The role of metabolically generated heat in the thermoregulation of a mammal (Mus musculus) and an amphibian (Rana pipiens)

ABSTRACT

In the face of natural fluctuations in environmental temperatures, animals engage in thermoregulation to maximize their fitness within their natural environments. Since temperature has such a pervasive impact on the rate of important biochemical processes, the type of thermoregulatory strategy adopted by a given organism can have significant implications with regards to its energy balance and life history. In this study, we investigate two major classes of thermoregulatory strategies - endothermy and ectothermy - via a representative mammal (the house mouse, *Mus musculus*) and a representative amphibian (the northern leopard frog, *Rana pipiens*). Using respirometry, we determine whether our test species are capable of using metabolic heat to thermoregulate. We find that the mouse exhibits elevated rates of oxygen consumption at cold temperatures (6.88 ± 0.50 mL O_2 hr^{-1} g^{-1}) which are significantly greater than rates at warm control temperatures (2.83 ± 0.23 mL O_2 hr^{-1} g^{-1}), which provides support for our hypothesis that mice, which are mammals and therefore endotherms, are able to use their metabolic heat in thermoregulation. This corresponds well with the primary literature, and we conclude that mammals are able to use metabolic heat to thermoregulate in the temperature range between their lower lethal temperature limit and their thermoneutral zone. In comparison, the frog does not exhibit significantly different rates of oxygen consumption at cold (0.14 ± 0.07 mL O_2 hr^{-1} g^{-1}) and warm (0.16 ± 0.04 mL O_2 hr^{-1} g^{-1}) temperatures, supporting our hypothesis that frogs, which are amphibians and therefore ectotherms, are not able to use their metabolic heat in thermoregulation. This result in conjunction with our literature review allows us to conclude that the metabolic rate in amphibians tends to increase with temperature, making it likely that our hypothesis is correct.

INTRODUCTION

In nature, animals are subject to variation in a number of environmental factors. Temperature variation in particular can have a significant impact on organismal functions, such as those related to metabolism and enzyme kinetics. By directly influencing diffusion and reaction rates, increases in body temperature produce increases in biochemical activity, and thus, metabolic rate, which is the sum of biochemical reactions within an organism. However, temperature can also affect the configuration and stability of proteins, and at very high temperatures, proteins become denatured and unable to function correctly. Thus, thermoregulation is required to maintain body temperature within optimal ranges.

One of the two key determinants of body temperature is the sum of heat fluxes between the organism and its environment, which occurs through processes such as conduction, convection, evaporation, and radiation. In turn, these processes can be influenced behaviourally or physiologically by animals as part of their thermoregulatory strategy. For example, animals actively avoid extreme temperatures in favour of environments with more desirable temperatures, in order to decrease the thermal energy lost or gained from the environment. Where avoidance is not possible, other strategies come into play. For example, in low ambient temperatures, animals may decrease heat loss to the environment by increasing the thickness or density of insulating materials such as fat or fur, or by decreasing their exposed surface area by changing their body posture or huddling together with other members of the same species.

The second determinant of body temperature is through the generation of internal heat as a byproduct of metabolism. While all organisms generate small amounts of heat through metabolism, differences arise regarding whether animals are able to use metabolic heat as part of their thermoregulatory strategy. Broadly speaking, ectotherms are those animals such as amphibians, reptiles, and most invertebrates, that are unable to alter their metabolic rate in response to changes in ambient temperature. These animals thermoregulate only through behavioural and physiological strategies such as those mentioned previously (Aravena *et al*. 2014 p.4468). As a result, body temperatures in these animals tend to fluctuate according to changes in ambient temperature, which produces predictable changes in organism function (i.e. sluggish activity at low temperatures, and increased activity at higher temperatures).

In contrast, endotherms such as mammals and birds are animals that, in addition to behavioural and physiological strategies, can use energy produced by metabolism to maintain body temperatures elevated above ambient temperature. This is accomplished by increasing the rate of ATP usage, such as by shivering to increase the frequency of skeletal muscle contractions, or by reducing the effectiveness of ATP production, such as in mammalian brown adipose tissue, which is specialized to generate large amounts of heat through the uncoupling of oxidative phosphorylation. Although energetically very expensive, endothermy allows organisms to maintain their bodies at temperatures conducive to optimal enzyme function. Because of this,

endotherms are able to function at approximately the same activity level within a large range of external temperatures, and are able to sustain high levels growth and muscular activity (Clarke and Pörtner 2010 p.704). Particularly in polar and temperate regions where ambient temperature experiences considerable daily and seasonal fluctuations, this is essential to prevent long and periods of non-activity during which the organism may die due to an inability to obtain energy or escape from predators (Buckley *et al*. 2012 p.873).

