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# Evaluating the Energetic Value of Lepidoptera Using Bomb Calorimetry

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## Abstract

Lepidopterans are a core resource for many of North America's insectivorous bats. These predators consume Lepidoptera of varying sizes, and some bat species remove the wings of lepidopteran prey prior to consumption. Selection of larger prey and subsequent wing removal may allow bats to optimize the energetic value afforded by lepidopteran prey. To explore the relationships between caloric yield, body size, and wing presence, laboratory-reared *Trichoplusia ni* moths were grouped into large and small size classes. Wings were removed from half of the moths in each size class. Bomb calorimetry was used to determine the gross heat (cal/g) of moths in each treatment. To account for potential differences in energetic value among species, specimens of *Malacosoma americanum*, *Halysidota tessellaris*, and *Iridopsis sp.* moths were also combusted. Larvae of *M. americanum* were field-collected in April 2012 and reared in the laboratory. Adult *H. tessellaris* and *Iridopsis sp.* moths were wild-caught using an illuminated substrate at Mammoth Cave National Park in June - July 2015. No differences were detected for size class or wing condition of *T. ni* ( $P \geq 0.05$ ). Additionally, no differences were detected in the caloric yields of the various lepidopteran species, except between *Ma. americanum* and *Iridopsis sp.* ( $P = 0.03$ ). These results suggest that lepidopteran prey of various species and sizes may be of similar prey quality, and that the removal of wings by bats may be unrelated to caloric yield. Even so, we believe the lack of differences detected in this study indicate that our approach was likely too coarse of a method to capture subtle energetic differences among lepidopteran prey. Future studies including additional insect orders will clarify the potential limitations of conducting prey quality studies by bomb calorimetry.

## Introduction

Lepidoptera are a core resource for many of North America's insectivorous bats, and have been detected in the diets of all Kentucky bat species tested (Lacki et al. 2007). The gleaner species *Myotis septentrionalis* and *Corynorhinus rafinesquii* are lepidopteran specialists, with this prey taxon representing nearly 50% of the diet of *M. septentrionalis* (Dodd et al. 2012) and more than 80% of the diet of *C. rafinesquii* (Lacki and Dodd 2011). Lepidoptera are also common in the diets of more generalist predators, including *M. lucifugus*, *M.*

*sodalis*, and *Perimyotis subflavus*. Although *M. lucifugus* and *M. sodalis* may consume diverse diets, these species often rely on lepidopteran prey (Brack and LaVal 1985, Whitaker 2004, Feldhamer et al. 2009, Clare et al. 2014). The generalist predator *P. subflavus* opportunistically consumes soft-bodied arthropods, including lepidopterans (Whitaker 2004, Lacki et al. 2007, Dodd et al. 2014).

The ubiquity of Lepidoptera as a prey resource for insectivorous bats is thought to be a consequence of high digestive efficiency. The carbohydrate chitin, which

forms arthropods' hard exoskeletons, is difficult for most mammals to digest (Strobel et al. 2013). However, some bat species have the ability to optimize digestion of arthropod prey due to specialized gastrointestinal microflora (Strobel et al. 2013, Whitaker et al. 2004). These bats, including *M. septentrionalis*, *M. lucifugus*, *M. sodalis*, and *P. subflavus*, host chitinase-producing bacteria in the digestive tract (Whitaker et al. 2004). The enzyme chitinase promotes the breakdown of chitin, but does not allow it to be completely digested. As a result, insects with high chitin levels have low digestive efficiency (Barclay et al. 1991).

Some bats (e.g., *Corynorhinus* species) reject lepidopteran body parts such as the legs and wings (Lacki and Dodd 2011). This behavior may be a result of low palatability, but is thought to be due to low digestibility of these chitin-rich structures (Barclay et al. 1991). Smaller moths have lower digestive efficiency, likely due to the increased difficulty of removing indigestible or unpalatable structures from small prey (Barclay et al. 1991). Although larger moths are more digestible, it is not yet clear whether selection of larger moths affords a caloric benefit.

