The Metabolic Rates of Giant Panda and Red Panda

A Thesis

Submitted to the Faculty

of

Drexel University

by

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in partial fulfillment of the

requirements for the degree

of

Doctor of Philosophy

June 2015

Dedication

For James R. Spotila,

my great mentor.

Acknowledgement

First of all, I would like thank Dr. James R. Spotila who is my mentor, the career guide, and friend. Without him, I could not finish my research. I thank all my other committee members as well, Susan Kilham, Frank Paladino, Harold Avery, Kenneth Lacovara and Michael O'Connor.

I give special thanks to Benjamin Kilham, the bear expert, who helped me understand the behaviors of black bear; John Spotila and Sheri Yi from Global Cause Foundation, they managed all the research activities; Ken Nagy and John Speakman, who gave me a lot of useful advice on DLW technique; Edward Standora who helped me on metabolic rate study methods; Rong Hou who helped me in coordinating with different people at the Panda Base.

I thank my lab mates, Aliki Panagopoulou, Abby Dominy, Samir Patel, Gabi Blanco, Jack Suss, Carlos Mario Orrego, Kate Walsh, Lori Lester, Jake Owens, Steve Pearson, Jules Winters, Dane Ward, Kevin Smith, Yi Hu, Noga Neeman, Tom Radzio, Ryan Rebozo, Marilyn Sobel and Kathryn Christopher who always gave me supports when I needed it. I thank the staff from Panda Base who helped me to finish all the experiments, Zhihe Zhang, Xiangming Huang, Jinchao Lan, Zhi Yang, Li Luo, Songrui Liu, Wenjun Huang, Xiaolin Yang, Kongju Wu, Jincang He and Mingchao Yang.

I thank Guan Yin and Jingyong Xu, from Chengdu University of Technology, who helped me analyzing DLW samples. I also thank Tian Shi, Siqiang Yang and Yue Xie,

from Sichuan Agricultural University, who helped me taking goat blood samples for calibrating the mass spectrometer.

Finally, I want to thank my families, especially my parents, who gave me support and encouraged me to study abroad.

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Abstract

The giant panda (*Ailuropoda melanoleuca*) is one of the most endangered vertebrates and an iconic symbol of conservation. While progress has been made in understanding its genome, landscape ecology and anthropogenic effects on its populations, many aspects of its biology are unknown. I measured resting metabolic rate of the giant panda and compared it to metabolic rates of other large mammals. The giant panda has a thermal neutral zone that ranges from at least 9 to 26 ℃. Its metabolic rate is 6.0% to 44.3% below that predicted by regressions of mass vs. metabolic rate in the literature and that may be due to its strictly herbivorous bamboo diet. These results raise questions about the universal predictive power of physiological relationships such as the metabolic theory of ecology that attempt to predict ecological relationships based on metabolism and body size without considering other variables. For these predictions to be useful and truly reflective of the study animal, basic metabolic data are needed on more large mammals and animals with varied diets, before reliable predictive theories can be formulated.

I also measured the field metabolic rate of giant panda. The results showed that FMR varied between individuals despite the active time for each individual being very similar. The water-loop of the giant panda is much faster than predicted. The mean daily energy expenditure (DEE) was 21,592 KJ, (SD = 13,323) per day. That means there is enough bamboo in 1 km^2 of the Wolong Reserve to provide food for 81,726 panda days.

I measured the resting metabolic rate of red panda as well. There was no difference in metabolic rate between male and female red pandas. The RMR of red panda was higher in the winter than in the summer. McNab measured 2 red pandas in 1987 and found a very low metabolic rate, 0.153 ml/g/h. In my 17 red panda experiments, 0.204 ml/g/h was the lowest value that I measured. The average in summer (0.290 ml/g/h) was twice as high as McNab's mean. The red panda has a higher metabolic rate than previously measured.

CHAPTER 1: INTRODUCTION

The giant panda (*Ailuropoda melanoleuca*) is a mysterious creature that has enthralled the public since Père Armand David first discovered it for Western science in 1869. The giant panda is one of the most endangered vertebrates and an iconic symbol of conservation. It is now the focus of intense conservation efforts in China and there has been considerable success in breeding giant pandas in captivity and in studying their genome (Li et al., 2010). The main long-range goal of captive breeding efforts is to reintroduce giant pandas back into their wild habitat so that they can survive and breed, and reestablish viable populations in protected reserves. However, we lack a basic understanding of the physiological ecology of the giant panda that is necessary for ensuring that there are sufficient resources for the animals and that they will be able to adapt to the effects of climate change that are and will be occurring in their reserves (Tuanmu et al., 2013). Due to these reasons my research focused on the metabolic rates of the giant panda and red panda (*Ailurus fulgens*). Knowing the metabolic rate of these animals is the first step in understanding their energetic requirements or food requirements, which is needed before we proceed with the reintroduction project.

Giant pandas are large mammals and red pandas are small mammals. It is known that body mass is one of the most important factors affecting metabolic rate. There is a positive relationship between metabolic rate and body mass, and a negative relationship between metabolic rate per unit mass and body mass. It is the classic body size vs. metabolic rate theory of Kleiber (1961). This is because small animals have less ability to maintain a constant body temperature than large animals because they are more closely tied to the environment through heat exchange (Porter and Gates, 1969). Therefore, we need to know how body mass affects the metabolic rates of pandas.

Sieg et al. (2009) discuss the relationship between body size and metabolic rate. There has been much discussion about a power law in which the slope of metabolic rate to body mass relationship is either 0.67 or 0.75 (White and Seymour, 2003; O'Connor et al., 2007). Brown and others state that the slope is 0.75 and that this is a universal law in ecology- the metabolic law of ecology (Brown and Richard, 2012). However, Sieg et al. (2009) demonstrate that 0.75 is not always in the 95% confidence intervals of slope estimates and clade-specific slopes vary from 0.5 to 0.85, depending on the clade and regression model. For example, the slope of Soricids is 0.527, the slope of Chiropterans is 0.872, the slope of Rodents is 0.533, the slope of Primates is 0.775 and the slope of Marsupials is 0.757. So Sieg et al. suggest that there is no set approach to analyzing allometric data, and disagree with the existence of universal scaling of metabolic allometry in mammals. Finally, they suggest new measurements of mammalian basal metabolism should be done in order to better understand the application of particular analytical models to allometry data.

The metabolic rates of bears

The closest relatives of the giant pandas are the bears. Therefore, we should be able to estimate the metabolic rate of giant pandas from the metabolic rates of bears. However, there are no good data on that subject. There are only a few papers about the metabolic rates of bears. Most of them discuss the metabolic rates of hibernating animals. Watts (1988a) did an experiment on hibernating grizzly bears (*Ursus arctos horribilis*). Metabolic rate averaged 68% of predicted values from Kleiber (1975) for basal (resting) metabolism, 2788 kcal/day (-22℃) and 3779 kcal/day (-16℃). Watts also did experiments on polar bears (*Ursus maritimus*) and black bears (*Ursus americanus*) in 1987 and 1988 (Watts et al., 1987; Watts and Cuyler, 1988b). But, all of the experiments were on hibernating animals.

In 1981, Best's paper, on physiological indices of activity and metabolism in the polar bear, showed the relationship between metabolic rate and cardiac frequency and walking speed. He used a power law equation to calculate the metabolic rate of the bear and estimated that the energy cost per step per kg would increase if walking speed increased and that it would decrease with body size increase.

McNab (1992) measured the rate of oxygen consumption in two female sloth bears (*Melursus ursinus*) at ambient temperatures of 9-38.5 ℃. Measurements of resting sloth bears were not obtained at temperatures < 9 ℃. At temperatures near 10 ℃ the bears became active and it was not possible to measure basal metabolic rate. The mean basal rate of metabolism was 0.126 ml/g/h O_2. The rate of metabolism during activity ranged from 0.300 to 0.713 ml/g/h O_2, which was 2.4-5.7 times the basal metabolic rate. Immediately after eating, the bears had a rate of metabolism ca. 1.3 times the basal metabolic rate (ca. 0.160 ml/g/h) O_2. McNab stated that vertebrate-eating bears, in contrast, have higher basal rates, even when denning, but he provided no data. The thick fur coat of the sloth bear may partly compensate for its low basal rate.

Giant pandas

No one has measured the metabolic rate of giant pandas (*Ailuropoda melanoleuca*). However, some papers discussed the related question of panda's activity and energy. Han's (2001) paper measured daily activities of five pandas in Wolong Wu-Yi-Peng Reserve during 1981-1982. He used variance analysis as the index for outdoor activities of giant pandas. The results show that season and sex have significant influence on daily activities. Sex of the animal also had a great influence on yearly activities.

Zhou et al. (1990) measured the digestion of giant pandas. In that paper, they studied two pandas and measured total food intake, excretion, and total digestion. In addition, the authors also measured the percentage of crude protein, crude fat, crude fiber and nitrogen free extract digestion. Protein has the highest digestion rate, then fat, and nitrogen free extract. Fiber has the lowest digestion rate. According to their experience in feeding pandas, fiber can increase the activity of stomach and intestine. It is good for panda digestion. If pandas reduce the intake of fiber, the digestion efficiency also is reduced.

Wei (2000a) analyzed the energy flow through populations of both giant panda and red panda (*Ailurus fulgens*). The results indicate that energy flow through the giant panda population is higher than that through the red panda population. Giant panda energy flow is $1.86*10^6$ kJ/km^2 ·yr, 2.9 times as much as the red panda ($6.34*10^5$ kJ/km^2 ·yr). Though useable food resources of giant pandas and red pandas made up only 12.79% and 3.93% of total above ground standing biomass, energy intake and energy flow through populations of the two species each year accounted for 0.01% to 1.0% of the usable food biomass or above ground standing biomass. Wei (2000a) concluded that bamboo resources would not be a limiting factor for the population increases of both pandas unless the bamboo reached its reproductive age.

Red pandas

Wei (2000b) also studied the seasonal energy utilization of bamboo by red pandas. The results show that energy digestibility is high in summer-autumn, intermediate in spring, and low in winter. These variations correlated positively with the nutrients of bamboo. The daily metabolic energy requirement measured varied from 2603.3 kJ in the spring to 3139.8 kJ in the summer-autumn to 2740.8 kJ in the winter. In spring, the new leaves are just beginning to form and newly formed leaves are only a small proportion of the leaves on the branches. Leaves are less nutritious in this season than in the summer-autumn. However, the red panda can get supplemental energy to meet its requirements through feeding on shoots during this season, similar to the giant panda, because shoots are the most nutritious parts of bamboo. In the summer-autumn, when the leaves are most nutritious, the animals digested and assimilated more energy from leaves and exhibited the highest energy digestibility. During this period, the red panda digested 20.87% more energy than in the spring and 12.89% more than in winter to meet its energy requirements. During the winter, as leaves begin to turn yellow and die, their nutritional value declines, and the energy digestive efficiency decreased to the lowest.

McNab (1988) measured the resting metabolic rates of two red pandas from a zoo. He found that red pandas decreased metabolic rate at low environmental temperatures without reduced body temperature. Red panda has a low rate of metabolism, which was 0.153 ml/g/h oxygen consumption at ambient temperature between 25 ℃ and 36 ℃. This is 39% of Kleiber's prediction. As the temperature falls from 25 ℃ to 19 ℃, the metabolic rate increases. However, when the temperature drops below 19 ℃, the metabolic rate decreases to a minimum of 0.074 ml/g/h between 0 ℃ and 10 ℃. This metabolic rate equals 19% of the value expected from Kleiber and 48% of the rate measured between 25 ℃ and 36 ℃. However, the metabolic rates that McNab measured were also much below those predicted by allometric equations of metabolic rate vs. body size in mammals (Sieg et al., 2009).

Methods of studying MR

Flow-through respirometry method

All flow-through systems for measuring animal respiration measure the CO_2 and O_2 concentration changes made by an organism from an air stream by gas analyzers to get metabolism information. This method can have accurate data measurement, but the flow rate must be known, the gas analyzers must be accurate and recently calibrated, and the effects of the system's configuration must be known. Moreover, an animal must be put into an area such as a chamber from which air can be pumped at a known flow rate and sampled for gas analysis. This method can be used to measure the animal's metabolic rate with good precision by using flow-through systems. A mask or a chamber or a burrow, which is made by the animal itself, is needed for flow-through systems. Because the experiment will pull air through the chamber, the downstream flow rates have to be measured. In addition, the flow measurements will be influenced by the water vapor that is in the air stream, added by the animal, and also by the O_2 consumed and the CO_2 produced by the animal. Likewise, O_2 will be diluted by water vapor and CO_2, and CO_2 will be diluted by water vapor and enriched by the extraction of O_2 consumed by the animal. Because of these reasons the air samples must be dry when they go into the analyzer (Lighton, 2008).

Double labeled water

The double labeled water method (DLWM) was first described by Lifson et al. (1955) It can be used for measuring the metabolic rates of free range mammals and birds accurately and reliably. It is based on the different pathways of hydrogen and oxygen in water overlap of organisms. Hydrogen is only lost by water loss. However, oxygen is lost by both CO_2 loss and H_2O loss, so the oxygen in a given quantity of H_2O will be lost more quickly than the hydrogen. In other words, oxygen has the higher loss rate, and the difference between oxygen and hydrogen decline per unit time is the metabolic rate. (Lighton, 2008)

The DLWM has been used successfully on rats, bats, birds, reptiles, small and large mammals. The daily energy expenditure of root voles (*Microtus oeconomus*), rat (*Rattus norvegicus*), shrew (*Sorex araneus*) and chipmunk (*Tamias stratus*) are now known (Lee and Lifson, 1992; Poppitt et al., 1993; Careau et al., 2012; Careau et al., 2013; Szafrańska et al., 2014). The same is true for several species of birds (Williams and Prints, 1986; Visser et al., 2000; Welcker et al., 2013). The field metabolic rates (FMR) of two species of bats (*Pipistrellus pipistrellus* and *Eptesicus fuscus*) indicate some variation between individuals (Speakman and Racey, 1988; Kurta et al., 1990). For reptiles, the FMR of the desert lizard (*Ctenophorus nuchalis*) differs with season (Nagy, 1995). However, there is no seasonal difference in FMR of juvenile green turtles (*Chelonia* *mydas*) (Southward et al., 2006). Leatherback turtles (*Dernochelys coriaces*) have low FMR so they avoid overheating in the tropics (Wallace et al., 2005).

The daily energy expenditure (DEE) of two kinds of working dog, hunting dog and Inuit sled dog, (*Canis familiaris*) varies from 6647 KJ/day to 16600 KJ/day (Gerth et al., 2010; Ahlstrøm et al., 2011). Junghans et al. (1997) studied goats (African dwarf goat) with two isotope methods, ^{13}C bicarbonate (^{13}C-M) and doubly labeled water method (DLWM). They report that ^{13}C-M is good for measuring short-term (6-12 hours) FMR. The DLWM is good for a long-term FMR study. Black-tailed deer (*Thylogale billardierii)* have about three times higher FMR (1.063 ml/g/h CO_2) than kangaroos (*Macropus giganteus*) (0.369 ml/g/h CO_2) (Nagy et al., 1990). For marine animals, the DLWM gives a higher DEE reading for fur seal (*Callorhinus ursinus*) than a respiratory system (Dalton et al., 2014). The DLWM gave accurate measurements of the FMR of the grey seal (*Halichoerus grypus*) (Sparling et al., 2008). Variation in FMR among individual walrus (*Odobenus rosmarus*) depends upon activity levels (Acquarone et al., 2006). The DLWM has been widely used in energetic studies of hundreds of men, women and children (Kashiwazaki et al., 1995; Black, 1996; Johnston et al., 2007; Djafarian et al., 2010; Ojiambo et al., 2013). Recently this technique has also been used on fruit flies (*Drosophila melanogaster*), a very small insect (Piper, et al., 2014).

Heart rate methods

Boothby (1915) and Krogh and Lindhard (1917) demonstrated that there is a linear relationship between heart rate and oxygen consumption. The heart rate can be obtained by telemetry on free-ranging animals. One needs to derive a calibration equation for the species. The elements in the equation include: 1. Cardiac stroke volume-the amount of blood pumped per heartbeat. 2. Oxygen content of arterial blood. 3. Oxygen content of mixed venous blood. The equation is oxygen consumption = heart rate * Cardiac stroke volume * (Oxygen content of arterial blood - Oxygen content of mixed venous blood). This method relies on the premise that a change in heart rate is a major component in the response of the cardiovascular system of a species to an increase in the demand for oxygen. So it is a linear relationship between heart rate and oxygen consumption, the latter could be used to determine the oxygen consumption (Butler et al., 2004).

Methods in this study

Each method has its own disadvantages. For example, in the respiration system, the gas analyzer is very sensitive and needs care to make it work reliably (Lighton, 2008). You have to be very careful to deal with air temperature, air pressure, air flow rate and in removing vapor. Expenditure and isotope sustaining time are the two major issues for doubly labeled water method (Butler et al., 2004). The price of doubly labeled water is around 200 USD per gram, and 15 grams are needed for each giant panda. The cost is 3000 USD per giant panda. Meanwhile, you need to resample before isotopes run out, or you will lose 3000 USD. The biggest problem of the heart rate method is that you need to derive a calibration equation for each new species (Butler et al., 2004), and the relationship is difficult to develop between activity and heart rate because it is a nonlinear relationship.

Objectives of the Dissertation

In this dissertation I sought to measure the resting metabolic rates of giant pandas and red pandas. I also tested the DLW method to determine if that method was appropriate for measuring the metabolic rate of active giant pandas in the field. In Chapter 2 I present the data and discuss the results for the resting metabolic rate of the giant panda. In chapter 3 I present the field metabolic rate for giant pandas in the Chengdu Research Base of Giant Panda Breeding in Sichuan Province, China. The DLW method worked for giant pandas, but there were some problems that need to be considered in taking that method into the field. In Chapter 4 I present the results for the resting metabolic rates of red pandas. The results differ from those of McNab. Finally, in Chapter 5 I review the findings of the experiments and discuss conservation implications.

List of References

Acquarone, Mario., E. W. Born and J. R. Speakman. 2006. Field metabolic rates of walrus (*Odobenus rosmarus*) measured by the doubly labeled water method. Aquatic Mammals **32**: 363-369.

Ahlstrøm, Ø., P. Redman and J. Speakman. 2011. Energy expenditure and water turnover in hunting dogs in winter conditions. British Journal of Nutrition **106**: 158-161.

Best, R.C., K. Ronald and N. A. Oritsland. 1981. Physiological indices of activity and metabolism in the polar bear. Comparative Biochemistry and Physiology **69:** 177-185

Black, A. E., Coward, W. A., Cole, T. J. and Prentice, A. M. 1996. Human energy expenditure in affluent societies: an analysis of 574 doubly-labeled water measurements. European Journal of Clinical Nutrition **50**: 72-92.

Boothby, W. M. 1915. A determination of the circulation rate in man at rest and at work: the regulation of the circulation. The American Journal of Physiology **37**: 383-517.

Brown, H. B. and M. S. Richard. 2012. The metabolic theory of ecology and its central equation. In Richard M. S., J. H. Brown and A. Kodric-Brown. Metabolic Ecology: a scaling approach, 21-33.American Journal of Physiology **37**: 383-417.

Butler, P. J., J. A. Green, I. L. Boyd, and J. R. Speakman. 2004. Measuring metabolic rate in the field: the pro and cons of the doubly labeled water and heart rate methods. Functional Ecology **18**: 168-183.

Careau, V., P. Bergeron, D. Garant, D. Réale, J. R. Speakman and M. M. Humphries. 2012. The energetic and survival costs of growth in free-ranging chipmunks. Oecologia **171**: 11-23.

Careau, V., D. Réale, D. Garant, F. Pelletier, J. R. Speakman and M. M. Humphries. 2013. Context-dependent correlation between resting metabolic rate and daily energy expenditure in wild chipmunks. The Journal of Experimental Biology **216** : 1-9.