Classification of organisms based on thermoregulatory strategy is essential for understanding their physiology and behaviour. In this study, we investigate whether small mammals and small amphibians are able to use their metabolic heat to thermoregulate. From the preceding discussion, we know that endotherms are those animals that can thermoregulate using internal heat from metabolism, while ectotherms cannot. Thus, I predict that mammals - which are endotherms - will be able to thermoregulate using metabolic heat, while amphibians - which are ectotherms - will not.

In this paper, we use the house mouse (*Mus musculus*) as our representative mammal, and the northern leopard frog (*Rana pipiens*) as our representative amphibian. These species were chosen for their similar size, which simplifies our comparison by accounting for allometric effects. Since oxygen consumption rate is directly proportional to metabolic rate, we will compare the metabolism of our test animals under different temperature conditions by using respirometry to measure oxygen consumption rates at cold and warm temperatures.

MATERIALS AND METHODS

Test Subjects

Twelve each of the northern leopard frog (*Rana pipiens*) and house mouse (*Mus musculus*) were obtained for this experiment from the Animal Care Facility at the University of British Columbia. Mice were 3-month-old virgin females kept in groups of 6 each, while the frogs were adults of mixed sex. Prior to this study, none of the animals had ever been experimented upon. Animals experienced a photoperiod of 12 hours of light alternating with 12 hours of dark (LD12:12). Animals were maintained in indoor constant-temperature environments held at 22°C for mice and 8-10°C for frogs. These and conditions were chosen to mimic conditions that these animals would normally experience in the wild. Six hours before trials began, animals were brought to the location of experimentation, which was held at 20 to 21°C.

Food (standard commercial pellets) and water were provided *ad libitum* to all animals until the time of experimentation, because, as noted in Hudson and Scott (1979 p.209), this can help minimize the possibility of mice entering torpor when exposed to temperatures below 20°C. As a result, mice cannot be guaranteed to have been post-absorptive, and this may have influenced our absolute values of oxygen consumption rates and affected the variance of our data. However, since the main focus of this study is to observe the relative magnitude and valence of changes in metabolic rate in response to different ambient temperatures, instead of determining absolute values for oxygen consumption rate, this will not have any effect on our overall discussion of whether small mammals and amphibians are able to thermoregulate using metabolic heat.

The frogs were also provided food and water *ad libitum*; however, as they appeared to be hibernating, they were never observed consuming any food, and can be assumed to have been post-absorptive at the time of experimentation.

Experimental Design

Metabolic rate was estimated by animals' rates of oxygen consumption, which were obtained through respirometry following the procedure outlined in the Biology 363 Lab Manual. Animal chambers were constructed using 300 mL glass jars covered with dark material to minimize the effect of external stimulation on animal behaviour. At the base of each animal chamber, isolated to prevent contact with test animals' skin, excess soda lime was placed to sequester any carbon dioxide released by the animal during the experiment. Each animal chamber was connected to a syringe through which pure O_2 could be dispensed, and was also connected to a manometer, which in turn was connected to a compensation chamber partially

filled with water to provide a constant-humidity, constant-pressure reference for monitoring pressure changes in the animal chamber.

Cold and warm temperature treatments were created by placing animal chambers within ice baths, or by leaving them at room temperature (22°C). Assuming that the six hours provided for frogs to acclimate to room temperature was sufficient, room-temperature chambers served as controls for both mice and frogs, while the cold-temperature chambers served as the cold treatment. Test animals were placed singly in animal chambers and allowed to acclimate to treatment temperatures for 10 minutes. While mice were acclimated with animal chambers left unsealed as specified in the BIOL 363 Lab Manual, preliminary tests showed that better results were obtained for frogs when acclimation occurred in closed chambers. Thus, the acclimation procedure was modified and all frogs were acclimated in closed chambers with supplemental oxygen provided at replacement levels.

Following acclimation, pure oxygen was flushed through the animal chambers, which were then sealed. Thus, during experimentation, animals were exposed to hyperoxic conditions (i.e. PO_2 > 21%). The time taken for a set volume (2 mL for frogs, 30 mL for mice) of pure oxygen to be consumed was recorded. Without removing animals from the experimental set-up, as many successive measurements of oxygen consumption rate were obtained as possible within the 2-hour window with which animals were ethically permitted to remain within the animal chambers. Each animal was only used once and at a single temperature treatment, and was weighed upon trial completion. During each trial, the temperatures of test chambers were monitored. For mice, the temperature in the cold chamber averaged 13 ± 1°C while the warm chamber averaged 26 ± 1°C. For frogs, the cold chamber averaged 8 ± 1°C and the warm chamber averaged 24 ± 1°C.

