



## Review

Integrating physiology, behavior, and energetics: Biologging in a free-living arctic hibernator<sup>☆</sup>Cory T. Williams<sup>a</sup>, Brian M. Barnes<sup>b</sup>, C. Loren Buck<sup>a,\*</sup><sup>a</sup> Center for Bioengineering Innovation and Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ 86011, USA<sup>b</sup> Institute of Arctic Biology, University of Alaska Fairbanks, Fairbanks, AK 99775, USA

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## ABSTRACT

The use of animal-borne instruments (ABIs), including biologgers and biotransmitters, has played an integral role in advancing our understanding of adjustments made by animals in their physiology and behavior across their annual and daily cycles and in response to weather and environmental change. Here, we review our research employing body temperature (T<sub>b</sub>), light, and acceleration biologgers to measure patterns of physiology and behavior of a free-living, semi-fossorial hibernator, the arctic ground squirrel (*Urocitellus parryii*). We have used these devices to address a variety of physiological, ecological, and evolutionary questions within the fields of hibernation physiology, phenology, behavioral ecology, and chronobiology. We have also combined biologging with other approaches, such as endocrinology and tracking the thermal environment, to provide insights into the physiological mechanisms that underlie fundamental questions in biology including physiological performance trade-offs, timing and functional energetics. Finally, we explore the practical and methodological considerations that need to be addressed in biologging studies of free-living vertebrates and discuss future technological advancements that will increase the power and potential of biologging as a tool for assessing physiological function in dynamic and changing environments.

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**Abbreviations:** ABIs, animal-borne instruments; AGS, arctic ground squirrel; ECG, electrocardiogram; T<sub>b</sub>, body temperature; SCN, suprachiasmatic nucleus; T<sub>3</sub>, triiodothyronine; T<sub>4</sub>, thyroxine.

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## 1. Introduction

Determining how individuals function within and interact with their environment is critical to understand behavioral and physiological adaptations and plasticity and by extension, limits to organismal responses to environmental change. The importance of understanding animal–environment interactions and physiological function is evidenced by the emergence of a new field in biology, known as “conservation physiology”, which seeks to incorporate this knowledge into ecological models designed to predict population, community and ecosystem-level responses to environmental and land use change (Cooke et al., 2013). The physiological mechanisms that underlie phenotypic plasticity remain elusive, however, and therefore represent a significant impediment to predicting the ecological effects of a changing climate (Denny and Helmuth, 2009).

Controlled laboratory experiments will always play an instrumental role in environmental physiology, but only by investigating physiological function and behavior in free-living individuals will we be able to fully understand how individuals regulate various aspects of their annual cycle including, as examples, reproduction, migration, molt, and hibernation (Bartholomew, 1986). It is well known that captivity can result in psychological and physiological stresses in animals as evidenced by impaired function of the hypothalamus–pituitary–adrenal axis, depressed immune function, reduced growth rates, and disturbed reproductive cycling (e.g., Romero and Wingfield, 1999; Morgan and Tromborg, 2007; Buehler et al., 2008). Limitations in our ability to replicate natural habitat and natural diets in captive situations constrain the usefulness of data collected in a laboratory setting to develop a thorough understanding of physiological responses to environmental challenges under natural conditions. For these reasons, studies of free-living animals are central to understanding how individuals alter their physiology and behavior across their annual cycle and in response to unpredictable environmental change.

Biologging and biotelemetry, which involve the collection of data from animal-borne instruments (hereafter: ABIs), are advancing our fundamental understanding of physiological adaptation and responsiveness to environmental change. Specifically, this technology allows us to answer questions regarding physiology, behavior, and ecology of free-living animals under natural conditions that would have previously been limited to tests on model organisms under controlled conditions. To date, the greatest impact of ABIs in ecology and environmental physiology has been through the use of devices that enable quantitative measurement of animal movement through space and time, particularly via global positioning systems (GPS), satellite telemetry, and geolocators (Rutz and Hays, 2009; Cagnacci et al., 2010). However, a wide variety of ABIs have been developed that measure a range of physiological, behavioral, and environmental parameters including, but not limited to, body temperature (T<sub>b</sub>), heart rate, acceleration, pressure (depth), salinity, light, heat flux, EEG, and PO<sub>2</sub> (Butler et al., 2004; Block, 2005; Vysotski et al., 2006; McDonald and Ponganis, 2013). Some of these parameters, such as heart rate and acceleration, correlate strongly with metabolic rate which provides insight into how metabolism and daily energy expenditure are influenced by both intrinsic and extrinsic factors, including weather (Green et al., 2009; Halsey et al., 2011). Combining biologging with other techniques, such as endocrinology and immunology, allows for an integrative approach to understanding how physiology mitigates the effects of environmental change and influences behavior, performance, and ultimately lifetime fitness (Wingfield et al., 1997; Semeniuk et al., 2009). Thus, the use and further development of ABIs is critical to understanding the complex interactions between physiology, behavior, climate, and the environment (Evans et al., 2016).