Dalton, A. J. M., D. A. S. Rosen and A. W. Trites. 2014. Season and time of day affect the ability of accelerometry and the doubly labeled water methods to measure energy expenditure in northern fur seals (*Callorhinus ursinus*). Journal of Experimental Marine Biology and Ecology **452**: 125-136.

Djafarian, K., D. M. Jackson, E. Milne, P. Roger and J. R. Speakman. 2010. Doubly labeled water: Multi-point and two-point methods in pre-school children. International Journal of Pediatric Obesity **5**: 102-110.

Gerth, N., P. Redman, J. Speakman, S. Jackson and J. M. Starck. 2010. Energy metabolism of Inuit sled dogs. Journal of Comparative Physiology **180**:577-589.

Han, C., Y. Cao, C. Tang, G. Zhang and D. Li. 2001. Application of quantitative method I and the relation between giant panda's outdoor activities and the sex, age, and surroundings. Journal of Biomathematics **16**: 462-467.

Johnston, S. L., S. S. Erwin, D. M. Souter, B. J. Tolkamp, J. M. Yearley, I. J. Gordon, A. W. Illius, I. Kyriazakis and J. R. Speakman. 2006. Intake compensates for resting metabolic rate variation in female c57bl/6j mice fed high-fat diets. Obesity **15**: 1-7.

Junghans, P., M. Derno, M. Gehre, R Höfling, P. Kowski, G. Strauch, W. Jentsch, J. Voigt and U. Henning. 1997. Calorimetric validation of ^{13}C bicarbonate and doubly labeled water method for determining the energy expenditure in goats. Zeitschrift für Ernährungswissenchaft **36**: 268-272.

Kashiwazaki, H., Y. Dejima, J. Orias-Rivera and W. A. Coward. 1995. Energy expenditure determined by the doubly labeled water method in Bolivian Aymara living in a high altitude agropastoral community. The American Journal of Clinical Nutrition **62**: 901-910.

Kleiber, M. 1932. Body size and metabolism. Hilgardia **6**: 315-353

Kleiber, M. 1961. The Fire of Life. New York London, John Wiley & Sons.

Kleiber, M. 1975. Metabolic turnover rate: A physiological meaning of the metabolic rate per unit body weight. Journal of Theoretical Biology **53**: 199-204.

Krogh, A. and J. Lindhard. 1917. A comparison between voluntary and electrically induced muscular work in man. Journal of Physiology **51**: 182-201.

Kurta, A., T. H. Kunz and K. A. Nagy. 1990. Energetics and water flux of free-ranging big brown bats (*Eptesicus fuscus*) during pregnancy and lactation. Journal of Mammalogy **71**: 59-65.

Lee, J. S., N. Lifson. 1960. Measurement of total energy and material balance in rats by means of doubly labeled water. American Journal of Physiology **199**: 238-242.

Li, R., W.Fan, G. Tian, H. Zhu, L. He, J. Cai, Q. Huang, Q. Cai, B. Li, Y. Bai, Z. Zhang, Y. Zhang, W. Wang, J. Li, F. Wei, H. Li, M. Jian, J. Li, Z. Zhang, R. Nielsen, D. Li, W. Gu, Z. Yang, Z. Xuan, O. A. Ryder, F. C. Leung, Y. Zhou, J. Cao, X. Sun, Y. Fu, X. Fang, X. Guo, B. Wang, R. Hou, F. Shen, B. Mu, P. Ni, R. Lin, W. Qian, G. Wang, C. Yu, W. Nie, J. Wang, Z. Wu, H. Liang, J. Min, Q. Wu, S. Cheng, J.

Ruan, M.Wang, Z. Shi, M. Wen, B. Liu, X. Ren, H. Zheng, D. Dong, K. Cook, G. Shan, H. Zhang, C. Kosiol, X. Xie, Z. Lu, H. Zheng, Y. Li, C. C. Steiner, T. T. Lam, S. Lin, Q. Zhang, G. Li, J. Tian, T. Gong, H. Liu, D. Zhang, L. Fang, C. Ye, J. Zhang, W. Hu, A. Xu, Y. Ren, G. Zhang, M. W. Bruford, Q. Li, L. Ma, Y. Guo, N. An, Y. Hu, Y. Zheng, Y. Shi, Z. Li, Q. Liu, Y. Chen, J. Zhao, N. Qu, S. Zhao, F. Tian, X. Wang, H. Wang, L. Xu, X. Liu, T. Vinar, Y. Wang, T. Lam, S. Yiu, S. Liu, H. Zhang, D. Li, Y. Huang, X. Wang, G. Yang, Z. Jiang, J. Wang, N. Qin, L. Li, J. Li, L. Bolund, K. Kristiansen, G. K. Wong, M. Olson, X. Zhang, S. Li, H. Yang, J. Wang, and J. Wang. 2010. The sequence and de novo assembly of the giant panda genome. Nature **463**: 311-317.

Lifson, N., G. B. Gordon and R. McClintock. 1955. Measurement of total carbon dioxide production by mean of $D_2{}^{18}O$. Journal of Applied Physiology **7**: 704-710.

Lighton, J. B. 2008. Measuring Metabolic Rates. Oxford University Press, Oxford, England.

McNab, B. 1988. Energy conservation in a tree-kangaroo and the red panda. Physiological Zoology **61**: 280-292.

McNab, B. 1992. Rate of metabolism in the termite-eating sloth bear. Journal of Mammalogy **73**:168-172.

Nagy, K. A. and S. D. Bradshaw. 1995. Energetics, osmoregulation, and food consumption by free-living desert lizards, *Ctenophorus* (*Amphibolurus nuchalis*). Amphibia-Reptilia **16**: 25-35.

Nagy, K. A., G. D. Sanson and N. K. Jacobsen. 1990. Comparative field energetics of two macropod marsupials and ruminant. Australian Wildlife Research **17**: 591-599.

O'Connor, M. P., S. J. Kemp, S. J. Agosta, F. Hansen, A. E. Sieg, B. P. Wallace , N. M. McNair and A. E. Dunham. 2007. Reconsidering the mechanistic basis of the metabolic theory of ecology. Oikos **116**: 1058-1072.

Ojiambo, R., A. R. Gibson, K. Konstabel, D. E. Lieberman, J. R. Speakman, J. J. Reilly and Y. P. Pitsiladis. 2013. Free-living physical activity and energy expenditure of rural children and adolescents in the Nandi region of Kenya. Annals of Human Biology **40**:318-323.

Piper, M. D. W., C. Selman, J. R. Speakman and L. Partridge. 2014. Using Doubly-Labeled Water to measure energy expenditure in an important small ectotherm *Drosophila melanogaster*. Journal of Genetics and Genomics **41**: 505-512.

Poppitt, S. D., J. R. Speakman and P. A. Racey. 1993. The energetics of reproduction in the common shrew (*Sorex araneus*): A comparison of indirect calorimetry and the doubly labeled water method. Physiological Zoology **66**: 964-982.

Porter, W. P. and D. M. Gates. 1969. Thermodynamic equilibria of animals with environment. Ecological Monographs **39**: 227-244.

Sieg, A.E., P. O. Michael, N. M. James, W. G. Bruce, J.A. Salvatore and E. D. Arthur. 2009. Mammalian metabolic allometry: do intraspecific variation, phylogeny, and regression models matter. The American Naturalist **174**: 720-733.

Southwood, A. L., R. D. Reina, V. S. Jones, J. R. Speakman and D. R. Jones. 2006. Seasonal metabolism of juvenile green turtles (*Chelonia mydas*) at Heron Island, Australia. Canadian Journal of Zoology **84**: 125-135.

Sparling, C. E., D. Thompson, M. A. Fedak, S. L. Gallon and J. R. Speakman. 2008. Estimating field metabolic rates of pinnipeds: doubly labeled water gets the seal of approval. Functional Ecology **22**: 245-254.

Speakman, J. R., and P. A. Racey. 1988. Validation of the doubly labeled water technique in small insectivorous bats by comparison with indirect calorimetry. Physiological Zoology **61**: 514-526.

Szafrańska, P. A., K. Zub, M. Wieczorek, A. Książek, J. R. Speakman and M. Konarzewski. 2014. Shaving increases daily energy expenditure in free-living root voles. The Journal of Experimental Biology **217**: 3964-3967.

Tuanmu, M. N., A. Vina, J. A. Winkler, Y. Li, W. Xu, Z. Ouyang and J. Liu. 2013. Climate-change impacts on understory bamboo species and giant pandas in China's Qinling Mountains. Nature Climate Change **3**: 249-253.

Visser, G. H., P. E. Boon and H. A. J. Meijer. 2000. Validation of the doubly labeled water method in Japanese Quail *Coturnix c. japonica* chicks: is there an effect of growth rate? Journal of Comparative Physiology **170**: 365-372.

Wallace, B. P., C. L. Williams, F. V. Paladino, S. J. Morreale, R. T. Lindstrom and J. R. Spotila. 2005. Bioenergetics and diving activity of interesting leatherback turtles *Dermochelys coriacea* at Parque Nacional Marino Las Baulas, Costa Rica. The Journal of Experimental Biology **208**: 3873-3884.

Watts, P. D., N. A. Øritsland and R. J. Hurst. 1987. Standard metabolic rate of polar bears under simulated denning conditions. Physiological Zoology **60**: 687-691.

Watts, P. and C. Cuyler. 1988b. Metabolism of the black bear under simulated denning conditions. Acta Physiologica Scandinavica **134**: 149-152.

Watts, P. D. and C. Jonkel. 1988a. Energetic cost of winter dormancy in grizzly bear. Journal of Wildlife Management **52**: 654-656.

Wei, F., Z. Wang and Z. Feng. 2000a. Energy Flow through gopulations of giant pandas and red pandas in Yele Natural Reserve. Acta Zoologica Sinica **46**: 287-294.

Wei, F., Z. Wang, Z. Feng, M. Li and A. Zhou. 2000b. Seasonal energy utilization in bamboo by the red panda. Zoo Biology **19**: 27-33.

Welcker, J., O. Chastel, G. W. Gabrielsen, J. Guillaumin, A. S. Kitaysky, J. R. Speakman, Y. Tremblay and C. Bech. 2013. Thyoid hormones correlate with basal metabolic rate but not field metabolic rate in a wild bird species. Plos One **8**: 1-7.

White, C. R. and R. S. Seymour. 2003. Mammalian basal metabolic rate is proportional to body mass. Proceedings of the Academy of National Science USA **100**: 4046-4049.

Williams, J. B. and A. Prints. 1986. Energetics of growth in nestling savannah sparrows: a comparison of doubly labeled water and laboratory estimates. Cooper Ornithological Society **88**: 74-83.

Zhou, X., L. Wang, Q. Zhou, X. Zhang and Y. Chen. 1990. Diet test of giant panda in fuzhou zoo. Sichuan Journal of Zoology **9**: 43-44.

CHAPTER 2: RESTING METABOLIC RATES OF THE GIANT PANDA, A HERBIVOROUS CARNIVORE

Introduction

The giant panda (*Ailuropoda melanoleuca*) is a mysterious creature that has enthralled the public since Père Armand David first discovered it for Western science in 1869. It is now the focus of intense conservation efforts in China and there has been considerable success in breeding giant pandas in captivity and in studying their genome (Li et al., 2010). Schaller et al. (1985) documented the feeding habits, home range, behavior and basic population biology of the giant panda in the Wolong Reserve in Sichuan Province, China. Since then there has been considerable research on population genetics and anthropogenic impacts on the giant panda (Liu et al., 2001; Zhu et al., 2013). However, there is still much that is unknown about its basic biology and ecology.

The main long-range goal of captive breeding efforts is to reintroduce giant pandas back into their wild habitat so that they can survive and breed, and reestablish viable wild populations in protected reserves. However, we lack a basic understanding of the physiological ecology of the giant panda that is necessary for ensuring that there are sufficient resources for the animals and that they will be able to adapt to the effects of climate change that are and will be occurring in their reserves (Tuanmu et al., 2013). For example, we need to know; what are the energetic requirements of the giant panda and how are those requirements manifested in its habitat requirements? Giant pandas are thought to prefer habitats with dense conifer forests at higher altitude (Qi et al., 2009; Qi et al., 2011). However, is that because of physiological constraints due to energetics and temperature tolerance, or is it due to the presence of human activity at lower elevations (Liu et al., 2001, Qi et al., 2011)? Ecology is based on the exchange of energy and matter between an animal and its environment (Gates, 1962). From the animal's perspective their ecology is basically metabolic and ecological interactions that are based on the biological processing of energy and naturally available materials (Brown et al., 2012). Therefore, we cannot proceed with reintroduction of giant panda until we know the metabolic requirements of the giant panda. The giant panda metabolic rate will give resource and reserve designers or managers basic data to determine how much food must be available and how that might change seasonally. We must design reserves that have sufficient food reserves for summer, fall, winter and spring conditions, which may place widely different energetic constraints on these animals, as well as provide differing standing biomass of forage available to eat.

The study of vertebrate metabolism has produced important insights into the role of body size in the biology of animals and has led to continuing controversy about the scaling relationship between body size and metabolic rate (White and Seymour, 2005; Sieg et al., 2009). It has also led to development of the metabolic theory of ecology (MTE) that aims to provide mathematical equations for the mechanistic underpinnings of ecology

(West et al., 1997; Brown et al., 2004) relating how body size and temperature, through their effect on metabolic rate, affect rates and timing of ecological processes. However, the MTE has been controversial and there are continuing discussions of the proper scaling exponent in the allometric equation of body size vs. basal metabolic rate ($BMR = aM^b$) in mammals where M is body mass. Is $b = 2/3$ or ¾ ? (Kleiber, 1961; White and Seymour, 2003; O'Connor et al., 2007). One of the problems in this discussion has been the paucity of data from large mammals. More than 80% of values in the mouse to elephant regression analyses have been from small mammals and small and large mammals may lie on different regression lines for body mass vs. metabolic rate (Heusner, 1991). Therefore, it is important to obtain accurate measurements of metabolic rates in large mammals, despite the difficulty in studying such mammals in the laboratory. Ecological theory is only as strong as the data that underlie its equations.

The giant panda provides an interesting case for a large mammal. It is a member of the Family Ursidae related to omnivorous bears (O'Brien et al., 1985; Hu, 2000; Ellis et al., 2006). Its digestive system is that of a carnivore, but its diet is primarily herbivorous (Li et al., 2010). It is specialized for eating primarily one plant type, bamboo. We might expect that it would have a metabolic rate below that expected for a mammal of its size based on its diet of bamboo leaves and stalks since mammals such as sloths (*Bradypus griseus, Choloepus hoffmanni*) that feed extensively on leaves have low basal metabolic rates (McNab, 1978) and sloth bears (*Melursus ursinus*) that eat termites also have a lower than expected metabolic rate (McNab, 1992). Therefore, the object of this experiment was to measure the BMR of the giant panda, both to obtain knowledge of its physiology and to add data to test the MTE. Giant pandas are rare and not readily available for physiological studies. However, I was fortunate to be able to measure the metabolic rate of 9 giant pandas at the Research Base of Giant Panda Breeding in Chengdu, China. The focus of this study was to determine the standard metabolism of resting giant pandas within a range of temperatures.

Methods

Giant panda acquisition and maintenance

I studied giant pandas (*Ailuropoda melanoleuca*) at the Research Base of Giant Panda Breeding (Panda Base) (www.panda.org.cn) and conducted all experiments in cooperation with the research, veterinary and husbandry staff there. The Research Base of Giant Panda Breeding was a nonprofit organization with offices in Chengdu, Sichuan Province, China. It was a center for wildlife research, giant panda captive breeding, conservation education, and educational tourism. It was not possible to carry out metabolic studies of giant pandas in the past because they were very rare and most zoos only had one or two individuals. I was fortunate that the Panda Base had 107 giant pandas and I was allowed to use 9 of them for these experiments under very close veterinary supervision. The staff there had unique experience in breeding and husbandry of giant pandas based on more than 30 years of research. Giant pandas lived in their normal enclosures and ate a diet composed primarily of bamboo supplemented with foods such as apples and “panda cake”, a biscuit made of a mixture of grains with vitamins. I transported pandas to the laboratory for each experiment.

Metabolic rate experimental design

I measured metabolic rate during two seasons, summer and winter. Because there was no effective air temperature-control room at the Panda Base I had to use natural air temperature change during the seasons to study pandas under warm and cool conditions. I did that to assess the thermal neutral zone of the giant panda. However, according to the husbandry rules of the Panda Base, giant pandas should not be exposed to temperatures greater than 25℃. Past experience showed that if giant pandas experienced temperatures above 25℃, they became heat stressed and experienced health problem. So in our experiment, I attempted to keep the maximum experimental temperature at 25.0℃. In winter I could not obtain an experimental temperature below 9.1℃.

I studied 5 giant pandas during each season, including young animals (1-2 years old), sub adults and adults. One adult was studied twice. Because giant pandas are diurnal, I conducted all experiments during night hours (2200-0400). Giant pandas were weighed before and after each experiment.

Our goal was to measure the BMR of these animals keeping in mind the criteria of Kleiber (1961) that the animals be post-absorptive and at rest. Normally I would fast the animals for 24 h before an experiment. However, past experience at the Panda Base indicated that if giant pandas did not eat for 24 h they became restless and agitated, paced around their enclosures and were very active. Therefore, animals fasted for 12 h before an experiment, but could drink water. Some animals did pass feces during experiments so they may have been digesting vegetation. Speakman et al., (1993) stated that it is not always possible to adhere completely to the Kleiber criteria in studies on wild animals and that it is necessary to trade off the strict adherence to arbitrary rules with the constraints of reality for the species under study. Even Kleiber (1961) stated that measurement of a true BMR was probably only possible in humans. Many authors use the term standard metabolic rate or resting metabolic rate rather than BMR for non-human animals. I believe that our measurements of the resting metabolic rate (RMR) of giant pandas are as close to BMR as it is possible to obtain under realistic conditions.

I measured metabolic rate in a Plexiglas chamber using a flow through system to measure oxygen consumption and carbon dioxide production. The chamber was 1.5 m * 1.5 m * 2.0 m and constructed of 2.0 cm Plexiglas with a steel frame for added strength. One side of the chamber was a door held by steel hinges, sealed with a rubber gasket and closed with metal latches (Figure 1). There were three 2.5 cm holes with 60 cm long tubing attached to avoid backflow for air intake at the bottom right side of the chamber. There was one 2.5 cm exit hole at the top left side of the chamber that connected to spiral-wound tubing leading to a Flowkit -500 mass flow system (Sable Systems International). A subsample of air went from the Flowkit pump to a FOXBOX oxygen and carbon dioxide analyzer (Sable Systems International). The three air intake holes and one air exit hole eliminated negative pressure in the system. The placement of the holes reduced air stagnation and two small battery operated fans in the chamber assured that the air was well mixed. Six 24-gauge Cu-Co thermocouples (+/- 0.05℃) located inside the chamber on the top, right side, left side, back side, and in the mouth of the air intake and exit holes measured chamber temperatures.

The Sable System Flowkit used a precision mass flow sensor with a rotary pump controlled by a microprocessor to control air flow rate to within 2 % of reading. The Flowkit pump's air flow was set at 150L/min. After leaving the Flowkit pump, air was subsampled though a small plastic tube and drawn into the FOXBOX system at a rate of 200 ml/min. The subsample went through a relative humidity meter and temperature meter before it entered the gas analyzers. Sample air passed through the CO_2 analyzer and then a drierite (anhydrous calcium sulfate (gypsum) with cobalt (II) chloride added as a color indicator) column before entering the O_2 analyzer to remove water vapor which would interfere with the fuel cell in the oxygen analyzer. The accuracy of the Sable System Foxbox was 0.1 % for O_2 over a range of 2-100% and 1 % for CO_2 over a range of 0-5% when calibrated used using calibration gas (14.93 % O_2, 3.99 % CO_2) from Dalian Special Gas Industry Company and tested by National Institute of Measurement and Testing Technology. 100 % dry N_2 and room air were also used to calibrate the system. I converted gas measurements to standard temperature and pressure dry (STPD).