The relationships between caloric yield, body size, and wing presence are poorly understood. Thus, our objectives were: (1) explore the relationships between caloric yield, body size, and wing presence by determining the mean gross heat (cal/g) generated across large, small, winged, and wingless representatives of a model lepidopteran species (*Trichoplusia ni*), (2) investigate potential differences in energetic value among species by using bomb calorimetry to combust *Malacosoma americanum*, *Halysidota tessellaris*, and *Iridopsis* sp. moths, and (3) evaluate the overall viability of bomb calorimetry as a

method of conducting prey quality studies.

## Methods

*Malacosoma americanum* tents and larvae were field-collected in April 2012 at Mammoth Cave National Park (N 37° 11.83', W 86°04.50'). Tents (n = 1-3) were placed in plastic housing (32 cm × 26 cm × 9 cm) lined with paper towels to absorb moisture and provide substrate. The developing insects were supplied ad libitum with fresh, field-collected *Prunus* sp. foliage. Throughout the three-week rearing process, some tents were disposed of to maintain hygienic conditions. Pupae were subsequently removed from plastic housing and placed individually in plastic diet cups (30 ml) until emergence. Adult moths were flash-frozen within 24 hr of emergence; adult moths (in diet cups) were submerged in liquid nitrogen for 5-10 seconds, and immediately stored in a -80°C freezer.

Larvae of *T. ni* were reared communally from 25 eggs on 110 g of a pinto bean-based diet in a 240 ml Styrofoam cup kept at ambient conditions (Evenden and Haynes 2001). Other details of the rearing methods are described by Shorey and Hale (1965). Pupae were separated, sexed, placed individually in diet cups (30 ml), and flash-frozen in liquid nitrogen within 24 hr of adult emergence. Specimens were then stored in a -20°C freezer. Adult *T. ni* were divided into large and small size classes (individual masses of  $118 \pm 0.80$  and  $87 \pm 0.69$  mg, respectively), and wings were removed from half of the moths in each size class.

Wild-caught moths were collected from June - July 2015 at the Mammoth Cave International Center for Science and Learning (N 37° 12.44', W 86° 7.93'). A cotton sheet was hung vertically and stretched taut at ground level; the sheet was

illuminated between approximately 2000 and 2300 hours with a 10 w black light and electrical harness (Universal Light Trap, Bioquip Products, Rancho Dominguez, CA, USA) (Figure 1). Lepidoptera attracted to the sheet were collected in plastic diet cups and immediately placed on ice. Specimens were temporarily stored at -18°C and transferred to -80°C within 7 d. Although numerous taxa were collected, *H. tessellaris* and *Iridopsis sp.* were selected for combustion due to their ready abundance and conspicuous appearance (Covell 2005).

To prepare for combustion, all frozen Lepidoptera were transferred to open, heat-resistant vials and dried in a 55°C oven for approximately 24 hr. Specimens were consolidated by treatment (Table 1) and ground with a mortar and pestle for 30-60 seconds until a coarse powder was attained. A Parr 1281 Oxygen Bomb Calorimeter (Parr Instrument Company, Moline, IL, USA) was calibrated daily using a 1.0 g benzoic acid



**Figure 1:** Cotton sheet deployed at Mammoth Cave National Park to sample Lepidoptera, illuminated by 10 w black lights with electrical harnesses.

**Table 1:** Summary of *Trichoplusia ni*, *Malacosoma americanum*, *Halysidota tessellaris*, and *Iridopsis sp.* treatments. The treatment marked with an asterisk was not included in the initial comparison of small vs. large-bodied and winged vs. wingless *T. ni*, but was included in the comparison of species. N = number of samples combusted per treatment.