Here, we review our use of ABIs that measure temperature, light, and acceleration in a free-living semi-fossorial hibernator, the arctic ground squirrel (hereafter: AGS; *Urocitellus parryii*). First we provide a brief overview of the practical and methodological issues associated with the use of these particular ABIs. Then, we use our work on AGSs as an

example to illustrate the diversity of physiological and ecological questions that can be addressed using these relatively simple devices. We also demonstrate how combining biologging with endocrinology can provide insight into the functional mechanisms that underlie individual differences in behavior and physiology. The promise and power of ABIs is enormous and continued technological advancements of ABIs will undoubtedly provide for a more integrative understanding of the physiological and behavioral mechanisms that underlie vertebrate responses to environmental change.

## 2. ABIs — practical and methodological considerations

According to “Moore’s law”, which is actually an empirical observation, the number of transistors on an integrated circuit doubles approximately every 18 months and this has important implications for processing speeds and memory (Schaller, 1997). Battery technology is also improving, though at a much slower rate, such that the battery has become the greatest limiting technical factor in terms of continuing to reduce the size, mass and lifespan of ABIs. Nevertheless, the combined evolution of processing speed, storage capacity and battery technology has resulted in modern devices that are much smaller and better performing than their predecessors of just a few years ago. As a result, technologies that were once only suitable for deployment on relatively large animals, such as accelerometry, are now being used on increasingly smaller organisms (Rutz and Hays, 2009; Brown et al., 2013). This has led to widespread use of ABIs, but there are a variety of practical and methodological considerations that need to be addressed when initiating an ABI-study. These considerations include cost and robustness of the device, sampling resolution, method of attachment, capture/handling effects, effects of the device itself, and methods of analysis. Despite the continued progress in miniaturizing and improving ABI technology, the commercial demand is relatively small and thus further development/refinement of this technology requires continued support from funding agencies. The high cost of cutting-edge ABIs also means that many researchers continue to use older, more affordable technologies. Selection of an appropriate sampling interval, resolution and method of analyses is also very important and dependent on the question being addressed; this subject is too lengthy to discuss here, but see Ropert-Coudert and Wilson (2004).

Whether an ABI is worn externally or implanted is an important consideration. In general, we suggest following the taxon-specific guidelines provided by various societies (birds: Fair and Jones, 2010; fish: Nickum, 2004; mammals: Sikes and Gannon, 2011; reptiles and amphibians: Beaupre et al., 2004). Physical characteristics of the device such as its size, shape, mass, and buoyancy require important consideration and are dependent upon the size and ecology of the species; drag and buoyancy, for example, are much more important than mass for aquatic animals (Ponganis, 2007). Mass is much more important for flying birds and bats than it is for animals that use terrestrial locomotion (Barron et al., 2010; Rojas et al., 2010; O’Mara et al., 2014). It should also be noted that even within a particular taxonomic group, some species may be more sensitive to capture/handling stress and/or carrying the ABIs, and therefore attempts should be made to validate a lack of deleterious effects on natural physiology and behavior of a given species (e.g., Whidden et al., 2007; Jepsen et al., 2015). In some cases the decision regarding implantation or external-mounting will depend on the ABI itself. ABIs that measure environmental parameters (e.g., light) will obviously need to be mounted externally whereas some ABIs that measure physiological variables (e.g., PO<sub>2</sub>) must be implanted; however, many ABIs can be either mounted externally or implanted. One should not assume that external devices are necessarily preferred simply because they avoid invasive surgeries as some externally-affixed ABIs may be more likely to cause negative behavioral effects (Saraux et al., 2011). In a recent meta-analysis of biologging effects in birds, White et al. (2013) found that externally attached, but not implanted ABIs, were consistently detrimental to the birds. They concluded that