Statistical analysis

I used a fitting linear model (LM) in program R (R Development Core Team 2011). The LM consisted of the interactions between two fixed factors that included age and sex with temperature and mass as covariates. I used model comparison and simplification to remove factors that were not significantly related to RMR. The final linear model contained the effects of mass and age. I accepted $P \leq 0.05$ as a statistically significant difference.

Results

The resting metabolic rate (RMR) of the giant panda ranged from 0.127 ml/g/h to 0.242 ml/g/h (Figure 2). The LMER model ANOVA (Table 1) indicated that there was a statistically significant effect of age (df = 2, 9; F = 80.16; P = 0.002), mass (df = 1, 9; F = 17.22; P = 0.025) and an interaction between sex and age (df = 1, 9; F = 37.29; P = 0.009). The RMR of adult and sub-adult giant pandas was 0.150 ml/g/h (range = 0.126 ml/g/h to 0.189 ml/g/h) and RMR for young giant pandas was 0.204 ml/g/h (range = 0.183 ml/g/h to 0.225 ml/g/h. There was no difference in RMR between males and females, and no difference in RMR due to environmental temperature and season. The effect of mass is clear in Figure 2 and mass and age are obviously related. Because both sub-adult pandas were female the effect of sex was not significant. The interaction of age and sex was coincident and may also be related to the small sample size. The RQ data were variable. Values ranged from 0.59 to 0.85 (Table 2).

Discussion

There were differences in metabolic rate between young cub and adult giant pandas**.** In our study both age and mass affected RMR. Age affects RMR because young animals have higher RMR than adults (Karasov, 2007). However, the masses of young giant panda cubs were from 63 kg to 68 kg, smaller than those of adults at 78 kg to 132 kg. Therefore, mass also was important in determining the RMR. Although the effects of mass and age were confounded, the statistical significance of the effects suggested that age had a greater effect than mass on RMR.

There was no difference in metabolic rate between male and female giant pandas. Some mammals have behavioral and physiological differences between males and females that cause differences in RMR due to hormones (McNab, 2000, Henry, 2005, Black et al., 1996). For example, in humans, males have higher BMR and active metabolic rate than females, but female margays (*Leopardus wiedii*) have higher BMR than males. Captive and wild male giant pandas are more active in the daytime than female giant pandas (Liu et al, 2002). Female giant pandas have more restricted habitat requirements than males (Qi et al., 2011). Those differences would probably be reflected in their active metabolic rates. There was no difference in activity of males and females in our metabolic chamber. Both sexes were quiescent. Therefore, there was no difference in their RMR.

Temperature is an important factor affecting RMR. Mammals have a thermal neutral zone in which animals have a minimum RMR. Below that zone metabolic rate increases due to thermoregulation. Above that zone metabolic rate increases due to a loss in the ability of the animal to cool its body temperature by behavioral and physiological means (Withers, 1992). In our experiment, there was no significant difference in metabolic rates of giant pandas at environmental temperatures between 9.1℃ and 26.5℃. Therefore, these temperatures were within the thermal neutral zone. There was no indication that the animals were more active at these temperatures and they showed no signs of behavioral stress.

The RQ data were variable. An animal digesting fat has a RQ of 0.7, an animal digesting carbohydrates has an RQ of 1.0 and an animal digesting protein has an RQ of 0.8-0.9. RQ values lower than 0.7 are often considered to be in error (Livesey and Elia, 1988). However, low RQs are not unusual for metabolic studies of animals. For example, studies on birds (King, 1957), hibernating black bear (*Ursus americanus*) (Nelson, 1973), pig (Thorbek, 1974; Chwalibog, et al., 2002), white rat (Wang, et al., 1975), and green turtle (*Chelonia mydas*) (Jackson, 1985) report some RQs below 0.7. Low RQs also occur in humans (Owen et al., 1998). Benedict (1932) and Benedict and Lee (1938) discussed this problem and suggested that low RQs were due to fat transformation into carbohydrate through the process of gluconeogenesis. The RQ for gluconeogenesis is 0.4 (Schutz, 1997) so if this occurs in an animal the resulting RQ would be below 0.7. Walsberg and Wolf (1995) suggest that RQ below 0.7 may be due to incomplete oxidation of fat and non-pulmonary CO_2 loss. It may also be due to incomplete ketone oxidation and the loss of oxidation products through urine or breath (Schutz and Ravussin, 1980). I cannot be sure of the cause of RQ below 0.7 in some of our giant pandas. Additional studies are needed to clarify that situation. Elevated RQs above 0.7 were probably due to animals that were not post-absorptive. Giant pandas in this study were only fasted for 12 h due to veterinary concerns and the passage time for bamboo through the gut may be longer than this fasting period. There was a tradeoff between fasting them longer and having them get active due to hunger, and limiting the time of fasting so that they were resting. In order to be sure giant pandas are post-absorptive a longer fast would be needed. The limitation in numbers of animals available for experimentation and the restlessness of animals that are hungry will make it very difficult to obtain such data.

Contrary to our expectations, based on its diet of bamboo leaves and stalks (McNab, 1978), the metabolic rate of the giant panda was similar to that of other mammals of the same size. I compared the resting metabolic rate in ml/h (MR) of the giant panda to those reported for 21 other mammals ranging in size from 50 kg to 193 kg taken from Sieg et al. (2009) (Table 2). The metabolic rate of the giant panda was higher than that of the sloth bear (McNab, 1992) but lower than that of the tiger (*Panthera tigris*), lion (*Panthera leo*), cow (*Bos taurus*), and eland (*Taurotragus oryx*) (McNab, 2000; Taylor and Lyman, 1967; Taylor et al., 1969). At similar mass, humans have a similar RMR as young (2 year old) giant pandas. Dolphins and sea lions have higher metabolic rates (Williams et al., 2001; Rosen and Trites, 2002). This is because they were not as quiet as land animals during the metabolic experiments. In addition, water has a much higher heat transfer rate than air and the higher rate of heat loss in water requires a higher RMR (Spotila, 1980).

I plotted the MRs of the giant panda and the 21 other large mammals (Table 3) from Sieg et al. (2009) (Figure 3). The regression line through those data ($\log_{10}$ (MR) = 0.8227 Log_{10} (Mass) + 0.2359, $r^2 = 0.57$; $P = 0.000$) was almost the same as that of Sieg et al. (2009) for carnivores/ungulates/pangolins (Fereuungulata) (Waddell et al., 1999; Springer et al., 2005). Both of those lines were above the line calculated from all 695 mammals in their data set. That supports their conclusion that phylogenetic relationships affect the body size- metabolic rate regression and that there is not a single universal metabolic rate-body mass scaling relationship in mammals.

Giant panda metabolic rates were 6.0% to 44.3 % below those predicted by the Fereuungulata regression line (Table 3). White and Seymour (2005) suggest that the presence of large herbivores in a data set will elevate the scaling exponent because large herbivores are less likely to be post-absorptive when metabolic rate is measured. In support of this suggestion our data indicated that even if some of our giant pandas were not post-absorptive, their metabolic rates were still below predicted values for mammals of similar size. Therefore, a combination of phylogenetic relationships and physiological factors affect the metabolic rate of individual species and no one predictive line can account for all variation in the body size-metabolism relationship among mammals.

The proponents of the MTE (West and Brown 2005; Allen and Gillooly 2007; Moses et al., 2008) indicate that minimization of transport costs of metabolites to metabolically active tissues require a scaling exponent of ¾ for metabolism. However, our data on the metabolic rate of the giant panda in comparison to allometric analysis of the metabolic rates of large mammals (Sieg et al., 2009) indicates that the MTE has extremely limited predictive power. It does not accurately predict the metabolic rate of a giant panda. Inherent variation in the metabolic processes of individual species such as the giant panda, an animal with the digestive system of a carnivore that has an herbivorous diet, affects the slope estimate for the relationship between body size and metabolic rate in a group of mammals. Only with more detailed studies of the metabolism of additional species of mammals, both herbivores and carnivores, will we clarify any potential universal theories for mammalian physiological ecology. For example, there are no data on the RMR of species such as the black bear (*Ursus americana*), a common inhabitant of eastern North America, except for hibernating individuals (Watts et al., 1987; Watts and Cuyler, 1988a; Watts and Jonkel, 1988b). Yet bears are the closest relatives of the giant panda and knowledge of their metabolic rates will clarify whether the lower than expected metabolic rate of giant pandas is unique to their digestive physiology or is common to closely related members of their phylogenetic clade. Before we can have confidence in major theories about metabolic ecology we need to acquire more data on large animals because it is apparent that understanding the basis for variation between individuals and species is important in understanding animals in the laboratory and the real world.

List of References

Allen, A. P., and J. F. Gillooly. 2007. The mechanistic basis of the metabolic theory of ecology. Oikos **116**: 1073–1077.

Benedict, F. G. and R. C. Lee. 1938. Hibernation and Marmot Physiology. Carnegie Institution of Washington, Washington, D. C., 102-134.

Benedict, F. G. 1998. The physiology of large reptiles. Carnegie Institution of Washington, Washington, D. C., 409-416.

Black, A. E., Coward, W. A., Cole, T. J. and Prentice, A. M. 1996. Human energy expenditure in affluent societies: an analysis of 574 doubly-labeled water measurements. European Journal of Clinical Nutrition **50**: 72-92.

Brown, J. H., J. F. Gillooly, A. P. Allen, V. M. Savage and G. B. West. 2004. Toward a metabolic theory of ecology. Ecology **85**: 1771-1789.

Brown, H. B. and M. S. Richard. 2012. The metabolic theory of ecology and its central equation. In Richard M. S., J. H. Brown and A. Kodric-Brown. Metabolic Ecology: a scaling approach, 21-33.

Chwalibog, A., A-H. Tauson and G. Thorbek. 2002. Energy metabolism and substrate oxidation in pigs during feeding, starvation and re-feeding. Journal of Animal Physiology and Animal Nutrition **88**: 101-112.

Ellis, S., W. Pan, Z. Xie, and D. E. Wildt. 2006. The giant panda as a social, biological and conservation phenomenon. Chapter 1, 1-16, In: Giant pandas: Biology, veterinary medicineand management.(eds. D. E. Wildt, A. Zhang, H. Zhang, D. L. Janssen, and S. Ellis) Cambridge University Press, New York.

Gates, D. M. 1962. Energy exchange in the biosphere. Harper and Row, N. Y.

Henry, C. 2005. Basal metabolic rate studies in humans: measurement and development of new equations. Public Health Nutrition **8**: 1133-1152.

Heusner, A. A. 1991. Size and power in mammals. Journal of Experimental Biology **160**: 25-54.

Hu, J. 2000. Review on the classification and population ecology of the giant panda. Zoological Research **21**: 28-34.

Jackson, D. C. 1985. Respiration and respiratory control in the green turtle, *Chelonia mydas*. Copeia **1985**, 664-671.

Karasov, W.H. 2007. Physiological Ecology, pp 656-660.Princeton University Press, New Jersey,

King, J. R. 1957. Comments on the theory of indirect calorimetry as applied to birds. Northwest Science **31**: 155-169.

Kleiber, M. 1961. The Fire of Life: an introduction to animal energetics. Wiley, N. Y.

Li, R., W.Fan, G. Tian, H. Zhu, L. He, J. Cai, Q. Huang, Q. Cai, B. Li, Y. Bai, Z. Zhang, Y. Zhang, W. Wang, J. Li, F. Wei, H. Li, M. Jian, J. Li, Z. Zhang, R. Nielsen, D. Li, W. Gu, Z. Yang, Z. Xuan, O. A. Ryder, F. C. Leung, Y. Zhou, J. Cao, X. Sun, Y. Fu, X. Fang, X. Guo, B. Wang, R. Hou, F. Shen, B. Mu, P. Ni, R. Lin, W. Qian, G. Wang, C. Yu, W. Nie, J. Wang, Z. Wu, H. Liang, J. Min, Q. Wu, S. Cheng, J. Ruan, M.Wang, Z. Shi, M. Wen, B. Liu, X. Ren, H. Zheng, D. Dong, K. Cook, G. Shan, H. Zhang, C. Kosiol, X. Xie, Z. Lu, H. Zheng, Y. Li, C. C. Steiner, T. T. Lam, S. Lin, Q. Zhang, G. Li, J. Tian, T. Gong, H. Liu, D. Zhang, L. Fang, C. Ye, J. Zhang, W. Hu, A. Xu, Y. Ren, G. Zhang, M. W. Bruford, Q. Li, L. Ma, Y. Guo, N. An, Y. Hu, Y. Zheng, Y. Shi, Z. Li, Q. Liu, Y. Chen, J. Zhao, N. Qu, S. Zhao, F. Tian, X. Wang, H. Wang, L. Xu, X. Liu, T. Vinar, Y. Wang, T. Lam, S. Yiu, S. Liu, H. Zhang, D. Li, Y. Huang, X. Wang, G. Yang, Z. Jiang, J. Wang, N. Qin, L. Li, J. Li, L. Bolund, K. Kristiansen, G. K. Wong, M. Olson, X. Zhang, S. Li, H. Yang, J. Wang, and J. Wang. 2010. The sequence and de novo assembly of the giant panda genome. Nature **463**: 311-317.

Liu, J., M. Linderman, Z. Ouyang, L. An, J. Yang and H. Zhang. 2001. Ecological degradation in protected areas: the case of Wolong Nature Reserve for giant pandas. Science **292**: 98-101.

Liu, D., G. Zhang, R. Wei, H. Zhang, J. Fang and R. Sun. 2002. Effects of sex and age in the behavior of captive giant pandas (*Ailuropoda melanoleuca*). Acta Zoologica Sinica **48**: 585-590.

Livesey, G. and M. Elia.1988. Estimation of energy expenditure, net carbohydrate utilization, and net fat oxidation and synthesis by indirect calorimetry: evaluation of errors with specialreference to the detailed composition of fuels. American Society for Clinical Nutrition **47**: 608-628.

Moses, M. E., C. Hou, W. H. Woodruff, G. B. West, J. C. Nekola, W. Zuo, and J. H. Brown.2008. Revisiting a model of ontogenetic growth: estimating model parameters from theory and data. American Naturalist **171**: 632–645.

McNab, B. K. 1978. Energetics of arboreal folivores: physiological problems and ecological consequences of feeding on an ubiquitous food supply. Pp 153-162. In: The Ecology of Arboreal Folivores (ed. G. G. Montgomery), Smithsonian Institution Press, Washington D.C.

McNab, B. K. 1992. Rate of metabolism in the termite-eating sloth bear (*Ursus ursinus*). Journal of Mammalogy **73**: 168-172.

McNab, B. K. 2000. The standard energetics of mammalian carnivores: Felidae and Hyaenidae. Canadian Journal of Zoology **78**: 2227-2239.

Nelson, R. A., H. W. Wahner, J. D. Jones, R. D. Ellefson and P. E. Zollman. 1973. Metabolism of bears before, during, and after winter sleep. American Journal of Physiology **224**: 491-496.

O'Brien, S. J., W. G. Nash, D. E. Wildt, M. E. Bush and R. E. Benveniste. 1985. A molecular solution to the riddle of the giant panda's phylogeny. Nature **317**: 140-144.

O'Connor, M. P., S. J. Kemp, S. J. Agosta, F. Hansen, A. E. Sieg, B. P. Wallace , N. M. McNairand A. E. Dunham. 2007. Reconsidering the mechanistic basis of the metabolic theory of ecology. Oikos **116**: 1058-1072.

Owen, O. E., K. L. Smalley, D. A. D'Alessio, M. A. Mozzoli and E. K. Dawson. 1998. Protein,fat, and carbothydrate requirements during starvation: anaplerosis and cataplerosis. The American Journal of Clinical Nutrition **68**: 12-34.

Qi, D., Y. Hu, X. Gu, M. Li and F. Wei. 2009. Ecological niche modeling of the sympatric giant and red pandas on a mountain-range scale. Biodiversity and Conservation **18**: 2127-2141.

Qi, D., S. Zhang, Z. Zhang, Y. Hu, X. Yang, H. Wang and F. We. 2011. Different habitat preferences of male and female giant pandas. Journal of Zoology **285**: 205-214.

Rosen, D. A. S. and A.W. Trites. 2002. Cost of transport in stellar sea lions, *Eumetopias jubatus*. Marine Mammal Science **18**: 513-524.

Schaller, G. B., J. Hu, W. Pan and J. Zhu. 1985. Giant pandas of Wolong. University of Chicago Press.

Schutz, Y. and E. Ravussin. 1980. Respiratory quotients lower than 0.7. The American Journal of Clinical Nutrition **33**: 1317-1319.

Schutz, Y. 1997. On problems of calculating energy expenditure and substrate utilization from respiratory exchange data. Energy Metabolism **36**: 255-262.

Sieg, A. E., M. P. O'Connor, J. N. McNair, B. W. Grant, S. J. Agosta and A. E. Dunham. 2009. Mammalian metabolic allometry: do intraspecific variation, phylogeny, and regression models matter. The American Naturalist **174**: 720-733.

Speakman, J. R., R. M. McDevitt and K. R. Cole. 1993. Measurement of basal metabolic rates:don't lose sight of reality in the quest for comparability. Physiological Zoology **66**: 1045-1049.

Spotila, J. R. 1980. Constraints of body size and environment on the temperature regulation of dinosaurs. In: Thomas, R.D.K. and E.C. Olson. A cold look at the warm-blooded dinosaurs. Westview Press, Boulder.

Springer, M. S., W. J. Murphy, E. Eizirik and S. J. O'Breien. 2005. Molecular evidence for majorplacental clades. In: Rose, K. D. and J.D. Archibald. The rise of placental mammals: origins and relationships of the major extant clades. John Hopkins University Press, Baltimore, 37-49

Taylor, C. R. and C.P. Lyman. 1967. A comparative study of the environmental physiology of an East African antelope, the eland, and the Hereford steer. Physiological Zoology **40**: 280-295.

Taylor, C. R., D. Robertshaw and R. Hofmann. 1969. Thermal panting: a comparison of wildebeest and zebu cattle. American Journal of Physiology **217**: 907-910.

Thorbek, G. 1974. Energy metabolism in fasting pigs at different live weight as influenced by temperature. In: Menke, K. H., H-J. Lantzsch and J. Reichel. Energy metabolism of farm animals. EAAP Publish, Stuttgart, 147-150.

Tuanmu, M. N., A. Vina, J. A. Winkler, Y. Li, W. Xu, Z. Ouyang and J. Liu. 2013. Climate-change impacts on understory bamboo species and giant pandas in China's Qinling Mountains. Nature Climate Change **3**: 249-253.

Waddell, P. J., Y. Cao, J. Hauf and M. Hasegawa. 1999. Using novel phylogenetic methods to evaluate mammalian mtDNA, including amino acid-invariant sites-logdet plus site stripping, to determine internal conflicts in the data, with special reference to the positions of hedgehog, armadillo, and elephant. Systematic Biology **48**: 31-53.

Walsberg, G. E. and B. O. Wolf. 1995. Variation in the respiratory quotient of birds and implications for indirect calorimetry using measurements of carbon dioxide production. The Journal of Experimental Biology **198**: 213-219.

Wang, L. C. H. and R. E. Peter. 1975. Metabolic and respiratory responses during Helox-induced hypothermia in the white rat. American Journal of Physiology **229**: 890-895.

Watts, P. D., N. A. Øritsland and R. J. Hurst. 1987. Standard metabolic rate of polar bears under simulated denning conditions. Physiological Zoology **60**: 687-691.

