in the middle and not significantly different from each other (Fig. 4).

Immature Metabolic Rates

Multiple regression analysis yielded the following equation relating immature firebrat $\dot{V}_{O_2}$ (ml h^{-1}) to temperature (°C) and mass (g):

$$\text{Log}_{10}\dot{V}_{O_2} = -1.781(\pm 0.061) + 0.046(\pm 0.001)\ \text{Temperature} - 01.000(\pm 0.020)\ \text{Log}_{10}\text{Mass}$$

($F_{2,133} = 2294.4$, $p < 0.0001$, $r^2 = 0.9718$). Coefficients for temperature and Log_{10}Mass were highly significant ($p < 0.0001$). In comparison, immature silverfish $\dot{V}_{O_2}$ had the following relationship with temperature (°C) and mass (g):

$$Log_{10}\dot{V}_{O_2} = -1.935(\pm 0.074) + 0.041(\pm 0.001)\ \text{Temperature} - 0.877(\pm 0.025)\ Log_{10}\text{Mass}$$

($F_{2,139} = 1179.6$, $p < 0.0001$, $r^2 = 0.9444$). Coefficients for temperature and Log_{10}Mass were highly significant ($p < 0.0001$). Similar to the adults, the interaction term (Temperature*Log_{10}Mass) was not included because it contributed nothing to the models, generating no change in r^2 for firebrats and silverfish. In addition to understanding the effects of both predictors together, we also wanted to understand the effects of either temperature or mass alone. Therefore, we adjusted all immature $\dot{V}_{O_2}$ to 25°C. After adjusting for temperature, the following equation related immature firebrat adjusted-$\dot{V}_{O_2}$ to mass (g):

$$Log_{10}\dot{V}_{O_2\,@\,25^\circ C} = -0.640(\pm 0.056) + 1.000(\pm 0.020)\ Log_{10}\text{Mass}$$

($F_{1,134} = 2539.3$, $p < 0.0001$, $r^2 = 0.9499$) (Fig. 5). Immature silverfish adjusted $\dot{V}_{O_2}$ was related to mass (g) by the following equation:

$$Log_{10}\dot{V}_{O_2\,@\,25^\circ C} = -0.914(\pm 0.068) + 0.877(\pm 0.025)\ Log_{10}\text{Mass}$$

($F_{1,140} = 1277.3$, $p < 0.0001$, $r^2 = 0.9012$) (Fig. 5). Analysis of covariance (species as main effect, mass as covariate) reveled a significant interaction between species and mass, indicating that firebrat and silverfish $\dot{V}_{O_2}$ scale differently with mass ($F_{1,274} = 3.89$, $p < 0.0001$). Some studies have reported metabolic rates only in terms of μW, therefore, we have also reported the power function relating immature firebrats metabolic rate (μW) to mass (g):

$$MR\ [\mu W] = 1100.0(\pm 291.1)\ Mass^{0.969(\pm 0.0490)}$$

($F_{1,134} = 959.9$, $p < 0.0001$, $r^2 = 0.8775$). For immature silverfish, the following equation related metabolic rate (μW) to mass (g):

$$MR\ [\mu W] = 840.4(\pm 244.3)\ Mass^{0.909(\pm 0.059)}$$

($F_{1,140} = 610.0$, $p < 0.0001$, $r^2 = 0.8133$).

In addition, we calculated the effects of temperature on mass specific $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}) for immature firebrats:

$$Log_{10}\dot{V}_{O_2} = -1.780(\pm 0.026) + 0.046(\pm 0.001)\ Temperature$$

($F_{1,134} = 2181.9$, $p < 0.0001$, $r^2 = 0.9421$) (Fig. 6). Temperature had the following effect on immature silverfish mass specific $\dot{V}_{O_2}$:

$$Log_{10}\dot{V}_{O_2} = -1.604(\pm 0.035) + 0.041(\pm 0.001)\ Temperature$$

($F_{1,140} = 1012.8$, $p < 0.0001$, $r^2 = 0.8777$) (Fig. 6). Analysis of covariance (species as main effect, temperature as covariate) revealed a significant interaction between species and temperature, indicating that firebrats and silverfish mass specific $\dot{V}_{O_2}$ scale differently with temperature ($F_{1,274} = 8.06$, $p < 0.0049$).

Immature Q_{10} and Respiratory Quotient

The same method used to calculate adult Q_{10} was also used to calculate immature Q_{10}. Immature firebrat and silverfish Q_{10}s were 2.86(±0.07) and 2.57(±0.08), respectively. Because the linear relationship between mass specific $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}) fits the data well for both species ($r^2 > 0.87$, Fig. 6), average Q_{10}s can be assumed to be constant across the measured temperature range.

Mean immature firebrat respiratory quotient (RQ) was plotted against temperature for both species (Fig. 7). Analysis of variance found RQ to be significantly different across the measured temperature range for both firebrats ($F_{4,131} = 10.4$, $p < 0.0001$) and silverfish ($F_{4,137} = 14.4$, $p < 0.0001$). The results of the LSD test found RQ at 10°C to be significantly lower than all other temperatures for both species. In addition, the other temperatures showed complex relationships best understood graphically (Fig. 7).

Comparison between Ages and Species

In addition to comparing mass specific $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}) between adults and immatures for both species across a range of temperatures, we also compared all groups (species and age) at specific temperatures. We adjusted each mass specific $\dot{V}_{O_2}$ measurement to 25°C (average

preferred temperature of the silverfish, Sweetman, 1939), and 37°C (average preferred temperature of firebrats, Adams, 1933) and used analysis of variance at each temperature to detect differences. There were significant differences in mass specific $\dot{V}_{O_2}$ between the species-age groups at 25°C ($F_{3,620} = 49.6$, $p < 0.0001$) and 37°C ($F_{3,620} = 174.8$, $p < 0.0001$), and the LSD test was used to compare means. Briefly, at 25°C adult silverfish and adult firebrats did not differ from one another, but the immatures of both species had significantly greater $\dot{V}_{O_2}$ than adults and were significantly different from each other (Table 1). At 37°C, immature silverfish and firebrats did not have significantly different $\dot{V}_{O_2}$; however, immatures had significantly greater $\dot{V}_{O_2}$ than both adult silverfish and firebrats which were significantly different from each other (Table 1).

Comparisons between Thysanura and Other Arthropods

To compare our results with those presented for other insects and arthropods, we adjusted all metabolic rates (μW) to 25°C using the above equations which relate temperature to $\dot{V}_{O_2}$. Despite the differences in $\dot{V}_{O_2}$ we detected earlier, we also pooled all of our data for adults and immatures of both species into a combined set we called Thysanura. In this way we could determine if Thysanura as a group showed any differences with values reported for ticks and other arthropods (ants, beetles, spiders) by Lighton and Fielden (1995). Mass (g) related to the metabolic rate (μW) of Thysanura by the following equation:

$$MR\ [\mu W] = 699.4(\pm 49.8)\ Mass^{0.875(\pm 0.019)}$$

($F_{1,622} = 6094.0$, $p < 0.0001$, $r^2 = 0.9074$) (Fig. 8). After log-transforming the above equation, we used analysis of covariance to compare the slope (mass scaling exponent) with ticks and other arthropods as reported by Lighton and Fielden (1995). When compared with ticks, analysis of covariance revealed a significant interaction between the group (Thysanura vs. ticks) and mass, indicating that thysanurans and ticks scale differently with mass, with thysanurans scaling approximately 1.59 times more with mass ($F_{1,628} = 14.64$, $p = 0.0001$) (Fig. 8). Similarly, when compared with other arthropods, analysis of covariance revealed a significant interaction between the group (Thysanura vs. other arthropods) and mass, indicating that Thysanurans and other arthropods scale differently with mass, with thysanurans scaling approximately 1.12 times more with mass ($F_{1,703} = 19.74$, $p < 0.0001$) (Fig. 8). Due to the differences in scaling factors, comparisons among the groups are difficult to make. However, visual examination of the plot of metabolic rate (μW) versus mass (g) reveals no overlap in the 95% confidence intervals of Thysanura with ticks or other arthropods for the range of masses measured, with ticks having the lowest metabolic rates, followed by thysanurans, and then other arthropods (Fig. 8).

Discussion

One complication that is typically encountered when measuring the standard metabolic rate of any ectothermic animal is how to prevent and/or control for activity/movement. Movement can cause significant changes in rates of O_2 consumption, making comparisons among individuals and species nearly impossible (Bartholomew et al., 1985; Lighton and Duncan, 1995; Lighton and Feener, 1989). In this study, we were able to negate the effects of movement, by videotaping all individuals during incubation and only including those whose movement did not significantly affect $\dot{V}_{O_2}$. Unfortunately, few authors have adequately

addressed and accounted for movement in their studies, making comparisons between data sets very difficult. Edwards and Nutting (1950) reported a mass specific $\dot{V}_{O_2}$ for firebrats approximately twice as great as the values we report here across a similar range of temperatures. Differences between their findings and ours are likely due to a large amount of movement during their experiments, as indicated by the wide range of $\dot{V}_{O_2}$ values they reported (see Edwards and Nutting, 1950). In addition, we also compared individuals who were discarded from our experiments due to movement with the range of $\dot{V}_{O_2}$ values reported by Edwards and Nutting (1950). This comparison revealed all individuals we discarded to fall within the range of $\dot{V}_{O_2}$ values reported by Edwards and Nutting (1950), indicating that there was probably considerable movement during their experiments.

Another complication we encountered was how to measure and compare different life stages of an ametabolous insect. For insects with a determinant number of life stages or different castes, it is much easier to know where to divide and form groups for comparative purposes. Despite this problem, our data suggested two groups (adults and immatures) which were modeled independently. The biology of the firebrat also supported this division. Adams (1933) noted that eggs were first reported in a colony with an average mass of 0.00920g. Because sexual maturation can lead to changes in metabolic rates in holometabolous insects (Vogt and Appel, 1999), we divided our animals into adults and immatures at a similar mass of 0.00700g (Table 1). We chose this mass to be sure that we not only captured sexually mature individuals, but we would also capture those individuals beginning sexual maturation. The two groups were significantly different, which led us to model both groups (adults and immatures) independently for each species.

The relationship between mass-specific $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}) and temperature was strong ($r^2 >$ 0.87) for immature and adult silverfish and firebrats. In addition, each age-species combination was affected in a significantly different way by temperature. Specifically, temperature had a 23-24% greater effect on immature mass-specific $\dot{V}_{O_2}$ when compared with adults for both species (Fig. 1). The difference observed is supported by Lindsay's (1940) findings that adult silverfish could survive several months at 2°C while immatures could only survive 2 days at the same temperature. This suggests that immatures are more sensitive to temperature, possibly because of the difference in energy expenditure between immatures (growth and development) and adults (maintenance and reproduction). Comparisons between adult and immature firebrats and silverfish were simplified by adjusting all mass specific $\dot{V}_{O_2}$ values to 25°C (optimum temperature of silverfish) and 37°C (optimum temperature of firebrat) and using analysis of variance to compare (Table 1). At both temperatures immatures had significantly higher mass specific $\dot{V}_{O_2}$. We believe this could be due to different selective factors on different ages. Adults were likely selected for survival and longevity (lower metabolism) whereas immatures were likely selected to rapidly grow and develop (higher metabolism).

In addition, firebrat mass specific $\dot{V}_{O_2}$ showed an 11-12% stronger relationship with temperature than silverfish mass specific $\dot{V}_{O_2}$. For adults, this is illustrated in Fig. 3 and the respective Q_{10} values of 2.32 for firebrats and 2.07 for silverfish. For immatures, this is illustrated in Fig. 6 and the respective Q_{10} values of 2.86 for firebrats and 2.57 for silverfish. One possible explanation for the higher Q_{10} values seen in firebrats in comparison to silverfish could be the ability of firebrats to actively absorb water from the air (Beament et al., 1964; Noble-Nesbit, 1969, 1970, 1975). Active water absorption is likely energy expensive, especially at higher temperatures where water loss would be the greatest and the partial pressure gradient

between outside air and air inside the insect rectum the greatest (Hadley, 1994). Although ineffective at the relative humidity of this study (0%), this behavior would likely lead to elevated metabolic rates at higher temperatures, explaining the higher Q_{10} values seen in firebrats.

Metabolic rates of all animals tested were also adjusted to 25°C so the effects of mass could be compared (Fig.2, Fig. 5). Log-mass scaling factors ranged from 0.7932 (firebrat adults) to 0.9999 (firebrat immatures). However, no patterns were detected when adults and immatures of firebrats and silverfish were compared to one another. When $\dot{V}_{O_2}$ values were converted to μW, mass scaling factors for adult silverfish and immature firebrats and silverfish were greater than those reported by Lighton and Fielden (1995) for arthropods ($mass^{0.825}$), while adult firebrats were lower. The different mass scaling coefficients for different species-age groups (ranging from 0.753 to 0.969) offer some insight into the ongoing mass-scaling debate. The inter- and intra-specific variation along with the deviation from a 0.75 mass scaling coefficient (except adult firebrats) provide support for the cell-size model of mass scaling (Chown et al., 2007).

Respiratory quotients of both firebrats and silverfish displayed rather complex relationships with temperature (Fig. 4, Fig.7). In all ages and species, RQ was lowest at 10°C (0.544 - 0.639), where a high percentage of fat was likely being metabolized. At all other temperatures measured, it is likely that metabolism shifts from fat to a combination of fat and carbohydrates. The one exception is in immature silverfish, which show a sharp decline in RQ at 40°C (Fig. 7). This might be explained due to the long incubation period at such a high temperature for this stage. This extended incubation period could have led to the depletion of usable carbohydrate energy, forcing smaller silverfish to rely on their fat reserves for energy production. Our results show that the substrate being metabolized (carbohydrate, protein, fat) for

all thysanurans changes with temperature. However, our study only showed a change in substrate metabolism, not in diet. Therefore, long term exposure to different temperatures should be investigated to determine if diet changes to accommodate the observed shift in substrate metabolism.

In addition to comparisons made between ages and species, we also compared the pooled data for all thysanurans with data reported for ticks and other arthropods (ants, beetles, spiders) by Lighton and Fielden (1995). The relationships that we observed agree with the biology of each of the groups measured and support the idea that metabolic rates are good predictors of survivorship during starvation (Rixon and Stevenson, 1957). Ticks are capable of surviving extended periods of starvation and are known to live for years without feeding and therefore would be expected to have the lowest metabolic rates at any given mass (Needham and Teel, 1991). Other arthropods including spiders, beetles, and ants generally do not have the same capacity to survive extended periods of starvation and would therefore be expected to have the highest metabolic rates of the groups considered at any given mass (Klotz, 2008; Mallis, 2011). Finally, thysanurans would be expected to be in between both of these groups. Although they do not have the ability to survive periods of starvation for as long as ticks, they can survive longer without feeding than a majority of the other arthropods reported (Adams, 1933; Lindsay, 1940; Meek, 2011; Sweetman, 1938, 1939).

In conclusion, firebrat and silverfish $\dot{V}_{O_2}$ were significantly affected by both temperature and mass. The strength of these relationships differed between species and ages. This study represents the first use of modern technology to measure the metabolic rate of this basal order of insects, which includes monitoring and accounting for movement. In addition, this study also provides a guide for the division of insects which proceed through an indeterminate number of

instars. Our data also provide support for finding by Chown et al. (2007) who suggest that the 3/4-power law is not necessarily accurate across intraspecific groups. Finally, the results presented here should aid in the understanding of metabolism in other species with the capacity to survive extended periods of starvation.

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Table 1-Mass data for adult and immature firebrats and silverfish. Also, a comparison of mean mass specific $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}) for adult and immature firebrats and silverfish at 25 and 37°C. Means within columns which differ significantly according to the LSD test are indicated by different letters ($p < 0.05$).