Since the nature of the respirometer set-up is likely to have been stressful for the test subjects and affected oxygen consumption rate, the first measurement for mice was discarded. However, due to time constraints, the first measurement for frogs was retained. Thus, data obtained for frogs may have been confounded by stress.

Data Analysis

Data for this experiment were collected on February 25-26, 2016, by students enrolled in the 2015W2 section of Biology 363: Laboratory in Animal Physiology at the University of British Columbia, Canada. The majority (21/24) of trials included in this analysis took place on February 25th. However, to increase the statistical power of the analysis, data were randomly selected from trials occurring on February 26th to ensure that all sample sizes were the same (n = 6).

Treatment Temperature Determination

For each trial, multiple temperature readings were obtained throughout the experiment. These values were averaged to obtain an average temperature for that trial.

Finally, the temperatures of the six trials per treatment, per species, were averaged, and their 95% confidence intervals determined, to determine the average temperatures experienced by animals of a given species at each of the cold and warm treatments.

Standardization of Oxygen Consumption Rates

After discarding the first measurement (for mice, but not for frogs; see "Experimental Design"), the average oxygen consumption rate per trial was determined. These averages were then converted to STPD-standardized mass-specific oxygen consumption rates through the following formula:

$$\frac{volume\ O_2\ consumed\ (mL)}{consumption\ time\ (hr) \times organism\ mass\ (g)} \times STPD\ coefficient\ . \qquad (1)$$

Standardization by STPD was required to correct for the effects of temperature, ambient pressure, and water pressure on oxygen consumption.

Oxygen consumption rate is affected by body size: in general, larger organisms have higher metabolisms and therefore higher rates of oxygen consumption. Although some published papers (e.g. Feder 1982 p.24), have chosen to standardize their oxygen consumption rates by dividing them by mass raised to the power of an allometrically determined exponent, this was deemed unnecessary for the purposes of this paper since the animals used in the two treatments did not have masses that differed significantly from each other (34.2 ± 2.2g and 37.0 ± 3.6g for mice in the cold and warm treatments respectively; 43.3 ± 4.6g and 41.5 ± 5.6g for frogs in the cold and warm treatments respectively). Since the animals' masses were not significantly different between treatments, we therefore express oxygen consumption as a mass-specific value. This correction is required to ensure that any differences we observe between treatments are not caused by differences in body mass.

Statistical Analysis

For each species, oxygen consumption rates in each temperature treatment were averaged and plotted along with their 95% confidence intervals. If confidence intervals overlapped in any of their values (including endpoints), this was taken as an indication that the means were not significantly different from each other. Conversely, if the confidence intervals did not overlap, the means were considered significantly different.

RESULTS

Description of Results

Since the body masses of animals did not differ significantly between treatments (see "Data Analysis: Standardization of Oxygen Consumption Rates"), we can directly compare the mass-specific oxygen consumption rates of treatment groups.

Oxygen consumption rate of *Mus musculus* was significantly greater in the cold temperature (6.88 ± 0.50 mL O_2 hr^{-1} g^{-1}) than in the warm temperature (2.83 ± 0.23 mL O_2 hr^{-1} g^{-1}) (Figure 1). This difference represents a 140% increase in oxygen consumption rate in the cold treatment relative to control.

Oxygen consumption rate of *Rana pipiens* was not significantly different between the cold (0.14 ± 0.07 mL O_2 hr^{-1} g^{-1}) and warm (0.16 ± 0.04 mL O_2 hr^{-1} g^{-1}) temperatures (Figure 2). While the difference was not significant, it is worth noting that oxygen consumption rate was approximately 10% greater in the warm temperature control than at the colder temperature.