Watts, P. and C. Cuyler. 1988a. Metabolism of the black bear under simulated denning conditions. Acta Physiologica Scandinavica **134**: 149-152.

Watts, P. D. and C. Jonkel. 1988b. Energetic cost of winter dormancy in grizzly bear. Journal of Wildlife Management **52**: 654-656.

West, G. B., J. H. Brown and B. J. Enquist. 1997. A general model for the origin of allometric scaling laws in biology. Science **276**: 122-126.

West, G. B., and J. H. Brown. 2005. The origin of allometric scaling laws in biology from genomes to ecosystems: towards a quantitative unifying theory of biological structure and organization. Journal of Experimental Biology **208**: 1575–1592.

White, C. R. and R. S. Seymour. 2003. Mammalian basal metabolic rate is proportional to body mass. Proceedings of the Academy of National Science USA **100**: 4046-4049.

White, C. R. and R. S. Seymour. 2005. Allometric scaling of mammalian metabolism. The Journal of Experimental Biology **208**: 1611-1619.

Williams, T. M., J. Haun, R.W. Davis, L.A. Fuiman and S. Kohin. 2001. A killer appetite: metabolic consequences of carnivory in marine mammals. Comparative Biochemistry and Physiology Part A **129**: 785-796.

Withers, P. C. 1992. Comparative Animal Physiology. Saunders College Publishing, Florida,106-108.

Zhu, L., Y. Hu, D. Qi, H. Wu, X. Zhan, Z. Zhang, M. W. Bruford, J. Wang, X. Yang, X. Gu, L. Zhang, B. Zhang, S. Zhang and F. Wei. 2013. Genetic consequences of historical anthropogenic and ecological events on giant pandas. Ecology **94**: 2346-2357.

TABLES

Table 2.1. Results of fitting linear model Analysis of Variance on the effects of sex, age, mass and temperature on the metabolic rates of giant pandas. Age and sex were fixed factors and temperature and mass were covariates.

Analysis of Variance Table

Response: RMRO2

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Sex	1	0.000	0.000	5.03	0.111
Age	2	0.007	0.004	80.17	0.002 **
Mass	1	0.001	0.001	17.23	0.025 *
Temperature	1	0.000	0.000	0.05	0.840
Sex:Age	1	0.002	0.002	37.30	0.009 **
Residuals	3	0.000	0.000		

Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1

Table 2.2. Metabolic rates and RQs of giant pandas measured in a metabolic chamber at the Chengdu Research Base of Giant Panda Breeding in Chengdu, China. Metabolic rates were below predictions. A is adult, Y is juvenile, S is subadult, M is male and F is female

Studbook Number	Sex	Mass(g)	Age	Temperature(℃)	RMR CO2(ml/g/h)	RMR O2(ml/g/h)	RQ	RMR O2 Prediction (ml/g/h)	% below the prediction
386	M	132000	A	9.1	0.121	0.146	0.83	0.210	30.3
762	F	84000	S	9.2	0.107	0.126	0.85	0.227	44.3
761	F	78000	S	9.2	0.129	0.187	0.69	0.230	18.7
467	M	132000	A	12.8	0.113	0.147	0.77	0.210	29.9
491	F	102000	A	10.8	0.089	0.148	0.60	0.220	32.6
386	M	131000	A	26.5	0.104	0.144	0.72	0.210	31.4
814	F	67000	Y	25.0	0.125	0.208	0.60	0.237	11.8
813	F	67000	Y	24.9	0.136	0.198	0.69	0.237	16.6
815	M	63000	Y	25.5	0.133	0.225	0.59	0.239	6.0
820	F	68000	Y	25.3	0.140	0.183	0.77	0.236	22.5

Table 2.3. Metabolic rates of 21 large mammals compiled by Sieg et al (2009) and juvenile and adult giant pandas measured at the Chengdu Research Base of Giant Panda Breeding.

Number	Animal	Mass	RMR O2	Log10(Mass)	Log10(MR O2)
1	Jaguar	50400	0.222	4.70	4.05
2	White Tailed Deer	51190	0.226	4.71	4.06
3	Ribbon Seal	54700	0.363	4.74	4.30
4	Red Deer	58000	0.283	4.76	4.22
5	Giant Panda Young	66250	0.204	4.82	4.13
6	Sloth Bear	66957	0.126	4.83	3.93
7	Bighorn Sheep	67332	0.342	4.83	4.36
8	Homo	67650	0.198	4.83	4.13
9	American Badger	76020	0.300	4.88	4.36
10	Arabian Oryx	84100	0.221	4.92	4.27
11	Caribou	85000	0.346	4.93	4.47
12	Lion	98000	0.173	4.99	4.23
13	Water Buck	100000	0.267	5.00	4.43
14	Giant Panda Adult	109833	0.150	5.04	4.22
15	Llama	115000	0.164	5.06	4.28
16	Sea Lion	121833	0.350	5.09	4.63
17	Eland	125000	0.239	5.10	4.48
18	Tiger	137900	0.174	5.14	4.38
19	Wildebeest	140000	0.213	5.15	4.47
20	Harp Seal	150000	0.195	5.18	4.47
21	Bottlenosed Dolphin	165625	0.335	5.22	4.74
22	Ass	177500	0.164	5.25	4.46
23	Cow	193000	0.175	5.29	4.53

FIGURES

Figure 2.1. Metabolic chamber used to measure the metabolic rates of giant pandas at the Research Base of Giant Panda Breeding in Chengdu, China.

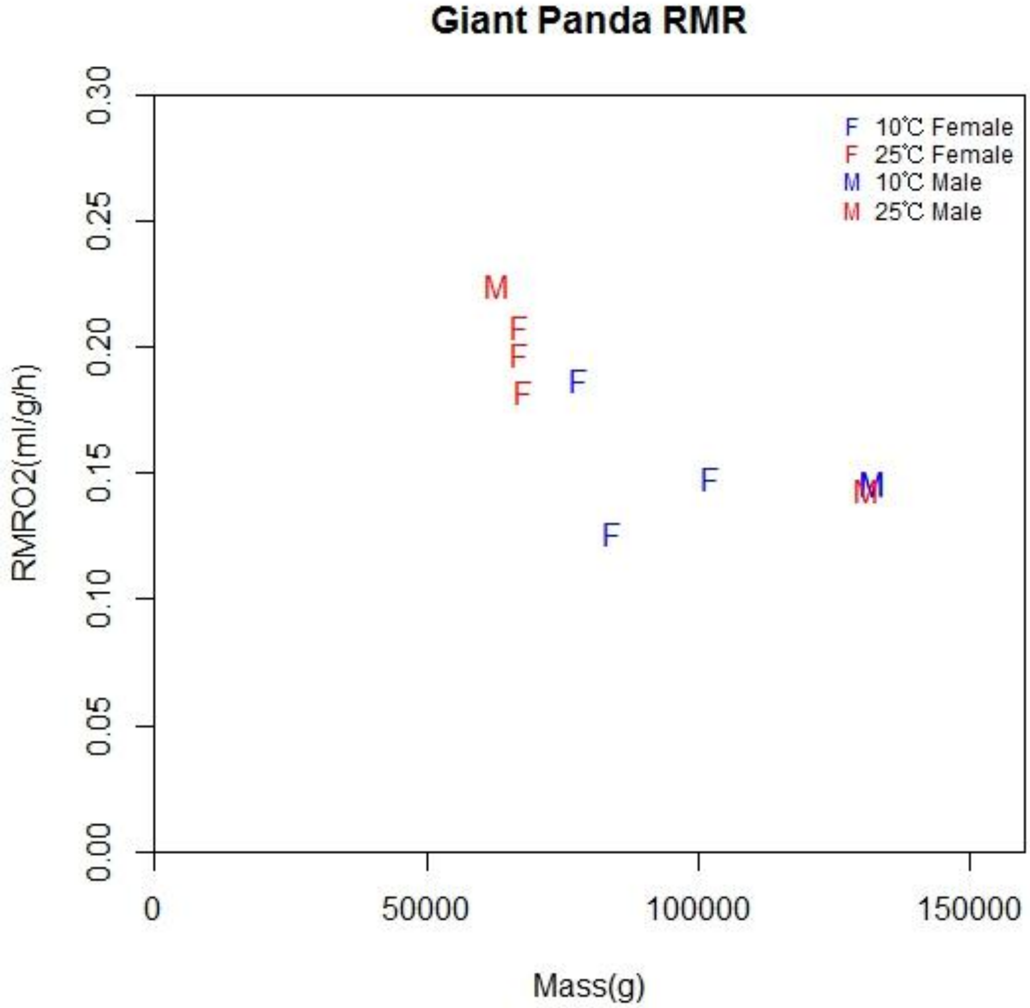

Figure 2.2. Metabolic rates of giant pandas measured at the Research Base of Giant Panda Breeding in Chengdu, China. Animals were at rest in a metabolic chamber at temperatures between 9.1 and 26.5 ℃. M represents males and F represents females.

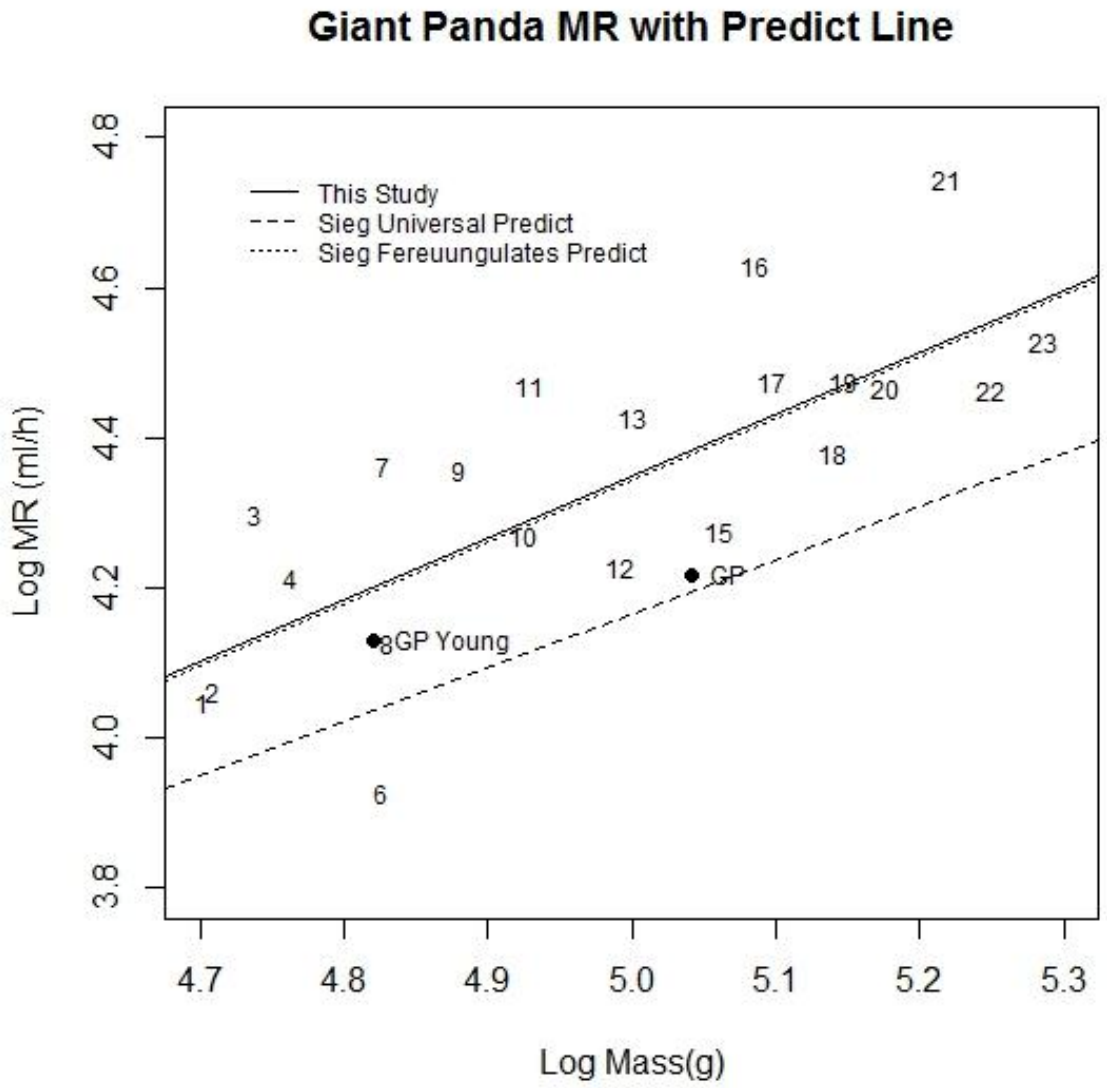

Figure 2.3. Relationship between body mass and metabolic rate in giant pandas and 21 other large mammals. GP represents giant panda. Regression lines for all mammals and for Ferreuungulate mammals are from Sieg et al. (2009). Solid line is regression line calculated by us with the addition of the giant panda.

CHAPTER 3: THE FIELD METABOLIC RATE OF GIANT PANDA IN CAPTIVITY USING DOUBLY LABELED WATER TECHNIQUE

Introduction

The captive population of giant pandas (*Ailuropoda melanoleuca*) in the world has increased over the last 30 years from about 6 to 350. However, the ultimate goal of giant panda captive breeding is to release pandas into the wild to supplement natural populations in nature reserves. No one has successfully introduced captive bred giant pandas into the wild. In order to do so it will be necessary to have a better understanding of their basic biological requirements. This includes knowledge of their energetics and food requirements. There are no reports of energetic research on giant pandas and only a few articles about habitat use and temperature tolerance (Qi et al., 2009; Qi et al., 2011). Recently I measured the resting metabolic rate (RMR) of nine pandas at two temperatures (Fei, 2015, Chapter 2) and compared it to metabolic rates of other large mammals. The giant panda has a thermal neutral zone that ranges from at least 9 to 26 ℃. Higher temperatures are stressful. Its metabolic rate is below that predicted by regressions of mass vs. metabolic rate in the literature and that may be related to its bamboo diet.

There are a few studies of metabolic rates of bears. However, most of them are on animals in hibernation (Watts et al., 1987; Watts and Cuyler, 1988a; Watts and

Jonkel, 1988b) so they are not useful in establishing the true metabolic cost for a bear in nature and cannot be extrapolated to the giant panda. McNab (1992) measured RMR of two female sloth bears in Florida. He found that their metabolic rates were much below those predicted by regression lines of metabolic rate vs. body size in mammals. The thick fur of sloth bear can decrease the heat loss rate and is one reason the sloth bear presents a low RMR. There is only one physiological study of an active polar bear. They did not measure the metabolic rate directly. However, they exercised the bear on a treadmill and found that cardiac frequency was linearly related to estimated metabolic rate. Best et al. (1981) estimated that the energy cost per step per kg would increase if walking speed increased and that it would decrease with body size increase.

There is a need to determine the metabolic cost of activity of the giant panda under natural conditions so that we can have a basis for determining the carrying capacity of natural reserves. If we know how much energy it costs a giant panda to go about its daily life and the amount of food (bamboo) available in its natural environment than we can calculate how many animals can live in a given area. He (2000) reports that wild giant pandas eat 13.14kg to 14.58 kg of bamboo leaves and stems a day or 43.74 kg of shoots a day. Chen (1998), Liu (2002) and Yang (2005) measured the protein digestion rate and fiber digestion rate of bamboo. The apparent digestibility of crude protein had a negative relationship for 1.5 to 2.5 year old giant pandas. However, it showed a positive relationship for 2.5 to 4.7 years old giant pandas (Liu et al., 2002). Gross energy density had a negative relationship with the apparent digestibility of crude protein (Yang et al., 2005). Giant pandas have a higher coarse cellulose digestion rate with mechanically ground bamboo that fresh bamboo (Chen et al., 1998). Energy intake and output should be in a dynamic balance. However, Schulz et al (1992) report that there can be a difference between energy intake in food and activity metabolic rate in humans and that the difference can be due to differences in accuracy in the two methods. Therefore, the best way to determine the metabolic cost of activity for an animal is to actually measure active metabolic rate in the field rather than rely on food intake and digestion efficiency measurements. Therefore, we need to measure the active metabolic rate of the giant panda to complete the circle from food to activity. The best way to do that is through the use of doubly labeled water (DLW) (Speakman, 1997).

The doubly labeled water method (DLWM) was first described by Lifson et al. (1955). It can be used accurately and reliably for measuring the metabolic rates of free range mammals and birds. The DLWM is based on the different pathways of hydrogen and oxygen in water consumed by organisms. Hydrogen is only lost by water loss. Oxygen is lost through both CO_2 loss and H_2O loss. So, the oxygen in a given quantity of H_2O will be lost more quickly than the hydrogen. In other words, oxygen has the higher loss rate, and the difference between oxygen and hydrogen loss per unit time allows calculation of the metabolic rate (Lighton, 2008).

The DLWM has been used successfully on rats, bats, birds, reptiles, small and large mammals. The daily energy expenditure of root voles (*Microtus oeconomus*), rat (*Rattus norvegicus*), shrew (*Sorex araneus*) and chipmunk (*Tamias stratus*) are now known (Lee and Lifson, 1992; Poppitt et al., 1993; Careau et al., 2012; Careau et al., 2013; Szafrańska et al., 2014). The same is true for several species of birds (Williams and Prints, 1986; Visser et al., 2000; Welcker et al., 2013). The field metabolic rates (FMR) of two species of bats (*Pipistrellus pipistrellus* and *Eptesicus fuscus*) indicate some variation between individuals (Speakman and Racey, 1988; Kurta et al., 1990). For reptiles, the FMR of the desert lizard (*Ctenophorus nuchalis*) differs with season (Nagy, 1995). However, there is no seasonal difference in FMR of juvenile green turtles (*Chelonia mydas*) (Southward et al., 2006). Leatherback (*Dernochelys coriaces*) turtles have low FMR so they avoid overheating in the tropics (Wallace et al., 2005).

The daily energy expenditure (DEE) of two kinds of working dog (hunting dog and Inuit sled dog, (*Canis familiaris*) varies from 6647 KJ/day to 16600 KJ/day (Gerth et al., 2010; Ahlstrøm et al., 2011). Junghans et al. (1997) studied goats (African dwarf goat) with two isotope methods, ^{13}C bicarbonate (^{13}C-M) and DLWM. They report that ^{13}C-M is good for measuring short-term (6-12 hours) FMR. The DLWM is good for a long-term FMR study. Black-tailed deer (*Thylogale billardierii)* have about three times higher FMR (0.369 ml/g/h CO_2) than kangaroos (*Macropus giganteus*) (1.063ml/g/h CO_2) (Nagy et al., 1990). For marine animals, fur seal (*Callorhinus ursinus*) the DLWM gives a higher DEE reading than a respiratory system (Dalton et al., 2014). The DLWM gave accurate measurements of the FMR of the grey seal (*Halichoerus grypus*) (Sparling et al., 2008). Variation in FMR among individual walrus (*Odobenus rosmarus*) depends upon activity levels (Acquarone et al., 2006). The DLWM has been widely used in energetic studies of hundreds of men, women and children (Kashiwazaki et al., 1995; Black, 1996; Johnston et al., 2007; Djafarian et al., 2010; Ojiambo et al., 2013). Recently this technique has also been used on fruit flies (*Drosophila melanogaster*), a very small insect (Piper, et al., 2014).

Here I report the successful use of the DLWM to measure the active metabolic rate of giant pandas under captive conditions at the Research Base of Giant Panda Breeding in Chengdu, China. Giant pandas under zoo like conditions have an active metabolic rate 2 times higher than resting metabolic rate measured in the laboratory.