Species	Age	n	Mass (g) Mean (±SE)	Minimum Mass (g)	Maximum Mass (g)	$\dot{V}_{O_2}$ at 25°C Mean (±SE)	$\dot{V}_{O_2}$ at 37°C Mean (±SE)
Firebrat	Adult	150	0.02154 (±0.00066)	0.00762	0.03647	0.204 (±0.004) A	0.562 (±0.010) A
	Immature	136	0.00252 (±0.00017)	0.00028	0.00647	0.236 (±0.005) B	0.831 (±0.016) C
Silverfish	Adult	196	0.02273 (±0.00067)	0.00713	0.04824	0.208 (±0.003) A	0.498 (±0.008) B
	Immature	142	0.00266 (±0.00017)	0.00029	0.00698	0.277 (±0.007) C	0.862 (±0.022) C

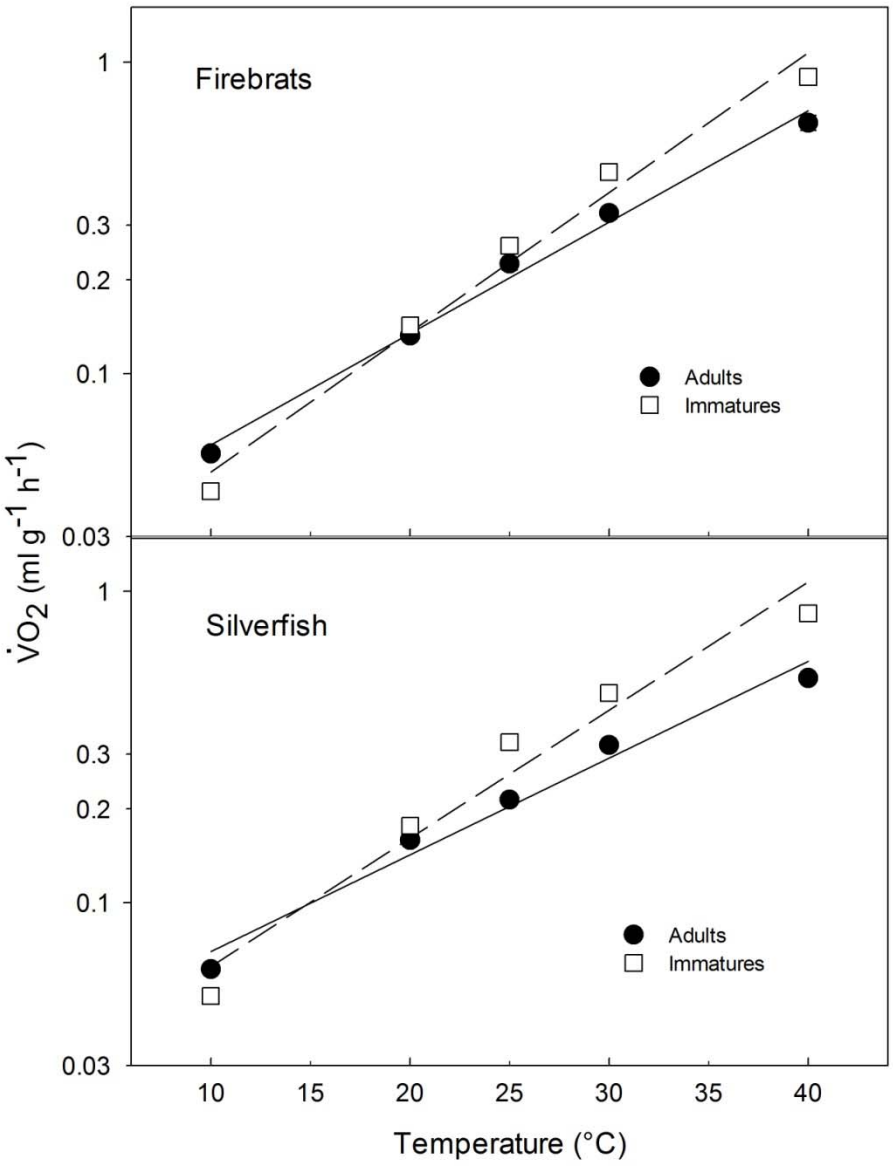

Fig. 1-Mass specific $\dot{V}_{O_2}$ for adult and immature firebrats and silverfish across a range of temperatures. See text for equations.

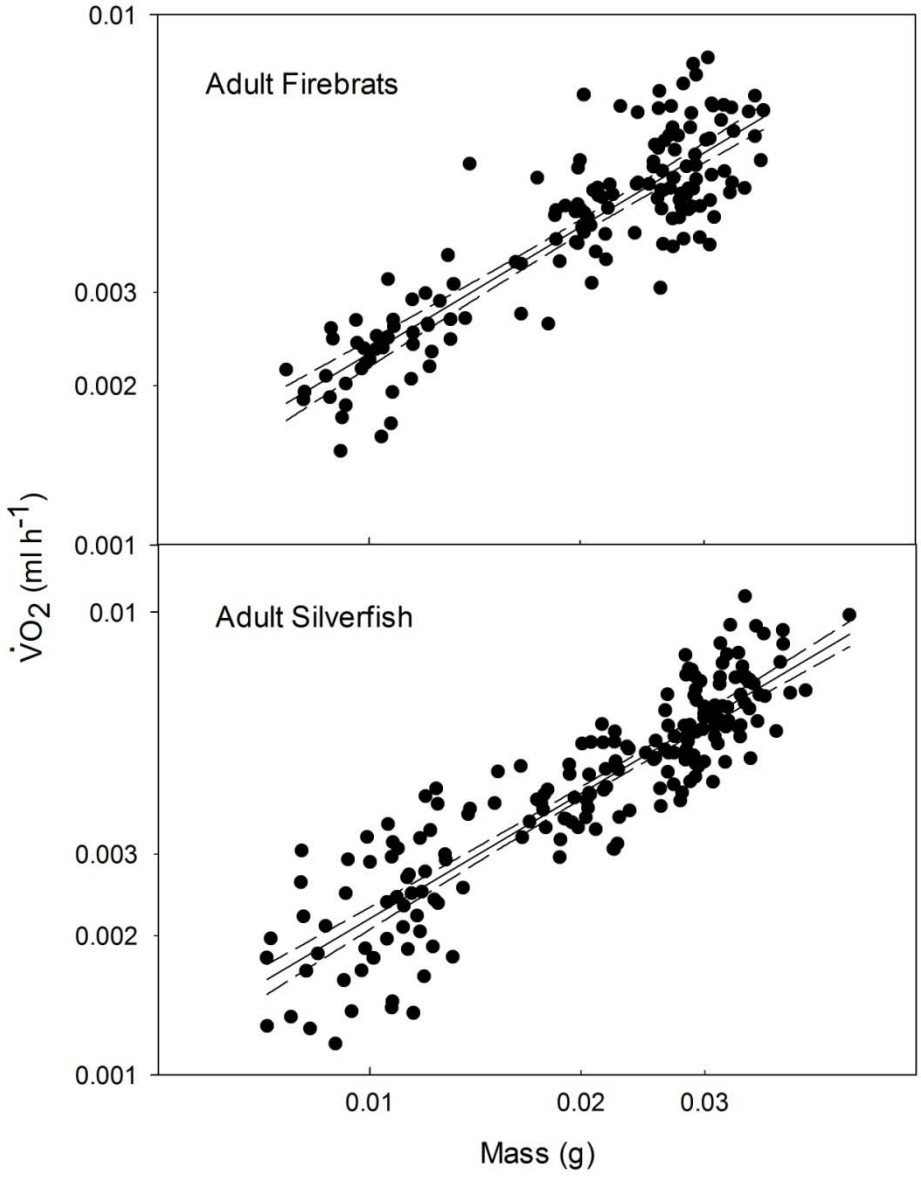

Fig. 2-Temperature adjusted $\dot{V}_{O_2}$ for a range of masses of adult firebrats and silverfish. See text for equations.

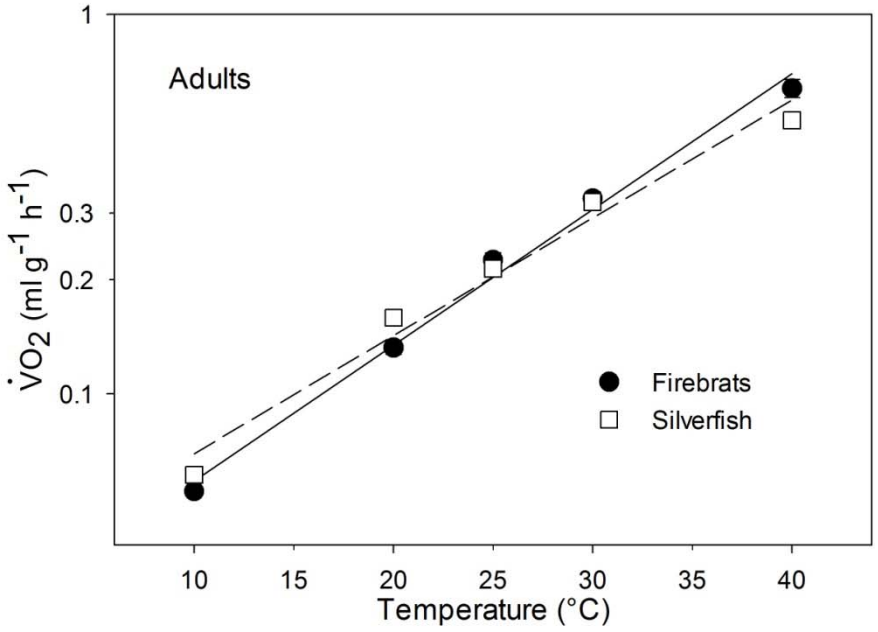

Fig. 3-Mass specific $\dot{V}_{O_2}$ for adult firebrats and adult silverfish across a range of temperatures. See text for equations.

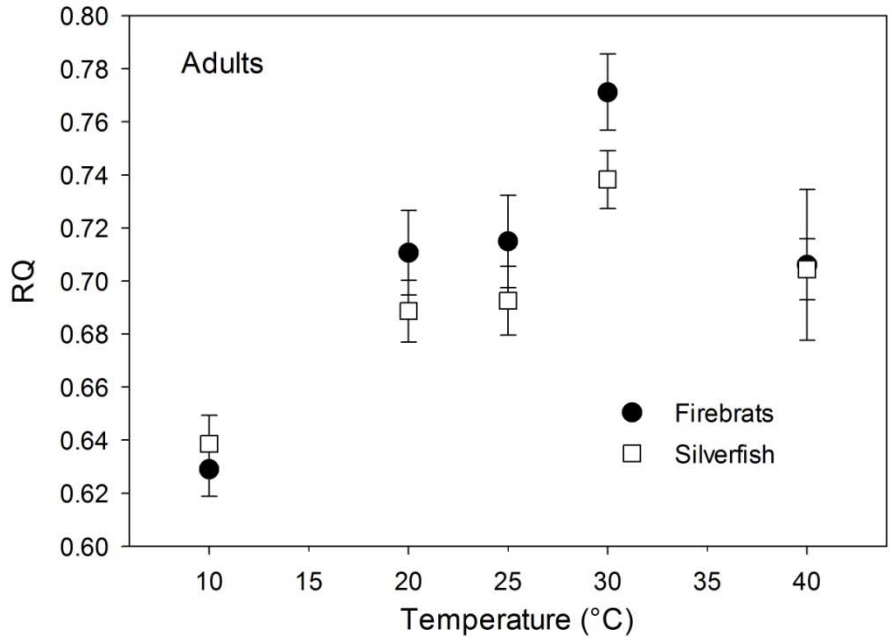

Fig. 4-RQ versus temperature for adult firebrats and adult silverfish.

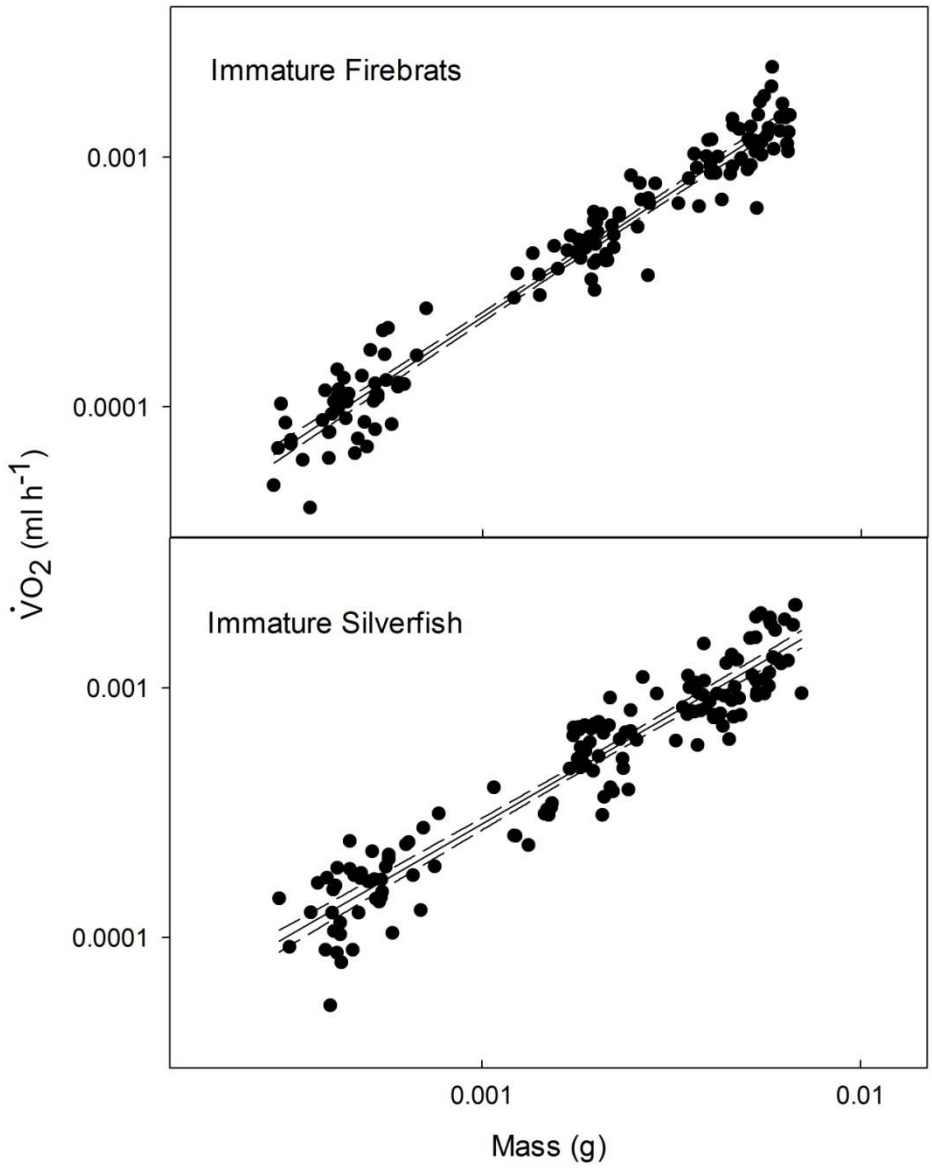

Fig. 5-Temperature adjusted $\dot{V}O2$ for a range of sizes of immature firebrats and silverfish. See text for equations.

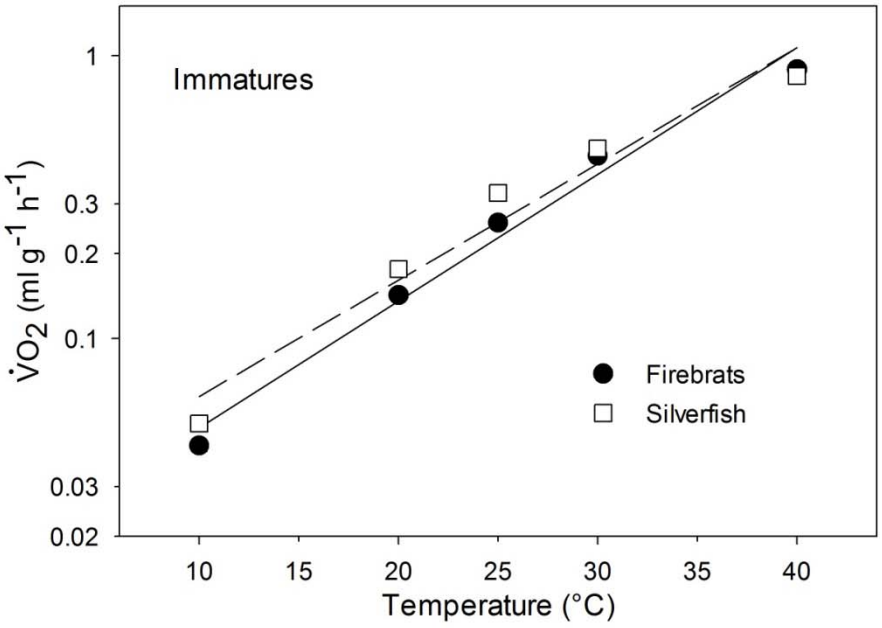

Fig. 6-Mass specific $\dot{V}_{O_2}$ for immature firebrats and immature silverfish across a range of temperatures. See text for equations.

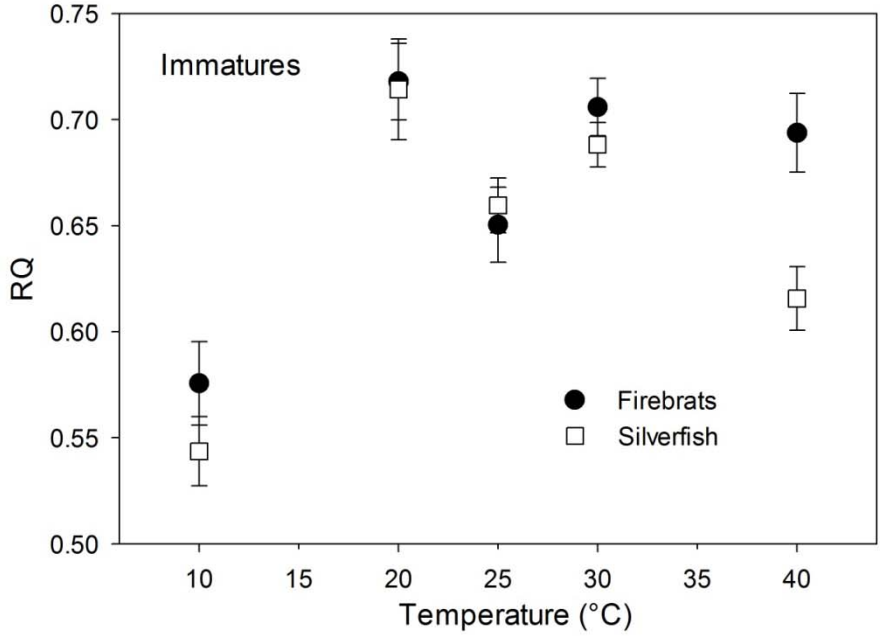

Fig. 7-RQ vs. Temperature for immature firebrats and immature silverfish.