implantation of ABIs is preferable to external attachment, provided the risk of mortality associated with the anesthesia and surgery required for implantation can be mitigated. We are not aware of a similar analysis for mammals, but when deploying collar-mounted devices it is crucial to be aware that episodes of growth or fattening must be accounted for in the design of the attachment to minimize irritation, abrasion, and infection (Sikes and Gannon, 2011). A collar that is affixed too loosely can also cause harm if it is prone to becoming caught on sticks, branches, or other foreign objects. The choice of whether an ABI is implanted or affixed-externally will also depend on how the device downloads and its battery life and memory capacity which influences the length of deployment (see “The future of biologging” below).

### 2.1. Measuring body temperature (T<sub>b</sub>)

On February 16, 1959, Dr. Robert Rausch crawled into the winter den of a black bear in Anchorage, Alaska and obtained a measurement of its rectal T<sub>b</sub> during hibernation (Hock, 1960). The single data point he obtained from this presumably nerve-racking event confirmed earlier studies of captive bears indicating that these large carnivores do not exhibit the substantial reductions in T<sub>b</sub> observed in small mammals (Hock, 1951; Lyman and Chatfield, 1956; Bartholomew and Cade, 1957; Hock, 1960). Needless to say, technological advances in biotelemetry and biologging have greatly increased our ability to obtain relatively continuous measurements of T<sub>b</sub> in free-living animals throughout their annual cycles (e.g., Boyer and Barnes, 1999; Körtner et al., 2000; Dausmann, 2005; McCafferty et al., 2015). ABIs that measure T<sub>b</sub> have played a particularly important role in studies designed to determine if, when and which species exhibit heterothermy, including hyperthermia (Fuller et al., 1999) and daily torpor and hibernation, and determining the conditions under which heterothermy is employed (reviewed in Ruf and Geiser, 2015). Recent studies have also begun to focus on the use of a controlled moderate decrease in T<sub>b</sub> as an energy-saving mechanism in animals typically regarded as strict endotherms (Arnold et al., 2004; Hetem et al., 2014). In addition, because daily cycles of T<sub>b</sub> are generated by the master circadian clock in the suprachiasmatic nucleus (SCN; Buhr et al., 2010), T<sub>b</sub> is a potentially useful proxy for assessing the persistence of rhythms in free-living individuals (Williams et al., 2012a).

Early laboratory studies of torpor and hibernation were reliant on captive animals because T<sub>b</sub> was measured using a thermocouple inserted into the rectum (e.g., Benedict and Lee, 1938) or implanted subcutaneously (Hock, 1951) and connected to a large external chart recorder that was not attached to the animal. While these studies provided important insight into the physiology of heterothermy in species that exhibit daily torpor or prolonged hibernation, they were limited to the laboratory because animals were tethered to the external data recorder. Further, the disruptive nature of the process meant that measured patterns of heterothermy were not absolutely indicative of natural patterns. Captivity itself can also influence thermoregulation in unexpected ways (Geiser et al., 2000). For example, captive hibernators will often go through a step-wise series of “test-drops” – short (< 24 h) bouts of torpor with consecutively lower T<sub>b</sub> interrupted by arousals to euthermic T<sub>b</sub> between each short torpor bout – prior to entering their first multiday torpor; these test-drops were postulated to be necessary for resetting or reprogramming the hypothalamic regions controlling thermoregulation (Strumwasser, 1958; Hammel, 1967; Luecke and South, 1972; Pivorun, 1976). However, these “test-drops” rarely occur in free-living animals and appear to be a side-effect of captivity itself (Sheriff et al., 2011), at least in some species.