Species	Size Class	Wings	Sample Weight (mg)	N
<i>T. ni</i>	Large	Yes	250	6
<i>T. ni</i>	Large	No	250	6
<i>T. ni</i>	Small	Yes	250	6
<i>T. ni</i>	Small	No	250	6
<i>T. ni</i> *	-	Yes	250	2
<i>Ma. americanum</i>	-	Yes	200-250	5
<i>Ma. americanum</i>	-	Yes	400-450	5
<i>Ma. americanum</i>	-	Yes	600-650	5
<i>Ma. americanum</i>	-	Yes	800-850	3
<i>H. tessellaris</i>	-	Yes	250	7
<i>Iridopsis sp.</i>	-	Yes	250	4

pellet (Parr Instrument Company, Moline, IL, USA). To determine whether sample weight affects gross heat generated by bomb calorimetry, we combusted *Ma. americanum* samples weighing 200-250 mg, 400-450 mg, 600-650 mg, and 800-850 mg. Following this assessment of methods, a standard sample weight of 250 mg was used for *T. ni*, *H. tessellaris*, and *Iridopsis sp.* treatments. The number of bomb calorimetry samples combusted was dependent upon the volume of processed lepidopteran material available for each treatment. All treatments were combusted according to instructions provided by the bomb calorimeter manufacturer.

We determined the mean gross heat  $\pm$  SE (cal/g) generated by the combustion of each treatment. A one-way analysis of variance (ANOVA) was used to test for differences between *Ma. americanum* sample weight



classes, and a 2×2 ANOVA was used to test for differences between *T. ni* treatments. To test for potential differences in energetic value among species, Wilcoxon Rank-Sum tests were used to make pairwise comparisons (using 250 mg samples).

## Results

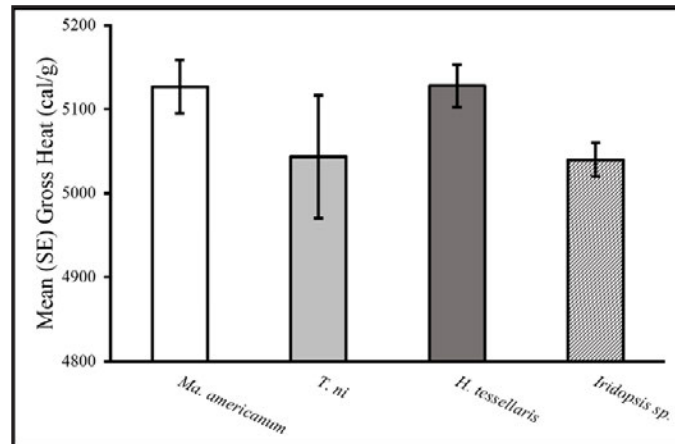
*Malacosoma americanum* was found to have a significantly greater caloric yield than *Iridopsis sp.* ( $W_{5,4} = 19, P = 0.03$ ), although no additional differences were detected between pairwise comparisons across species (Figure 2). No differences were detected between any *Ma. americanum* weight classes ( $F_{3,14} = 1.6, P > 0.05$ ) (Figure 3) or *T. ni* treatments ( $F_{3,23} = 0.86, P > 0.05$ ) (Figure 4).

## Discussion

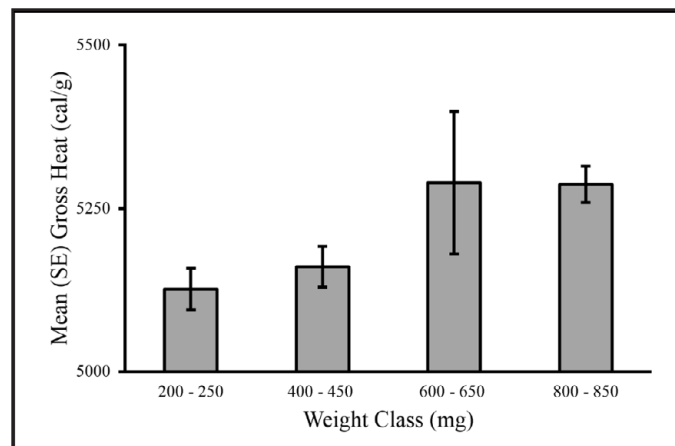
The lack of differences detected between *Ma. americanum* weight classes suggests the gross heat generated by combustion is likely not affected by sample weight. These data indicate that any sample weight (adhering to manufacturer's specifications for safe calorimeter usage) could be combusted effectively. Based on these findings, we recommend that future studies reduce sample weights to conserve raw material and maximize the number of combustion reactions possible.