Figures

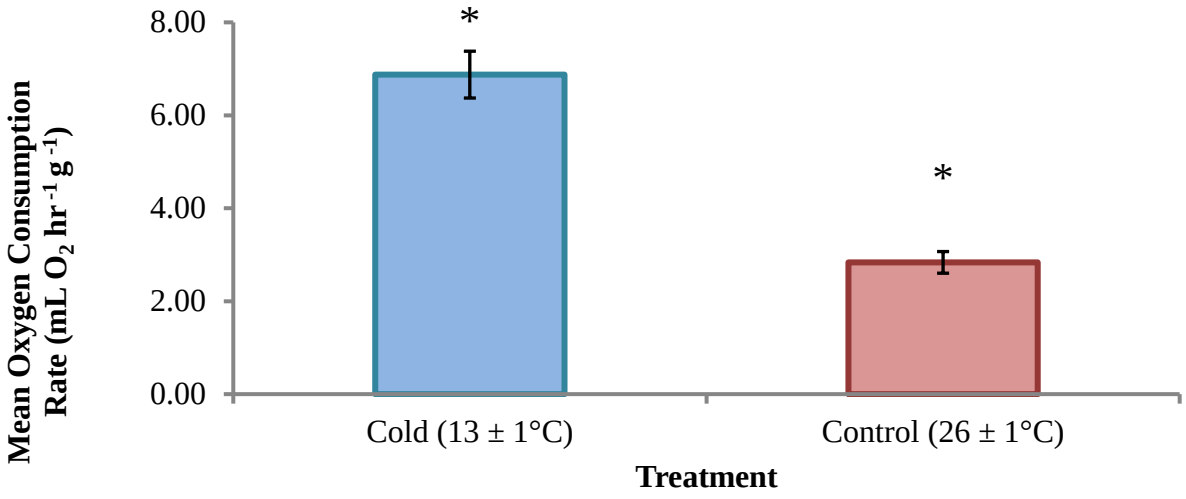

Figure 1. Mean oxygen consumption of the house mouse (*Mus musculus*) in different-temperature treatments (oxygen consumption rates and treatment temperatures expressed as mean ± 95% CI; n = 6 per treatment; * represents significant differences ($p < 0.05$) between means; mice were healthy 3-month-old virgin females weighing 31 - 45 g)

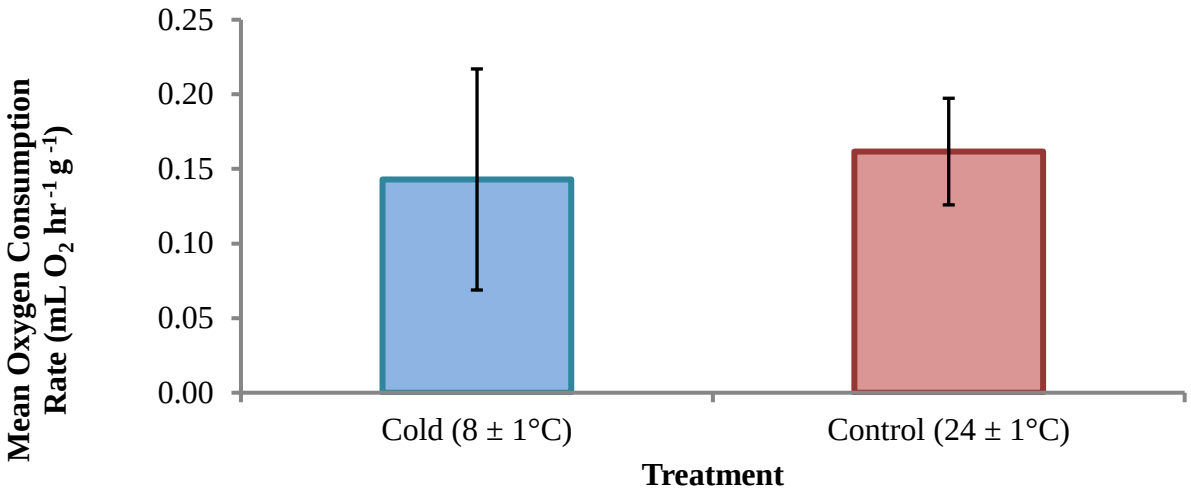

Figure 2. Mean oxygen consumption of the northern leopard frog (*Rana pipiens*) in different-temperature treatments (oxygen consumption rates and treatment temperatures expressed as mean ± 95% CI; n = 6 per treatment; frogs were healthy adults of mixed sex weighing 31 - 52 g)

DISCUSSION

Thermoregulation in Small Mammals

One hypothesis we tested was that small mammals are capable of thermoregulation using metabolic heat. Since mammals are classified as endotherms, then when they are in lower-temperature environments, they should be able to generate more internal heat to compensate for the greater difference between ambient and body temperatures; this increase should be achieved through increases in metabolic rate. Thus, metabolic rates in mammals are expected to correlate negatively with ambient temperature. Since oxygen consumption rate is directly proportional to metabolic rate, then when the external temperature decreases, oxygen consumption rate should increase. If small mammals are capable of thermoregulation using metabolic heat, then we predict mass-specific oxygen consumption rate to be significantly greater in the cold treatment than in the warm temperature control. Accordingly, our results show that *Mus musculus* has a significantly greater oxygen consumption rate at 13°C than at 26°C. This aligns with my predicted result, and provides evidence in support of my hypothesis. I conclude that small mammals are able to thermoregulate using changes in metabolic rate.