Methods

Giant panda acquisition and maintenance

I studied giant pandas at the Research Base of Giant Panda Breeding (Panda Base) (www.panda.org.cn) and conducted all experiments in cooperation with the research, veterinary and husbandry staff there. The Research Base of Giant Panda Breeding was a nonprofit organization with offices in Chengdu, Sichuan Province, China. It was a center for wildlife research, giant panda captive breeding, conservation education, and educational tourism. It has not been possible to carry out studies of the active metabolic rates of giant pandas in the past because they are very rare and most zoos only have one or two individuals. I was fortunate that the Panda Base had 107 giant pandas and I was allowed to use 8 of them for these experiments under very close veterinary supervision. The staff there had unique experience in breeding and husbandry of giant pandas based on more than 30 years of research. Giant pandas lived in their normal enclosures and ate a diet composed primarily of bamboo supplemented with foods such as apples and “panda cake”, a biscuit made of a mixture of grains with vitamins. The giant pandas were trained to present their forearm for blood sampling making it possible to obtain samples with minimum disturbance to the animal.

DLW experimental design

There was no information on equilibration time and water loop rate of giant pandas or similar animals. Therefore, I picked one giant panda to do a safety test to be sure that there was no harm to the animal and also to measure the equilibration time. I took a background blood sample first, and then injected 10.12 g of doubly labeled water (Sigma-Aldrich deuterium oxide-18 99%D 75%O18) mixed with physiological saline. The dose depended upon mass. In that way, I obtained 80 p.p.m. oxide-18 above the background level at equilibration time. After two physiological half-lives the concentration would be 20 p.p.m. above background level, which was the minimum concentration that the mass spectrometer could accurately measure. The elimination half-life is 5 days for a 50 kg mammal or larger (Speakman, 1997). That meant that there would be about 10 efficacious experimental days. After injection, I took blood samples every 2 h for 8 h to measure the equilibration time. Then, I took blood samples after 3 days, 5 days and 10 days to measure the physiological half-life. I sealed all blood samples in individual glass tubes using an alcohol burner, placed them in a bigger PVC tube with cotton to protect them and stored them in a freezer at -40℃.

For a normal experiment, I took a background blood sample first and injected 10.15g to 12.56g DLW depending on the mass of the panda. After 5 h equilibration time, I took a sample. After 3 days and 5 days I took additional blood samples, treating them as before.

I also set up a video camera for each experimental animal to record its behavior during the experimental period in summer. The camera operated 24 h a day from the beginning of the first blood sample to the end of taking the last blood sample. I used this record to calculate the active time of the animal. The camera was not available for winter experiments.

Sample analysis and calculation

Samples were tested and analyzed by the Laboratory of Isotope Geology at Chengdu University of Technology (formerly: Chengdu College of Geology).

I used the two-sample technique (Speakman, 1997) to calculate the CO_2 production. I used 0.9 as RQ to predict oxygen consumption. I used Nagy (1999) to predict the FMR and food requirement. Microsoft Excel was used to store data and for calculations.

Results

I did 8 experiments on 7 individuals in two different seasons. One giant panda did not cooperate. It would not let us take blood samples after the DLW injection. Therefore, I obtained 7 valid results for 6 animals. The CO_2 production ranged from 0.265 ml/g/h - 0.628 ml/g/h (Mean = 0.426 ml/g/h; SD = 0.277) in the winter (Mean temperature = 8.6 ℃, range = 5 ℃ to 18 ℃ during experiment time) and 0.126 ml/g/h - 0.404 ml/g/h (Mean = 0.256 ml/g/h; SD = 0.126) in the summer (Mean temperature = 25.2 ℃, range = 22 ℃ to 33 ℃ during experiment time).

The O_2 consumption was 0.379 ml/g/h - 0.628 ml/g/h (Mean = 0.513 ml/g/h; SD = 0.308) in the winter and 0.180 ml/g/h - 0.577 ml/g/h (Mean = 0.284 ml/g/h; SD = 0.140) in the summer. I used 0.9 as the FMR RQ to convert oxygen consumption to kilojoules. The average DEE was 21,592 KJ/day (SD =13,323, range = 9,401 KJ/day to 47,716 KJ/day). Total body water percentage was 66.4% to 75.7%. Water turnover rate was 15.52 kg/day (SD = 4.44) or 17.45% of TBW/day (SD = 3.15%). Isotope half-life was 2.28 days to 3.81 days. There was considerable individual variation in these values (Table 3.1).

The camera was not available during the winter. However, according to my observations, giant pandas were active about 40% of the time during the DLW experiments. Based on video camera recordings, giant pandas were active 30.3% to 34.0% in the summer experiments. The daily time table was very regular for each giant panda, no matter the season. They usually were active from 0730 to 1200. This was the most active time during the day, but they would rest periodically during that time. Then they would rest until 1600 with short periods of activity. After that they were awake, ate for about 1 or 2 h and walked around a little. After 1900, they would sleep until 0700. However, most giant pandas would wake up once or twice to eat some bamboo during the night. There was no direct relationship between the activity times of the giant pandas and their active metabolic rates.

Discussion

The active metabolic rates of giant pandas varied between individuals despite the active time for each individual being very similar. The active time was greatly influenced by husbandry practices. Keepers usually cleaned the large cage of a giant panda inside a building and gave the animal new bamboo at 0730. At that time the giant panda was allowed out into the enclosure. However, if the temperature was greater than 25℃ in the summer, keepers would keep giant pandas inside the building so that they did not get heat stressed. Husbandry experience indicated that giant pandas would have health problems after long exposure to temperatures higher than 25℃. The keepers would add new bamboo in the afternoon around 1600 and call the giant panda back to the cage. Giant pandas were less active in the summer than in the winter. In the summer, giant pandas rarely walked around. They just ate and drank water, or just found a cool spot to rest. In winter, they actively moved around in the enclosure. They climbed trees, played with each other, made scent marks on trees and other objects and even watched people who were watching them. I recorded their active time but was unable to measure the magnitude of activity. That was the reason why they had similar activity times but quite different FMRs. The difference in FMR in summer and winter was illustrated by looking at the same individual. The panda Shishi had a much higher active metabolic rate in summer than that in winter (Table 3.1).

The mean DEE of 21,592 KJ, SD = 13323) per day was similar to the estimation of daily digestible energy intake (17,222 KJ to 28,329KJ) of wild giant pandas (He et al., 2000). It was not surprising that some individuals had lower FMR because they were captive animals living in a small area of an enclosure. However, all of them were similar to the prediction from Nagy's et al. (1999) equation. I calculated that giant pandas would have to eat about 13.13 (SD = 8.10) kg of bamboo to support the metabolic rates that I measured (He et al., 2000). Giant pandas at the Panda Base usually ate around 15 kg to 20 kg bamboo per day (unpublished data). Giant pandas in the Xiangling Mountains eat 13.14 kg to 14.48 kg of bamboo a day (He et al., 2000). However, the prediction from Nagy's (1999) equation about food requirement was smaller than what the giant pandas actually ate. So the prediction was not adequate for this special carnivore with an herbivorous diet. The diet of the giant panda is 99% bamboo and bamboo has a large water content and contains a lot of fiber. Giant pandas have a carnivore's digestive system, which has a limited ability to digest cell walls. So bamboo is a low energy source for them. Giant pandas need to eat more bamboo than predicted to get enough energy intake.

The water-loop of the giant panda is much faster than predicted. Speakman (1997) predicted that a 50 kg or larger animal would have a 5-day half-life for doubly labeled water. However, a 100 kg giant panda had a rate twice as high as the prediction. This was probably due to their special diet, 99% bamboo. Giant pandas do not drink water very often, but they do drink a lot each time. They will continue drinking for 2 to 3 min (personal observation). In addition, the giant panda has a different kidney type from other bears (Hu, 2000). The kidney of the giant panda is formed by 6 to 11 renal lobes. Each renal lobe is comprised of 2-3 primary small kidneys, which is an archaic type. A bears' kidney is a duplex kidney, build up by many renculus (Li, 2003).

Comparing the FMR of the giant panda to those of 10 similar sized mammals (Hudson et al. 2013) it appears that it is lower than most similar sizes mammals, like the seal (*Arctocephalus gazella*), deer (*Odocoileus hemionus* and *Cervus elaphus*), oryx (*Oryx leucoryx*) and kangaroo (*Macropus giganteus*) However, it is higher than that of the reindeer (*Rangifer tarandus*). It is not surprising that the FMR of my giant pandas are lower than that of most similar sized mammals, since they were in a captive zoo-like environment and are not very active. The low FMR of the reindeer may have been because they were in an energy conserving mode due to cold conditions in the field.

It does not appear that bamboo is the limiting factor in the number of giant pandas that can live in a given nature reserve. For example, in the Yele Nature Reserve, there is 1,634,529.3 kg of bamboo (*Bashania spanostachya*) per km^2 (Wei et al., 2000). Based on our FMR measurements and digestive efficiency measurements (He et al., 2000) a giant panda needs to eat about 20 kg of bamboo a day. That means there is enough bamboo in 1 km^2 of the Reserve to provide food for 81,726 panda days. Assuming that the giant pandas eat no more than ½ of the standing crop of bamboo than that would provide about 40,000 food days for a standing crop of 110 pandas in a year. If they used only 10% of the bamboo resources a year, then 1 km^2 would support 22 giant pandas, and that is for just one species of bamboo in that reserve. However, the home range of a giant panda is usually 3.0 to 6.0 km^2 (Schaller et al., 1985). So there must be limitations in the biology of the giant panda that go beyond the food supply, since that size home range provides a density of giant pandas that is 1 to 2 % of what would be supported by food alone.

Based on food supply the 2000 km^2 Wolong Nature Reserve, with 50% undisturbed area (Liu et al., 2001; Linderman et al., 2005) could support 22,000 giant pandas if all of the area is covered with bamboo, but only 166 to 333 giant pandas based on home range. In fact the estimated number of giant pandas living in the reserve was 143 reported by State Forestry Administration of the People' Republic of China 2003. If the disturbed area was rehabilitated as giant panda habitat the number of giant pandas in the Reserve could be doubled. Therefore, more data are needed on both home range and FMR of giant pandas in the wild under natural conditions if we are to obtain a better estimate of the carrying capacity of nature reserves.

List of References

Acquarone, M., E. W. Born and J. R. Speakman. 2006. Field metabolic rates of walrus (*Odobenus rosmarus*) measured by the doubly labeled water method. Aquatic Mammals **32**: 363-369.

Ahlstrøm, Ø., P. Redman and J. Speakman. 2011. Energy expenditure and water turnover in hunting dogs in winter conditions. British Journal of Nutrition. **106**: 158-161.

Black, A. E., Coward, W. A., Cole, T. J. and Prentice, A. M. 1996. Human energy expenditure in affluent societies: an analysis of 574 doubly-labeled water measurements. European Journal of Clinical Nutrition **50**: 72-92.

Best, R., K. Ronald and N. A. Øritsland. 1981. Physiological indices of activity and metabolism in the polar bear. Comparative Biochemistry and Physiology **69**: 177-185.

Careau, V., P. Bergeron, D. Garant, D. Réale, J. R. Speakman and M. M. Humphries. 2012. The Energetic and Survival Costs of Growth in Free-ranging Chipmunks. Oecologia **171**: 11-23.

Careau, V., D. Réale, D. Garant, F. Pelletier, J. R. Speakman and M. M. Humphries. 2013. Context-dependent correlation between resting metabolic rate and daily energy expenditure in wild chipmunks. The Journal of Experimental Biology **216** : 1-9

Chen, Y., N. Weng, X. Zou, A. Wang and D. Zheng. 1998. Comparative Study on Digestion Rates of Coarse Cellulose between Combined Bamboo Powder Feed and Regular Feed for Giant Panda. Journal of Northeast Forestry University **26**: 36-38.

Dalton, A. J. M., D. A. S. Rosen and A. W. Trites. 2014. Season and time of day affect the ability of accelerometry and the doubly labeled water methods to measure energy expenditure in northern fur seals (*Callorhinus ursinus*). Journal of Experimental Marine Biology and Ecology **452**: 125-136.

Djafarian, K., D. M. Jackson, E. Milne, P. Roger and J. R. Speakman. 2010. Doubly labeled water: Multi-point and two-point methods in pre-school children. International Journal of Pediatric Obesity **5**: 102-110.

Fei, Y. 2015. The metabolic rate of giant panda and red panda, chapter 2. In: Fei, Y., The metabolism of giant pandas and red pandas. Ph.D. Dissertation, Drexel University, Philadelphia.

Gerth, N., P. Redman, J. Speakman, S. Jackson and J. M. Starck. 2010. Energy metabolism of Inuit sled dogs. Journal of Comparative Physiology **180**:577-589.

He, L., F. Wei, Z. Wang, Z. Feng, A. Zhou, P. Tang and J. Hu. 2000. Nutritive and energetic strategy of giant pandas in Xiangling Mountains. Acta Ecologica Sinica **20**: 177-183.

Hu, J. 2000. Review on the classification and population ecology of the giant panda. Zoological Research **21**: 28-34.

Hudson, L. N., N. J. B. Isaac and D. C. Reuman. 2013. The relationship between body mass and field metabolic rate among individual birds and mammals. Journal of Animal Ecology **82**: 1009-1020.

Johnston, S. L., S. S. Erwin, D. M. Souter, B. J. Tolkamp, J. M. Yearley, I. J. Gordon, A. W. Illius, I. Kyriazakis and J. R. Speakman. 2006. Intake compensates for resting metabolic rate variation in female c57bl/6j mice fed high-fat diets. Obesity **15**: 1-7.

Junghans, P., M. Derno, M. Gehre, R Höfling, P. Kowski, G. Strauch, W. Jentsch, J. Voigt and U. Henning. 1997. Calorimetric validation of ^{13}C bicarbonate and doubly labeled water method for determining the energy expenditure in goats. Zeitschrift für Ernährungswissenchaft **36**: 268-272.

Kashiwazaki, H., Y. Dejima, J. Orias-Rivera and W. A. Coward. 1995. Energy expenditure determined by the doubly labeled water method in Bolivian Aymara living in a high altitude agropastoral community. The American Journal of Clinical Nutrition **62**: 901-910.

Kurta, A., T. H. Kunz and K. A. Nagy. 1990. Energetics and water flux of free-ranging big brown bats (*Eptesicus fuscus*) during pregnancy and lactation. Journal of Mammalogy **71**: 59-65.

Lee, J. S., N. Lifson. 1960. Measurement of total energy and material balance in rats by means of doubly labeled water. American Journal of Physiology **199**: 238-242.

Li, T. 2003. Giant panda evolution research progress. Science & Technology Progress and Policy **1**:271-275.

Lifson, N., G. B. Gordon and R. McClintock. 1955. Measurement of total carbon dioxide production by mean of $D_2{}^{18}O$. Journal of Applied Physiology **7**: 704-710.

Lighton, J. B. 2008. Measuring Metabolic Rates. Oxford University Press, Oxford, England.

Linderman. M., S. Bearer, L. An, Y. Tan, Z. Ouyang and J. Liu. 2005. The effects of understory bamboo on broad-scale estimates of giant panda habitat. Biological Conservation **121**: 383-390.

Liu, X., M. Li, R. Wei, J. Yu, D. Hu, Z. Yang and X. Huang. 2002. Study on apparent digestibility of crude protein by captive sub-adult giant panda. Chinese Journal of Applied & Environmental Biology **8**: 174-178.

Liu, J., M. Linderman, Z. Ouyang, L. An, J. Yang and H. Zhang. 2001. Ecological degradation in protected areas: the case of Wolong Nature Reserve for giant pandas. Science **292**: 98-101.

McNab, B. K. 1992. Rate of metabolism in the termite-eating sloth bear (*Ursus ursinus*). Journal of Mammalogy **73**: 168-172.

Nagy, K. A. and S. D. Bradshaw. 1995. Energetics, osmoregulation, and food consumption by free-living desert lizards, *Ctenophorus* (*Amphibolurus nuchalis*). Amphibia-Reptilia **16**: 25-35.

Nagy, K. A., G. D. Sanson and N. K. Jacobsen. 1990. Comparative field energetics of two macropod marsupials and ruminant. Australian Wildlife Research **17**: 591-599.

Nagy, K. A., I. A. Girard and T. K. Brown. 1999. Energetics of free-ranging mammals, reptiles, and birds. Annual Review of Nutrition **19**: 247-277.

Ojiambo, R., A. R. Gibson, K. Konstabel, D. E. Lieberman, J. R. Speakman, J. J. Reilly and Y. P. Pitsiladis. 2013. Free-living physical activity and energy expenditure of rural children and adolescents in the Nandi region of Kenya. Annals of Human Biology **40**:318-323.

Piper, M. D. W., C. Selman, J. R. Speakman and L. Partridge. 2014. Using Doubly-Labeled Water to measure energy expenditure in an important small ectotherm *Drosophila melanogaster*. Journal of Genetics and Genomics **41**: 505-512.

Poppitt, S. D., J. R. Speakman and P. A. Racey. 1993. The energetics of reproduction in the common shrew (*Sorex araneus*): A comparison of indirect calorimetry and the doubly labeled water method. Physiological Zoology **66**: 964-982.

Qi, D., Y. Hu, X. Gu, M. Li and F. Wei. 2009. Ecological niche modeling of the sympatric giant and red pandas on a mountain-range scale. Biodiversity and Conservation **18**: 2127-2141.

Qi, D., S. Zhang, Z. Zhang, Y. Hu, X. Yang, H. Wang and F. We. 2011. Different habitat preferences of male and female giant pandas. Journal of Zoology **285**: 205-214.

Schaller, G. B., J. Hu, W. Pan and J. Zhu. 1985. Giant pandas of Wolong. University of Chicago Press.

Schulz, L. O., S. Alger, I. Harper, J. H. Wilmore and E. Ravussin. 1992. Energy expenditure of elite female runners measured by respiratory chamber and doubly labeled water. Journal of Applied Physiology **72**: 23-28.

Southwood, A. L., R. D. Reina, V. S. Jones, J. R. Speakman and D. R. Jones. 2006. Seasonal metabolism of juvenile green turtles (*Chelonia mydas*) at Heron Island, Australia. Canadian Journal of Zoology **84**: 125-135.

Sparling, C. E., D. Thompson, M. A. Fedak, S. L. Gallon and J. R. Speakman. 2008. Estimating field metabolic rates of pinnipeds: doubly labeled water gets the seal of approval. Functional Ecology **22**: 245-254.

Speakman, J. R. 1997. Doubly labelled water theory and practice. Chapman and Hall. London. UK.

Speakman, J. R., and P. A. Racey. 1988. Validation of the doubly labeled water technique in small insectivorous bats by comparison with indirect calorimetry. Physiological Zoology **61**: 514-526.

Szafrańska, P. A., K. Zub, M. Wieczorek, A. Książek, J. R. Speakman and M.

Konarzewski. 2014. Shaving increases daily energy expenditure in free-living root voles. The Journal of Experimental Biology **217**: 3964-3967.

Visser, G. H., P. E. Boon and H. A. J. Meijer. 2000. Validation of the doubly labeled water method in Japanese Quail *Coturnix c. japonica* chicks: is there an effect of growth rate? Journal of Comparative Physiology **170**: 365-372.

Wallace, B. P., C. L. Williams, F. V. Paladino, S. J. Morreale, R. T. Lindstrom and J. R. Spotila. 2005.Bioenergetics and diving activity of interesting leatherback turtles *Dermochelys coriacea* at Parque Nacional Marino Las Baulas, Costa Rica. The Journal of Experimental Biology **208**: 3873-3884.

Watts, P. D., N. A. Øritsland and R. J. Hurst. 1987. Standard metabolic rate of polar bears under simulated denning conditions. Physiological Zoology **60**: 687-691.

Watts, P. and C. Cuyler. 1988a. Metabolism of the black bear under simulated denning conditions. Acta Physiologica Scandinavica **134**: 149-152.

Watts, P. D. and C. Jonkel. 1988b. Energetic cost of winter dormancy in grizzly bear. Journal of Wildlife Management **52**: 654-656.