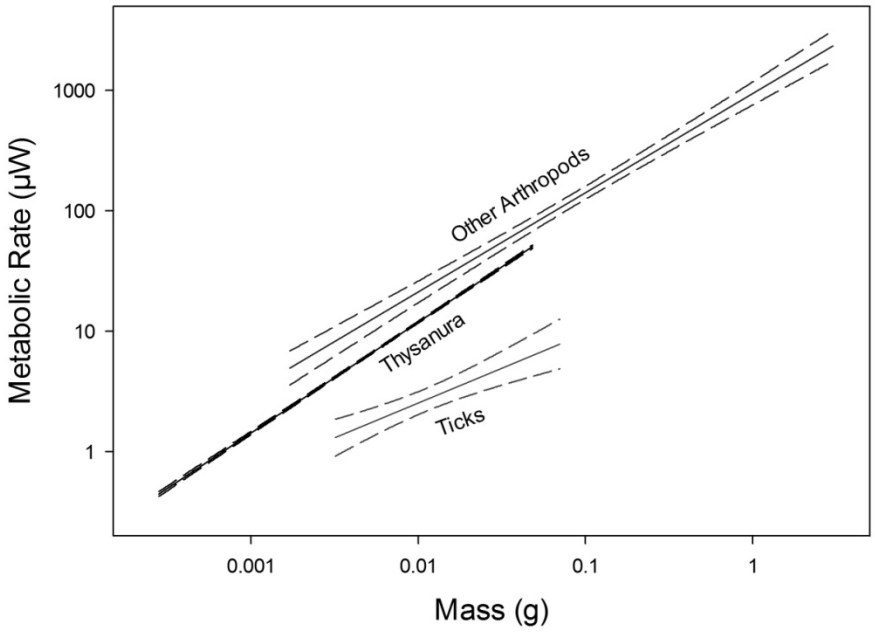

Fig. 8-Metabolic rate (µW) versus mass (g) for Thysanura, ticks and other arthropods (ants, beetles, spiders).

Chapter 3

Effects of starvation and molting on the metabolic rate of the common bed bug, *Cimex lectularius* L.

Abstract

The common bed bug, *Cimex lectularius* L., is a common hematophagous pest in the urban environment. The recent resurgence has been attributed to a number of factors, one of which is their ability to survive extended periods of starvation. However, the relationship between starvation and metabolism in bed bugs has received little attention. To better understand this relationship we measured the metabolism of all life stages for > 900 h after feeding (starvation). Measurements were also made around molting for the immature life stages, which occurs only after a blood meal. Closed system respirometry was used to measure metabolic rates of all life stages (including mated and unmated adults). Starvation and molting had significant effects on the metabolism of the common bed bug. Metabolic rate ($\dot{V}_{O_2}$, ml g^{-1} h^{-1}) declined significantly with the period of starvation for adults. Similarly, $\dot{V}_{O_2}$ declined significantly with the post molting period for immatures (used to standardize all immature life stages). In some cases, the decline in $\dot{V}_{O_2}$ after feeding/molting was as great as 86%. In most life stages, the ratio of $\dot{V}_{CO_2}$ to $\dot{V}_{O_2}$ (respiratory quotient, RQ) declined over time, indicating a gradual shift in metabolic substrate from protein (blood) to fat. Finally, daily percent change in body mass was also modeled. All results are discussed in terms of the life history of the

common bed bug as well as compared with other species capable of surviving extended periods of starvation.

Introduction

Starvation plays a critical role in the survival of all animals. During starvation, most animals are incapable of producing their own energy and therefore must rely on previously acquired and stored energy. The ability of an organism to survive starvation is determined primarily by two factors: the amount of energy available at the onset of starvation and the rate of energy use during starvation (metabolism) (Wang et al., 2006). Of these two factors, only metabolic rate can be modified by organisms entering unplanned starvation. Previous studies have shown that metabolic rates can predict an organisms ability to survive during starvation, assuming energy-use is the only influencing factor (DeVries and Appel, 2013; Rixon and Stevenson, 1957). The rate of energy usage is entirely dependent on the metabolic rate of the organism, and species having lower metabolic rates are capable of surviving longer during starvation (Rixon and Stevenson, 1957). Comparisons among ectothermic species are generally made using the standard metabolic rate (SMR), a measure of the metabolic rate of an ectothermic species which is in a post-absorptive state (no longer digesting food), at rest, and at a define temperature (DeVries and Appel, 2013; Lighton and Fielden, 1995; Moyes and Schulte, 2008; Vogt and Appel, 1999). Unfortunately, ectothermic species which commonly experience prolonged periods of starvation are extremely difficult to define as being post-absorptive. Furthermore, these species often experience metabolic depression during periods of starvation, making standardized metabolic measurements even more difficult because these depressions are

not always consistent among species (Bradley et al., 2003; Schimpf et al., 2012; Zwicky and Wigglesworth, 1956).

In addition to starvation, molting may also have large effects on the metabolism of ectothermic species. Molting is required for growth and development and is present across a number of animal taxa. Molting is a very costly energetic process due to its associated events (hormone production, apolysis, digestion of old cuticle, and production of new cuticle) (Chapman, 1998). In addition, this process must be relatively quick to avoid both predation and desiccation. Due to the energy cost associated with molting, it is often closely associated with feeding. Therefore, it is important to consider molting and starvation together when measuring metabolism in any non-mature ectothermic animals.

At present, few ectothermic species have had their metabolism fully characterized while starving or while molting. Zwicky and Wigglesworth (1956) measured the metabolic rate of *Rhodnius prolixus* Stål during periods of feeding, molting, and starvation. Their results indicated large increases in metabolism after feeding and during molting, followed by a rapid decline after molting. Bradley et al. (2003) also measured the metabolism of *R. prolixus* after feeding, finding similar results to those of Zwicky and Wigglesworth (1956). However, Bradley et al.'s (2003) use of CO_2 to measure metabolic rates complicates their results because CO_2 production is driven by metabolic substrate, which likely changes during starvation (Sinclair et al., 2011). Defur (1990) found a peak in metabolism during molting for the blue crab, *Callinectes sapidus* Rathbun. Finally, Secor and Diamond (1997) measured and characterized the change in metabolism of the Burmese python, *Python molurus* L., after feeding on meals of various sizes. The effects of starvation and molting on the metabolism of the common bed bug, *Cimex lectularius* L., have not been measured.

The common bed bug is an increasing problem to humans in the 21st century. Bed bugs are ectoparasites of endothermic species, with humans as their primary host (Usinger, 1966). Their presence in homes leads to bites and skin irritations, and can result in lasting psychological and financial problems for their victims (Delaunay et al., 2011; Goddard and deShazo, 2009; Reinhardt and Siva-Jothy, 2007). In addition to insecticide resistance and a cryptic lifestyle, bed bug success can be largely attributed to their ability to survive for extended periods without feeding (Romero, 2011; Romero et al., 2007; Yoon et al., 2008; Zhu et al., 2010). The length of survival depends on life stage, temperature, and relative humidity and can range from a month to over a year (Usinger, 1966). Bed bugs cycle through successive feeding and molting events, with a blood meal required for molting to occur (Usinger, 1966). Reproduction is also closely associated with feeding; a blood meal is required for continued egg and sperm production (Usinger, 1966). While experiencing long term starvation, bed bugs display changes in behavior which include a reluctance to aggregate over time (Olson et al., 2009) and decreased activity during the scotophase (Romero et al., 2010). These behaviors likely prolong bed bug survival by reducing non-essential energy costs (aggregation/random host searching) and focusing more energy into stimulated host searches (Olson et al., 2009; Reis and Miler, 2011; Romero et al., 2010). However, it is still unclear what occurs metabolically to accompany these behavioral changes to allow bed bugs to survive extended periods of starvation. Bed bug metabolism has received very little attention; Mellanby (1932) and Rao (1973) reported the only studies on bed bug metabolism. However, neither of these studies addressed changes in metabolic rate during starvation and molting.

Due to the lack of information on ectothermic animal metabolic response to starvation and molting, the increased presence of bed bugs as a major pest in the urban environment, and

the extraordinary ability of bed bugs to survive starvation, we determined that a complete analysis of the effects of starvation and molting on bed bug metabolism was necessary. In this study we measured the metabolic rate of bed bugs for > 900 h of starvation. This period included molting and hatching in nymphal bed bugs. Changes in O_2 consumption, CO_2 production, and respiratory quotient (RQ) were calculated. We corrected for bed bug movement so only individuals that exhibited little to no movement were included in the study; movement can significantly impact respiratory measurements (Bartholomew et al., 1985; Lighton and Duncan, 1995; Lighton and Feener, 1989). In addition, the effects of starvation on body loss were determined. The information provided by this study is discussed in context with the life history of the bed bug.

Materials and Methods

Experimental Animals

Insecticide susceptible bed bugs obtained from the insect control research center (Baltimore, MD, USA) were reared by at the University of Minnesota, Twin Cities, MN, USA, as described by Olson et al. (2009). Briefly, bugs were reared at 23±2^{o}C, 55±5% RH, and 14:10 (L:D) in 0.5 L plastic jars which contained filter paper harborage and a mesh top to allow ventilation. Bugs were fed human blood (1:1 combination of red blood cells and plasma) purchased from the American Red Cross (ST. Paul, MN, USA) that had expired for human use, using an artificial Parafilm® membrane (American National Can, Chicago, IL, USA).

Bed bugs were shipped to Auburn, AL, USA, via overnight delivery immediately after feeding, when needed. Once the bed bugs arrived, they were placed into respirometry chambers to await experiments. While not being tested, bed bugs were kept in identical conditions as

above. Prior to testing, bed bugs were allowed at least 24h to acclimate to the respirometry chambers (see below).

Respirometry Measurements

Different instars were received and tested in a randomized order by instar. Once received, bed bugs were immediately placed into respirometry chambers consturcted of 3 ml plastic syringes that contained one small (0.775x0.375 mm^2) piece of cardstock paper to provide harborage and help limit bed bug movement. The syringes had 6 holes (1.4 mm diameter) drilled past the last gradation. After the 24 h acclimation period, bed bugs were weighed to the nearest 10^{-5} g using a Mettler-Toledo AX205 digital balance (Mettler-Toledo GmbH, Greifensee, Switzerland). The syringes were placed on a manifold where dry, CO_2 free air was forced through at a rate of 230 ml/min for a minimum of 6 min. This process purged all CO_2 and H_2O from the respirometry chambers. After purging was complete, a 26 gauge needle was attached to the respirometry chamber and the volume was adjusted to a known level (depending on the life stage and starvation time) by pushing the plunger past the drilled hole to the desired volume. The respirometry chamber was then sealed by attaching a rubber stopper (size 000) to the needle. Each syringe was then placed into an incubator at 25^{o}C. Incubation times varied widely (2-30 h), depending on the size (mass) of the bed bug and the post-feeding period. The incubator was illuminated with a red light (20W) and animals were monitored for movement by a SONY® DCR-SX85 video camera (Sony Corporation, Minato, Tokyo, Japan). Video recordings (MPEGs) were reviewed manually a rate of 1 min of video per second of viewing time using Windows® Media Player Classic version 6.4.9.1. After incubation was complete, an air sample from each respirometry chamber was injected into the respirometry system (described below)

which permitted measurement of O_2 consumption and CO_2 production. Following testing, each animal was weighed again to the nearest 10^{-5} g. To adjust for any possible leakages, especially during the longer incubation periods, we used control syringes with every group tested. These syringes were treated identically to the experimental syringes except they contained no animals. After injections were complete, any residual leakage of O_2 or CO_2 into or out of the control syringes was used to adjust the values in the experimental syringes. On average, CO_2 leakage was less than 5% of the total measured CO_2 in experimental syringes and oxygen showed no changes.

The respirometry system used to measure O_2 and CO_2 was similar to that described by DeVries and Appel (2013) and operates as follows: prior to entering the respirometry chamber, air was forced into a Whatman purge-gas generator (Whatman Inc., Haverhill, MA, USA) where the air is scrubbed of both CO_2 and H_2O. Next, the air free of CO_2 and H_2O was forced into a 340+ L mixing tank and then into an open manifold, where it equalized to atmospheric pressure. The air was then pulled through a Drierite®-Ascarite®-Drierite® column (W.A. Hammond Drierite Company LTD., Xenia, OH, USA; Thomas Scientific, Swedesboro, NJ, USA) to ensure that all water and CO_2 had been removed. Next, the air was pulled past an injection port, where air samples were injected from each respirometry chamber after incubation was complete. This air then flowed through a Li-6251 CO_2 analyzer (LI-COR Inc., Lincoln, NE, USA) and another Drierite®-Ascarite®-Drierite® column, to remove all CO_2 and water before measuring O_2. Next, the air traveled to a Sable Systems Oxzilla II O_2 analyzer (Sable Systems, Henderson, NV, USA) and a Sable Systems mass flow system MFS2 (Sable Systems, Henderson, NV, USA), which pulled air through the system at a rate of 100 ml min^{-1} at STP. Data were recorded and analyzed using Datacan V acquisition and analysis software (Sable Systems, Henderson, NV,

USA). The exact time of incubation (from the removal of the respirometry chamber from the manifold to when the air sample was injected) was recorded for each bed bug.

Repeated Measures Testing Schedule

Bed bug O_2 consumption and CO_2 production were measured daily from the day after they were received until 9 days post feeding, after which they were tested intermittently for 30 d, although this varied depending on survival. Exceptions to this schedule included adult females, first instars, and second instars, which were tested for less than 33 days because of high mortality. Also, first instars were measured beginning with their egg stage and they were measured 2 or more days apart because of the extended time required to produce measureable results. Approximately 10 replicates of each instar (1, 2, 3, 4, 5, unmated males, unmated females, mated males, mated females) were measured repeatedly. Preliminary results revealed no differences between those bugs which had been tested multiple times and those which were only tested once, so when bed bugs died, they were replaced by bed bugs of the same age which had been starved an equal amount of time and allowed at least 24 h to acclimate to the respirometry chamber. This ensured that sample sizes remained relatively consistent throughout the experiments.

Metabolic Calculations

Several additional variables were calculated from the recorded data. Metabolic rate was measured and is reported as $\dot{V}_{O_2}$ in ml^{-1} g h^{-1} (mass specific). Respiratory quotient (RQ) was calculated by dividing total CO_2 production by total O_2 consumption and is reported as a unit-less value. Finally, to assess the changes in body composition changes to mass were reported as

percent mass change per day (%MC d^{-1}). Using a percentage for this measurement standardizes the measurement for all life stages and allows for comparisons among all life stages.

Data Analysis

Due to the complex relationship between starvation and metabolism ($\dot{V}_{O_2}$), we were unable to mathematically model this relationship. However, a detailed description of the pattern of metabolic decline was used to understand the effects of starvation on bed bug metabolic rate ($\dot{V}_{O_2}$). Student t-tests were used to compare mated male and female $\dot{V}_{O_2}$ at several specific times post feeding. Analysis of variance (ANOVA) was used to compare $\dot{V}_{O_2}$ among all nymphal instars and unmated adults at several specific times post molting. Respiratory quotients (RQ) and percent mass change per day (%MC d^{-1}) are also reported and compared among life stages at specific time post feeding or post molting using either a t-test or analysis of variance.

Finally, it is important to note that when referencing a life stage (e.g., third instars) the fed previous instar (e.g., fed second instars) and molted current instars (e.g., molted third instars) are treated as one because we were interested in understanding the effects of starvation on metabolic rate, and all life stages (except adults) molt directly after feeding. Mated adults males, females, and first instars (eggs) were the exceptions. Unmated adults were obtained from fifth instars which were isolated after feeding but before molting to ensure no mating occurred.

Results

Activity

Preliminary results indicated that movement of < 50 mm h^{-1} did not significantly affect bed bug metabolism ($p = 0.2398$). Therefore, all bed bugs which moved less than 50 mm h^{-1}

were included in the study. When given at least 24 h to acclimate to their respirometry chamber, bed bugs generally had little to no movement; and we discarded only 22 injections representing 2.1% of all injections.

Mated Adults-Metabolic Rates

Mated adult males and females were modeled separately from unmated adult males, unmated adult females, and all nymphal instars because they were the only life stages measured which did not molt after a blood meal. The relationships between metabolic rate and starvation time can be described as a curvilinear decline (Fig. 1a). However, closer examination reveal several periods during starvation which do fit a curvilinear pattern (Fig. 1a). The general pattern can be described as a peak in $\dot{V}_{O_2}$ 0-48 h after feeding, followed by a rapid decline in metabolism until 168 h (males) or 192 h (females) of starvation. At those respective times, both sexes show a roughly 48 h period of stable $\dot{V}_{O_2}$. After these times, mated male $\dot{V}_{O_2}$ began to decline again, returning to the original curvilinear decline. Mated female $\dot{V}_{O_2}$ did not decline and remained relatively constant until 400 h (17 d) when the last measurement was recorded. After this time a large number (50%) of mated females had died preventing further testing.

A t-test to compare mated adult male and female $\dot{V}_{O_2}$ at specific times during starvation. After 48 h (first recorded metabolic rate), females had significantly greater $\dot{V}_{O_2}$ than males ($t = 2.35$, $df = 20$, $p = 0.0294$) (Table 1), however, at 192 h post feeding, there were no significant difference between the sexes. After 370 h post feeding we observed a 59.5% reduction in $\dot{V}_{O_2}$ for adult males and a 53.0% reduction in $\dot{V}_{O_2}$ for adult females (Table 1). Adult females also had significantly greater $\dot{V}_{O_2}$ than males ($t = 2.83$, $df = 18$, $p = 0.0112$) (Table 1).