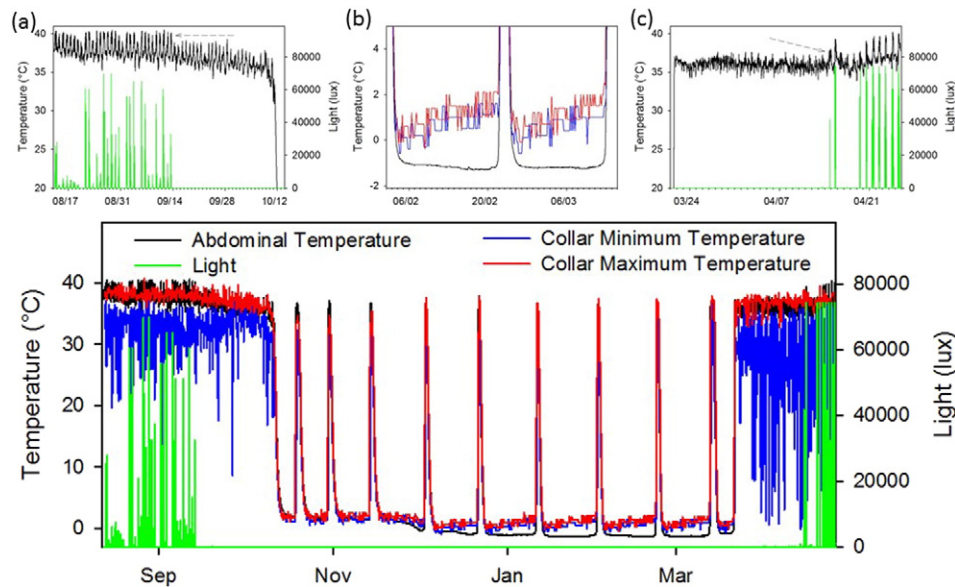
In the late 80s and early 90s, field researchers began to deploy newly developed and smaller T<sub>b</sub> loggers and transmitters that could be either surgically implanted into the abdominal cavity or affixed to a collar (e.g., Young, 1990; Brigham, 1992; Michener, 1992). Widespread use of these devices has led to a variety of discoveries, from supercooling of body fluids in hibernating AGS (Barnes, 1989) to hibernation in tropical primates (Dausmann et al., 2004) to a species of dormouse that can

hibernate for 11 months of the year when there is insufficient food for reproduction (Hoelzl et al., 2015). Biologging of T<sub>b</sub> has also had important conservation implications; mortality associated with white nose syndrome in bats, for example, has been shown to be associated with the increased energetic costs of frequent arousals from torpor, which, in turn, is driven by hypotonic dehydration associated with wing damage (Warnecke et al., 2013).

Technological advances are leading to the development of even smaller devices with a range of capabilities (see [The future of biologging](#) below). Nevertheless, as previously mentioned, a key consideration for many field biologists is cost of device. For example, despite their fairly poor resolution and relatively small memory capacity, Thermochron iButtons (Maxim Integrated, Dallas, TX, USA), which first became available almost 2 decades ago, remain the most commonly deployed device for measuring T<sub>b</sub> in small mammals. The largest capacity iButton that measures across the range of T<sub>b</sub> characteristic of heterotherms (DS1922L) can collect only 4096 datapoints at its highest resolution setting (0.125 °C) and 8192 datapoints at the low resolution setting (0.5 °C). Preference for iButtons stems from their low cost (~\$55/device) and relatively long battery life (~5 years); cost is low because these devices have commercial applications and are manufactured in bulk. To accommodate the use of these devices on smaller animals, particularly bats, researchers have developed methods of modifying the devices to decrease their size and mass (Robert and Thompson, 2003; Lovegrove, 2009) and to insulate them because they emit ultrasonic noise (Willis et al., 2009). Much smaller temperature sensitive transponders have also been developed, but these devices must be in close proximity to an external reader (~1 m) and the error in measured values increases at low T<sub>b</sub> (Wacker et al., 2012; Langer and Fietz, 2014).

In our research, we have used modified TidBits (12–13 g, Stowaway model TBICU32-05 + 44, coated weight 14–15 g, Onset Computer Corp, Pocasset, MA) which have greater memory and resolution (32,520 datapoints with 0.2 °C resolution), as well as the smaller iButtons (3 g; 4–5 g coated), with our selection of devices being dependent on our research question; the modified TidBits are no longer being produced, however. To assess whether very low amplitude circadian rhythms persist during deep torpor we have also recently deployed very high capacity, high resolution dataloggers (TidBit v2, Onset Computer Corp) that can measure 42,000 datapoints at 0.02 °C resolution (Fig. 1). These devices are more expensive (\$132) and quite large (23 g) such that they are not generally suitable for small mammals. Again, the selection of an appropriate ABI will depend on the question being addressed.