Wei, F., Z. Wang and Z. Feng. 2000. Energy flow through populations of giant pandas and red pandas in Yele Natural Reserve. Acta Zoologica Sinica **46**: 287-294.

Welcker, J., O. Chastel, G. W. Gabrielsen, J. Guillaumin, A. S. Kitaysky, J. R. Speakman, Y. Tremblay and C. Bech. 2013. Thyoid hormones correlate with basal metabolic rate but not field metabolic rate in a wild bird species. Plos One **8**: 1-7

Williams, J. B. and A. Prints. 1986. Energetics of growth in nestling savannah sparrows: a comparison of doubly labeled water and laboratory estimates. Cooper Ornithological Society **88**: 74-83.

Yang, C., X. Zou and G. Zhang. 2005. Effects of Protein Level and Energy Density of Concentrated Diets on Digestibility of Adult Giant Pandas. Scientia Silvae Sinicae **41**: 119-125.

TABLES

Table 3.1. M in Sex means male and F means female. W in season means winter and S means summer.

Name	Studbook Number	Sex	Mass (kg)	Season	Average Temperature (℃)	High (℃)	Low (℃)	Study Duration (day)	D2 Background (ppm)	O18 Background (ppm)	Nd (mol)	No (mol)	Nd/No	kd	ko	kd/ko
Shishi	467	M	132	W	8.9	17	5	2.75	148.45	1988.17	5671.33	5539.46	1.0238	0.0108	0.0128	0.8429
Qiyuan	491	F	102	W	8.3	18	5	5.50	147.65	1985.73	3861.97	3752.99	1.0290	0.0076	0.0086	0.8778
Meilan	649	M	114	S	25.9	32	22	4.81	152.59	1993.14	4712.26	4544.81	1.0368	0.0090	0.0104	0.8656
Shishi	467	M	136	S	25.9	32	22	4.83	146.13	1984.39	5597.90	5187.36	1.0791	0.0119	0.0128	0.9295
Qiubang	574	M	138	S	24.5	31	22	4.80	146.81	1983.81	5509.94	5314.54	1.0368	0.0127	0.0138	0.9223
Wuyi	630	M	122	S	25.0	31	22	4.80	148.72	1986.31	4484.69	4288.52	1.0457	0.0088	0.0097	0.9022
Xiongbang	540	M	132	S	24.5	33	22	4.80	149.82	1988.67	5457.22	5287.02	1.0322	0.0109	0.0123	0.8840

Table 3.2. M in Sex means male and F means female. W in season means winter and S means summer. Activity time in the winter is by personal observing. 0.9 was used as RQ to predict oxygen consumption and FMR in KJ/day. Nagy 1999 body mass vs. FMR equation was used to predict giant panda FMR based on mass.

Name	Studbook Number	Sex	Mass (kg)	Season	Average Temperature (℃)	Active Time (%)	FMR CO2 (ml/g/h)	FMR O2 (ml/g/h)	FMR (KJ/day)	Predict FMR (KJ/day)	Water loss (kg/day)	Water Turnover (%/day)	Total Body Water (%)	Half Life (day)
Shishi	467	M	132	W	8.9	40.0	0.658	0.731	47717	27641	16.93	16.93	75.7	2.95
Qiyuan	491	F	102	W	8.3	40.0	0.265	0.295	14876	22875	8.89	13.13	66.4	3.81
Meilan	649	M	114	S	25.9	34.0	0.404	0.449	25301	24821	12.76	15.56	72.0	3.21
Shishi	467	M	136	S	25.9	32.4	0.126	0.140	9401	28253	19.22	20.53	68.8	2.44
Qiubang	574	M	138	S	24.5	30.3	0.177	0.197	13450	28557	21.08	21.97	69.5	2.28
Wuyi	630	M	122	S	25.0	33.9	0.193	0.215	12959	26088	11.77	15.20	63.4	3.29
Xiongbang	540	M	132	S	24.5	33.6	0.378	0.420	27440	27641	17.99	18.85	72.3	2.65

Table 3.3. M in Sex means male and F means female. W in season means winter and S means summer.

Name	Studbook Number	Sex	Mass (kg)	Season	Water loss (kg/day)	Water Turnover (%/day)	Total Body Water (%)	Half Life (day)
Shishi	467	M	132	W	16.93	16.93	75.7	2.95
Qiyuan	491	F	102	W	8.89	13.13	66.4	3.81
Meilan	649	M	114	S	12.76	15.56	72.0	3.21
Shishi	467	M	136	S	19.22	20.53	68.8	2.44
Qiubang	574	M	138	S	21.08	21.97	69.5	2.28
Wuyi	630	M	122	S	11.77	15.20	63.4	3.29
Xiongbang	540	M	132	S	17.99	18.85	72.3	2.65

Table 3.4. Field metabolic rates of giant panda and other large mammals.

FMR of Giant Panda with Other Similar Size Mammal						
Genus	Species	Common name	Mass (kg)	SD	FMR (KJ/day/kg)	SD
Arctocephalus	gazella	fur seal	43.51	2.58	417.74	66.78
Lama	glama	camelid	48.00	6.62	297.53	57.98
Odocoileus	hemionus	mule deer	53.03	12.52	654.30	191.11
Macropus	giganteus	grey kangaroo	60.80	0.00	176.48	0.00
Phocarctos	hookeri	sea lion	61.03	18.09	264.02	64.36
Rangifer	tarandus	rein deer	74.93	4.61	106.88	55.87
Oryx	leucoryx	oryx	84.10	13.86	196.00	73.33
Cervus	elaphus	red deer	107.50	1.86	234.87	20.13
Pongo	pygmaeus	orangutan	113.91	36.10	472.90	28.84
Ailuropoda	melanoleuca	giant panda	125.14	13.21	172.85	100.69
Odobenus	rosmarus	walrus	1310.00	84.85	292.87	58.05

Table 3.5. Giant panda food requirement of the FMR is from his study. Digestibility, food energy and water content of bamboo are from He et al., 2000. The Nagy prediction equation is from Nagy, 1999.

Giant panda bamboo requirment calculation										
								Nagy (1999) prediction		
Studbook Number	FMR (KJ/day)	Digestibility (%)	Energy intake (KJ)	Food energy (KJ/kg)	Intake dry matter (kg)	Water content of bamboo (%)	Bamboo intake (kg)	Mammalian carnivores (kg)	Mammalian frugivores (kg)	Herbivores (kg)
467	47717	18.14	263046	18528.5	14.20	51.07	29.01	9.47	24.10	15.91
491	14876	18.14	82007	18528.5	4.43	51.07	9.05	2.95	7.51	4.96
649	25301	18.14	139475	18528.5	7.53	51.07	15.38	5.02	12.78	8.43
467	9401	18.14	51826	18528.5	2.80	51.07	5.72	1.87	4.75	3.13
574	13450	18.14	74147	18528.5	4.00	51.07	8.18	2.67	6.79	4.48
630	12959	18.14	71440	18528.5	3.86	51.07	7.88	2.57	6.55	4.32
540	27440	18.14	151268	18528.5	8.16	51.07	16.69	5.44	13.86	9.15

CHAPTER 4: THE METABOLIC RATE OF THE RED PANDA, *AILURUS FULGENS*, A DIETARY BAMBOO SPECIALIST

Introduction

The red panda (*Ailurus fulgens*) (Cuvier, 1825) is called a “panda”, but molecular and chromosomal data place it in its own family, Ailuridae. It is related to weasels, otters, raccoons, kinkajous and skunks that are all members of the superfamily Musteloidea (Flynn, et al. 2000; Nie, et al 2002). The red panda has a similar diet, primarily bamboo, and shares the same habitat as the giant panda, *Ailuropoda melanoleuca* (Wei, et al., 2000). The false thumb (carpal bone) of the red panda evolved as an adaptation to climbing and secondarily developed for item manipulation. Thus, its adaptation for eating bamboo is a case of convergent evolution with the giant panda (Salesa et al., 2006). Ecological studies of the red panda have defined its home range and seasonal activity in different nature reserves (Reid, et al. 1991; Wei et al. 2000; Zhang, et al. 2009) and described habitat use and separation between the red panda and the giant panda (Wei et al. 2000). Threats to its survival and conservation have been known for some time (Yonzon and Hunter 1991). However, there appears to be a high level of genetic diversity in the red panda populations in Sichuan and Yunnan Provinces (Su, et al. 2001).

There are considerable efforts underway to understand the ecology of the red panda and to increase its populations in natural reserves. Yet it is difficult to design an effective strategy for red panda reintroduction if we do not understand its basic biology. Surprisingly there are few studies on the physiology of the red panda. In addition, we do not know the thermoregulatory adaptations that allow this animal to function in the mountains at 2800-3000 m during winter and summer. Ultimately what is needed is knowledge of its biophysical ecology and climate space (Porter and Gates 1969). The basis for such analyses is information on the metabolic rate of the red panda.

In one of a series of classic studies McNab (1988) measured the resting metabolic rates of two red pandas from a zoo. He found that red pandas decreased metabolic rate at low environmental temperatures without reduced body temperature. However, the metabolic rates that McNab measured were much below those predicted by allometric equations of metabolic rate vs. body size in mammals (Sieg et al., 2009). In addition, his descriptions of his experimental animals suggest that they were very docile, perhaps even lethargic. That is quite unlike red pandas at the Research Base of Giant Panda Breeding (Panda Base) in Chengdu, Sichuan Province China (www.panda.org.cn) that live in large enclosures and are very active. They are anything but docile and are difficult to restrain. Therefore, I measured the resting metabolic rate (RMR) of red pandas during winter and summer at the Panda Base to obtain information on animals that were living under more natural conditions than in McNab's study. My measurements indicated that red pandas have metabolic rates similar to those expected from allometric relationships and that red pandas elevate their metabolic rates at low temperatures.

Methods

Red panda acquisition and maintenance

I studied red pandas at the Research Base of Giant Panda Breeding (Panda Base) in Chengdu, China (www.panda.org.cn) and conducted all experiments in cooperation with the research, veterinary and husbandry staff there. The Research Base of Giant Panda Breeding was a nonprofit organization with offices in Chengdu, Sichuan Province, China. It was a center for wildlife research, giant panda and red panda captive breeding, conservation education, and educational tourism. Because red pandas are rare and most zoos only have a few individuals there have not been many animals available for metabolic studies. I was fortunate that the Panda Base had more than 100 red pandas and I was allowed to use 17 (all adults) of them for these experiments under very close veterinary supervision. The staff there had unique experience in breeding and husbandry of giant pandas and red pandas based on more than 30 years of research. Red pandas lived in their normal enclosures with access to large outside areas and ate a diet composed primarily of bamboo supplemented with foods such as apples and "panda cake", a biscuit made of a mixture of grains with vitamins. I transported red pandas to the laboratory for each experiment.

Metabolic rate experimental design

I measured metabolic rate during two seasons, summer and winter. Because there was no effective air temperature-control room at the Panda Base I had to use natural air temperature change during the seasons to study the red pandas under warm and cool conditions. I did that to assess the thermal neutral zone of the red panda.

I studied 10 red pandas in summer and 7 red pandas in winter. Because red pandas are diurnal, I conducted all experiments during night hours (2200-0400). Red pandas were weighed before and after each experiment. My goal was to measure the basal metabolic rate (BMR) of these animals keeping in mind the criteria of Kleiber (1961) that the animals be post-absorptive and at rest. Speakman et al., (1993) stated that it is not always possible to adhere completely to the Kleiber criteria in studies on wild animals and that it is necessary to take into consideration the constraints of reality for the species under study. Even Kleiber (1961) stated that measurement of a true BMR was probably only possible in humans. So many authors use the term standard metabolic rate or resting metabolic rate (RMR) rather than BMR for non-human animals. I believe that my measurements of the resting metabolic rate (RMR) of red pandas were as close to BMR as it was possible to obtain under realistic conditions because I fasted the animals for 24 h before an experiment and the animals were at rest and not active in the metabolism chamber.

I measured metabolic rate in a Plexiglas chamber using a flow through system to measure oxygen consumption and carbon dioxide production. The chamber was 1 m * 1 m * 1 m and constructed of 2.0 cm Plexiglas with a steel frame for added strength. One side of the chamber was a door held by steel hinges, sealed with a rubber gasket and closed with metal latches (Figure 1). There were three 2.5 cm holes, with 60 cm long tubing attached to avoid backflow, for air intake at the bottom right side of the chamber. There was one 2.5 cm exit hole at the top left side of the chamber that connected to spiral-wound tubing leading to a Flowkit -500 mass flow system (Sable Systems International). A subsample of air went from the Flowkit pump to a FOXBOX oxygen and carbon dioxide analyzer (Sable Systems International). The three air intake holes and one air exit hole eliminated negative pressure in the system. The placement of the holes reduced air stagnation and two small battery operated fans in the chamber assured that the air was well mixed. Six 24-gauge Cu-Co thermocouples (+/- 0.05℃) located inside the chamber on the top, right side, left side, back side, and in the mouth of the air intake and exit holes measured chamber temperatures.

The Sable System Flowkit used a precision mass flow sensor with a rotary pump controlled by a microprocessor to control air flow rate to within 2 % of reading. The Flowkit pump's air flow was set at 25 L/min. After leaving the Flowkit pump, air was subsampled though a small plastic tube and drawn into the FOXBOX system at a rate of 200 ml/min. The subsample went through a relative humidity meter and temperature meter before it entered the gas analyzers. Sample air passed through the CO_2 analyzer and then a drierite column before entering the O_2 analyzer. Since water vapor would interfere with the fuel cell in the oxygen analyzer I removed the water before it entered that analyzer. The accuracy of the Sable System Foxbox was 0.1 % for O_2 over a range of 2-100% and 1.0 % of span for CO_2 over a range of 0-5%). I used calibration gas (14.93 % O_2, 3.99 % CO_2) from Dalian Special Gas Industry Company and tested by National Institute of Measurement and Testing Technology, 100 % dry N_2 and room air to calibrate the system. I converted gas measurements to standard temperature and pressure (STP).

Statistical analysis

I used a fitting linear model (LM) in program R (R Development Core Team 2011). The LM consisted of the interactions between two fixed factors that included season and sex with temperature and mass as covariates. I used model comparison and simplification to remove factors that were not significantly related to RMR. The final linear model contained the effects of season and temperature. I accepted $P \leq 0.05$ as a statistically significant difference.

Results

The resting metabolic rate (RMR) of the red panda ranged from 0.204 ml/h•g to 0.406 ml/h•g (Figure 2). The LMER model ANOVA (Table 1) indicated that there was a statistically significant effect of season (df = 1, 11; F = 28.149; P = 0.000) and temperature (df = 1, 11; F = 6.541; P = 0.027). The RMR of red pandas was 0.290 ml/h/g (range = 0.204 ml/h•g to 0.342 ml/h•g) in the summer (temperature range from 15.3℃ to 20.2℃);

and it was 0.361 ml/h/g (range = 0.331 ml/h•g to 0.406 ml/h·g) in the winter (temperature range from 5.3℃ to 9.1℃). There was no difference in RMR between males and females, and no difference in RMR due to mass.

Discussion

There was no difference in metabolic rate between male and female red pandas. Some mammals have behavioral and physiological differences between males and females that cause differences in RMR. For example, in humans, males have higher BMR and active metabolic rate than females, but female margays (*Leopardus wiedii*) have higher BMR than males (McNab, 2000; Henry, 2005; Black et al., 1996). Those differences would probably be reflected in their active metabolic rates. There was no difference in activity of males and females in my metabolic chamber. Both sexes were quiescent. Therefore, there was no difference in their RMR.

Temperature is an important factor affecting RMR. Mammals have a thermal neutral zone in which animals have a minimum RMR. Below that zone metabolic rate increases due to thermoregulation. Above that zone metabolic rate increases due to a loss in the ability of the animal to cool its body temperature by behavioral and physiological means (Withers, 1992). In my experiments, metabolic rates of red pandas were statistically significantly higher in winter at environmental temperatures between 5.3℃ to 7.6℃ than in summer at environmental temperatures of 15.5℃ to 20.2℃. Temperatures in summer season were probably within the thermal neutral zone, but winter temperatures were below the thermal neutral zone. There was no difference in activity by red pandas in the chamber during winter and summer. However, I expect that red pandas will reduce their activity to conserve heat during winter in nature. Two red pandas spent more time resting in winter than summer in the Wolong Nature Reserve, Sichuan Province, China (Reid et al. 1991). Energy digestibility of bamboo, *Bashania spanostachya*, in the Yele Nature Reserve, Sichuan Province is low in winter and red pandas take in more energy in summer-autumn than in winter (Wei et al. 2000). Therefore, red pandas should reduce their activity in winter to conserve energy and thermoregulation.

Species comparisons

Contrary to my expectations based on its major diet of bamboo leaves and stalks and McNab's data from two red pandas (McNab, 1988) the metabolic rate of the red panda was similar to that of other mammals of the same size. I compared the resting metabolic rate in ml/h (MR) of the giant panda to those reported for 49 other mammals ranging in size from 2010 g to 10,550 g taken from Sieg et al. (2009) (Table 2). The metabolic rate of the red panda was higher than that of the some similar mass species such as the chimpanzee (*Pan troglodytes*), crab-eating fox (*Cerdocyon thous*), eyra cat (*Puma yagouaroundi*), and plains vizcacha (*Lagostomus maximus*); but lower than that of the raccoon (*Procyon lotor*), golden-mantled howling monkey (*Alouatta palliata*), Bornean orangutan (*Pongo pygmaeus*), culpeo (*Lycalopex culpaeus*), North American porcupine (*Erethizon dorsatum*), Guinea baboon (*Papio papio*) and lowland paca (*Cuniculus paca*) (Bruhn, 1934; Milton et al., 1979; Kohl, 1980; Hennemann, et al., 1983; McNab, 1995; Fournier and Thomas, 1999; McNab, 2000; Fournier and Thomas, 1999; Silva et al., 2004; Arends and McNab, 2001). There was no pattern in the metabolic rates of those animals because there was too much variation between species. Taxonomic, physiological and environmental differences are not adequately explained by current theories (Glazier 2005;

Sieg et al. 2009). Diverse adaptations in thermoregulation and metabolism have evolved in the context of physiological, biochemical and ecological constraints.

McNab's red pandas

McNab measured 2 red pandas in 1987 and found a very low metabolic rate, 0.153 ml/g/h. The data of McNab were well below those in my study and well below the values predicted by the regression line in Sieg et al. (2009). In my 17 red panda experiments, 0.204 ml/g/h was the lowest value that I measured. The average in summer (0.290 ml/g/h) was twice as high as McNab's mean. It is possible that his red pandas had a subclinical condition that lowered their metabolic rate. McNab stated that the red pandas could be grasped by their tails to move them around. Normal red pandas are not docile. They are easily frightened and aggressive. When you get close to them, they will shout and warn you. If you try to touch them, they will scratch and bite. So you must be very careful and have thick gloves and thick sleeves for protection, when you transfer them. Nevertheless, once in the metabolic chamber the red pandas in my experiments settled down and rested peacefully. The lowering of metabolic rate that McNab's red pandas underwent without a drop in body temperature also suggests that the animals may have been ill. That is not to state that McNab's experiments were poorly done. Rather they were groundbreaking. However, my experiment shows the value of a larger sample size, more natural conditions in the red panda enclosures at the Panda Base and a better understanding of the normal biology of the animal.