Mated Adults-Respiratory Quotient

The respiratory quotient (RQ) decreased over time for both mated adult males and females. RQ showed a linear relationship with time post feeding (Fig. 1b, Table 2). A more detailed examination of the residuals from the regression reveals that these equations are only suitable for understanding that there is a general decline in RQ with time.

In addition, we used a t-test to compare mated adult males and female RQ at specific times during starvation. The t-test revealed no differences in RQ between sexes at any of the times compared (48, 192, 370 h of starvation). However, it should be noted that at 370 h male RQ was almost significantly higher than female RQ ($p = 0.0618$).

Mated Adults-Change in Body Mass

Percentage of mass change per day (% MC d^{-1}) showed different relationships with starvation time between mated males and females. The relationship for mated males showed a gradual decline in the rate of body mass change with starvation time (Fig. 1c). This trend displayed a similar pattern to the change in $\dot{V}_{O_2}$ with starvation time for males. However, females displayed a different relationship which is characterized by a steep increase in % MC d^{-1} at 144 h of starvation (Fig. 1c). It is also worth noting that female % MC d^{-1} remained higher than % MC d^{-1} in males for the duration of the experiments. Female mass change included the mass lost due to egg production.

We also used a t-test to compare mated adult males and female % MC d^{-1} at specific times during starvation (same used for metabolism and RQ). Significant differences were detected at 192 h post feeding ($t = 4.18$, $df = 18$, $p = 0.0006$) and 370 h post feeding ($t = 7.23$, $df = 18$, $p < 0.0001$).

Unmated Adults and Nymphal Instars-Metabolic Rates

The relationships between unmated adult and nymphal instar metabolic rates and time post molting is a curvilinear decline after a large peak in metabolic rate at time 0 h (during molting, Fig. 2a, 3a, and 4a). In general, there is a curvilinear relationship after a large peak in metabolic rate at time 0 h (during molting). However, similar to adults, closer examination of the plots of $\dot{V}_{O_2}$ versus time reveals several areas which do not fit the curvilinear relationship (Fig. 2a, 3a, 4a). Generally, these periods occurred 100-150 h after molting. During this period, $\dot{V}_{O_2}$ remained relatively constant, returning to a curvilinear relationship immediately thereafter.

Due to the similarity among all life stages which molted after feeding, we developed an ideal curve, depicting the general effects of time post molting on $\dot{V}_{O_2}$ (Fig. 5). We determined six important events in $\dot{V}_{O_2}$ that occur over the time leading up to and following molting. The first step is an initial increase in metabolic rate leading up to molting (Fig. 5, a) following feeding. This is followed by a peak in $\dot{V}_{O_2}$ when the bed bug molts (Fig. 5, b). Next, a rapid decline in $\dot{V}_{O_2}$ declines rapidly (Fig. 5, c) until reaching the initial plateau (Fig. 5, d). The initial plateau usually occurs between 100-150 h post molting (slightly later in unmated adult females). After this plateau, $\dot{V}_{O_2}$ declines gradually (Fig. 5, e) until reaching the starvation plateau (Fig. 5, f). The starvation plateau will persist until either the bed bug feeds and repeats the cycle or dies. Unmated adult males had a small initial plateau, but their metabolic decline was linear before and after the plateau (Fig. 2a), unlike the other stages which declined in a curvilinear fashion. In addition, first instars which hatched (Fig. 4) had a similar pattern but never reached the starvation plateau (Fig. 5, f) due to mortality.

We also used analysis of variance to compare mean $\dot{V}_{O_2}$ values among stages at several times after molting. The times selected represented important times depicted on the ideal curve (Fig. 5). Between -9 and 11 h post molting (molting, b on Fig. 5), analysis of variance revealed life stage to have a significant effect on $\dot{V}_{O_2}$ ($F_{6,52} = 3.38$, p=0.0068). Using Tukey's test, the only significant difference was between first instars and second and fourth instars (Table 3). At 128-155 h post-molting (initial plateau, d on Fig. 5), we observed a decrease in $\dot{V}_{O_2}$ between 30% (first instars) and 71% (Adult unmated females). Analysis of variance revealed $\dot{V}_{O_2}$ to be significantly different among life stages ($F_{6,55} = 12.48$, $p < 0.0001$). Using Tukey's test, we began to see a complex division of life stages (Table 3). However, some general trends began to develop at this time. There was a division between the smaller nymphal instars (1-2) and unmated adult males compared with the larger nymphal instars (4-5) and unmated adult females; third instars were intermediate between the two groups. After 216-248 h post molting (beginning of starvation plateau, between e and f on Fig. 5), metabolic rates declined 51.8% (first Instars) to 78.8% (fourth instars) from the maximum (molting) $\dot{V}_{O_2}$. Analysis of variance revealed $\dot{V}_{O_2}$ was significantly different among life stages ($F_{6,56} = 15.50$, $p < 0.0001$). Using Tukey's test, the groups became more rigidly established with third instars still being an intermediate between groups. Finally, we compared $\dot{V}_{O_2}$ for all life stages between 400-800 h post molting (extended starvation, f on Fig. 5). A wide time range was used because little to no change in $\dot{V}_{O_2}$ occurred during this time span and it was necessary to encompass all life stages (all tested at different times over this period). A 64.2% (second instars) to 87.0% (fourth instars) decrease in metabolic rate from the maximum (molting) $\dot{V}_{O_2}$ was observed (first instars not measured due to mortality). Analysis of variance revealed $\dot{V}_{O_2}$ was significantly different among life stages ($F_{5,62} = 17.74$, $p < 0.0001$). Further analysis using Tukey's test revealed three groups

with second instars having the highest $\dot{V}_{O_2}$, third instars being an intermediate group, and fourth instars, firth instars, unmated adult males, and unmated adult females composing a lower group (Table 3).

It is also important to compare both mated and unmated adults. This is somewhat complicated because of the molting observed in unmated adults (Fig. 2a). However, when evaluated visually the overall pattern of $\dot{V}_{O_2}$ over time post feeding or molting is similar between mated and unmated adult males but different between unmated and mated adult females (Fig. 1a, 2a). Mated and unmated males both decrease in similar fashions over time. However, unmated females show a steep decline after molting and maintain a low metabolic rate throughout the majority of starvation whereas mated adult females maintain a relatively high metabolic rate during starvation.

Unmated Adults and Nymphal Instars-Respiratory Quotient

Respiratory quotient (RQ) declined linearly with time post molting for all nymphal instars and unmated adults (Fig. 2b, 3b, 4b; Table 4). However, a detailed examination of the plot of RQ versus time and the residuals from the regression indicated that these equations are only suitable for understanding that there is a decline in RQ with time, and not for predicting RQ at a particular time post molting.

Analysis of variance was also used to test for differences in RQ between life stages at different critical times after molting (see above). There were significant differences ($p < 0.014$) in RQ at all times. Further analysis using Tukey's test revealed a general trend after molting of younger life stages having lower RQ than older life stages, although results did vary (Table 5).

Unmated Adults and Nymphal Instars-Change in Body Mass

Percentage of mass change per day (% MC d^{-1}) declined with starvation time for all nymphal instars and unmated adults (Fig. 2c, 3c, 4c). However, high variation in % MC d^{-1} prevented us from modeling this change. Further examination of the plot of % MC d^{-1} versus time post molting reveal the decline in % MC d^{-1} to be similar to that of $\dot{V}_{O_2}$ versus time post feeding for most life stages.

Analysis of variance was also used to test for differences in % MC d^{-1} at different critical times after molting (see above). Significant differences were only detected at 216-248 h ($p = 0.0002$) and 400-800 h ($p < 0.0001$). At these times the older life stages generally showed less change in body mass than younger life stages (Table 6).

Discussion

Starvation resulted in a strong and varied reduction in the metabolic rate of all life stages of bed bugs. The periods identified correlate well to biological processes which occur after feeding in the common bed bug. For mated adults, the peak in $\dot{V}_{O_2}$ after feeding can be explained by the high enzymatic functions and biological processes necessary for food digestion (Fig. 1a). The rapid decline following feeding is most likely do to the reduction in enzymatic activity after digestion (Fig. 1a). The short plateau period observed in males (168 h) and females (192 h) is not yet fully understood, but it occurs at approximately the time bed bugs would normally begin searching for another blood meal (Mellanby, 1939; Reinhardt and Siva-Jothy, 2007; Usinger, 1966). After this plateau, a general decline occurs where we believe bed bugs reduce their metabolism in an effort to conserve energy and prolong survival during starvation. The general pattern described here is similar to that seen in immature stages, but does not include

molting (see below for a description of this pattern). When mated adult males and females are compared, we identified some distinct differences between the sexes (Fig. 1a). These figures revealed that as time progressed, mated adult females tended to have higher $\dot{V}_{O_2}$ (Fig. 1a). In addition, $\dot{V}_{O_2}$ was significantly greater for mated adult females at 48 h and 370 h post feeding (Table 1). These results indicate females are likely allocating a large portion of energy into reproduction (probably egg production) which is energetically costly (Hayward and Gillooly, 2011; Ronn et al., 2006).

We also found a strong relationship between molting and the metabolism of all immature and unmated life stages. All life stages of bed bugs which molt, do so following a blood meal (Usinger, 1966). This allowed us to standardize all of the curves by plotting $\dot{V}_{O_2}$ versus post molting time (as opposed to time post-feeding in mated adults). The ideal curve relating time post molting to metabolic rate begins with a sharp increase in $\dot{V}_{O_2}$ (a) leading to the molt (b). Molting (b) was energetically costly in all life stages, although this is not surprising because of the requirements of molting such as hormone production, apolysis, digestion of old cuticle, production of new cuticle, and ecdysis (Chapman, 1998; Zwicky and Wigglesworth, 1956). After molting, $\dot{V}_{O_2}$ declined rapidly (c) until reaching the initial plateau (d). At this time, major processing (concentrating) of the blood meal has begun to slow and the energy requirements for molting are also declining because the major molting processes have already occurred. The initial plateau occurred from ≈ 100-150 h post molting, but for most life stages this was ≈ 8-9 d post-feeding, although this did vary between life stages as some took longer to molt than others. This is also an important time period because bed bugs are reported to feed approximately every 7 d (Mellanby, 1939; Reinhardt and Siva-Jothy, 2007; Usinger, 1966). The initial plateau probably represents the time that a majority of the blood meal has been processed (metabolized)

which is why the initial plateau occurs slightly past the maximum time bed bugs generally wait to seek a blood meal. Bed bug metabolism appears to have been selected to maintain at this level for a short time (2-3 d). This selection would allow bed bugs to maintain a higher metabolic rate for rapid host finding, but only if a continual food supply is available. If a blood meal is not found, bed bug metabolism will continue to decline (e) leading to the starvation plateau (f). The second decline is likely due to a gradual reduction in non-essential metabolic processes prior to entering the starvation plateau. This reduction in non-essential metabolic functions likely serves to increase the time bed bugs can survive between blood meals. The starvation plateau persisted with minimal change for the duration of the experiments or until death. Although the ideal curve is a good reference for most immature or unmated life stages, it does not accurately describe unmated adult males (Fig. 2a). Unmated adult males have a linear decline in $\dot{V}_{O_2}$ after molting until reaching the starvation plateau. Unmated adult males likely maintain elevated metabolic rates so they are capable of quickly responding to any female stimuli they encounter. Because reproduction is male controlled in bed bugs (Usinger, 1966), it is likely that a strong drive to mate as soon as possible has evolved, possibly leading to elevated metabolic rates after molting in unmated males.

To reinforce the observed visual trends in $\dot{V}_{O_2}$, we also compared all life stages which molted using analysis of variance at several times post molting (Table 3). This comparison provides a more quantifiable comparison of $\dot{V}_{O_2}$ of different life stages at several important times post molting (described above; Fig. 5). The differences observed between molting and hatching suggest that molting is more energetically expensive than hatching, although differences were only found between first instars (hatch) and second and fourth instars (molt) (Table 3). At all other times post mottling there is a general division between the smaller nymphal instars (1-2)

compared with the larger nymphal instars (4-5) and unmated adult females; third instars were intermediate between the two groups and unmated adult males belonged to different groups at different times (Table 3). These differences indicate that smaller instars maintain higher metabolic rates throughout starvation, although it is unclear why. One hypothesis is that smaller bed bugs would likely die of desiccation earlier than larger bed bugs because they lose water at a faster rate (Benoit et al., 2007). If this were the case, it would be to their advantage to maintain a metabolic rate that could potentially produce metabolic water, likely through fat metabolism (Mellanby, 1942). It is also worth noting that our results correlate with the values of survivorship for different nymphal instars. Smaller instars that maintain higher $\dot{V}_{O_2}$ during starvation have the shortest lifespans while larger instars that maintain higher $\dot{V}_{O_2}$ during starvation have the longest lifespans (Polanco et al., 2011; Usinger, 1966). We therefore hypothesize that unmated adult female bed bugs would survive the longest period of starvation compared with the other life stages. This would be an evolutionarily beneficial strategy because females do not control the mating cycle (Usinger, 1966). Evolutionarily, the ultimate objective for female bed bugs is to feed then reproduce and thus unmated females would be best served (from a fitness standpoint) to feed and wait for males to find them and mate so they have maximal energy available to produce eggs once mated. In addition, the link between feeding and molting seen in bed bugs is also beneficial to survival. This linkage allows molting, a very energy expensive process, to only occur when energy is available (after a blood meal). Without this strategy, bed bugs in an environment with varying levels of host availability that molted twice after a single blood meal might run out of energy before being able to feed again. This strategy also correlates nicely with the proposed evolutionary hypothesis of bed bugs originally feeding on cave dwelling bats (Usinger, 1966). Under this hypothesis, bed bugs would be

subjected to extended periods without food because many species of bats are known to commonly migrate between roosting sites throughout the year, often changing sites do solely to the presence of parasites (Fleming and Eby, 2003; Lewis, 1995; Reckardt and Kerth, 2007). This rapid movement of hosts would favor bed bugs which conserved energy and only molted when food was available.

The differences observed between mated and unmated adults lie primarily with the females. Mating status had little effect on male metabolism (Fig. 1a, 2a). This is not surprising because once mature, males have few restrictions which limit them from mating. However, mating status did have an effect on female metabolism. Mated females maintained elevated metabolic rates throughout starvation in comparison to unmated females which had one of the lowest metabolic rates of all life stages tested (Fig. 1a, 2a). The difference observed is likely due to the egg production seen only in mated adult females. Unmated females cannot produce viable offspring yet and therefore would benefit to reduce energy use until they can start producing eggs.

The curvilinear relationships between $\dot{V}_{O_2}$ and starvation or molting time make very interesting comparisons with a number of different animal taxa. The easiest comparison can be made between all bed bug life stages which molt and the life stages of *R. prolixus* which molt. Similar to bed bugs, *R. prolixus* is a hematophagous ectoparasite of mammals and birds and its later instars require a blood meal to molt (Roberts et al., 2009). Zwicky and Wigglesworth (1956) generated a curve relating $\dot{V}_{O_2}$ (ml h^{-1}) of *R. prolixus* to time post feeding (molting). The pattern they observed was similar to that described here (Fig. 5). Zwicky and Wigglesworth (1956) found a peak in $\dot{V}_{O_2}$ during molting which is likely due to the high energetic requirements during molting (Chapman, 1998). In addition, they found an extended period with little change

in $\dot{V}_{O_2}$, similar to our starvation plateau (f, Fig. 5). Both species have indeterminate times between meals and likely have lower metabolic rates in an effort to conserve energy. However, there were two important differences. First, the period to reach maximal $\dot{V}_{O_2}$ was considerably longer for *R. prolixus* (> 14 d after feeding) than the common bed bug (4-6 d after feeding). Second, they did not find an initial plateau (d, Fig. 5), which suggests that an initial plateau in $\dot{V}_{O_2}$ may be specific to hematophagous insects with similar life histories to bed bugs, or possibly bed bugs alone. In addition to the insect *R. prolixus*, there are also strong similarities in $\dot{V}_{O_2}$ and the molting cycle between bed bugs and another arthropod, the blue crab, *Callinectes sapidus* Rathbun (Defur, 1990). Both species have maximal metabolic rates during molting. This suggests that molting has similar effects on the metabolism of all arthropods and a peak in metabolism during this time is likely a common event. Another species worth comparing with the bed bug is the Burmese python, *Python molurus* (L.). Burmese pythons obviously share no close relationship with bed bugs in terms of phylogeny, however their feeding strategy and ectothermic nature warrant a comparison with bed bugs (Greene, 1997). Secor and Diamond (1997) reported a peak in oxygen consumption from 24 to 48 h after feeding. Bed bugs showed a similar peak in $\dot{V}_{O_2}$ between 0h and 48 h after feeding (Fig. 1a). A peak in $\dot{V}_{O_2}$ following feeding is not uncommon among animals (Benedict, 1932; Brody, 1945 as cited by Secor and Diamond, 1997) and our data show that bed bugs are no exception. In addition, it is likely that meal size would affect $\dot{V}_{O_2}$ in adult bed bugs similar to how it affected pythons, but this hypothesis was not tested in the present study (Secor and Diamond, 1997). After the peak in $\dot{V}_{O_2}$ following feeding, Burmese python $\dot{V}_{O_2}$ declines until returning to their baseline SMR in a curvilinear fashion (Secor and Diamond, 1997). The pattern of decline seen in Burmese pythons is very similar that seen in bed bugs (Fig. 1a), only lacking a plateau period.