In addition to selecting the most appropriate device, one must consider whether to a) surgically implant the device in the abdominal cavity, b) implant the T<sub>b</sub> logger/transmitter subcutaneously, or c) affix the device adjacent to the skin. Several studies have demonstrated that skin temperature closely tracks core T<sub>b</sub> during heterothermy although ambient temperature can have slight, but significant effects on skin temperature (Barclay et al., 1996; Körtner and Geiser, 2000; Dausmann, 2005). Differences between core T<sub>b</sub> and skin temperature are also expected to be evident during interbout arousals during hibernation with the periphery warming later and cooling faster, although this difference is likely not detectable at the sampling resolution used in most field studies. Skin temperature is also likely to be much less reflective of core T<sub>b</sub> in animals exposed to highly fluctuating environmental conditions. Even for core T<sub>b</sub>, no gold standard exists for measuring deep-body temperature though measurements should be from sites that track temperature changes in the central volume (Taylor et al., 2014). Variation in T<sub>b</sub> can be substantial both within the body and over time, particularly in species for which the heterothermic response involves peripheral cooling, reductions in thermal core volume, and/or temporary abandonment of high core T<sub>b</sub> (Eichhorn et al., 2011). Overall, the determination of whether to use an implanted or externally mounted T<sub>b</sub> logger will depend on the question(s) being addressed, the potential for bias due to methods of attachment or surgery, and which approach does the least harm to the animal.



**Fig. 1.** Minimum (blue line) and maximum (red line) collar temperature both closely track core Tb (black line), as assessed using an abdominally-implanted temperature logger, throughout the heterothermic interval of hibernation. The light measurements (green line) indicate when the AGS is above vs. below ground. The more variable collar temperatures reflect the lower resolution of the collar mounted logger relative to the implanted Tb logger ( $\pm 0.5$  °C vs.  $\pm 0.02$  °C resolution). Abdominal Tb can be used to assess the timing of immergence in the fall, because the amplitude of the daily Tb rhythm decreases after the final day spent above ground (arrow in panel a). Abdominal Tb can also be used to determine the timing of emergence because a daily Tb rhythm is initiated with exposure to light (arrow in panel c). Variation in collar temperature is much higher than in abdominal Tb when animals are euthermic and therefore, unlike core Tb, is not useful in assessing the intervals of below-ground euthermicity that precede and follow hibernation; however, this can be assessed with a collar-mounted logger that measures both light and temperature. Abdominal Tb remains stable throughout torpor bouts when the animal is thermogenic whereas collar temperature is slightly warmer and exhibits a slight rise across the torpor bout (panel b).

## 2.2. Light loggers and accelerometers – quantifying activity and energy expenditure

Light sensitive radio-transmitters were first utilized to measure patterns of above-ground activity by Hut et al. (1999) who showed that European ground squirrels (*Spermophilus citellus*) are below ground in early morning and evening and thus do not perceive the rapid natural light–dark transitions that occur at civil twilight. Long et al. (2005) subsequently used these devices, in combination with Tb loggers, to understand how environmental conditions and activity patterns influenced core Tb in free-living AGS. Despite the utility of light-sensitive radio-transmitters (reviewed in Long et al., 2007), use of these devices never became widespread, likely because of their large size (~10 g) and expense, as well as the significant challenges associated with the continuous collection of telemetry data in heavily vegetated or topographically complex environments. However, these issues have been overcome with the development of lightweight and rugged light loggers that were originally designed for the geolocation of migratory birds based on the timing of dawn and dusk (Phillips et al., 2004). We have used these devices affixed to collars to measure patterns of above-ground activity in AGS (Williams et al., 2012b) as well as patterns of nest use in an arboreal rodent, the red squirrel (*Sciurus vulgaris*; Williams et al., 2014b). Our experience to date suggests that most of these devices are not sensitive enough for measuring detailed activity patterns in nocturnal rodents.