Validity of Experimental Protocol: *Mus musculus*

In order to determine whether the methods used in this paper were valid, I compared our results to values taken from primary literature. To warrant inclusion in our review, studies were required to have been conducted under controlled laboratory settings, and to have sufficiently large ($n \geq 3$) sample sizes.

For *Mus musculus*, my comparison only included studies that reported resting oxygen consumption rates. Basal metabolic rates were also considered acceptable since, like with resting metabolic rate, these measurements require test subjects to be at rest. A total of five studies were selected for use in this comparison, and their results are summarized in Table 1. There are three notable differences between the methods we used and the methods used in these five studies. The first is that our study was the only one where test subjects were not guaranteed to have been post-absorptive. In comparison, of the four surveyed studies that commented on their subjects' absorptive status, all four had used post-absorptive animals. The second difference is in the type of experimental set-up used. Four of the five studies employed open flow respirometry in their measurement of oxygen consumption rate, while only one used closed respirometry as we did. Finally, the mice used in our study were larger than the ones used in all of the other studies. Despite these differences, our value for mass-specific oxygen consumption is well within the range of the values reported in literature. Thus, it is likely that our methods were appropriate for the determination of mass-specific oxygen consumption rates in mice.

Table 1. Literature comparison of values for mass-specific oxygen consumption of *Mus musculus*						
Study	**Oxygen Consumption (mL O_2 hr^{-1} g^{-1})**	**Mass (g)**	**Experimental Temperature (°C)**	**Sample size**	**Subject Characteristics**	**Additional Notes**
Present study	2.83 ± 0.23 (mean ± 95% CI)	37.0 ± 3.6 (mean ± 95% CI)	26 ± 1 (mean ± 95% CI)	6	Female, virgin, 3 months old, not post-absorptive	Closed respirometry
Martin *et al.* 1980 p.521 (Table 1: singles)	3.28 ± 0.42 (mean ± 95% CI)	26.5 (mean)	25 (mean)	6	Mixed sex, none pregnant or lactating, post-absorptive	Closed respirometry
Tomlinson *et al.* 2007 p.647 (Fig. 1: normothermic mice)	2.29 ± 0.14 (mean ± 95% CI)	16.1 (mean)	30 (mean)	4	Male, post-absorptive	Open flow respirometry
Selman *et al.* 2001 p.780 (Table 1)	1.14*and 1.57** (means)	31.2* and 34.2 ** (means)	30 (mean)	9* and 10**	Female, between 18-20 wks, effectively post-absorptive	Open flow respirometry * Low food consumption strain ** High food consumption strain
Speakman and McQueenie 1996 p.753 (Table 1: control)	2.26 (mean) (basal oxygen consumption)	24.8 ± 1.1 (mean ± 95% CI)	28 (mean)	9	Female, virgin, effectively post-absorptive	Open flow respirometry
MacAvoy *et al.* 2012 p.986 (text description)	3.51† and 3.28†† (means) (basal oxygen consumption)	N/A	N/A	3 per strain	Female	Open flow respirometry † BALB/c strain females †† CBA/J strain females

Internal Validity of Conclusions: Thermoregulation in Small Mammals

The results from our study supported the hypothesis that small mammals are able to thermoregulate using metabolic heat. I surveyed the literature in order to determine whether other support for this conclusion existed. Studies were included if they compared the metabolic rate of a mammal(s) under different manipulations of ambient temperature (i.e. temperature that did not vary from daily or seasonal changes). Although metabolic rate can be measured accurately in a number of ways, the papers included in this survey all investigated metabolic rate via oxygen consumption rates. Studies were only used if conducted under controlled laboratory settings.