Time post-feeding (molting) caused a general decline in RQ for mated adults (Fig. 1b) and all life stages which molted except first instars (Fig 2b, 3b, 4b). For all life stages, RQ declined linearly with starvation indicating a gradual shift in metabolic substrate from protein to fat (Table 2, 4). This shift also indicates that bed bugs use protein as their major fuel source, only shifting to fat when necessary (protein is used up). Mellanby (1932) also found protein to be the main fuel source used by bed bugs, however, he did not report any shift in metabolic substrate with starvation. No differences in RQ were found at any of the times post feeding used to compare mated adults. However, a trend in RQ at 370 h post feeding suggest that females were possibly metabolizing a greater proportion of fat. This is also supported by the greater slope in the equation relating RQ to starvation time for females (Table 2). If female RQ is indeed lower at this time, it is likely due to their higher metabolism and energy expenditure in reproduction (egg production). Analysis of variance revealed some significant differences in RQ between the life stages which molted, with larger instars generally having higher RQ for a longer period of time after molting (Table 5). This relationship suggests that the blood meals acquired by older instars last for longer periods because older instars do not resort to fat metabolism as early as the younger instars. Also, the younger instars tended to have higher metabolic rates (Table 3) which would lead to a faster depletion of protein and necessitate an earlier shift to fat. First instar RQ showed no relationship with time post-hatching (Fig. 4b). This suggests that first instars are only metabolizing fat/lipid throughout their within-stadia development.

Time post-feeding (molting) had a significant effect on changes in bed bug body mass (% MC d^{-1}). Mated adult females had significantly greater % MC d^{-1} than mated adult males. The differences found between mated adults are not surprising because the change in female weight also included eggs produced. The greater change in female weight correlates well with the low

survival time of mated adult females (50% died after 17 d), which was much lower than adult males (>50 % alive after 35 d). These mortality numbers make sence in light of the greater metabolic rate (Fig. 1a, Table 1), i.e. the faster change in metabolic substrate from protein to fat (Fig. 1b, Table 2), and the greater % MC d^{-1}. Although the survival time we report is much lower than reported by Usinger (1966), he also reported adult females (presumably mated) as having one of the lowest mean survival times when compared to other instars at 27°C. However, Polanco et al. (2011) found females capable of surviving as long as all other instars for several strains. The difference reported by Polanco et al. (2011) may be a result of measuring recently emerged and likely unmated females, a group which we show to have a much lower metabolic rate, likely caused by the lack of egg production. All immature stages had a peak in % MC d^{-1} during molting (Fig. 2c, 3c, 4c). Molting leads to large changes in mass due to both the loss of the exoskeleton and evaporative water loss due to the teneral state of the insect following molting (Wigglesworth and Gillett, 1936). Once bed bugs (all life stages) initiate their starvation metabolism (f, Fig. 5), changes in % MC d^{-1} become minimal. At this time, a majority of the change in mass is due to water loss and minimal excretion (due to basal metabolism, not blood processing). These values remain relatively low because bed bugs are excellent at conserving water (Benoit et al., 2007). However, as time post-molting increased, the older instars showed a pattern of less % MC d^{-1}. In light of bed bug biology, this makes since because these instars also lose less water than the younger instars (Benoit et al., 2007). One correlation we observed was bed bugs with a mass change above 4 % tended to survive for the least amount of time (personal observation). This can be seen by the short duration of testing by those life stages (first instars, second instars, and mated adult females).

In conclusion, bed bug metabolic rates ($\dot{V}_{O_2}$) were significantly affected by time post-feeding and time post-molting. Adult metabolic rates had a curvilinear relationship with starvation time. This relationship was similar to that seen in Burmese pythons, a drastically different animal sharing only a similar feeding strategy. Metabolic rates of all bed bug life stages which molted showed curvilinear relationships with time post-molting. An ideal curve was developed for these life stages which depicted and described the changes in metabolism associated with molting and subsequent starvation. In addition, these life stages also shared some similarities with two other arthropods: *R. prolixus* and *C. sapidus*, which also showed peaks in metabolic rate during molting. Respiratory quotients declined as time progressed in all life stages indicating a shift in metabolic substrate (protein to fat), except first instars which did not appear to change metabolic substrate during starvation. Percent mass change per day also declined with time post feeding/post molting and showed an interesting relationship with survival.

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Table 1-Comparison of mean (±SE) mass specific $\dot{V}_{O_2}$ (ml h^{-1} g^{-1}) values over a range of starvation times between mated adult males and females. Sample sizes are bracketed. *indicates significant difference between sexes at the p=0.05 level.

Time Post Feeding (h)	Adult Male (mated)	Adult Female (mated)
48	0.611(±0.020) [14]*	0.7199(±0.0513) [8]*
192	0.395(±0.019) [17]	0.3673 (±0.0395) [4]
370	0.248(±0.014) [16] *	0.3384(±0.0313) [4]*
840	0.150(±0.010) [11]	-

Table 2-Linear regression equations for mated adult male and female bed bugs relating respiratory quotient (RQ) to time post feeding (h). Equations are fitted for the specified post feeding time ranges (h).

Life Stage	Equation	d.f.	F	p	r^2	Time range (h)
Adult Male (mated)	RQ = 0.715(±0.014) - 0.000145(±0.000038)Time	11	14.3	0.0031	0.5646	48-840
Adult Female (mated)	RQ = 0.724(±0.023) - 0.000382(±0.000099)Time	7	14.8	0.0063	0.6791	48-360

Table 3-Comparison of mean (±SE) mass specific $\dot{V}_{O_2}$ (ml h^{-1} g^{-1}) values for immature bed bugs over a range of post molting times. Sample sizes are bracketed. Means with the same letter within each column are not significantly different at the p = 0.05 level.

Life Stage	Time Post Molting (h)			
	-9 – 11 h	128-155 h	216-248 h	400-800
First Instars	0.544(±0.076) [7] A	0.379(±0.027) [7] AB	0.262(±0.010) [7] AB	-
Second Instars	0.838(±0.028) [7] B	0.413(±0.020) [7] A	0.307(±0.020) [7] A	0.300(±0.013) [7] A
third Instars	0.723(±0.035) [10] AB	0.369(±0.030) [10] ABC	0.224(±0.019) [10] BC	0.215(±0.020) [22] B
Fourth Instars	0.829(±0.046) [9] B	0.288(±0.014) [8] CD	0.176(±0.010) [9] C	0.107(±0.013) [10] C
Fifth Instars	0.762(±0.061) [10] AB	0.318(±0.019) [10] BC	0.192(±0.014) [10] C	0.118(±0.011) [9] C
Adult Males (Unmated)	0.702(±0.019) [6] AB	0.376(±0.017) [10] AB	0.300(±0.014) [10] A	0.108(±0.006) [10] C
Adult Females (Unmated)	0.702(±0.050) [9] AB	0.207(±0.007) [10] D	0.171(±0.010) [10] C	0.101(±0.005) [10] C

Table 4-Linear regression equations for unmated adult and nymphal instar bed bugs relating respiratory quotient (RQ) to time post molting (h). Equations are fitted for the specified post molting time ranges (h).

Life Stage	Equation	d.f.	F	p	r^2	Time range (h)
First Instar	-	-	-	-	-	-
Second Instar	RQ = 0.646(±0.006)-0.000246(±0.000027)Time	11	82.6	<0.0001	0.8825	(-33)-543
Third Instar	RQ = 0.606(±0.020)-0.000217(±0.000074)Time	11	8.7	0.0130	0.4430	(-76)-716
Fourth Instar	RQ = 0.636(±0.012)-0.000085(±0.000036)Time	12	5.7	0.0344	0.3218	(-17)-931
Fifth Instar	RQ = 0.688(±0.007)-0.000145(±0.000023)Time	12	40.5	<0.0001	0.7714	(-26)-922
Adult Male (Unmated)	RQ = 0.659(±0.009)-0.000097(±0.000027)Time	9	13.2	0.0055	0.5942	(-89)-859
Adult Female (Unmated)	RQ = 0.642(±0.008)-0.000067(±0.000026)Time	9	6.8	0.0283	0.4308	(-85)-863

Table 5-Comparison of mean (±SE) RQ for immature bed bugs over a range of post molting times. Sample sizes are bracketed. Means with the same letter within each column are not significantly different at the p = 0.05 level.

Life Stage	Time Post Molting (h)			
	-9 – 11 h	128-155 h	216-248 h	400-800
First Instars	0.582(±0.025) [7] A	0.567(±0.017) [7] AB	0.577(±0.022) [7] AB	-
Second Instars	0.633(±0.008) [7] AB	0.607(±0.027) [7] A	0.605(±0.015) [7] AB	0.501(±0.010) [7] A
third Instars	0.584(±0.026) [10] A	0.492(±0.027) [10] B	0.549(±0.033) [10] B	0.499(±0.024) [22] A
Fourth Instars	0.631(±0.010) [9] AB	0.563(±0.018) [8] AB	0.642(±0.020) [9] AB	0.563(±0.028) [10] AB
Fifth Instars	0.669(±0.017) [10] B	0.643(±0.013) [10] A	0.630(±0.016) [10] AB	0.580(±0.025) [9] AB
Adult Males (Unmated)	0.639(±0.006) [6] AB	0.623(±0.011) [10] A	0.658(±0.027) [10] A	0.657(±0.022) [10] B
Adult Females (Unmated)	0.647(±0.009) [9] AB	0.620(±0.010) [10] A	0.655(±0.026) [10] AB	0.633(±0.012) [10] B

Table 6-Comparison of mean percent mass change per day (% MC d^{-1}) for immature bed bugs over a range of post molting times. Sample sizes are bracketed. Means with the same letter within each column are not significantly different at the p = 0.05 level.

Life Stage	Time Post Molting (h)			
	-9 – 11 h	128-155 h	216-248 h	400-800
First Instars	7.99(±1.02) [7] A	3.93(±0.68) [7] A	3.69(±0.32) [7] AB	-
Second Instars	9.92(±2.02) [7] A	5.37(±2.15) [7] A	4.25(±0.43) [7] A	2.81(±0.27) [7] A
third Instars	7.54(±0.90) [10] A	5.38(±1.01) [10] A	2.04(±0.31) [10] BC	2.29(±0.15) [22] A
Fourth Instars	8.85(±1.78) [9] A	2.83(±0.49) [8] A	2.53(±0.33) [9] AB	1.15(±0.07) [10] B
Fifth Instars	11.32(±1.51) [10] A	2.25(±0.28) [10] A	1.88(±0.31) [10] C	1.38(±0.15) [9] B
Adult Males (Unmated)	6.95(±0.71) [6] A	2.70(±0.32) [10] A	2.52(±0.39) [10] AB	1.18(±0.11) [10] B
Adult Females (Unmated)	8.16(±0.82) [9] A	2.34(±0.35) [10] A	1.92(±0.48) [10] C	0.84(±0.04) [10] B

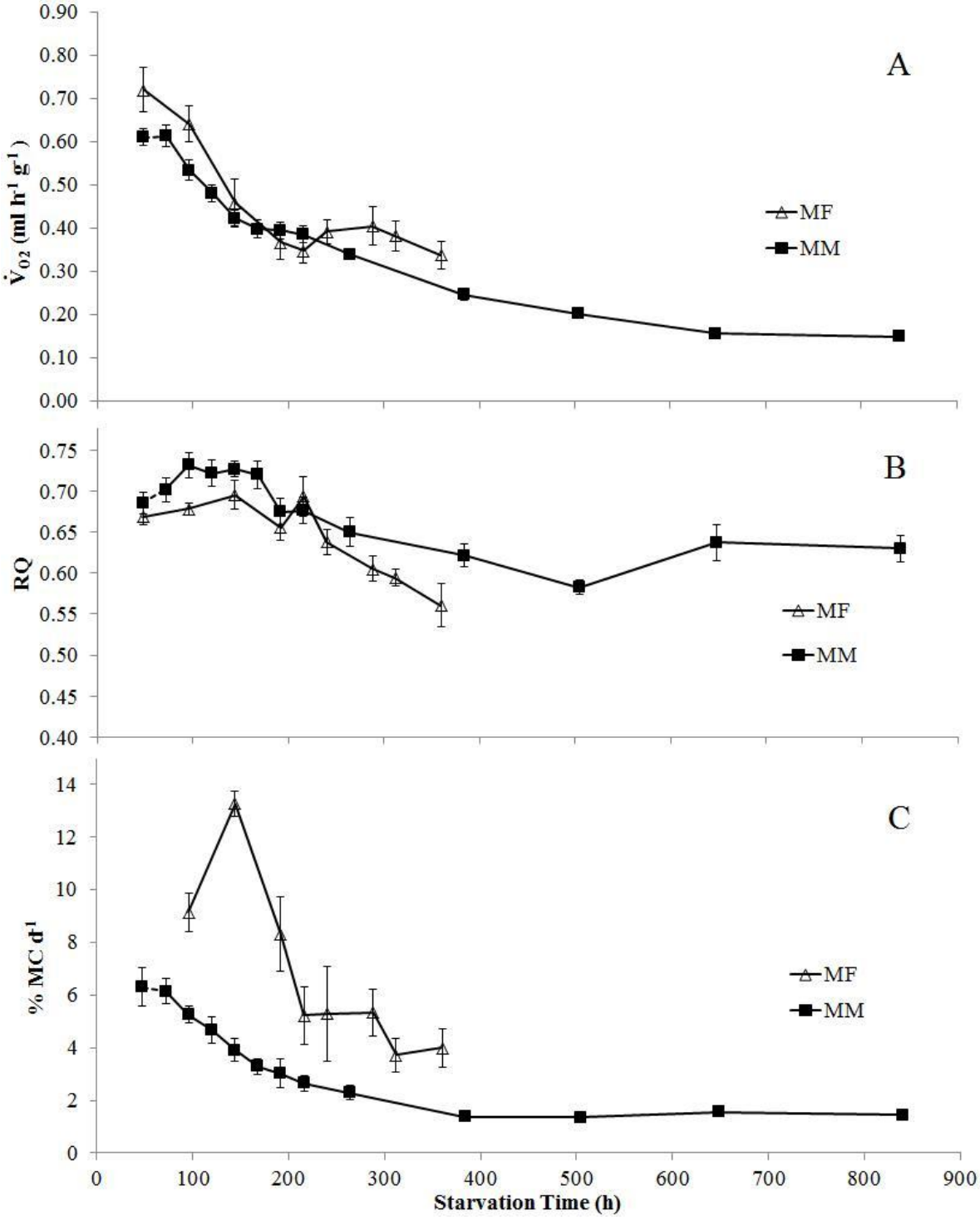

Fig. 1-Effects of starvation time (h) on several factors for mated adult males (MM) and mated adult females (MF). Factors include (A) $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}), (B) RQ, and (C) percent mass change per day (% MC d^{-1}). All values reported are mean±SE.

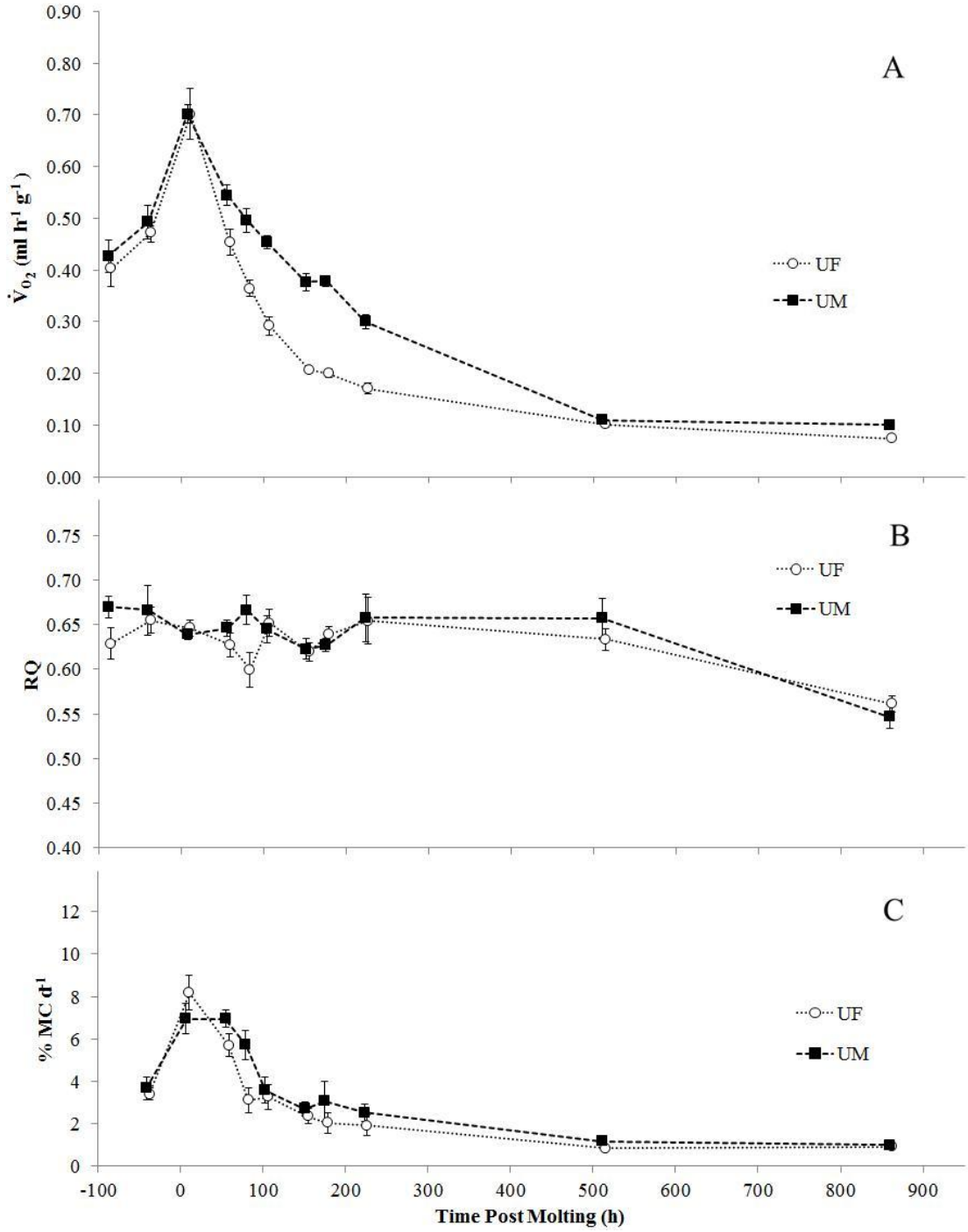

Fig. 2-Effects of time post molting (h) on several factors for unmated adult males (UM), unmated adult females (UF), fifth instar bed bug nymphs (5), and fourth instar bed bug nymphs (4). Factors include (A) $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}), (B) RQ, and (C) percent mass change per day (% MC d^{-1}). All values reported are mean±SE.