Metabolic scaling

I plotted the RMRs of the red panda and the 49 other similar size mammals from Sieg et al. (2009) (Figure 3). The regression line through those data ($\log_{10}$ (MR) = 1.1641 Log_{10}

(Mass) - 1.0771, $r^2 = 0.74$; $P = 0.000$) was different from that of Sieg et al. (2009) for carnivores/ungulates/pangolins (Fereuungulata) (Waddell et al., 1999; Springer et al., 2005). The new regression line was steeper than that both of the Fereuungulata and universal regression lines, which were calculated from all 695 mammals in their data set. That supports their conclusion that phylogenetic relationships affect the body size-metabolic rate regression and that there is not a single universal metabolic rate-body mass scaling relationship in mammals (Sieg. et al., 2009). In addition, different mass domains of mammals show different regression scaling (Clarke et al, 2010). Small mammals and large mammals may have different scaling relationships (Glazier, 2005). This is due to the variation of RMR within or among animal species. Metabolic rate is greatly affected by temperature, activity, food digestion and so on. Temperature and activity are the two major factors related to RMR. They are often well controlled during the experiment. However, some other important factors like growth or age may not be controlled. In Sieg's 695 mammal species dataset, there are some species that were not adults so they would not provide the metabolic rate for an adult of that size. In addition, some species exhibit sexual differences in RMR. That will increase the variation in metabolic data for a species. Furthermore, factors such as light intensity, season, geographical location, nutritional level, parasitic infection and exposure to air or water will also affect the metabolic status of experimental animals. Therefore, it is hard to truly measure BMR of wild animals. White laboratory rats and humans may be the only animals that will provide a true BMR because they are adjusted to a stable laboratory environment (Kleiber, 1961; Speakman et al., 1993).

The data sets used to calculate MR vs. body mass regressions, typically have more small size mammals than large size mammals and metabolic scaling is steeper in large than in small mammals. In addition, a combination of phylogenetic relationships and physiological factors affect the metabolic rate of individual species and no one predictive line can account for all variation in the body size-metabolism relationship among mammals (Glazier 2005). More research should focus on RMR variation within and among species. These experiments will have to take into account the myriad factors that affect metabolism in a species and the repeatability of measurements on an individual animal under the same test conditions. Davy et al. (2014) discuss the importance of repeatability and rank repeatability in behavioral experiments. Past studies of RMR indicate that there is considerable variability between individuals of the same species in an experiment. That suggests that differences in RMR may be subject to natural selection. Future metabolic studies should determine the repeatability of RMR within an individual and the rank repeatability of RMR between individuals within a test group. Such measurements will help to refine our estimates of RMR vs. body mass for a given species and perhaps reduce the inter- specific variation in in those estimates.

List of References

Arends, A., and B.K. McNab. 2001. The comparative energetics of 'caviomorph' rodents. Comparative Biochemistry and Physiology Part A. **130**: 105-122.

Black, A. E., Coward, W. A., Cole, T. J. and Prentice, A. M. 1996. Human energy expenditure in affluent societies: an analysis of 574 doubly-labeled water measurements. European Journal of Clinical Nutrition **50**: 72-92.

Bruhn, J. M. 1934. The respiratory metabolism of infrahuman primates. American Journal of Physiology **110**: 477-484.

Clarke, A., P. Rothery and N. J. B. Isaac. 2010. Scaling of basal metabolic rate with body mass and temperature in mammals. Journal of Animal Ecology **79**: 610-619.

Davy, C. M., J. E. Paterson and A. E. Leifso. 2014. When righting is wrong: performance measures require rank repeatability for estimates of individual fitness. Animal Behavior **93**: 15-23.

Flynn, J. J., M. A. Nedbal, J. W. Dragoo and R. L. Honeycutt. 2000. Whence the red panda?. Molecular Phylogenetics and Evolution **17**: 190-199.

Fournier, F., and D.W. Thomas. 1999. Thermoregulation and repeatability of oxygen-consumption measurements in winter-acclimatized North American porcupines (*Erethizon dorsatum*). Canadian Journal of Zoology **77**: 194-202.

Glazier, D. S. 2005. Beyond the ‘3/4-power law’: variation in the intra-and interspecific scaling of metabolic rate in animals. Biological Reviews **80**: 611-662.

Hennemann, W. W., S.D. Thompson, and M.J. Konecny. 1983. Metabolism of crab-eating foxes, Cerdocyon thous: ecological influences on the energetics of canids. Physiological Zoology **56**: 319-324.

Henry, C. 2005. Basal metabolic rate studies in humans: measurement and development of new equations. Public Health Nutrition **8**: 1133-1152.

Kleiber, M. 1961. The Fire of Life: an introduction to animal energetics. Wiley, N. Y.

Kohl, H. 1980. Temperaturregulation, stoffwechsel und Nierenfunktion beim chinchilla (Chinchilla Laniger Molina, 1782) und beim viscacha (Lagostomus Maximus Brookes, 1828). Zoologische Jahrbücher. Abteilung für allgemeine Zoologie und Physiologie der Tiere **84**: 472-501.

McNab, B. K. 1988. Energy conservation in a Tree-Kangaroo (*Dendrolagus matschiei*) and the Red Panda (*Ailurus fulgens*). Physiological Zoology **61**: 280-292

McNab, B. K. 1995. Energy expenditure and conservation in frugivorous and mixed-diet carnivorans. Journal of Mammalogy **76**: 206-222.

McNab, B. K. 2000. The standard energetics of mammalian carnivores: Felidae and Hyaenidae. Canadian Journal of Zoology **78**: 2227-2239.

Milton, K., T.M. Casey, K.K. Casey. 1979. The basal metabolism of mantled howler monkeys (*Alouatta palliata*). Journal of Mammalogy **60**: 373-376.

Nie, W., J. Wang, P. C. M. O'Brien, B. Fu, T. Ying, M. A. Ferguson-Smith and F. Yang. 2002. The genome phylogeny of domestic cat, red panda and five mustelid species revealed by comparative chromosome painting and G-banding. Chromosome Research **10**: 209-222.

Porter, W. P. and David M. Gates. 1969. Thermodynamic Equilibria of Animals with Environment. Ecological Monographs **39**: 227-244.

Reid, D. G. 1991. Ecology of the red panda *Ailurus fulgens* in the Wolong Reserve, China. Journal of Zoology **225**: 347-364.

Salesa, M. J., M. Antón, S. Peigné and J. Morales. 2006. Evidence of a false thumb in a fossil carnivore clarifies the evolution of pandas. PNAS **103**: 379-382.

Sieg, A. E., M. P. O'Connor, J. N. McNair, B. W. Grant, S. J. Agosta and A. E. Dunham. 2009. Mammalian metabolic allometry: do intraspecific variation, phylogeny, and regression models matter. The American Naturalist **174**: 720-733.

Silva, S. I., F.M. Jaksic, and F. Bozinovic. 2004. Interplay between metabolic rate and diet quality in the South American fox, *Pseudalopex culpaeus*. Comparative Biochemistry and Physiology Part A **137**: 33-38.

Speakman, J. R., R. M. McDevitt and K. R. Cole. 1993. Measurement of basal metabolic rates: don't lose sight of reality in the quest for comparability. Physiological Zoology **66**: 1045-1049.

Springer, M. S., W. J. Murphy, E. Eizirik and S. J. O'Breien. 2005. Molecular evidence for majorplacental clades. In: Rose, K. D. and J.D. Archibald. The rise of placental mammals: origins and relationships of the major extant clades. John Hopkins University Press, Baltimore, 37-49.

Su, B., Y. Fu, Y. Wang, L. Jin and R. Chakraborty. 2001. Genetic diversity and population history of the red panda (*Ailurus fulgens*) as inferred from mitochondrial DNA sequence variations. Molecular Biology and Evolution **18**: 1070-1076.

Waddell, P. J., Y. Cao, J. Hauf and M. Hasegawa. 1999. Using novel phylogenetic methods to evaluate mammalian mtDNA, including amino acid-invariant sites-logdet plus site stripping, to determine internal conflicts in the data, with special reference to the positions of hedgehog, armadillo, and elephant. Systematic Biology **48**: 31-53.

Wei, F., Z. Feng, Z. Wang and J. Hu. 2000. Habitat use and separation between the giant panda and the red panda. Journal of Mammalogy **81**: 448-455.

Withers, P. C. 1992. Comparative Animal Physiology. Saunders College Publishing, Florida, 106-108.

Yonzon, P. B., and M. L. Hunter Jr. 1991. Conservation of the Red Panda *Ailurus fulgens*. Biological Conservation **57**: 1-11.

Zhang Z., J. Hu, J. Yang, M. Li and F. Wei. 2009. Food habits and space-use of red pandas Ailurus fulgens in the Fengtongzhai Nature Reserve, China: food effects and behavioural responses. Acta Theriologica **54**: 225–234.

TABLES

Table 4.1. Results of fitting linear model Analysis of Variance on the effects of sex, mass and temperature on the metabolic rates of red pandas. Age and sex were fixed factors and temperature and mass were covariates.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Sex	1	0.001	0.001	1.62	0.230	
Season	1	0.020	0.020	28.15	0.000	***
Temperature	1	0.005	0.005	6.54	0.027	*
Mass	1	0.001	0.001	1.40	0.262	
Sex:Season	1	0.000	0.000	0.01	0.917	
Residuals	11	0.008	0.001			
Signif. codes: 0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1						

Table 4.2. Metabolic rates and RQs of red pandas measured in a metabolic chamber at the Chengdu Research Base of Giant Panda Breeding in Chengdu, China. Metabolic rates were below predictions.

Animal Number	Sex	Mass (g)	Temperature (℃)	RMR CO2 (ml/g/h)	RMR O2 (ml/g/h)	RQ	RMR O2 Prediction (ml/g/h)	% below the prediction
1	M	5490	19.0	0.224	0.290	0.77	0.369	21.41
2	M	5660	18.2	0.228	0.279	0.82	0.367	24.00
3	F	5540	19.9	0.231	0.342	0.68	0.369	7.26
4	F	5750	20.2	0.211	0.299	0.71	0.366	18.24
5	M	5260	18.5	0.306	0.304	1.01	0.372	18.34
6	F	6320	15.9	0.235	0.299	0.79	0.360	17.10
7	M	7150	17.3	0.238	0.290	0.82	0.352	17.64
8	F	7380	15.5	0.191	0.204	0.94	0.350	41.83
9	F	6780	16.3	0.199	0.286	0.70	0.356	19.50
10	M	5780	17.6	0.256	0.311	0.82	0.366	15.12
11	M	5590	7.6	0.247	0.359	0.69	0.368	2.50
12	M	4420	7.9	0.354	0.406	0.87	0.384	-5.72
13	F	6770	9.1	0.266	0.366	0.73	0.356	-2.74
14	M	5500	8.3	0.298	0.354	0.84	0.369	4.13
15	M	5570	5.5	0.289	0.353	0.82	0.368	4.09
16	F	5920	5.3	0.293	0.331	0.88	0.364	9.14
17	F	8290	7.0	0.272	0.360	0.75	0.343	-4.91

Table 4.3. Metabolic rates of 49 large mammals compiled by Sieg et al (2009) and red pandas measured at the Chengdu Research Base of Giant Panda Breeding.

Number	Animal	Genus	Species	Mass (g)	RMR O2 (ml/g/h)	Log10 (Mass)	Log10 (MR O2)
1	Small-toothed palm civet	Arctogalidia	trivirgata	2010	0.275	3.30	2.74
2	Southern viscacha	Lagidium	viscacia	2056	0.340	3.31	2.84
3	Striped civet	Fossa	fossana	2260	0.401	3.35	2.96
4	South African Springhare	Pedetes	capensis	2300	0.341	3.36	2.89
5	Common brown lemur	Eulemur	fulvus	2330	0.139	3.37	2.51
6	Kinkajou	Potos	flavus	2406	0.334	3.38	2.91
7	Jamaican coney	Geocapromys	brownii	2456	0.300	3.39	2.87
8	Wildcat	Felis	silvestris	2617.8	0.180	3.42	2.67
9	Desmarest's hutia	Capromys	pilorides	2630	0.227	3.42	2.78
10	Groundhog	Marmota	monax	2660	0.270	3.42	2.86
11	Red-rumped agouti	Dasyprocta	leporina	2687	0.580	3.43	3.19
12	Tayra	Eira	barbara	2950	0.414	3.47	3.09
13	Red fox	Vulpes	vulpes	2965.3	0.488	3.47	3.16
14	Patas monkey	Erythrocebus	patas	3000	0.213	3.48	2.81
15	Verreaux's sifaka	Propithecus	verreauxi	3000	0.243	3.48	2.86
16	Asian palm civet	Paradoxurus	hermaphroditus	3160	0.241	3.50	2.88
17	Brazilian porcupine	Coendou	prehensilis	3280	0.282	3.52	2.97
18	Margay	Leopardus	wiedii	3550	0.283	3.55	3.00
19	White-nosed coati	Nasua	narica	3630	0.327	3.56	3.07
20	Yellow-bellied marmot	Marmota	flaviventris	3706.5	0.343	3.57	3.10
21	Collared mangabey	Cercocebus	torquatus	3750	0.428	3.57	3.21
22	Azara's agouti	Dasyprocta	azarae	3849	0.490	3.59	3.28
23	South American coati	Nasua	nasua	3850	0.260	3.59	3.00
24	Arctic fox	Vulpes	lagopus	3932.9	0.458	3.59	3.26
25	African palm civet	Nandinia	binotata	4270	0.202	3.63	2.94
26	Coypu	Myocastor	coypus	4325	0.710	3.64	3.49
27	Golden-mantled howling monkey	Alouatta	palliata	4670	0.428	3.67	3.30
28	Bornean orangutan	Pongo	pygmaeus	4970	0.305	3.70	3.18
29	Common chimpanzee	Pan	troglodytes	5020	0.280	3.70	3.15
30	Raccoon	Procyon	lotor	5385	0.387	3.73	3.32
31	Culpeo	Lycalopex	culpaeus	5418	0.888	3.73	3.68
32	Crab-eating fox	Cerdocyon	thous	5614	0.272	3.75	3.18
33	Red panda	Ailurus	fulgens	5740	0.153	3.76	2.94
34	North American porcupine	Erethizon	dorsatum	5974	0.476	3.78	3.45
35	Red panda this study	Ailurus	fulgens	6068.8	0.290	3.78	3.25
36	Eyra cat	Puma	yagouaroundi	6105	0.255	3.79	3.19
37	Guinea baboon	Papio	papio	6760	0.404	3.83	3.44
38	Plains viscacha	Lagostomus	maximus	6804.5	0.234	3.83	3.20
39	Lowland paca	Cuniculus	paca	6831.5	0.346	3.83	3.37
40	Black-backed jackal	Canis	mesomelas	7720	0.505	3.89	3.59
41	Aardwolf	Proteles	cristata	7928.2	0.254	3.90	3.30
42	Blue monkey	Cercopithecus	mitis	8500	0.399	3.93	3.53
43	European otter	Lutra	lutra	8671.4	0.555	3.94	3.68
44	Japanese macaque	Macaca	fuscata	9300	0.469	3.97	3.64
45	Bobcat	Lynx	rufus	9400	0.449	3.97	3.63
46	Olive baboon	Papio	anubis	9500	0.311	3.98	3.47
47	Raccoon dog	Nyctereutes	procyonoides	9800	0.409	3.99	3.60
48	Serval	Leptailurus	serval	10120	0.329	4.01	3.52
49	Coyote	Canis	latrans	10171.4	0.358	4.01	3.56
50	Mantled guereza	Colobus	guereza	10450	0.285	4.02	3.47
51	Gray wolf	Canis	lupus	10550	0.375	4.02	3.60

FIGURES

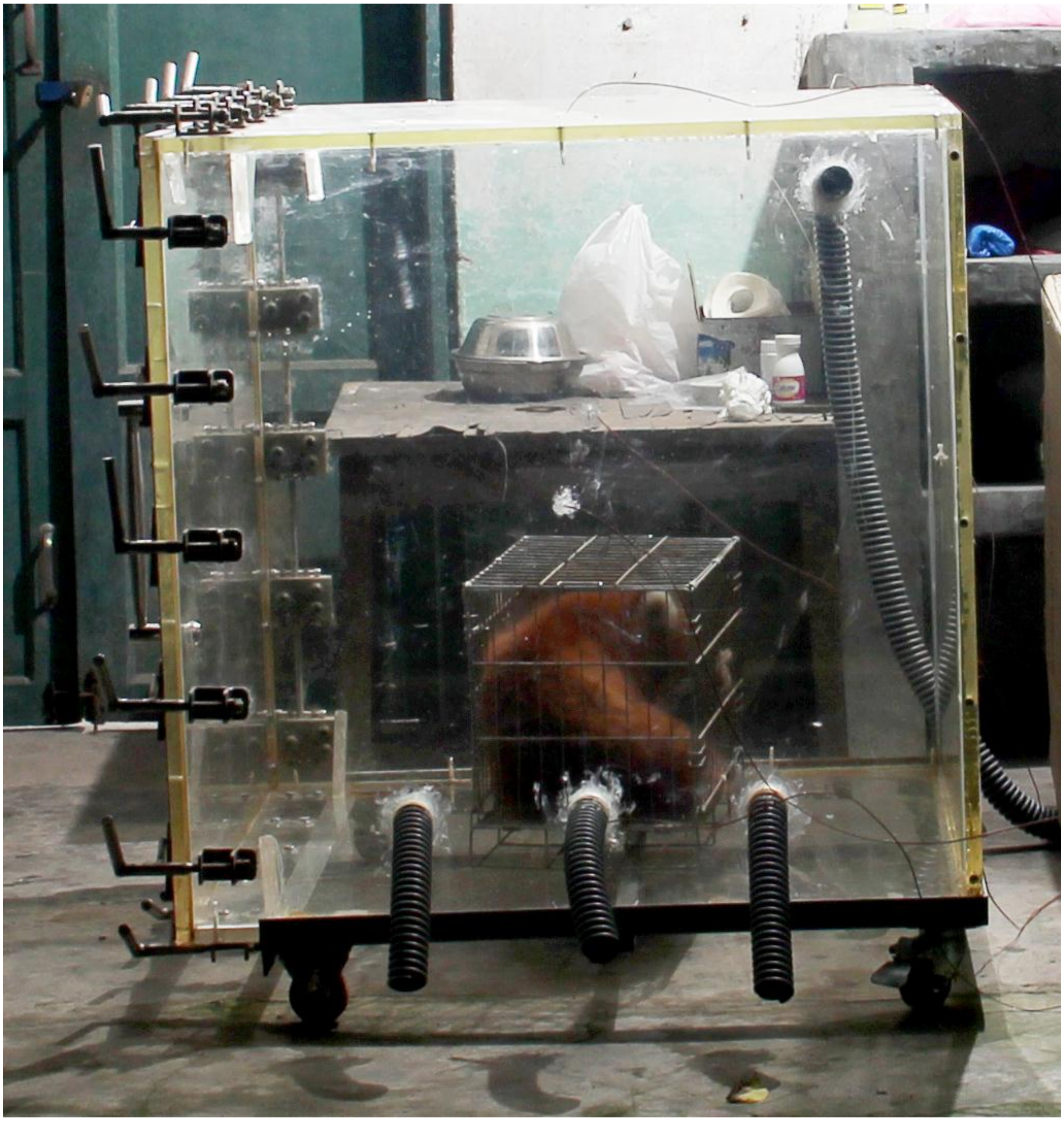

Figure 4.1. Metabolic chamber used to measure the metabolic rates of red pandas at the Research Base of Giant Panda Breeding in Chengdu, China.