Results from the six studies included in this review are summarized in Table 2. The mammals investigated in these papers reflect a respectable range of body masses (between 11 and 186 g), within which our test organism can be found. All but one of the studies report that when ambient temperature is decreased, metabolic rate increases in mammals, which supports my conclusion. The only exception was in Górecki *et al*. (1990). In this paper, below 30°C, metabolic rate behaved as expected, increasing in response to decreases in temperature. However, above 30°C, metabolic rate increased slightly with increasing temperature for both *Mus musculus* and *Mus spretus*. The reason for this anomaly is that the temperature range in this study exceeded 30°C, which is the thermoneutral temperature for mice (Speakman and Keijer 2013 p.5). Although this observation does not directly contradict my hypothesis, since it would be counterproductive for a thermoregulating animal to *increase* its metabolic heat production if ambient temperature had already exceeded the animal's ideal, thermoneutral temperature, it does suggest that my hypothesis may be incomplete. Instead, mammals are likely to thermoregulate through metabolic heat only when their body temperature is below their thermoneutral temperature range.

External Validity of Conclusions

Our experiment, as well as the studies reviewed in Table 2, were conducted under controlled laboratory conditions. Thus, while it is quite clear that small mammals are able to thermoregulate using metabolic heat under low temperature regimes simulated in laboratory conditions, it is less clear what occurs in animals' natural habitats when they are subject to seasonal and daily variations in temperature.

So how are mammals able to cope when exposed to extreme or prolonged cold in the wild? The high energetic cost of endothermy makes it unsustainable under certain conditions. In particular, when an animal's food consumption is low, or when the ambient temperature is low for a prolonged period, the metabolic cost of maintaining body temperature elevated well above ambient temperature becomes too great. One of the strategies that mammals have evolved for enhancing survival under such conditions is known as torpor, which describes the (relatively

short-term, in comparison to hibernation) depression of metabolism below basal metabolic rate, causing body temperature to decrease considerably until it reaches only a few degrees above ambient temperature (Swoap 2008 p.817). This strategy allows a great many small mammals to survive through periods of cold weather when food may be scarce. For example, torpor has been observed in bats (Currie *et al*. 2015 p.R34), mice (Hudson and Scott 1979 p.205) and shrews (Thompson *et al*. 2015 p.1), though notably not in rats (Yoda *et al*. 2000 p.R134).

Since it is such a common strategy, a better understanding of torpor will shed light on the energy balance and life history strategies of many small mammals. In particular, it would be interesting to investigate the precise physiological or environmental processes involved in the initiation and conclusion of torpor. Elucidating the mechanisms involved will help us determine to what extent the conclusions we reached in this study regarding mammalian thermoregulation correctly reflect the strategies used by mammals in their natural environments.

Table 2. Effect of increased temperature on metabolic rate in small mammals				
Study	**Species**	**Mass (g)**	**Effect of Decreased Temperature on Metabolic Rate**	**Temperature Range Studied (°C)**
Present Study	*Mus musculus* (house mouse)	35.6 ± 2.1 (mean ± 95% CI)	Increase	13 to 26
Martin *et al.* 1980 p.521 (Table 1: singles)	*Mus musculus* (house mouse)	26.5 (mean)	Increase	10 to 30
Yousef *et al.* 1971 p.711 (Table 1: control males and females)	*Tupaia chinesis* (tree shrew)	148.4 - 186.4 (range)	Increase	11 to 35
Fairfield 1948 p.358 (text description)	*Rattus norvegicus* (brown rat, Wistar strain)	N/A	Increase	20 to 35
Soriano *et al.* 2002 p.449-450 (Figs. 2 and 3)	*Sturnira erythromos* (hairy yellow-shouldered bat)	15.9 (mean)	Increase	10 to 38
	Tadarida brasiliensis (Mexican free-tailed bat)	11.0 (mean)	Increase	10 to 38
Baldo *et al.* 2015 p.115 (Fig. 2)	*Ctenomys talarum* (tuco-tucos)	121.24 - 142.46 (range)	Increase	5 to 35
Górecki *et al.* 1990 p.211 (Fig. 2)	*Mus musculus* (house mouse)	13.2 - 18.6 (range)	Mostly Increase	0 to 33
	Mus spretus (Algerian mouse)	21.8 ± 1.8 (mean ± 95% CI)	Mostly Increase	-5 to 33

Thermoregulation in Small Amphibians

The second hypothesis tested in this paper was that small amphibians are not capable of thermoregulation using metabolic heat. Although basal metabolic processes in amphibians do release a small amount of heat, we assume that the effect of this heat on body temperature is negligible, since, in ectotherms, the factor that has the greatest influence on body temperature is the temperature of the surrounding environment. Since amphibians are ectotherms, they should be unable to thermoregulate by adjusting their metabolic rates, and their metabolic rates should be independent of temperature. Thus, if small amphibians are not capable of thermoregulation using metabolic heat, then we predict that mass-specific oxygen consumption rate should not be significantly different between the cold treatment and the control. Our results show that the mass-specific oxygen consumption rate of *Rana pipiens* is not significantly different between ambient temperatures of 8°C and 24°C. This aligns with our predicted result, and provides evidence in support of our hypothesis. We conclude that small amphibians are not able to thermoregulate using metabolic heat.