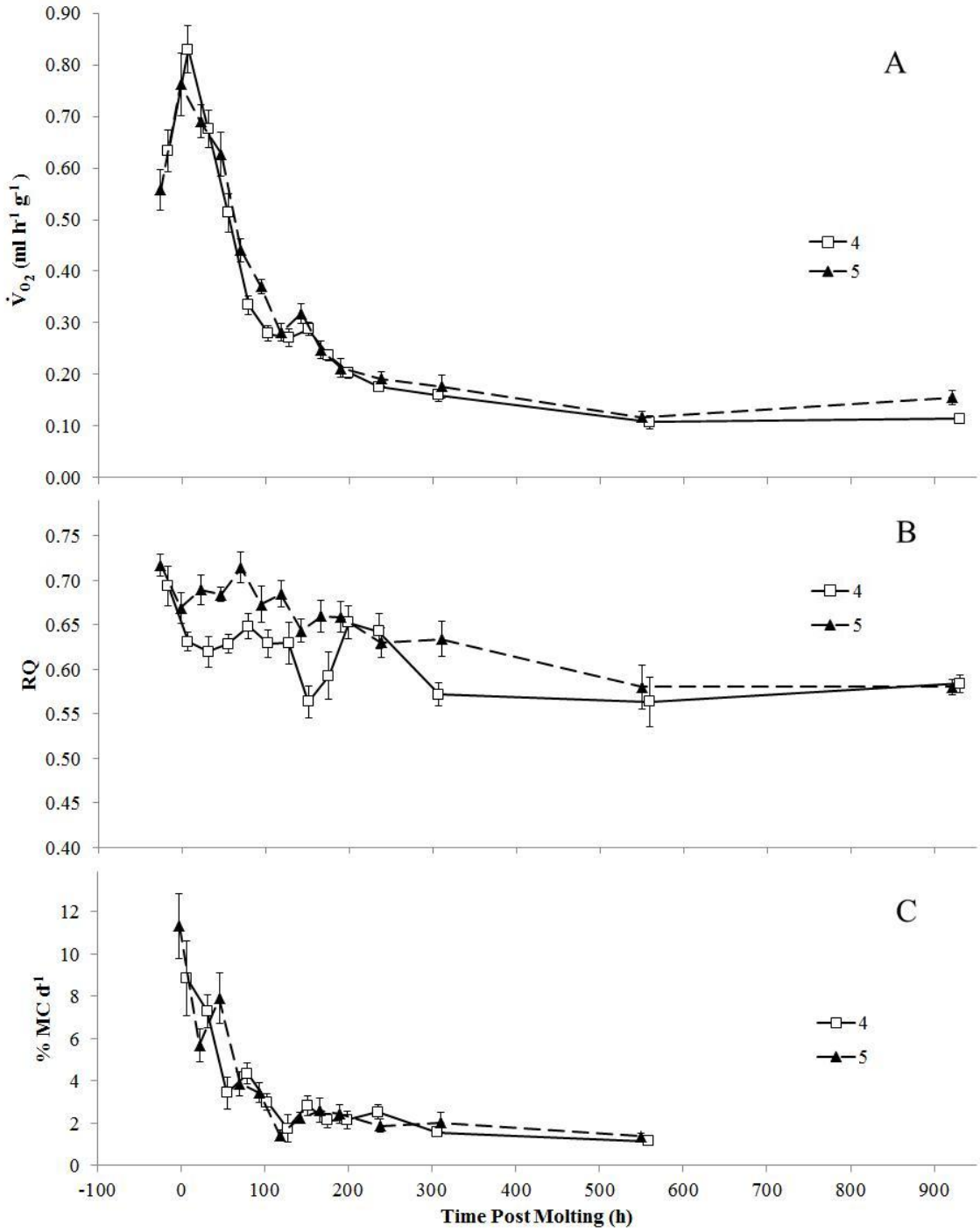

Fig. 3-Effects of time post molting (h) on several factors for fifth instar bed bug nymphs (5) and fourth instar bed bug nymphs (4). Factors include (A) $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}), (B) RQ, and (C) percent mass change per day (% MC d^{-1}). All values reported are mean±SE.

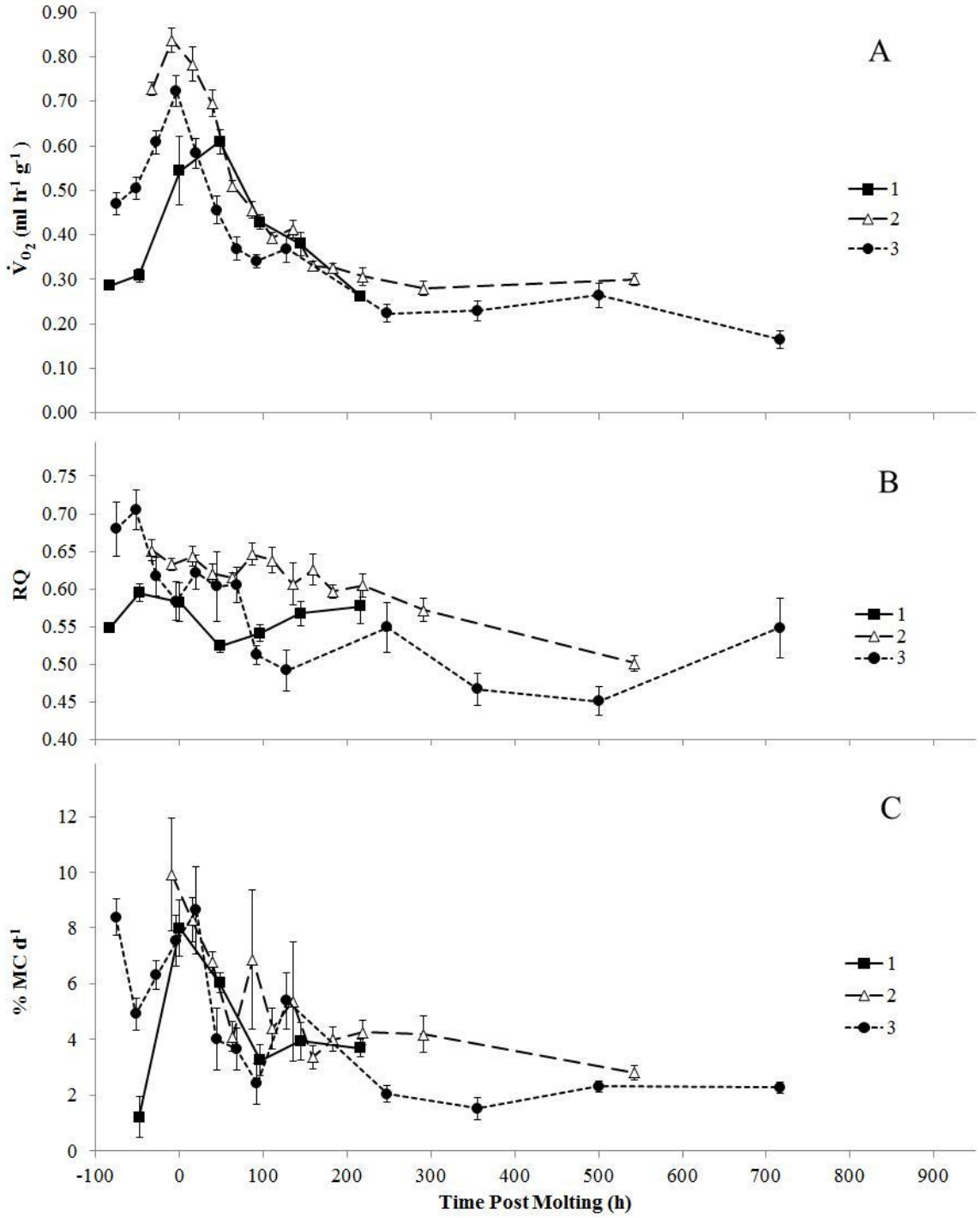

Fig. 4-Effects of time post molting (h) on several factors for third instar (3), second instar (2), and first instar (1) bed bug nymphs. Factors include (A) $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}), (B) RQ, and (C) percent mass change per day (% MC d^{-1}). All values reported are mean±SE.

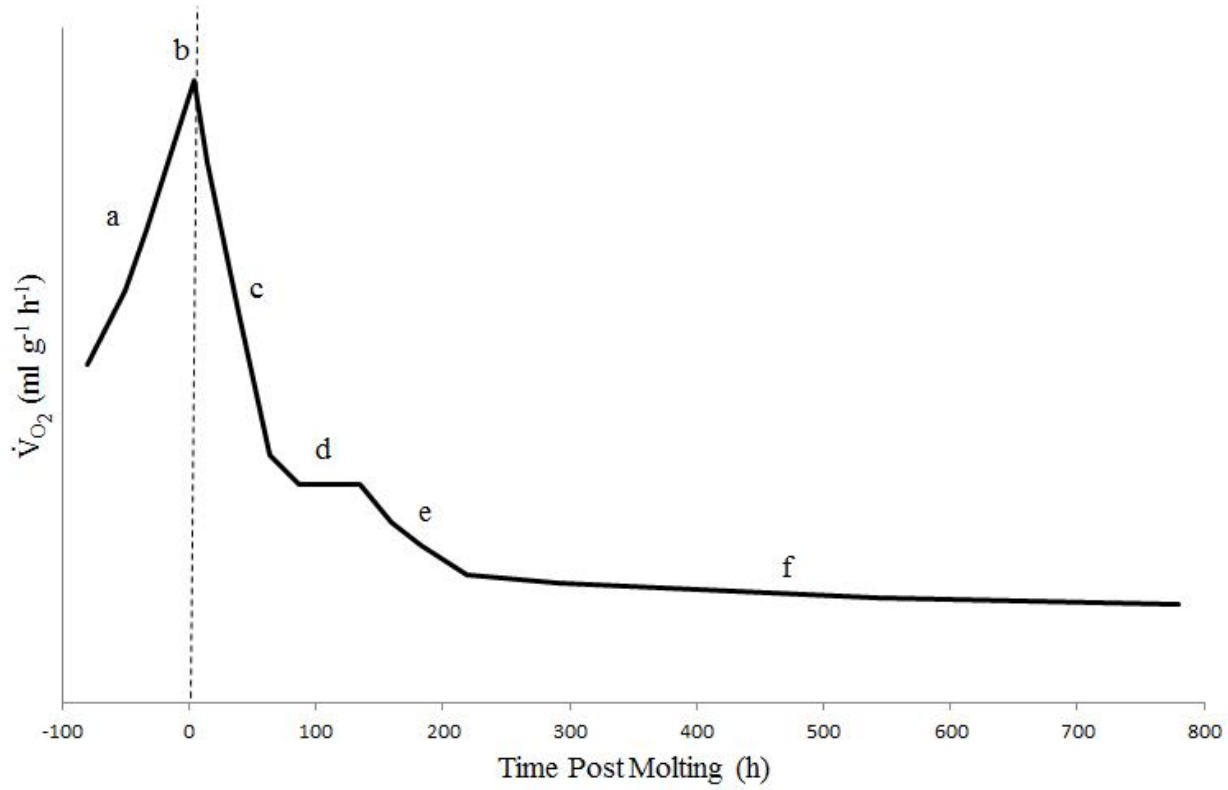

Fig. 5-Ideal curve relating $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}) to time post molting for life stages which molt. Different sections are labeled (a-f) and are discussed in the text.

Chapter 4

Standard metabolic rate of the common bed bug, *Cimex lectularius*: Effects of temperature, mass, and life stage

Abstract

Metabolic rates provide important information about the biology of organisms. For ectothermic species such as insects, factors such as temperature and mass heavily influence metabolism, but these effects differ considerably between species. In this study we examined the standard metabolic rate of the common bed bug, *Cimex lectularius* L. We used closed system respirometry and measured both O_2 consumption and CO_2 production across a range of temperatures (10, 20, 25, 30, 35°C) and life stages while also accounting for activity. Temperature had a stronger effect on the mass specific $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}) of mated males (Q_{10} = 3.29), mated females (Q_{10} = 3.19), unmated males (Q_{10} = 3.09), and nymphs that molted (first instars, Q_{10} = 3.05) than on unmated females (Q_{10} = 2.77) and nymphs that hatched (second through fifth instars, Q_{10} = 2.78). In addition, we found the respiratory quotient (RQ) to be constant across all temperatures and life stages. However, RQ was significantly lower in first instar nymphs than all other life stages of the bed bug. $\dot{V}_{O_2}$ (ml h^{-1}) scaled more with mass than values previously reported for other arthropods or that would be predicted by the 3/4-power law. The results are used to understand the biology and ecology of the common bed bug.

Introduction

Metabolic rates play an important role in understanding the biology of all animal life. Respiratory physiology has been used for years to understand the metabolism of a wide variety of arthropods including ants (Lighton, 1988; Vogt and Appel, 1999), beetles (Burges, 1960), cockroaches (Dingha et al., 2009), crickets (Hack, 1997), fleas (Fielden et al., 2001), moths (Schneiderman and Williams, 1953), spiders (Anderson, 1970), termites (Shelton and Appel, 2001), ticks (Fielden et al., 1999; Lighton and Fielden, 1995) and thysanurans (DeVries and Appel, 2013). Metabolic measurements provide useful information on the rate of energy expenditure and often incorporate the effects of temperature and mass (Lighton and Fielden, 1995; Vogt and Appel, 1999). Mass is of particular importance due to the ongoing debate regarding mass scaling. Specifically, the 3/4-power law is currently being challenged by other models such as the cell-size model which predicts a wider range of mass scaling coefficients (Chown et al., 2007; Glazier, 2005; Kozlowski et al., 2003; West et al., 1997; West et al., 2002). Metabolic rates also provide insight into what materials are being metabolized (Kells et al., 1999; Vogt and Appel, 1999). Even though metabolic rates have been described in a number of taxa, bed bugs have received limited attention.

Bed bugs present a very interesting biological system in the study of metabolism. First, bed bugs can survive a wide range of temperatures, despite the relatively constant temperature of their usual environment (Benoit et al., 2009; Kells and Goblirsch, 2011). In addition, Benoit (2007) showed bed bugs are very resistant to desiccation. Bed bugs are capable of surviving extended periods of starvation, with some reports indicating survival after more than a year without feeding (Usinger, 1966). Prolonged starvation has been shown to have significant impacts on their metabolism, with different life stages responding differently to starvation

(Chapter 3). In addition to their interesting biology, it is also important to gain a better understanding of bed bugs due to their status as a major pest in the urban environment, capable of causing both physical and psychological harm to their victims (Delaunay et al., 2011; Goddard and deShazo, 2009; Reinhardt and Siva-Jothy, 2007).

Despite the importance of bed bugs and the insight metabolic measurements could provide into their biology and life history, little information is currently available on bed bug metabolism. Mellanby (1932) performed the first study on bed bug metabolism, although little information on metabolic rates was presented. This study focused largely on the energy source used over time by examining bugs before and after starvation for their chemical composition. Rao (1973) also conducted a study on bed bug metabolism. This study indicated a difference in metabolic rate between mated and unmated bed bugs, but did not measure or discuss differences or similarities between any other life stages, focusing largely on the role of sperm in increasing metabolic rate. DeVries et al. (Chapter 3) conducted a study which provided insights into the effects of starvation on bed bug metabolism, but did not address the effects of temperature or mass. With the current lack of information on bed bug metabolism, we determined that a complete study of the effects of temperature, mass, and life stage on bed bug metabolism was necessary. In the present study we used closed system respirometry to measure the standard metabolic rate of the common bed bug across a range of temperatures, masses, and life stages. The results are related to bed bug biology and compared with other arthropods (DeVries and Appel, 2013; Lighton and Fielden, 1995).

Materials and Methods

Experimental Animals

Bed bugs were reared and maintained at the University of Minnesota, Twin Cities, MN as described by Olson et al. (2009). Briefly, we used an insecticide susceptible laboratory strain that was maintained at 23±2°C and 55±5% RH on a 14:10 light: dark cycle. Colonies were maintained in modified 0.5 l plastic jars with filter paper for harborage and a screen top to allow feeding. Bed bugs were fed human blood from the American Red Cross which had expired for medical use. Blood was fed weekly as a 1:1 combination of red blood cells and plasma.

