

# A comparison between point- and semi-continuous sampling for assessing body temperature in a free-ranging ectotherm

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## Abstract

We used intracoelomically implanted temperature dataloggers to obtain semi continuous body temperature data and establish monthly thermal profiles for free ranging rattlesnakes. We mimicked random and non random point sampling methods by selecting a single daily data point from all values or from restricted times of day to reflect common point sampling constraints. Thermal profiles generated from point sampling differed from those generated from semi continuous sampling, and this difference was more apparent when point sampling was non random. We conclude that semi continuous sampling provides a better estimate of thermal profiles, and that point sampling methods are highly sensitive to deviations from true randomness.

*Keywords:* Point sampling; Semi continuous sampling; Body temperature; Telemetry; iButton; Ectotherm; Rattlesnake; Reptile; *Crotalus atrox*

## 1. Introduction

Most physiological processes are highly temperature sensitive (Angilletta et al., 2002; Huey, 1982). Since ectotherms do not produce significant amounts of body heat, they are vulnerable to environmental constraints that either induce behavioral changes or alter physiologic performance. Therefore, studying the thermal biology of free ranging ectotherms is essential in understanding the relationship between environmental variation, habitat use, and organismal function. To explore this relationship, one must employ methods to measure variation in both environmental temperature and body temperature ( $T_b$ ). In particular, studying the variation in  $T_b$  within and among organisms is important because it impacts performance and may reflect seasonal, inter specific, or sex differences in thermoregulatory behavior, as well as potential environmental constraints.

A thorough study of the thermal biology of a species requires that a large volume of  $T_b$  data points be collected to ensure sufficient data to analyze the variation in  $T_b$  (Peterson et al., 1993). There are two general sampling methods employed by researchers in studying the thermal biology of free ranging ectotherms: point sampling and semi continuous sampling. We define point sampling as the collection of data by physically visiting study animals in the field, usually by sampling  $T_b$  with a cloacal thermometer (the “grab and jab” technique; Avery, 1982) or with temperature telemetry (e.g., Brain and Mitchell, 1999; Muchlinski et al., 1990; van Marken Lichtenbelt et al., 1997). We define semi continuous sampling as the use of automated data collection systems to log data at programmed time intervals (e.g., Beaupre, 1995; Beaupre and Beaupre, 1994; King et al., 1998; Peterson, 1987; Peterson and Dorcas, 1992). Most automated data collection systems require considerable equipment (e.g., telemetry receiver, frequency scanner, and either a tape recorder or a pulse interval timer and datalogger; Beaupre and Beaupre, 1994; Lutterschmidt et al., 1996;

Peterson, 1987). Since such approaches can be costly and have considerable spatial limitations (i.e., animals must be located relatively close to each other in order to collect automated data from multiple animals), researchers often employ point sampling instead of semi continuous sampling methods. Point sampling methods require direct researcher involvement for each data point. These techniques, whether thermometer or telemetry based, often result in fewer  $T_b$  samples. Furthermore, animal access and/or temporal restrictions (e.g., difficulties working during excessive midday temperatures or limited nighttime lighting) usually make point sampling a non random process.

The recent advent of miniature temperature dataloggers that can be attached to or surgically implanted inside an animal has facilitated semi continuous logging of  $T_b$  without many of the financial and spatial restrictions imposed by radiotelemetry based systems. With the removal of many of the restrictions previously associated with semi continuous temperature sampling, such sampling is now a viable alternative for many studies. While semi continuous temperature sampling is usually considered preferred over point sampling, a comparison of the two methods has not been conducted. In this paper, we examine the effect of semi continuous sampling versus point sampling on the  $T_b$  profiles of a free ranging ectotherm, the Western Diamond backed Rattlesnake (*Crotalus atrox*). We hypothesized that point sampling would introduce a bias into the data because point sampling typically involves substantially fewer data points collected at certain, non random times of the day. We tested this hypothesis by collecting semi continuous  $T_b$  values from a group of snakes and then comparing the mean, minimum, and maximum monthly  $T_b$ s to similar values calculated from only selected data points. The calculations based on a subset of data points mimic the data that would be acquired using point sampling techniques. Data subsets were created using both random and non random approaches to assess not only the effect of a limited number of data points, but also the effect of commonly encountered temporal constraints associated with point sampling techniques.