Validity of Experimental Protocol: *Rana pipiens*

In order to determine whether the methods used in this paper are valid, we compared our results to values present in the published primary literature. To warrant inclusion in our review, studies were required to have been conducted under controlled laboratory settings, and to have sufficiently large (≥3) sample sizes.

Our comparison of *Rana pipiens* oxygen consumption rates included only those studies that reported resting oxygen consumption rates. Additionally, studies reporting submerged oxygen consumption rates were omitted, due to the possibility that the activity, and therefore the metabolic rate, of *R. pipiens* at rest differs when submerged. (To illustrate this possibility, Hutchison and Dady (1964 p.157) reported a submerged oxygen consumption rate of 0.02015 O_2 hr^{-1} g^{-1} at 25°C, a value that is much lower than all of the studies included in our final comparison; see Table 2). A total of five studies were used in our final comparison, and their results are summarized in Table 3. Overall, our mean rate of oxygen consumption was considerably larger (between 1.3 and 4.5 times greater) than found in the primary literature. Like in our study, four of these five studies used closed respirometry for their oxygen consumption measurements. Thus, it is unlikely that differences in experimental set-up were the cause for this difference in values. Furthermore, since our frogs were similar in size and identical in post-absorptive status to the frogs used in the other studies, it is also unlikely that the differences are due to differences in test subject characteristics.

The most notable difference between our study and the other five is acclimation duration. In our study, frogs were only allowed 10 minutes to acclimate, while the studies included in my comparison all reported acclimation times of 1 week or greater. If *Rana pipiens* require longer

acclimation times than we provided, then the oxygen consumption rates we reported may not be valid. In order to test this possibility, follow-up studies are required, wherein the frogs will be allowed at least a week to acclimate to their treatment temperatures before their metabolic rates are measured.

Table 3. Literature comparison of values for mass-specific oxygen consumption of *Rana pipiens*							
Study	**Oxygen Consumption Rate (mL O_2 hr^{-1} g^{-1})**	**Mass (g)**	**Experimental Temperature (°C)**	**Acclima-tion Period**	**Sample Size**	**Test Subjects**	**Additional Notes**
Present study	0.16 ± 0.04 (mean ± 95% CI)	41.5 ± 5.6 (mean ± 95% CI)	24 ± 1 (mean ± 95% CI)	10 min	6	Mixed sex, adult, post-absorptive	Closed respirometry
Turney and Hutchison 1974 p.588 (Table 1: routine - controls for 1200)	0.12250 ± 0.00911 (mean ± 95% CI)	20 - 45 (range)	25 (mean)	1 week	25	Mixed sex, post-absorptive	Closed respirometry
McNabb 1969 p.278 (calculated from Fig. 2 regression equation for control)	0.0359 (mean)	N/A	18 (mean)	1 week	3	Mixed sex, post-absorptive	Open flow respirometry
Seymour 1973 p.107 (Table 4)	0.042 ± 0.023 (mean ± 95% CI)	38.39 (mean)	20 (mean)	N/A	6	Post-absorptive	Closed respirometry
Hillman and Withers 1979 p.2102 (taken from Fig. 1 for a 40g frog at rest)	0.09 (mean)	40 (mean)	25 to 27 (range)	N/A	12	Post-absorptive	Closed respirometry
Guimond and Hutchison 1968 p.181 (Table 1: LD16:8 frogs used)	0.12167*	31.62 (mean)	25 (mean)	2 weeks	8	N/A	Closed respirometry

* This paper reported mean cutaneous oxygen consumption as well as mean pulmonary oxygen consumption. This value reflects the sum of these means.

Validity of Conclusions: Thermoregulation in Small Amphibians

The results from our study supported the hypothesis that small amphibians are not able to thermoregulate using their metabolic heat. A survey of literature for amphibians was conducted using the same filtering requirements as in the mammalian survey.

Results from the eight studies included in the review are summarized in Table 4. All eight studies show that decreases in ambient temperature produce decreases in metabolic rate, contradicting our findings. This difference suggests a flaw in our methodology, particularly since the literature review not only covers a large scope of amphibian types, but also six species in the *Rana* genus, including *R. pipiens* itself.