Bed bugs were shipped to Auburn, AL overnight immediately after feeding in 1.5 ml centrifuge tubes with filter paper for harborage and a small hole in the top for air ventilation (screening was present to prevent escape). Once the bed bugs arrived, they were maintained in the same 1.5 ml centrifuge tubes in identical conditions as those used for rearing until testing. The filter paper was replaced as needed and bugs were sometimes divided into multiple 1.5 ml centrifuge tubes to prevent overcrowding.

Testing was performed on average between 216 to 264 h post feeding for mated adults (no molting) and 108 to 150 h post molting for all other life stages which molted. These times provided a short period of relatively little change in metabolic rate while the animals had not yet entered into a starvation metabolic rate (Chapter 3). The post-molting times were approximately equal to the post-feeding times that mated adults were tested at, allowing for comparison of all life stages, regardless of whether or not they molted.

Respirometry Equipment

After bed bugs reached the correct time either post-feeding or post-molting, they were placed into individual respirometry chambers constructed of 3 ml plastic syringes (Becton, Dickinson and Company, Rutherford, NJ, USA). Syringes were modified to allow air to flow through them while still preventing the bed bugs from escaping. This was done by drilling six 1.4 mm diameter holes in each syringe barrel past the last gradation where the plunger enters the barrel. Bed bugs were placed into individual syringes (respirometry chambers) along with a 0.32 cm^2 piece of cardstock paper (0.81 x 0.40 cm^2) and allowed to acclimate in the respirometry chamber overnight. After acclimation, the syringe plungers were adjusted so that the drilled holes remained open, but the syringe was sealed off to the environment. Syringes were then placed onto a manifold which pushed dry, CO_2 free air into the syringe tip, through the syringe, and out the drilled holes at the top to remove all CO2 and water. Air flowed into the manifold at a rate of 230 ml min^{-1} before entering the syringes. Immediately after purging, a 26 gauge intradermal bevel needle (Becton, Dickinson and Company, Rutherford, NJ, USA) was placed on the end of each syringe and the syringe volume was adjusted to 0.7 ml. Then the needle was inserted into a rubber stopper (size 000) to seal the respirometry chamber off from atmospheric gasses. Syringes were then placed into incubators (Thermo Fisher Scientific, Marietta, OH, USA) at one of 5 temperatures (10, 20, 25, 30, 35°C) and allowed to incubate for various times, depending on temperature and the number of animals being tested (2-18 h, except first instars which required more time). Animals were observed for movement under red light (20 W) using a SONY® DCR-SX85 video camera (Sony Corporation, Minato, Tokyo, Japan). Windows Media Player Classic version 6.4.9.1 (© 2002-2009) was used to manually review all videos for movement at a rate of approximately 1 min normal recording time per sec. When the syringes

had been allowed enough incubation time so that measureable changes in O_2 and CO_2 concentrations could be obtained, an air sample (0.5 ml) was injected from each syringe into the respirometry system which allowed us to measure O_2 depletion and CO_2 enrichment. Finally, the total time of incubation was recorded after the injection and bed bugs were weighted to the nearest 0.00001 g on a Mettler-Toledo AX205 digital balance (Mettler-Toledo GmbH, Greifensee, Switzerland). During every experiment we also measured two control syringes. These syringes went through the same procedure as the above experimental chambers, except they contained no animals. After injections, we were able to adjust all experimental syringes by subtracting any CO_2 or O_2 changes measured in the control syringes. We measured no change in O_2 in the control syringes and CO_2 accounted for less than 5% of the total CO_2 measured in the experimental syringes.

Measurements of O_2 consumption and CO_2 production from the injected air samples occurred via a respirometry system following the methods of DeVries and Appel (2013), Lighton (1991), and Vogt and Appel (1999). Room air was forced into a Whatman purge-gas generator (Whatman Inc., Haverhill, MA, USA) where both CO_2 and water were removed from the air. The air then passed through a 340 l mixing tank and into an open mixing tank (30 l) where the air equalized to atmospheric pressure. Next the air was pulled from the open mixing tank through a Drierite®-Ascarite®-Drierite® column (Drierite-W.A. Hammond Drierite Company LTD., Xenia, OH, USA; Ascarite-Thmoas Scientific, Swedesboro, NJ, USA) to remove any minute traces of water or CO_2. The air was then pulled through an injection port where air samples were injected after incubation was complete. The air then passed through a Li-6251 CO_2 analyzer (LI-COR Inc., Lincoln, NE, USA) and a Sable Systems Oxzilla II O_2 analyzer (Sable Systems, Henderson, NV, USA). The air finally passed through a Sable Systems mass flow system MFS2

(Sable Systems, Henderson, NV, USA), which controlled the air flow (pulled the air) at a rate of 100 mL/min at STP. All data were recorded and analyzed using Datacan V (Sable Systems, Henderson, NV, USA).

Metabolic Calculations

Using the recorded metabolic measurements, we also calculated several other important metabolic variables. Metabolic rates ($\dot{V}_{O_2}$) are reported as both ml h^{-1} and ml g^{-1} h^{-1} (mass specific). In addition, metabolic rates are also reported in μW, where 1 W is equal to 1 J s^{-1} and 1 ml of O_2 is equal to 20.1 J (Lighton and Wehner, 1993). Respiratory quotient (RQ) was calculated from metabolic measurements of O_2 and CO_2 and is the result of dividing total CO_2 production by total O_2 consumption. Q_{10} is a measure of the change in metabolic rate with a 10°C change in temperature. Q_{10} is calculated by multiplying the slope of the equation relating temperature to mass specific $\dot{V}_{O_2}$ (in log form) and then taking the antilogarithm of the product (Lighton, 1989). However, this is only the case if the equation relating $Log_{10}\dot{V}_{O_2}$ to temperature is linear.

Statistical Analysis

A t-test (PROC TTEST, SAS Institute., 1985) was used to determine what level of activity effected metabolic rate. Life stages were divided into new groups based on sex, mating status, and growth/development to aid in comparisons. Adults were divided into four groups: mated males (MM), mated females (MF), unmated males (UM), and unmated females (UF). Nymphs (immatures) were divided based on whether they hatched or molted. This resulted in two groups: nymphs that hatched (NH, first instars) and nymphs that molted (NM, second-fifth

instars). The relationships between metabolism, temperature, and mass were determined using multiple regression for all bed bug groups (PROC GLM, SAS Institute., 1985). Analysis of covariance (PROC GLM, SAS Institute., 1985) was used to assess the effects of bed bug group (main effect) and temperature (covariate) on metabolism. Analysis of variance (PROC GLM, SAS Institute., 1985) was used to compared mass specific oxygen consumption ($\dot{V}_{O_2}$, ml g^{-1} h^{-1}) and RQ between all bed bug groups. An LSD test was used to determine differences between the means. Least square regression was used to fit a power function to model MR (μW) versus mass for all bed bugs. This equation was also log-transformed into a linear equation and compared with mass scaling equations for other arthropods using analysis of covariance (arthropod group as main effect, mass as covariate). All means are reported with standard errors (±SE) and significance was determined at $\alpha < 0.05$.

Results

Activity

Bed bugs which had activity (movement) that significantly affected SMR measurements were removed from the study. Movement which significantly affected bed bug SMR was determined using a t-test comparing mass specific $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}) between individuals which did not move to those which moved between 1 and 50 mm h^{-1} at 25°C. This t-test revealed no significant differences in mass specific $\dot{V}_{O_2}$ due to movement under 50 mm h^{-1} for adults ($t_{37} = -1.49$, $p = 0.1066$) and nymphs ($t_{56} = 0.95$, $p = 0.3460$). This permitted inclusion of all bed bugs which moved less than 50 mm h^{-1}. When allowed enough time to acclimate, bed bugs showed little to no movement, requiring us to only remove 6 samples (1.3% of all tested) because of movement.

Adult Metabolic Rates

All adult groups were modeled separately throughout the study. Each adult group was only modeled for the effects of temperature on mass specific $\dot{V}_{O_2}$ due to the relatively small mass ranges within adult groups. Mated male mass specific $\dot{V}_{O_2}$ had the following relationship with temperature (°C):

$$MM\text{-}Log_{10}\dot{V}_{O_2} = -1.772(\pm 0.048) + 0.052(\pm 0.002)\ Temperature$$

($F_{1,46} = 785.4$, $p < 0.0001$, $r^2 = 0.9447$) (Fig. 1). Mated female mass specific $\dot{V}_{O_2}$ had the following relationship with temperature (°C):

$$MF\text{-}Log_{10}\dot{V}_{O_2} = -1.791(\pm 0.068) + 0.050(\pm 0.003)\ Temperature$$

($F_{1,43} = 337.5$, $p < 0.0001$, $r^2 = 0.8870$) (Fig. 1). Unmated male mass specific $\dot{V}_{O_2}$ had the following relationship with temperature (°C):

$$UM\text{-}Log_{10}\dot{V}_{O_2} = -1.713(\pm 0.043) + 0.049(\pm 0.002)\ Temperature$$

($F_{1,46} = 870.7$, $p < 0.0001$, $r^2 = 0.9498$) (Fig. 1). Unmated female mass specific $\dot{V}_{O_2}$ had the following relationship with temperature (°C):

$$UF\text{-}Log_{10}\dot{V}_{O_2} = -1.796(\pm 0.049) + 0.044(\pm 0.002)\ Temperature$$

($F_{1,45} = 550.8$, $p < 0.0001$, $r^2 = 0.9245$) (Fig. 1). Analysis of covariance (adult group as main effect, temperature as covariate) revealed no significance differences by which $\dot{V}_{O_2}$ of adult groups scaled with temperature ($F_{3,184} = 2.37$, $p = 0.0719$). However, closer examination revealed that the variable for unmated adult females was significant in the model ($p = 0.0414$), despite the overall interaction not being significant.

Adult Q_{10} and RQ

Mean Q_{10} were 3.29(±0.15) for mated males, 3.19(±0.20) for mated females, 3.09(±0.12) for unmated males, and 2.77(±0.12) for unmated females. Also, because the linear equations relating adult $Log_{10}\dot{V}_{O_2}$ to temperature are good fits ($r^2 > 0.88$ for all) Q_{10} can be assumed to be constant across the measured temperature range.

Respiratory quotient showed little change with temperature and therefore was not modeled. Average RQ is reported for each life group (Table 1). Analysis of variance revealed RQ was not significantly different among adult groups ($F_{3,184} = 0.1$, $p = 0.9568$).

Nymph Metabolic Rates

Both nymph groups (nymphs that molted and nymphs that hatched) were modeled separately throughout the study. Multiple regression analysis was used to determine the relationship between $\dot{V}_{O_2}$ (ml h^{-1}), temperature (°C), and mass (g) in nymphs that molted (NM):

$$\text{NM-}Log_{10}\dot{V}_{O_2} = -1.738(\pm0.056) + 0.045(\pm0.001)\ \text{Temperature} + 1.022(\pm0.019)\ Log_{10}\text{Mass}$$

($F_{2,211} = 2671.5$, $p < 0.0001$, $r^2 = 0.9620$). Coefficients for temperature and Log_{10}Mass were both highly significant ($p < 0.0001$). Nymphs that hatched only consisted of first instars and had a very narrow range of masses. This narrow range of masses prevented us from detecting any effects of mass. Therefore nymphs that hatched were not modeled using both variables.

In addition to determining the effects of both mass and temperature, we also assessed the effects of each variable on $\dot{V}_{O_2}$ individually. However, due to the narrow range of masses in first instars, we chose to only model bed bugs with mass as a group. Mass specific $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}) was regressed on temperature for both nymphal groups. Mass specific $\dot{V}_{O_2}$ of nymphs that molted had the following relationship with temperature:

$$NM\text{-}Log_{10}\dot{V}_{O_2} = -1.803(\pm 0.0231) + 0.044(\pm 0.001)\ Temperature$$

($F_{1,212} = 2659.6$, $p < 0.0001$, $r^2 = 0.9262$) (Fig. 2). Mass specific $\dot{V}_{O_2}$ of nymphs that hatched had the following relationship with temperature:

$$NH\text{-}Log_{10}\dot{V}_{O_2} = -1.844(\pm 0.045) + 0.048(\pm 0.002)\ Temperature$$

($F_{1,50} = 746.4$, $p < 0.0001$, $r^2 = 0.9372$) (Fig. 2). Analysis of covariance (molting versus hatching as main effect, temperature as covariate) revealed a significant interaction between molting/hatching and temperature ($F_{1,264} = 14.2$, $p = 0.0002$) indicating that mass specific $\dot{V}_{O_2}$ scales differently with temperature between bed bug nymphs that molt (second-fifth instars) and bed bug nymphs that hatch (1^{st} instars).

Nymph Q_{10} and RQ

Nymphs that molt had a Q_{10} of 2.78(±0.06) and nymphs that hatch had a Q_{10} of 3.05(±0.13). Due to the good fit of the linear equations relating $Log_{10}\dot{V}_{O_2}$ to temperature (r^2>0.92), these values can be assumed to be constant across the measured temperature range.

Respiratory quotient changed little with temperature and therefore was not modeled. Average RQ is reported for both nymphs that hatched and nymphs that molted (Table 1). A t-test revealed RQ was significantly different between nymphal groups (t_{264} = -7.6, $p < 0.0001$).

Comparison among Different Groups

We compared mass specific $\dot{V}_{O_2}$ between all adult and nymph groups. Analysis of covariance (groups as main effect, temperature as covariate) revealed a significant interaction between adult and nymph groups and temperature, with the interaction term (temperature * bed bug group) being highly significant ($F_{5,448}$ = 38.1, $p < 0.0001$). This indicated that mass specific $\dot{V}_{O_2}$ of different groups scale differently with temperature. When only mated adult males, mated adult females, unmated adult males, and nymphs that hatch (first instar) are compared, the interaction term (temperature * bed bug group) is not significant ($F_{3,189}$ = 0.53, $p = 0.6602$) indicating that mass specific $\dot{V}_{O_2}$ of these groups scale similarly with temperature. Similarly, when only unmated females and nymphs that molt (second through fifth instars) are compared, the interaction term (temperature * bed bug group) is not significant ($F_{1,259}$ = 0.00, $p = 0.9473$) indicating that mass specific $\dot{V}_{O_2}$ of these groups scale similarly with temperature.

We also compared mass specific $\dot{V}_{O_2}$ at 25°C. Using analysis of variance, we determined mass specific $\dot{V}_{O_2}$ at 25°C was significantly different among the groups ($F_{5,91}$ = 15.4, $p <$

0.0001). The LSD test for mean comparisons indicated that all immature bed bug groups and unmated adult females had significantly lower mass specific $\dot{V}_{O_2}$ than mated adults and unmated adult males (Table 1).

Respiratory quotient was also compared among all groups. Analysis of variance revealed a significant difference in RQ between groups ($F_{5,448} = 29.2$, $p < 0.0001$). The LSD test for mean comparisons revealed that RQ of nymphs that hatched (first instar) was significantly lower than all other bed bug groups stages (Table 1). In addition, all adult bed bug groups were significantly greater than nymphs that molt (Table1).

Comparison between Bed Bugs and Others Arthropods

In addition to examining the metabolic relationships within bed bugs, we also examined the relationships between bed bugs and other arthropod groups. All bed bug data were combined so we could compare bed bugs as a species with ticks and other arthropods (ants, beetles, spiders) reported by Lighton and Fielden (1995) and thysanurans, reported by DeVries and Appel (2013). Bed Bug mass related to $\dot{V}_{O_2}$ by the following equation:

$$\text{Log}_{10}\dot{V}_{O_{2@\,25^{\circ}C}} = -0.421(\pm 0.054) + 1.056(\pm 0.018)\ \text{Log}_{10}\text{Mass}$$

($F_{1,95} = 3517.2$, $p < 0.0001$, $r^2 = 0.9737$) (Fig. 3). Bed bug metabolic rates were also converted to μW and related to mass (g) by the following equation:

$$\text{MR}\ [\mu W] = 1485.1(\pm 740.3)\ \text{Mass}^{0.984(\pm 0.090)}$$

($F_{1,95} = 526.3$, $p < 0.0001$, $r^2 = 0.8471$). The effect of mass on metabolic rate (μW) was compared among bed bugs, ticks, thysanurans, and other arthropods (ants, beetles, spiders) (DeVries and Appel, 2013; Lighton and Fielden, 1995). Analysis of covariance (taxa as main effect, mass as covariate) revealed that bed bugs had a significantly higher mass scaling coefficient than all other taxa ($p < 0.0001$). Despite the difference in mass scaling, it is clear that bed bugs, similar to thysanurans, had mass specific metabolic rates which fell in between ticks (lower) and other arthropods (ants beetles, spiders; higher) (Fig. 4). However, it should be noted that bed bug metabolic rates were much closer to other arthropod metabolic rates than they were to tick metabolic rates (Fig. 4).

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Discussion

Activity is always important to consider when making metabolic measurements, due to the large effects it can have on O_2 consumption and CO_2 production (Bartholomew et al., 1985; Lighton and Feener, 1989; Lighton and Duncan, 1995). Bed bugs made this problem rather simple to negate, with < 2% total number of animals measured moving significantly. This is not surprising from an insect which spends a majority of it lifecycle concealed and motionless (Reinhardt and Siva-Jothy, 2007; Usinger, 1966).