## 2. Materials and methods

### 2.1. Field site

The field site is a  $1.5 \times 1.0$  km area of upland Sonoran Desert (elevation 800 900 m) located approximately 33 km NNE of Tucson, Arizona. The habitat consists of rocky volcanic hillsides and sandy plains with intermittent washes. During the snake active season, ambient temperatures typically range between  $5\text{--}30^\circ\text{C}$  in spring and fall (March through mid May and mid September through mid November) and  $20\text{--}40^\circ\text{C}$  in

summer (mid May through mid September). Additionally, a limited but reliable summer rainy season (approximately 10 cm of rain) occurs between mid July and early September.

### 2.2. Temperature dataloggers

We used implantable temperature dataloggers (Thermochron iButtons, Maxim, Dallas, TX) to collect semi continuous  $T_b$  from 10 female *C. atrox* that already had intracoelomic radiotransmitters (13 14 g, model SI 2T, Holohil, Carp, Ontario). These dataloggers can record up to 2048 data points at selected intervals. We programmed the dataloggers to record  $T_b$  ( $^\circ\text{C}$ ) every 2 h starting May 1 and ending October 15, 2002. To prepare dataloggers for implantation, we glued a 15 cm length of non absorbable surgical suture to them, coated them twice with heavy duty rubber coating (Plasti Dip, PDI, Inc., Circle Pines, Minnesota), and sterilized them overnight in benzalkonium chloride (Benz all, Xtrium Laboratories, Chicago, Illinois). We then implanted the dataloggers intracoelomically at approximately mid body, using the suture to secure the datalogger to the body wall. The snakes were collected, implanted, and released at their site of capture in mid April, allowing them considerable time to recover from surgery before the dataloggers began recording data. In late October, we collected the snakes, removed and downloaded the dataloggers, and re released the snakes. During the study, we lost two snakes due to premature radio transmitter failure, so the data presented in this paper are from eight snakes.

### 2.3. Calculations

The treatments we applied to the data are summarized for reference in Table 1. For each snake, we calculated monthly mean  $T_b$  values for May through October by averaging all  $T_b$  values for the given month. We also calculated the mean daily minimum  $T_b$  and the mean daily maximum  $T_b$  for each month by averaging the lowest and highest values, respectively, for each day of the month. In addition, we determined the absolute minimum and maximum  $T_b$ s recorded for each month. The data for the mean  $T_b$ , the mean daily minimum  $T_b$ , and the mean daily maximum  $T_b$  will, hereafter, be referred to as the “semi continuous mean” data, and the data for the absolute monthly extreme values will be referred to as the “semi continuous extreme” data.

Next, we calculated the  $T_b$  values that we might have obtained had we been using three different point sampling approaches. We constructed the first analysis to simulate what a researcher would obtain if he or she visited the field site once per day at truly randomized times of day. Using a random number generator in Microsoft Excel, we randomly selected one of the twelve

Table 1  
Descriptions of the treatments applied to the temperature data

Treatment	Data set
Semi continuous mean	Bihourly samples for the entire month
Semi continuous extreme	Bihourly samples for the entire month
Random	One randomly selected data point for each day of the month
Non random daytime	One daily data point randomly selected from daytime hours (0800 1600)
Non random active	One daily data point randomly selected from daytime hours (0800 1600) during spring and fall and nighttime hours (1600 0800) during summer

data points from each snake for each day. Using these data, the mean, extreme minimum, and extreme maximum monthly  $T_b$  values were calculated as above. These data will hereafter be referred to as the “random” data.