A likely explanation for this difference deals with the length of the acclimation period. In our study, we only allowed our frogs to acclimate to their new temperatures for 10 minutes before recording oxygen consumption rates. In the studies included in our review, animals were commonly given several hours or days to acclimate to their treatment temperatures, with the lowest reported acclimation period being 20 - 40 minutes, which is still significantly longer than the acclimation period we used in our experiment. Hutchison and Dady (1964 p.151) mention the importance of amphibian thermal history in their paper, noting that preliminary experimentation revealed how three weeks was the minimum time required for their test animals to fully acclimate to test temperatures. Although their study was not included in my review because it involved submerged oxygen consumption rates, their observation is still useful in confirming that long acclimation periods are required when studying oxygen consumption rate under different temperature regimes. Because of this, replication of the present study with a longer acclimation period is recommended.

It is worth noting that our practice of using mean oxygen consumption rate in analysis may have resulted in oxygen consumption rates not indicative of actual oxygen consumption, since our data do not reflect changes in oxygen consumption with time. When running the trial for our frog under cold treatment, we noticed that its oxygen consumption rate decreased with time (personal observation). Thus, it is likely that our observations occurred while the animals were still acclimating. Therefore, an alternative method of refining our protocol would be to use the same methodology, but to test animals for a longer period of time, and only to include oxygen consumption rates in analysis after they had stabilized after an initial period of change. This would be another way of ensuring that animals had acclimated fully.

Recall that my initial prediction was for temperature to have no effect on metabolic rate. In light of the results outlined in these eight review studies, my prediction is most likely incorrect. When coming up with my prediction, I assumed that the only way in which metabolic rate could change was if the animal itself was using it in thermoregulation. However, I failed to consider the effect that temperature itself has on enzyme activity, and therefore, on metabolic processes. Thus, it is likely that my hypothesis of amphibians being unable to actively thermoregulate by

using their metabolic heat is still correct, and that the apparently contradictory results from primary literature are due to the significant influence that ambient temperature has on basal metabolic rate.

Table 4. Effect of increased temperature on metabolic rate in small amphibians					
Study	**Species**	**Mass (g)**	**Effect of Decreased Temperature on Metabolic Rate**	**Temperature Range Studied (°C)**	**Acclimation Period**
Present Study	*Rana pipiens* (northern leopard frog)	42.4 ± 3.5 (mean ± 95% CI)	No change	8 to 24	10 min
Holzman and McManus 1973 p.835 (Table 1)	*Rana vergatipes* (carpenter frog)	2 - 12 (range)	Decrease	5 to 25	20 - 40 min
McAllister and Fitzpatrick 1989 p.440 (Table 1)	*Eurycea neotenes* (Texas salamander)	0.20 - 0.92 (range)	Decrease	5 to 25	1 - 2 hr
Finkler 2006 p. 104 (Fig. 1)	*Ambystoma texanum* (small-mouthed salamander)	8.95 - 10.98 (range)	Decrease	5 to 20	2 hr
Chiu and Tong 1979 p.552 (Table 1)	*Rana tigrina* (Chinese bullfrog)	45 - 60 (range)	Decrease	15 to 25	7 days
Homyack *et al.* 2010 p.145	*Plethodon cinereus* (eastern red-backed salamander)	0.63 ± 0.16 (mean ± 95% CI)	Decrease	10 to 30	N/A
Feder 1982 p.26 (Table 2)	*Rhinella marina* (form. *Bufo marinus*) (cane toad)	41.7 - 160.3 (range)	Decrease	15 to 35	7 - 8 days
	Ooeidozyga (syn. *Occidozyga*) *laevis* (common puddle frog)	3.6 - 15.0 (range)	Decrease	15 to 35	7 - 8 days
	Rana cancrivora (crab-eating frog)	4.7 - 36.2 (range)	Decrease	20 to 35	7 - 8 days
	Hylarana (form. *Rana*)	13.1 - 24.9	Decrease	15 to 35	7 - 8 days

	erythraea (common green frog)	(range)			
	Rana magna (ambiguous)	11.1 - 57.3 (range)	Decrease	20 to 30	7 - 8 days
Currens *et al.* 2002 p.491 (	*Ambystoma talpoideum* (mole salamander)	3.2 - 5.1 (range)	Decrease	10 to 15	N/A
Seymour 1973 p.107 (Table 4)	*Rana pipiens* (northern leopard frog)	38.39 (mean)	Decrease	10 to 30	N/A
	Bufo cognatus (Great Plains toad)	39.58 (mean)	Decrease	10 to 30	N/A
	Rana catesbeiana (American bullfrog)	43.55 (mean)	Decrease	10 to 30	N/A

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