Mass specific $\dot{V}_{O_2}$ showed a strong relationship with temperature for both adults and nymphs ($r^2 > 0.88$). Of the groups measured, the $\dot{V}_{O_2}$ of nymphs which molted (second-fifth instars) and unmated females proved to be less affected by temperature than the $\dot{V}_{O_2}$ of mated adults, unmated adult males, and first instars (Fig. 1, 2). This relationship is due in large part to the elevated metabolism of some groups at higher temperatures, because all bed bug groups showed very similar metabolism at 10°C. The cause behind elevated metabolism (mated adults,

unmated adult males, and first instars) or depressed metabolism (second-fifth instars and unmated adult females) is still unclear, but we hypothesize it is likely due to the evolutionary benefits of having high versus low metabolism for different groups and the impacts of metabolism on fitness. Nymphs which molt and unmated adult females have no need to use energy until feeding becomes an option and therefore might have lower mass specific metabolic rates at any given temperature when feeding stimulants are absent. However, mated adults and unmated adult males gain few fitness advantageous by sitting and waiting for food. Therefore elevated metabolic rates were likely selected for due to the benefits in mate location and reproduction likely associated with higher metabolism. First instar nymphs were also likely selected to have higher metabolic rates because they do not have the same capacity to survive starvation as other life stages, likely due to the lack of nutrients available upon hatching (Chapman, 1998; Usinger, 1966). Without these nutrients available, it would be more favorable to always be in an alert, ready to host-locate and feed state to capitalize upon any feeding opportunities. This hypothesis could be tested by assessing which life stages are the first to locate and begin feeding at various post-molting, post-hatching times. Differences in metabolic scaling with temperature within a species are neither new nor uncommon (DeVries and Appel, 2013; Vogt and Appel, 1999). This phenomenon has received little attention in large part because many studies only measure one life stage of insects/arthropods or do not compare different interspecific life stages.

In addition to comparing how different groups scale with temperature, we also compared mass specific $\dot{V}_{O_2}$ for all groups at 25°C, the temperature common bed bugs are usually found (Table 1). This comparison revealed a similar pattern as the scaling factors between mass specific $\dot{V}_{O_2}$ and temperature, likely due to the same reasons discussed above. However, nymphs

which hatched (first instars) had mass specific $\dot{V}_{O_2}$ values that were significantly lower than mated adults and unmated adult males and not siginificantly different from unmated adult females and nymphs that molted (Table 1). This suggests that despite a higher mass specific $\dot{V}_{O_2}$ scaling factor, first instars have likely been selected to conserve energy at a normal environmental temperature.

We also assessed the effects of mass on $\dot{V}_{O_2}$ at 25°C for bed bugs as a group (Fig. 3). Comparison between groups and mass-scaling were not possible due to the limit size range of each group (except nymphs which molted). The mass-scaling coefficient for all bed bugs ($Mass^{0.984}$) was greater than that reported by Lighton and Fielden (1995) ($mass^{0.825}$) for all arthropods and much greater than would be predicted by the 3/4-power rule. The mass scaling factor for bed bugs therefore provides support for the cell-size model of mass scaling (Chown et al., 2007).

Respiratory quotient (RQ) values are used to understand what metabolic substrate is being metabolized, with pure carbohydrate metabolism indicated by and RQ value of 1, pure protein metabolism indicated by and RQ value of 0.835, and pure fat metabolism indicated by an RQ value of 0.7 (Livesey and Elia, 1988). However, these values often differ considerably from measured values, which is why RQ is best understood by looking at differnces rather than comparing to the theoretical values. In the present study, RQ showed no change across the measured temperature range for any group. This indicates that the metabolic substrate being metabolized did not change from 10 to 40°C. However, this is not surprising because of the large blood (protein) meals bed bugs take (Usinger, 1966). Mellanby (1932) also found bed bugs to consume mostly protein at the same period post-feeding. We also compared mean RQ among the different groups and found first instars to have a significantly lower RQ than all other groups

(Table 1). This is also not surprising because first instars did not take a blood meal and therefore do not have the same protein available for metabolism as the other life stages (Usinger, 1966). Therefore, they appear to be relying on fat (vitellogenin) metabolism during this time.

Our results also provide more support for the use of standard metabolic rates to understand and possibly predict survival times during starvation (DeVries and Appel, 2013; Rixon and Stevenson, 1957; Schimpf et al., 2012). When compared with other arthropods (ants, beetles, spiders), ticks, and thysanurans, we found bed bugs to align similarly to thysanurans, above ticks and slightly below other arthropods (Fig. 4). These results make sense in light of the biology of these species. Bed bugs have been reported to survive > 1 year without feeding, similar to thysanurans (Lindsay, 1940; Mallis, 2011). However, bed bugs do not have the same capacity to survive extended periods of starvation as long as ticks (Needham and Teel, 1991), but they can generally survive starvation longer than most other arthropods (Mallis, 2011). It is also important to note that bed bugs were measured approximately 8-10 d after feeding. At this time, bed bug metabolism will have reached a plateau, but if they continue to starve their metabolic rates will continue to decline, sometimes > 50% (Chapter 3). Therefore, it is reasonable to assume that if tested again at a later time (25 d starved) bed bug metabolic rates would be much lower than currently reported and likely approaching the line representing ticks (Fig. 4).

Despite the observed relationship between standard metabolic rate and survival during starvation, it is still unclear what role metabolism plays in determining lifespan. Many authors have suggested that lower metabolism is indicative of a longer lifespan (Pearl, 1928), but this hypothesis has been increasingly challenged by ideas such as the free radical hypothesis (Dowling and Simmons, 2009; Harman, 1992). Niitepold and Hanski (2013) provide interspecific evidence suggesting that peak metabolic rate is positively correlated with lifespan.

However, their study was unable to find any relationship between standard (resting) metabolic rate and life span. Life span is still a very complicated variable and the role metabolic rate plays in determining this is still unknown. However, survival during starvation is somewhat less complicated and the evidence provided here and by Lighton and Fielden (1995) and DeVries and Appel (2013) suggest that lower metabolic rates provide an adaptive advantage to arthropods that face long periods of starvation. The relationship between standard metabolic rate and survival during starvation should be further investigated to determine if this relationship holds true for other arthropods and other ectothermic species.

In conclusion, metabolism had a strong relationship with temperature for all stages of the common bed bug. Metabolism also had a strong relationship with mass for bed bugs as a group. This information was useful in identifying and characterizing differences between groups, including unmated and mated adults. In addition, our results provide support for the cell-size theory of metabolic scaling, due to the strong deviation from the 3/4 mass scaling factor. Our study also suggests that standard metabolic rate has good potential to help understand and possibly predict longevity during starvation for other arthropods. The results and equations presented in this paper should be useful for future metabolic studies, particularly those dealing with species capable of surviving extended periods of starvation.

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Table 1 – Mean respiratory quotient (RQ) and mass specific $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}, at 25°C) for all bed bug groups. Means within columns which differ significantly according to the LSD test are indicated by different letters ($p < 0.05$).

	RQ		$\dot{V}_{O_2}$ at 25°C	
Bed Bug Group	N	Mean (±SE)	N	Mean (±SE)
Mated Male	48	0.64(±0.01) A	10	0.375 (±0.025) A
Mated Female	45	0.64(±0.01) A	9	0.305 (±0.023) B
Unmated Male	48	0.65(±0.01) A	10	0.336 (±0.014) AB
Unmated Female	47	0.65(±0.01) A	10	0.214 (±0.010) C
Nymphs that Molt	214	0.61(±0.01) B	40	0.245 (±0.008) C
Nymphs that Hatch	52	0.53(±0.01) C	18	0.239 (±0.014) C

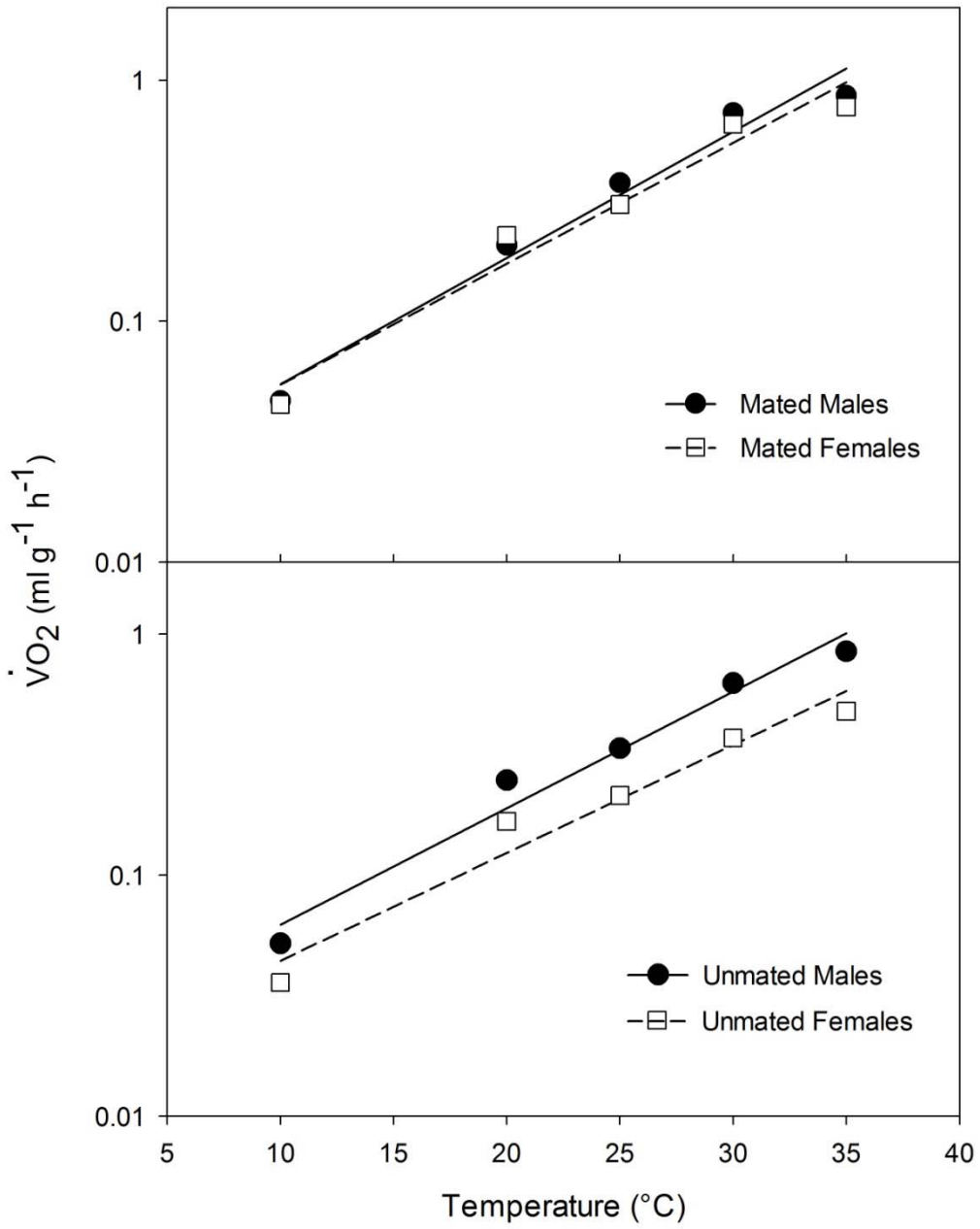

Fig. 1 – Mass specific $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}) for mated males (MM), mated females (MF), unmated males (UM), and unmated females (UF) across a range of temperatures. See text for equations.

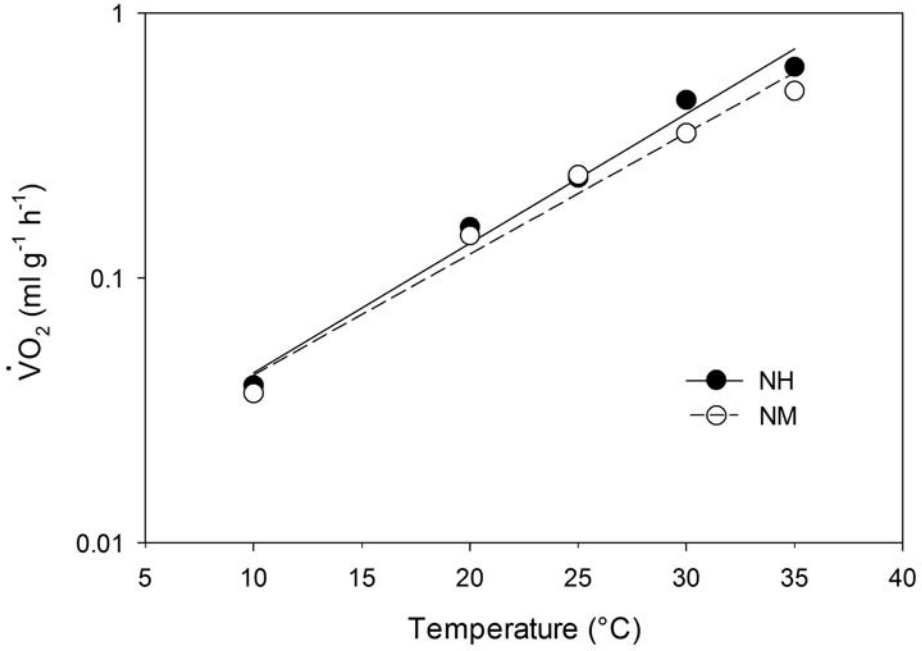

Fig. 2 – Mass specific $\dot{V}_{O_2}$ (ml g^{-1} h^{-1}) for nymphs which molt (NM, second through fifth instars) and nymphs which hatch (NH, first instars). See text for equations.

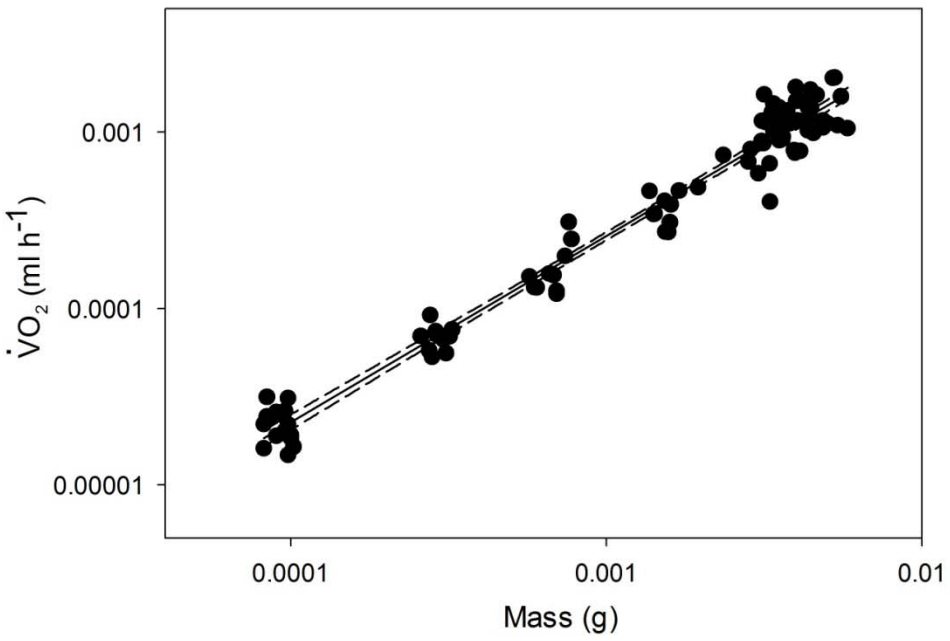

Fig. 3 – $\dot{V}_{O_2}$ versus mass for all bed bug groups (ml g^{-1} h^{-1}). See text for equation.

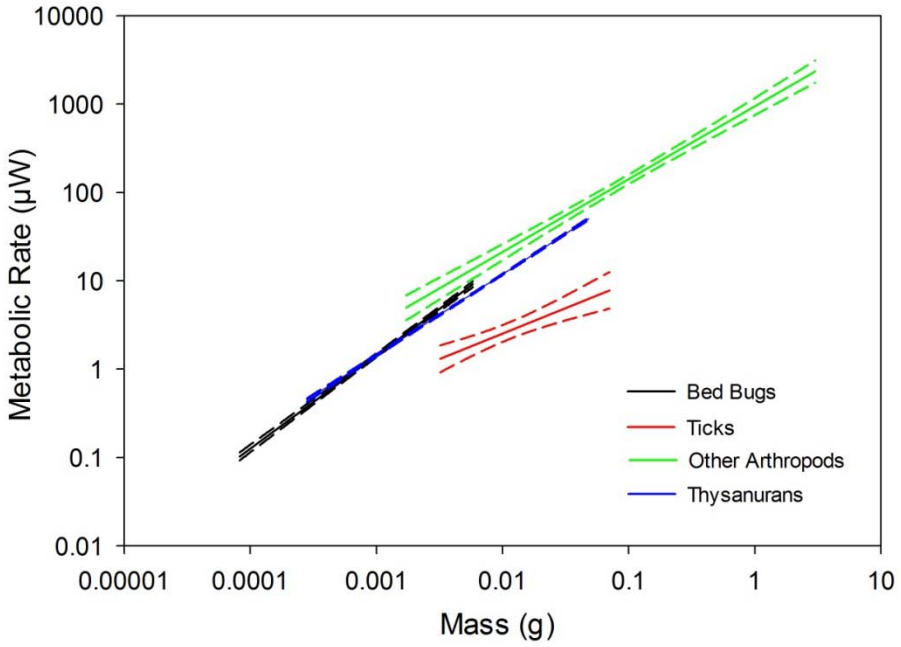

Fig. 4 – Metabolic rate (µW) versus mass (g) for bed bugs, thysanurans, ticks and other arthropods (ants, beetles, spiders).