In the second analysis, we attempted to mirror a situation in which a researcher obtains a single  $T_b$  measurement each day, only during daytime hours. We randomly selected one data point from each snake for each day between the times of 0600 and 1800. Using these data, the mean, extreme minimum, and maximum monthly  $T_b$  values were calculated as above. These data will hereafter be referred to as the “non random daytime” data.

In the final analysis, we examined the temperature profiles generated by randomly selecting a single  $T_b$  data point per day during the hours in which animal activity is most likely. At our field site, snake activity is highest during daylight hours in the spring and fall, and during nighttime in the hot summer months. Therefore, we randomly selected one  $T_b$  measurement per day for each snake, between the hours of 0600 and 1800 from May 1 to May 31 and Sept 15 to Oct 15, and between the hours of 1800 and 0600 from June 1 to Sept 14. Mean and extreme values were calculated as above. This analysis reflects not only the time when researchers are more likely to visit the field site, but also the limited time in which point sampling could occur if  $T_b$  was being collected using a cloacal thermometer. These data will hereafter be referred to as the “non random active” data.

#### 2.4. Data analysis

The data were subjected to tests for normality and heterogeneity of variances prior to inference. We analyzed the data with three repeated measures analyses of variance (RMANOVA), one each for the analyses of mean, minimum, and maximum monthly  $T_b$ . For each analysis, the  $T_b$  was the dependent variable, the treatment was the between subjects variable, and the month was the within subjects variable. The treatment factor in the RMANOVA on mean  $T_b$  had four levels: semi continuous mean, random, non random daytime, and non random active. The analyses of minimum and

maximum  $T_b$  also had a fifth level, semi continuous extreme. For each analysis, Mauchly’s Criterion for Sphericity was violated; we therefore used multivariate Wilks’ Lambda tests rather than univariate tests (O’Brian and Kaiser, 1985). Following RMANOVA, we conducted Tukey post hoc tests to determine whether treatment means differed from one another at each month. Alpha was set at 0.05 for all tests.

### 3. Results

The mean monthly  $T_b$  values for each data set are shown in Fig. 1. Mean monthly  $T_b$  varied significantly over the course of the active season (month effect: Wilk’s  $\lambda = 0.09$ ,  $F = 47.96$ ,  $p < 0.0001$ ). The treatment factor was significant ( $F = 3.03$ ,  $p = 0.05$ ), indicating that the temperature profiles generated by the four treatments differed. The month treatment interaction was not significant (Wilk’s  $\lambda = 0.55$ ,  $F = 1.09$ ,  $p = 0.38$ ), indicating that each treatment showed a similar change over time. During the hot summer months, it appears that random sampling approximated semi continuous sampling, while non random daytime sampling over estimated mean  $T_b$  and non random active sampling underestimated  $T_b$  (Fig. 1). This observation is supported by Tukey post hoc tests for July, which show that all treatments yielded significantly different values except the semi continuous mean and random sampling treatments. In August, non random active sampling yielded a significantly lower mean  $T_b$  than non random daytime sampling, indicating that values obtained from gathering  $T_b$  measurement at night versus during the day differ significantly. There were no differences in mean monthly  $T_b$  among treatment groups in the months of May, June, September, and October.

The minimum monthly  $T_b$  values for each data set are shown in Fig. 2. Minimum monthly  $T_b$  varied significantly over the course of the active season (month effect: Wilk’s  $\lambda = 0.19$ ,  $F = 25.74$ ,  $p < 0.0001$ ). The treatment factor was significant ( $F = 7.87$ ,  $p < 0.0001$ ), indicating that the temperature profiles generated by the four treatments differed. The month treatment interaction was not significant (Wilk’s  $\lambda = 0.63$ ,  $F = 0.78$ ,  $p = 0.73$ ),