Light loggers are useful in assessing timing and duration of above-ground activity (or outside of a nest), but they are not necessarily informative as to the intensity of activity of an animal while it is above-ground. To estimate intensity of activity, accelerometers, which typically measure acceleration along 3 orthogonal axes, are potentially useful (Wilson et al., 2006; Halsey et al., 2009; Brown et al., 2013). Use of accelerometers generally falls into two categories. The first approach involves quantifying fine-scale movements and body postures using high resolution sampling (>10 Hz) to deduce specific behaviors. This approach requires validation data (direct observations of behavior)

combined with an automated system to categorize waveform patterns and assign them to different behaviors; although manual assignment is sometimes used, a variety of machine-learning algorithms have been devised to convert waveforms to behavioral categories (reviewed in Brown et al., 2013). The second approach is to utilize accelerometry data as an index of energy expenditure. The relationship between body acceleration and oxygen consumption (as a proxy for metabolic rate) has been examined in a wide variety of taxa and, although the strength of the relationship varies somewhat, it has held across all species examined to date (reviewed in Brown et al., 2013). The most commonly used acceleration-based index of metabolic rate is overall dynamic body acceleration (ODBA), which involves removing the static effect of gravity for each axis and then summing the absolute values of dynamic acceleration for each axis (Wilson et al., 2006). Although ODBA is typically calculated using measurements of acceleration at a frequency of 10 Hz or higher, sampling frequencies as low as 1 Hz provide reliable estimates of energy expenditure, even in small animals (Halsey et al., 2009). While accelerometers have been long used in the study of medium to large-sized animals, including humans, reductions in the size of these devices have recently enabled deployments on small mammals. We are currently using axy-3 accelerometers (2 g; Technosmart, Italy) affixed to collars on free-living AGS; at a 10 Hz setting, we obtain up to 40 days of data on a fully charged battery.

## 2.3. The future of biologging

In many cases, researchers have switched from biotelemetry to biologging to limit practical difficulties related to interference in the reception of transmitted data and to reduce the mass or size of devices deployed by eliminating the battery demands and antenna associated with data transmission. The move to biologgers, in combination with technical advances that have greatly miniaturized circuit boards, memory, and batteries, have enabled the deployment of increasingly sophisticated devices on smaller organisms (Rutz and Hays, 2009). Further, the development of individual devices that can measure many

physiological and behavioral parameters simultaneously is providing greater insight into how animals take advantage of predictable change, as well as cope with environmental stochasticity (Wilson et al., 2008). However, a key limitation of biologgers is that the animal must be recaptured to obtain the data. This limitation is particularly severe for implanted devices, because a second surgery must be performed to recover the device.

In addition to biologgers and biotelemetry devices, a hybrid class of ABIs exists which archive the data onboard the device and later transmit data for retrieval. These devices, known as “pop-up tags”, are typically used on fish in marine systems where salt water prevents the transmission of data; the tags are buoyant and transmit data to satellites only after they are released from the animal and are floating on the water's surface (Musyl et al., 2011). Because the tags are not continuously transmitting data, they have reduced battery consumption relative to a typical transmitter. Similar temperature logging devices have now been developed that can be programmed and downloaded through organic tissue using RFID (radio-frequency identification) technology allowing data recovery without a secondary surgery (Weepit loggers, Alpha Mach, Sainte-Julie, Canada). Additionally, devices that transmit data via radio-waves rather than via satellite are now being utilized in laboratory experiments with rodents; these implanted devices are designed to measure a variety of physiological parameters including Tb, heart rate, ECG, and acceleration (e.g., MouseMonitor, Indus Industries, Texas, USA). These devices can be programmed and downloaded remotely and, importantly, the batteries can be charged inductively extending the life of the devices. Although the limited memory and, in particular, battery power, of these new inductively chargeable ABIs make them unsuitable for use on free-living animals, the development of devices with greater memory and battery life in the future will provide a powerful tool for measuring physiological function under field conditions.