We found no differences in energetic value between any *T. ni* treatment, suggesting that the removal of lepidopteran wings by bats may be unrelated to caloric yield. These results support the commonly accepted hypothesis that bats reject lepidopteran wings due to indigestibility (Barclay et al. 1991, Lacki and Dodd 2011). The lack of any significant differences between large and small *T. ni* indicates that caloric yield is independent of body size. However, *Ma. americanum* appears

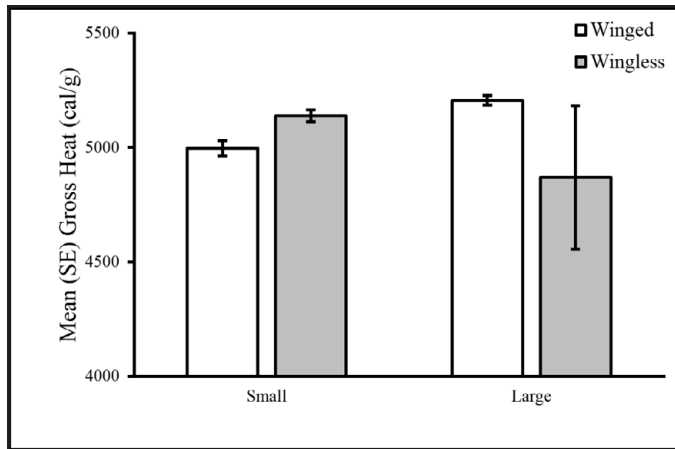
to have a significantly greater caloric yield than *Iridopsis sp.*, likely due to the larger body size of *Ma. americanum*. This explanation is supported by previously published literature regarding the energy density of fish; Glover et al. (2010) found



**Figure 2:** Mean gross heat  $\pm$  SE (cal/g) generated by combustion of coarsely ground samples of *Malacosoma americanum*, *Trichoplusia ni*, *Halysidota tessellaris*, and *Iridopsis sp.* using bomb calorimetry. We combusted five samples of *Ma. americanum*, twenty-six of *T. ni*, seven of *H. tessellaris*, and four of *Iridopsis sp.*



**Figure 3:** Mean gross heat  $\pm$  SE (cal/g) generated by combustion of coarsely ground *Malacosoma americanum* samples using bomb calorimetry. Five samples weighing 200-250 mg, 400-450 mg, and 600-650 mg, and three samples weighing 800-850 mg were combusted.



**Figure 4:** Mean gross heat  $\pm$  SE (cal/g) generated by combustion of coarsely ground *Trichoplusia ni* samples using bomb calorimetry. Six samples were combusted per treatment (small with wings present, small with wings removed, large with wings present, large with wings removed).

that the caloric yield of largemouth bass is directly related to body mass, with larger bass generally possessing greater energetic density.

Given that Lepidoptera are relatively soft-bodied (Freeman 1981), we suspect these prey may have comparatively less chitin than many insect orders, thus allowing predators to maximize digestive efficiency. Although it is likely that consuming Lepidoptera affords a digestive advantage, the similarity in energetic value among study species may suggest that lepidopteran prey of various species and sizes is of similar prey quality. However, based on the inconsistency of our results regarding caloric yield and body size, we believe the lack of differences detected in this study indicates our technique is likely too coarse of a method to capture subtle energetic differences among Lepidoptera. Future studies including additional insect orders will clarify the potential limitations of conducting prey quality studies by bomb calorimetry.

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