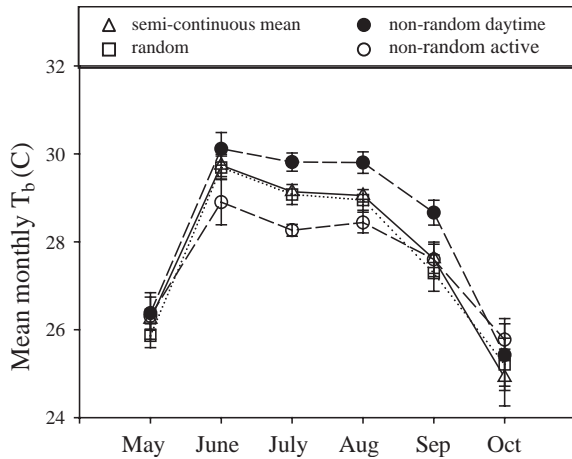


Fig. 1. Mean monthly body temperatures ( $T_b$ ) of female *C. atrox*. Values shown are mean monthly  $T_b$  ( $^{\circ}\text{C}$ ) of eight snakes  $\pm 1$  S.E.

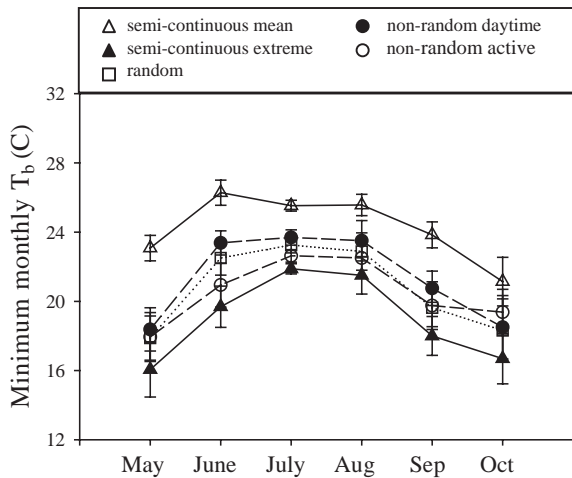


Fig. 2. Minimum monthly body temperatures ( $T_b$ ) of female *C. atrox*. Values shown are mean minimum monthly  $T_b$  ( $^{\circ}\text{C}$ ) of eight snakes  $\pm 1$  S.E.

indicating that each treatment showed a similar change over time. In general, all treatments tended to underestimate minimum  $T_b$  in comparison to the semi continuous mean treatment (Fig. 2). Tukey post hoc tests showed that the semi continuous extreme treatment yielded significantly lower  $T_b$  values than the semi continuous mean treatment in all months except August and October. In June, non random active sampling yielded a lower minimum  $T_b$  than semi continuous mean sampling, and in July, all three point sampling treatments yielded significantly lower minimum  $T_b$  values than semi continuous mean sampling. There were no differences in minimum monthly  $T_b$  among treatment groups in August and October.

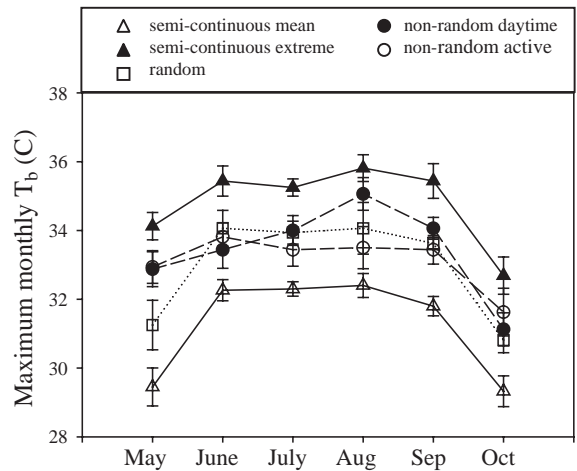


Fig. 3. Maximum monthly body temperatures ( $T_b$ ) of female *C. atrox*. Values shown are mean maximum monthly  $T_b$  ( $^{\circ}\text{C}$ ) of eight snakes  $\pm 1$  S.E.