### 3. Biologging in arctic ground squirrels

#### 3.1. Life history and hibernation physiology

AGSs are obligate hibernators that spend ~7–8 months of the year sequestered below ground in a hibernacula and have a relatively short ~4–5 month active season during which they reproduce, molt, and grow and fatten again for the subsequent hibernation bout (Buck and Barnes, 1999a). The presence of continuous permafrost in the Arctic means that, unlike for most other hibernators, the hibernacula of AGS lies within the active layer of soil which will freeze mid-way through their hibernation season reaching average temperatures of  $-10^{\circ}\text{C}$  that require AGS to defend a large thermal gradient between ambient temperature and Tb (Buck and Barnes, 1999b). This is in contrast with most small mammalian hibernators that thermoconform, with Tb approximating ambient temperature during torpor. Lab studies indicate that AGS are capable of maintaining steady state torpor at ambient temperatures as low as  $-26^{\circ}\text{C}$  via a 36-fold increase in metabolic rate during torpor associated with heat generation in brown adipose tissue (Richter et al., 2015). Abdominal Tb while animals are torpid, yet thermogenic, is consistently below  $0^{\circ}\text{C}$  (Buck et al., 2008) and some tissues and their fluids will even decrease to below their equilibrium freezing point and thus are supercooled (Barnes, 1989). Tb is not uniform during deep torpor, however, as animals will exhibit regional heterothermy with the head and thoracic region being warmer than the abdominal cavity and periphery (Barnes, 1989). This is evident in our data from free-living animals as collar temperatures (skin) exceed abdominal Tb during torpor (Fig. 1). This likely occurs because heat produced by brown adipose tissue, which is concentrated in periarterial regions in the thoracic cavity, is transferred by blood directly to the brain resulting in a relatively higher neck and brain temperature. Perhaps more surprisingly, we found that collar temperature appears to warm slightly across each torpor bout during the portion of the year that animals are continually thermogenic (Fig. 1b). This suggests that,

unlike abdominal Tb, heat production by brown fat may not be constant during deep torpor. Whether this indicates a slight change in brain temperature across the torpor bout requires further investigation. Similar to most small mammalian hibernators, torpor is interrupted by regular arousal intervals during which animals become euthermic for ~14 h (Buck et al., 2008; Fig. 1). Light logging at a frequency of once per minute indicates that animals do not broach the surface during these intervals of euthermia but rather remain sequestered within their below ground hibernacula throughout their 6–9 month hibernation season. However, female AGS will sometimes re-enter torpor and extend hibernation in the spring following particularly long euthermic intervals ( $>24$  h) which can include episodes of above-ground activity (CTW, BMB, CLB unpub. data).

#### 3.2. Phenology

Phenology is the study of the timing of annually recurring life-cycle events and how this timing is influenced by climate conditions. Most long-term phenological studies of hibernators rely on trapping data to infer when animals have completed hibernation. However, data may be inaccurate if animals have terminated hibernation but inclement weather reduces their activity and likelihood of being captured (Williams et al., 2014b). Additionally, some species of ground squirrel exhibit prolonged below-ground intervals of euthermia immediately prior to and following the heterothermic interval of hibernation (reviewed in Williams et al., 2014a). This is particularly important in male AGS, which average 7–9 and 15–25 days of below-ground euthermia in the fall and spring, respectively (Sheriff et al., 2011). In response to environmental variation or differences in gender (Barnes, 1996), hibernators may alter the amount of time spent torpid or the duration of these below-ground euthermic intervals; which approach is used has important implications for energetics as metabolic rate during torpor can be reduced by as much as 98% relative to basal euthermic levels (Ruf and Geiser, 2015). Conventional trapping approaches, however, cannot determine whether changes in the timing of fall emergence or spring emergence are associated with a reduction in the duration of the heterothermic interval versus the episodes of below-ground euthermia. For these reason, we have been using Tb as a means of assessing the timing of seasonally recurring life-cycle events and determining how this timing is influenced by spatial and temporal heterogeneity in environmental conditions.

In the fall, the amplitude and mean of the daily rhythm of Tb decrease when animals first enter their hibernacula (Fig. 1a; Williams et al., 2011); in males, but not females, this occurs well in advance of initiating torpor (~7–9 days; Sheriff et al., 2011). In the spring, Tb of euthermic AGS are arrhythmic until they emerge to the surface (Fig. 1c; Williams et al., 2012a, 2012b). Measuring Tb using implanted devices also provides a means of assessing if and when females give birth because core Tb decreases during gestation and increases by  $1-1.5^{\circ}\text{C}$  on the date of parturition (Williams et al., 2011). Although collecting collar temperature reflects core Tb data during heterothermy (Barclay et al., 1996; Dausmann, 2005; Fig. 1), we have found that collar Tb deviates substantially from abdominal Tb when animals are active in their burrows or on the surface (Fig. 1), and thus it is not a suitable means of estimating core Tb during the active season. This also means that collar Tb is not useful for determining the duration of the below-ground intervals of euthermia that precede and follow heterothermy. However, ABIs that measure both light and Tb, such as the Intigeo geolocators (1 g; Migrate Technology Ltd., Cambridge, UK) that we utilize, provide a means of capturing all of this information using a collar mounted device (Fig. 1). Furthermore, the light logging approach provides much more detailed data on time active on the surface. For example, males may emerge from hibernation but then reenter and remain in their burrow for several days if weather conditions are poor such that rates of thermal exchange (i.e., heat loss) on the surface are high (Williams et al., 2012b).