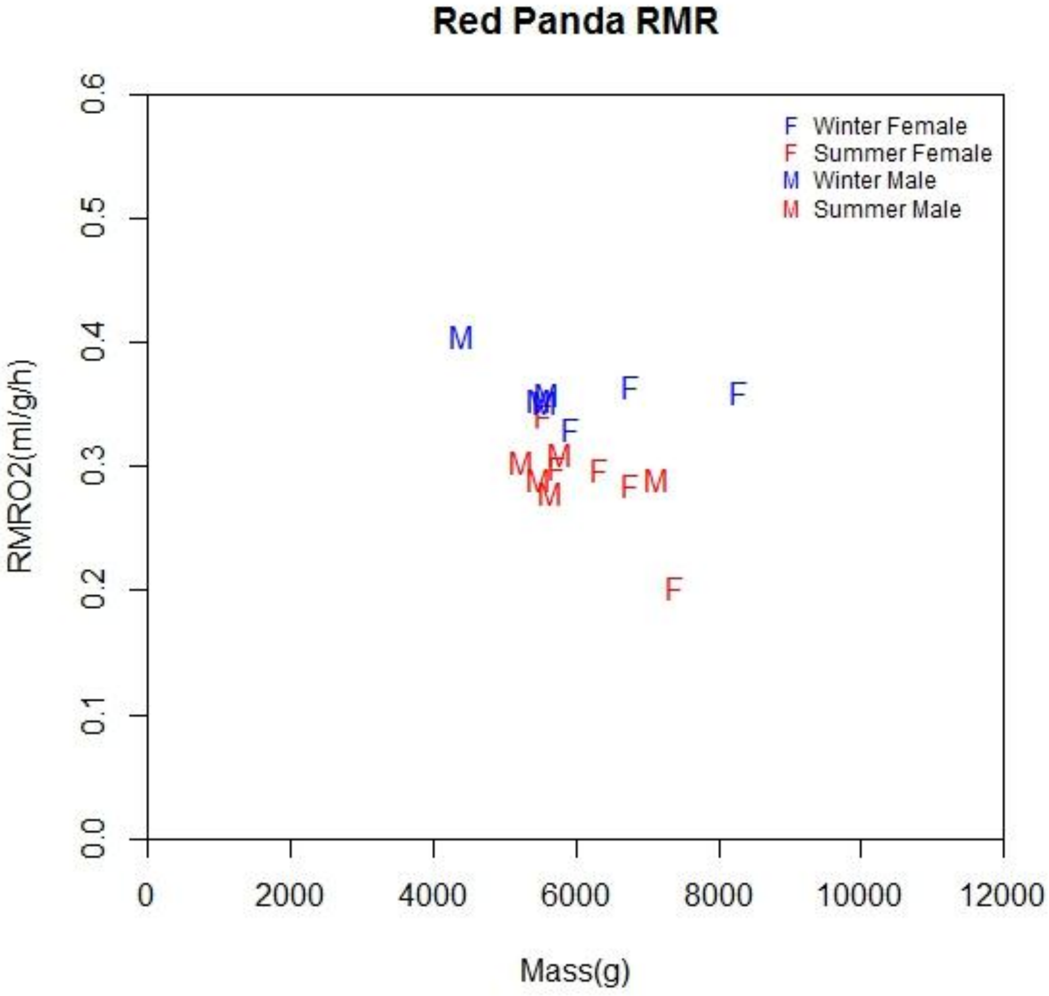

Figure 4.2. Metabolic rates of red pandas measured at the Research Base of Giant Panda Breeding in Chengdu, China. Animals were at rest in a metabolic chamber at temperatures between 5.3 and 20.2 ℃. M represents males and F represents females. Blue means RMR measured at winter time, and red is in the summer.

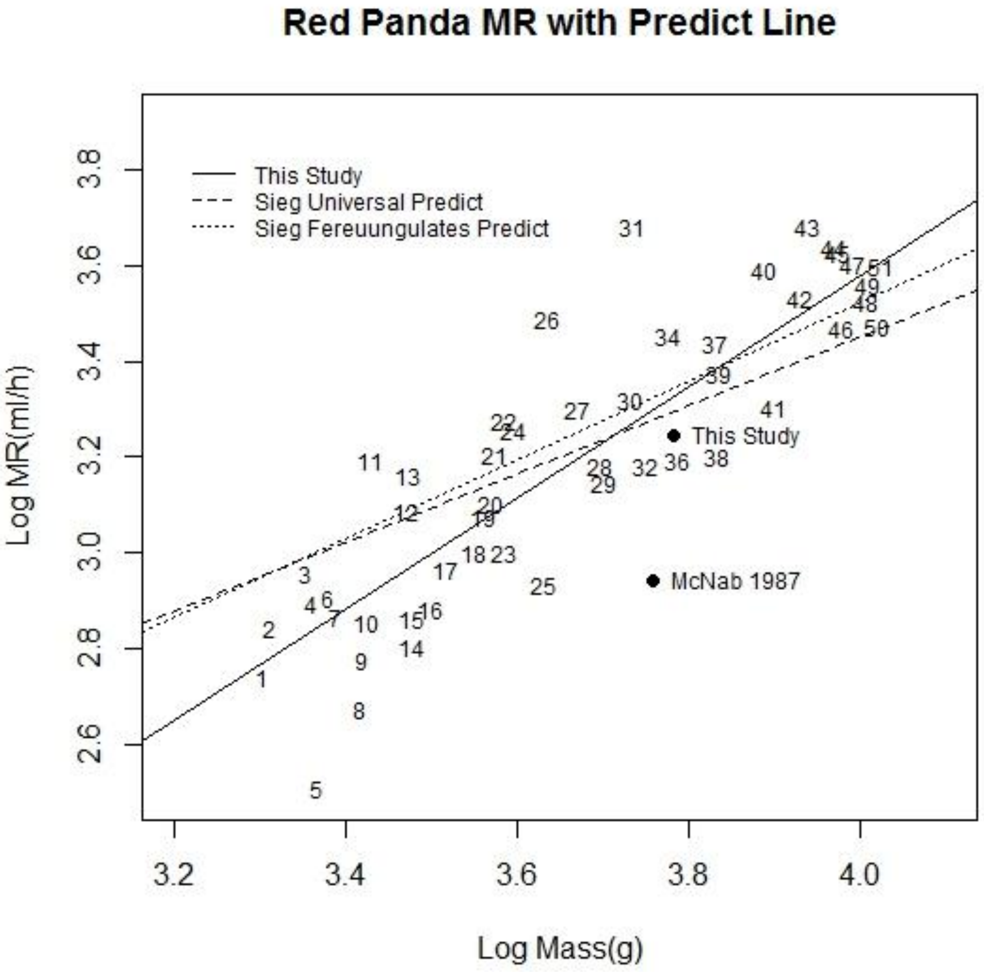

Figure 4.3. Relationship between body mass and metabolic rate in red pandas and 49 other large mammals. RP represents red panda. Regression lines for all mammals and for Ferreuungulate mammals are from Sieg et al. (2009). Solid line is regression line calculated by us with the addition of the red panda.

CHAPTER 5: CONCLUSIONS

I measured the metabolic rates of giant pandas at rest and when active in a zoo like setting. I also measured the metabolic rates of red pandas at rest. These data support the conclusion that bamboo should not be a limiting factor in the number of giant pandas in the wild.

RMR of giant pandas

There were differences in metabolic rates between young cub and adult giant pandas**.** In our study both age and mass affected RMR. Although the effects of mass and age were confounded, the statistical significance of the effects suggested that age had a greater effect than mass on RMR.

There was no difference in metabolic rate between male and female giant pandas. There was no difference in activity of males and females in our metabolic chamber. Both sexes were quiescent. Therefore, there was no difference in their RMR.

In my experiment, there was no difference in metabolic rates of giant pandas at environmental temperatures between 9.1℃ and 26.5℃. Therefore, these temperatures are within the thermal neutral zone. There was no indication that the animals were more active at these temperatures and they showed no signs of behavioral stress.

I compared the resting metabolic rate in ml/h (MR) of the giant panda to those reported for 21 other mammals ranging in size from 50 kg to 193 kg taken from Sieg et al. (2009). The metabolic rate of the giant panda was higher than that of the sloth bear (McNab, 1992) but lower than that of the tiger (*Panthera tigris*), lion (*Panthera leo*), cow (*Bos taurus*), and eland (*Taurotragus oryx*) (McNab, 2000; Taylor and Lyman, 1967;

Taylor et al., 1969).

I also plotted the MRs of the giant panda and the 21 other large mammals from Sieg et al. (2009). The regression line was almost the same as that of Sieg et al. (2009) for carnivores/ungulates/pangolins (Fereuungulata) (Waddell et al., 1999; Springer et al., 2005). Both of those lines were above the line calculated from all 695 mammals in their data set. That supports their conclusion that phylogenetic relationships affect the body size- metabolic rate regression and that there is not a single universal metabolic rate-body mass scaling relationship in mammals. Also, Giant panda metabolic rates were 6.0% to 44.3 % below those predicted by the Fereuungulata regression line. Therefore, a combination of phylogenetic relationships and physiological factors affect the metabolic rate of individual species and no one predictive line can account for all variation in the body size-metabolism relationship among mammals.

FMR of giant pandas

The active metabolic rates of giant pandas varied between individuals despite the active time for each individual being very similar. The active time was greatly influenced by husbandry practices. I recorded the active time of giant pandas but was unable to measure the magnitude of activity. That was the reason why they had similar activity times but quit difference FMRs.

The mean DEE of 21,592 KJ, SD = 13323) per day was similar to the estimation of daily digestible energy intake (17,222 KJ to 28,329KJ) of wild giant pandas (He et al., 2000). It was not surprising that some individuals had lower FMR because they were captive animals living in a small area of an enclosure. I calculated that giant pandas would have to eat about 13.13 (SD = 8.10) kg of bamboo to support the metabolic rates that I measured based on the work of He et al. (2000). Giant pandas at the Panda Base usually eat around 15 kg to 20 kg bamboo per day (unpublished data). Giant pandas in the Xiangling Mountains eat 13.14 kg to 14.48 kg of bamboo a day (He et al., 2000). However, the prediction from Nagy's (1999) equation about food requirement based on metabolic rate was smaller than what the giant pandas actually ate. So the prediction was not adequate for this special carnivore with an herbivorous diet.

The water-loop of the giant panda was much faster than predicted. Speakman (1997) predicted that a 50 kg or larger animal would have a 5-day half-life for doubly labeled water. However, a 100 kg giant panda had a rate twice as high as the prediction. This was probably due to their special diet, 99% bamboo. Giant pandas do not drink water very often, but they do drink a lot each time. They will continue drinking for 2 to 3 min (personal observation). In addition, the giant panda has a different kidney type from other bears (Hu, 2000).

Comparing the FMR of the giant panda to those of 10 similar sized mammals (Hudson et al. 2013) it appears that it is lower than most similar size mammals, like the seal (*Arctocephalus gazella*), deer (*Odocoileus hemionus* and *Cervus elaphus*), oryx (*Oryx leucoryx*) and kangaroo (*Macropus giganteus*) However, it is higher than that of the reindeer (*Rangifer tarandus*). It is not surprising that the FMR of my giant pandas were lower than that of most similar sized mammals, since they were in a captive zoo like environment and not very active.

It does not appear that bamboo is the limiting factor in the number of giant pandas that can live in a given nature reserve. For example, in the Yele Nature Reserve, there is 1,634,529.3 kg of bamboo (*Bashania spanostachya*) per km^2 (Wei et al., 2000). Based on our FMR measurements and digestive efficiency measurements (He et al., 2000) a giant panda needs to eat about 15-20 kg of bamboo a day. That means there is enough bamboo in 1 km^2 of the Reserve to provide food for 81,726 panda days. Assuming that the giant pandas eat no more than ½ of the standing crop of bamboo than that would provide about 40,000 food days for a standing crop of 110 pandas in a year. If they used only 10% of the bamboo resources a year, then 1 km^2 would support 22 giant pandas, and that is for just one species of bamboo in that reserve. However, the home range of a giant panda is usually 3.0 to 6.0 km^2 (Schaller et al., 1985) so there must be limitations in the biology of the giant panda that go beyond the food supply, since that size home range provides a density of giant pandas that is 1 to 2 % of what would be supported by food alone.

Based on food supply the 2000 km^2 Wolong Nature Reserve, with 50% undisturbed area (Liu et al., 2001; Linderman et al., 2005) could support 22,000 giant pandas if all of the area is covered with bamboo, but now only support 166 to 333 giant pandas based on home range. In fact the estimated number of giant pandas living in the reserve was 143 reported by state forestry administration of the People' Republic of China 2003. If the disturbed area was rehabilitated as giant panda habitat the number of giant pandas in the Reserve could be doubled. Therefore, more data are needed on both home range and FMR of giant pandas in the wild under natural conditions if we are to obtain a better estimate of the carrying capacity of nature reserves.

RMR of red panda

There is no difference of RMR of red panda between males and females. However, The RMR of red pandas was statistically significantly higher in winter at environmental temperatures between 5.3℃ to 7.6℃ than in summer at environmental temperatures of

15.5℃ to 20.2℃. Temperatures in summer season were probably within the thermal neutral zone, but winter temperatures were below the thermal neutral zone. The RMR of the red panda was similar to 49 other mammals ranging in size from 2010 g to 10,550 g taken from Sieg et al. (2009).

McNab (1988) measured 2 red pandas in 1987 and found a very low metabolic rate, 0.153 ml/g/h. The data of McNab were well below those in my study and well below the values predicted by the regression line in Sieg et al. (2009). It is possible that his red pandas had a subclinical condition that lowered their metabolic rate. The lowering of metabolic rate that McNab's red pandas underwent without a drop in body temperature also suggests that the animals may have been ill.

I plotted the RMRs of the red panda and the 49 other similar size mammals from Sieg et al. (2009). The new regression line was steeper than that both of the Fereuungulata and universal regression lines, which were calculated from all 695 mammals in their data set. That supports their conclusion that phylogenetic relationships affect the body size-metabolic rate regression and that there is not a single universal metabolic rate-body mass scaling relationship in mammals (Sieg et al., 2009). In addition, different mass domains of mammals show different regression scaling (Clarke et al., 2010). Small mammals and large mammals may have different scaling relationships (Glazier, 2005). This is due to the variation of RMR within or among animal species.

Conservation implications

Based on my research data, the FMR of captive giant pandas varies greatly between individuals. However, the active time for each individual is very similar. The active time was greatly influenced by husbandry practices. In order to reintroduce giant pandas to the wild, there should be changes in captive management. Giant pandas should have larger inside rooms with better cooling systems in the summer. In natural habitats summer temperature will be much cooler than in the modified environment of a zoo. Therefore, giant pandas should have large enclosures of several hectares in which they can be active under natural conditions. In this way, they will develop the ability to function in nature.

Because of the fast water turnover rate, giant panda may be sensitive to some medicines, and the concentration of drugs will drop quickly in the blood stream and tissues. Veterinarians should take this information into account when doing medical treatment on giant pandas. Husbandry staff should make sure giant pandas can get enough clean water to stay hydrated, especially in the summer.

In most natural reserves there is enough bamboo for the current populations of giant pandas. For example, in the Yele Reserve 1 km^2 would support at least 22 giant pandas. Other factors must limit the population. Perhaps it is home range as discussed above (Schaller et al., 1985). Perhaps it is habitat disturbance. If we can rehabilitate disturbed areas in nature reserves into giant panda habitat, the carrying capacity of nature reserves could be increased. Knowledge of the metabolic rate of giant pandas in nature reserves will help to establish the true limitations on panda populations and the cost to the giant pandas of living in disturbed and undisturbed areas of the reserves.

List of References

Clarke, A., P. Rothery and N. J. B. Isaac. 2010. Scaling of basal metabolic rate with body mass and temperature in mammals. Journal of Animal Ecology **79**: 610-619

Davy, C. M., J. E. Paterson and A. E. Leifso. 2014. When righting is wrong: performance measures require rank repeatability for estimates of individual fitness. Animal Behavior **93**: 15-23.

Glazier, D. S. 2005. Beyond the ‘3/4-power law’: variation in the intra-and interspecific scaling of metabolic rate in animals. Biological Reviews **80**: 611-662.

He, L., F. Wei, Z. Wang, Z. Feng, A. Zhou, P. Tang and J. Hu. 2000. Nutritive and energetic strategy of giant pandas in Xiangling Mountains. Acta Ecologica Sinica **20**: 177-183.

Hu, J. 2000. Review on the classification and population ecology of the giant panda. Zoological Research **21**: 28-34.

Hudson, L. N., N. J. B. Isaac and D. C. Reuman. 2013. The relationship between body mass and field metabolic rate among individual birds and mammals. Journal of Animal Ecology **82**: 1009-1020.

Linderman. M., S. Bearer, L. An, Y. Tan, Z. Ouyang and J. Liu. 2005. The effects of understory bamboo on broad-scal estimates of giant panda habitat. Biological Conservation **121**: 383-390.

Liu, J., M. Linderman, Z. Ouyang, L. An, J. Yang and H. Zhang. 2001. Ecological degradation in protected areas: the case of Wolong Nature Reserve for giant pandas. Science **292**: 98-101.

McNab, B. K. 1988. Energy conservation in a Tree-Kangaroo (dendrolagus matschiei) and the Red Panda (*Ailurus fulgens*). Physiological Zoology **61**: 280-292

McNab, B. K. 1992. Rate of metabolism in the termite-eating sloth bear (*Ursus ursinus*). Journal of Mammalogy **73**: 168-172.

Nagy, K. A., I. A. Girard and T. K. Brown. 1999. Energetics of free-ranging mammals, reptiles, and birds. Annual Review of Nutrition **19**: 247-277.

McNab, B. K. 2000. The standard energetics of mammalian carnivores: Felidae and Hyaenidae. Canadian Journal of Zoology **78**: 2227-2239.

Schaller, G. B., J. Hu, W. Pan and J. Zhu. 1985. Giant pandas of Wolong. University of Chicago Press.

Sieg, A. E., M. P. O'Connor, J. N. McNair, B. W. Grant, S. J. Agosta and A. E. Dunham. 2009. Mammalian metabolic allometry: do intraspecific variation, phylogeny, and regression models matter. The American Naturalist **174**: 720-733.

Speakman, J. R. 1997. Doubly labelled water theory and practice. Chapman and Hall. London. UK.

Springer, M. S., W. J. Murphy, E. Eizirik and S. J. O'Breien. 2005. Molecular evidence for major placental clades. In: Rose, K. D. and J.D. Archibald. The rise of placental mammals: origins and relationships of the major extant clades. John Hopkins University Press, Baltimore, 37-49.

Taylor, C. R. and C.P. Lyman. 1967. A comparative study of the environmental physiology of an East African antelope, the eland, and the Hereford steer. Physiological Zoology **40**: 280-295.

Taylor, C. R., D. Robertshaw and R. Hofmann. 1969. Thermal panting: a comparison of wildebeest and zebu cattle. American Journal of Physiology **217**: 907-910.

Waddell, P. J., Y. Cao, J. Hauf and M. Hasegawa. 1999. Using novel phylogenetic methods to evaluate mammalian mtDNA, including amino acid-invariant sites-logdet plus site stripping, to determine internal conflicts in the data, with special reference to the positions of hedgehog, armadillo, and elephant. Systematic Biology **48**: 31-53.

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Betz Chair in Environmental Science Fellowship

Publications

He, G., T. Wang, G. Yang, **Y. Fei**, Z. Zhang, C. Wang, Z. Yang, J. Lan, L. Luo and L. Liu. 2009. Sequence analysis of Bs-Ag2 gene from *Baylisascaris schroederi* of giant panda and evaluation of the efficacy of a recombinant Bs-Ag2 antigen in mice. Vaccine **27**: 3007-3011.

Fei, Y., H. Huang, G. Yang, Z. Zhang, C. Wang, Z. Yang, J. Lan, L. Luo and L. Liu. 2009. Identification of *Dirofilaria sp.* from *Ailurus fulgens* based on ITS-2 sequence. Veterinary Science in China **39**: 593-596.

Wang, T., G. He, G. Yang, **Y. Fei**, Z. Zhang, C. Wang, Z. Yang, J. Lan, L. Luo and L. Liu. 2008. Cloning, expression and evaluation of the efficacy of a recombinant *Baylisascaris schroederi* Bs-Ag3 antigen in mice. Vaccine **26**: 6919-6924.

Fei, Y., X. Zhang, J. Yuan, H. Ye and B. Zhu. 2006. Preliminary observation on feeding behavior of captive giant panda. Sichuan Journal of Zoology **25**: 80-83.