The maximum monthly  $T_b$  values are shown in Fig. 3. Maximum monthly  $T_b$  varied significantly over the course of the active season (month effect: Wilk's  $\lambda = 0.16$ ,  $F = 31.58$ ,  $p < 0.0001$ ). The treatment factor was significant ( $F = 21.49$ ,  $p < 0.0001$ ), indicating that the temperature profiles generated by the four treatments differed. The month treatment interaction was not significant (Wilk's  $\lambda = 0.57$ ,  $F = 0.96$ ,  $p = 0.52$ ), indicating that each treatment showed a similar change over time. There was a general trend for all treatments to overestimate maximum  $T_b$  in comparison to semi continuous mean sampling (Fig. 3). Tukey post hoc tests showed that the semi continuous extreme values overestimated maximum  $T_b$  relative to other analyses in all months. Both non random point sampling treatments overestimated maximum  $T_b$  in May, while all three point sampling methods overestimated it in July and September. In August, the non random daytime treatment overestimated  $T_b$ , and in October, the non random active treatment overestimated  $T_b$ .

#### 4. Discussion

When conducting studies of the thermal biology of free ranging ectotherms, two aspects of body temperature are of key interest: mean  $T_b$  and range of  $T_b$  (minimum to maximum  $T_b$ ). The mean  $T_b$  reflects the overall thermal experience of an animal and the range reflects variation, or the extent to which  $T_b$  is regulated. The variation in  $T_b$  typically provides a more accurate assessment of performance capabilities and thus better insight into thermal constraints of the environment (Huey, 1982). Further, the variation in  $T_b$  is an important variable in theoretical, experimental, and

evolutionary studies of the relationship between temperature and performance (reviewed in Angilletta et al., 2002). Due to the physiological importance of this distinction, it is essential that researchers accurately estimate both the mean  $T_b$  and variation in  $T_b$  of their study organism. The results of this study show that point sampling tended to yield  $T_b$  data that have similar seasonal trends, but quite different absolute values than those obtained using semi continuous sampling.

In terms of mean monthly  $T_b$ , obtaining one truly random  $T_b$  data point per day yields estimates that are nearly identical to the semi continuous means. However, it is unlikely that researchers conducting point sampling ever visit their sites in a truly random fashion, since most researchers visit their sites according to a habitual or logistical routine. Departures from randomness tended to influence the values of mean  $T_b$  in a predictable manner. Obtaining one point per day during daytime hours (non random daytime) tends to overestimate mean monthly  $T_b$  during the hot summer months, since this data set is devoid of data from the nighttime when  $T_b$ s are coolest. Contrarily, obtaining one point per day during nighttime (non random active) tends to underestimate mean monthly  $T_b$ , because this data set is devoid of the typically higher daytime  $T_b$  data. Despite being in burrows during the daytime in summer, the snakes had their highest  $T_b$  during the daylight hours. While our non random active data set is designed to reflect  $T_b$  estimates associated with point sampling methods that require access to the animals (e.g., grab and jab methods), real values from such methods would likely be even more obscured due to spatial restrictions that are not accounted for in our analysis.

In comparison to semi continuous mean sampling, all treatments underestimated minimum and overestimated maximum monthly  $T_b$ , resulting in a gross exaggeration (i.e., a 50–100% greater estimate) of temperature range (maximum minus minimum values, Table 2). This result is not surprising, since the semi continuous mean sampling assesses the average variation over the course of a single day, while all other analyses are estimating minimum and maximum temperatures from a data set covering a much longer period of time (one month). The semi continuous extreme data yield the most pronounced under and over estimates (and therefore greatest range), because these values are the absolute lowest and highest  $T_b$  values gleaned from this extended set of  $T_b$  values. While both daily and monthly variation in  $T_b$  are of value, daily variation in  $T_b$  provides a better estimate of thermoregulatory ability and the range of temperatures typically encountered. Variation in  $T_b$  calculated over an extended period of time (e.g., one month) can be significantly influenced by changing climatic or physiologic conditions. We contend that individual estimates of temperature variance used to assess thermoregulation are best calculated from data

Table 2

Mean monthly ranges of  $T_b$  values (mean maximum monthly  $T_b$  mean minimum monthly  $T_b$ ) of eight female *C. atrox*, generated from the five treatments

Treatment	Mean range of $T_b$ values (max min)					
	May	Jun	Jul	Aug	Sep	Oct
Semi continuous mean	6.4	6.0	6.8	6.8	7.9	8.2
Semi continuous extreme	18.1	15.8	13.4	14.3	17.4	16.0
Random	13.4	11.6	10.7	11.2	14.0	12.5
Non random daytime	14.5	10.1	10.3	11.6	13.7	12.3
Non random active	15.0	12.9	10.8	11.0	13.7	12.3

sets that address daily rather than monthly temperature variance. Admittedly, temperature extremes over a long duration can be of value in understanding the physiological tolerance of an animal; however all point sampling analyses failed to identify extreme temperatures as effectively as did the semi continuous extreme sampling technique (Figs. 2 and 3). Therefore, the semi continuous sampling data set provided better estimates of both thermoregulatory abilities as well as thermal tolerance in *C. atrox*.

In summary, estimates of the mean  $T_b$  and variation in  $T_b$  in free ranging ectotherms are best gained using methods that allow semi continuous collection of data. Methods for collecting semi continuous data include automated telemetry systems and temperature dataloggers that are attached to or implanted in the animal. While automated telemetry systems are used successfully in many studies, they have several disadvantages. Most systems are expensive, ranging from \$175 per device (Lutterschmidt et al., 1996) to over \$5000 per device (Peterson and Dorcas, 1992). They may also be subject to mechanical problems and breakdown (e.g. King et al., 1998). Most automated systems must be placed in a central, elevated location such that they can collect data from all study animals (Peterson and Dorcas, 1992). To circumvent this, some researchers employ multiple systems, which must be moved continually to remain within range of the animals (Beaupre and Beaupre, 1994). In situations such as these, the expense and logistical problems may be compounded.

We suggest that miniature temperature dataloggers allow researchers to circumvent some of these problems. Dataloggers such as iButtons are inexpensive, are easy to use, and are accurate within  $\pm 1^\circ\text{C}$  (Angilletta and Krochmal, 2003). They are relatively simple and thus less prone to equipment failure, and do not require movement of monitoring equipment in the field. They are small (17.4mm diameter and 5.9mm thick) and lightweight (3 g), allowing easy implantation into a wide variety of study species. Researchers have also modified iButtons to be even smaller and lighter for use in quite

small animals (Robert and Thompson, 2003). There are potential disadvantages to using iButtons, however. The limited memory of iButtons restricts thermal profiles to relatively short or infrequent temperature sampling, unless animals undergo repeated surgeries to replace the dataloggers. Also, data cannot be retrieved until the iButton is removed, so data can be lost if animals are either preyed upon or lost (e.g. due to radiotransmitter failure as in this study). Finally, the sampling interval cannot be changed during the study, as it can be using automated telemetry systems (Beaupre and Beaupre, 1994).

We have shown in a free ranging ectotherm that point sampling of a single, non random, daily  $T_b$  yields a skewed mean and a greater variance in  $T_b$  values when compared to semi continuous sampling. In most point sampling studies, researchers do not visit their field sites daily, so the error may actually be even greater in many studies that employ point sampling. With the advent of low cost miniature temperature dataloggers, researchers have an opportunity to implement semi continuous sampling of  $T_b$  and gain more accurate and precise thermal profiles of their study organisms. When designing a study to collect  $T_b$ , investigators must carefully choose the means by which the data will be collected, the frequency and duration of data collection, and the analysis that will be used in order to maximize their ability to address their hypotheses. If point sampling is used, investigators must cautiously assess any deviations from randomness to determine whether such deviations will negatively impact the goals of their study.

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