Hibernation is obligate in AGS, with the timing of initiation and termination of torpor governed by an endogenous circannual clock (reviewed in Williams et al., 2014a). Nevertheless, hibernators have been shown to exhibit phenotypic plasticity in the timing of annually recurring life-cycle events (Williams et al., 2014a). We have been examining how AGS respond to spatial and annual variation in environmental conditions, particularly with respect to variation in the timing of snow cover patterns which influence access to forage in the spring and fall (Sheriff et al., 2011, 2013, 2015). Our research has focused on two populations near Toolik Field Station (68° 38' N, 149° 38' W) that are only ~20 km apart but differ substantially in their snow cover regime. We have found that females at Toolik Lake terminate heterothermy an average of 11 days later than squirrels at Atigun River, where snow melt occurs an average of 27 days earlier (Sheriff et al., 2011). Delaying the termination of hibernation results in delayed parturition (13 days) such that juveniles have less time to grow and fatten prior to hibernation (Fig. 2a–b; Williams et al., 2012c); this situation is exacerbated by conditions in the fall as snow cover also accumulates 14 days earlier at Toolik Lake (Sheriff et al., 2015). While females appear to be plastic in their spring phenology, there are no site-differences in when heterothermy is terminated in males; however, the below-ground euthermic interval that follows heterothermy is, on average, 10 days longer in males at the high snow cover site (Sheriff et al., 2011, 2013).

In addition to variation in timing between sites and between years, individuals from the same location can differ substantially in their timing within a year (Fig. 2); females from the same site can differ in both the timing and duration of their active season (Fig. 3). For females, individuals that terminate hibernation and give birth earlier tend to enter hibernation earlier the subsequent fall (Fig. 2c). Additionally, evidence is now emerging that females will enter hibernation earlier if they lose their litter during early lactation (Fig. 3). Thus, the onset of hibernation in females is driven by an endogenous clock but is also influenced by carry-over effects from hibernation and reproduction. In contrast, the initiation of hibernation in males is not affected by conditions during the prior spring. Altogether, it is clear that annual timing in the AGS is sensitive to environmental conditions but sensitivity is a sex-dependent trait. More work is needed however, to elucidate the mechanisms that underlie this sensitivity as well as to define the limits of phenotypic plasticity. The contribution of genetic variation to individual differences in timing is also unknown. Ultimately, climate-driven changes in phenology are likely to occur through microevolution, in addition to phenotypic plasticity. Phenotypic plasticity itself has an underlying genetic basis and thus plasticity may also evolve under climate change (Chown et al., 2010).

### 3.2.1. Predicting phenology under climate change

Climate change is predicted to be more rapid and more severe in the Arctic compared to most regions of the world. Specifically, the Arctic is predicted to warm more rapidly (Ding et al., 2014), have reduced sea ice (Notz and Marotzke, 2012), increased atmospheric humidity and precipitation (Kopec et al., 2016), and expansion of deciduous woody shrubs (Sturm et al., 2001). Physiological plasticity and the potential for microevolution are important components of population resilience and can potentially be useful in predicting the 'winners' and 'losers' under climate change (Somero, 2010). However, like many species, AGS are likely to be impacted by climate change in a number of ways making clear predictions difficult (Wheeler and Hik, 2013).

Snow cover directly affects access to food for AGS and is therefore an important driver of spring phenology (Sheriff et al., 2011). Warming temperatures could lead to earlier snow melt resulting in advances in the timing of reproduction. This is likely to be beneficial to hibernators as it provides increased time for juveniles to grow and fatten prior to their first hibernation (Morton and Sherman, 1978; Williams et al., 2014a). In the Rocky Mountains of Colorado, for example, yellow-bellied marmots (*Marmota flaviventris*) have advanced their phenology by almost 30 days over the past three decades and this has led to higher population densities (Ozgul et al., 2010). However, the Arctic is also predicted to

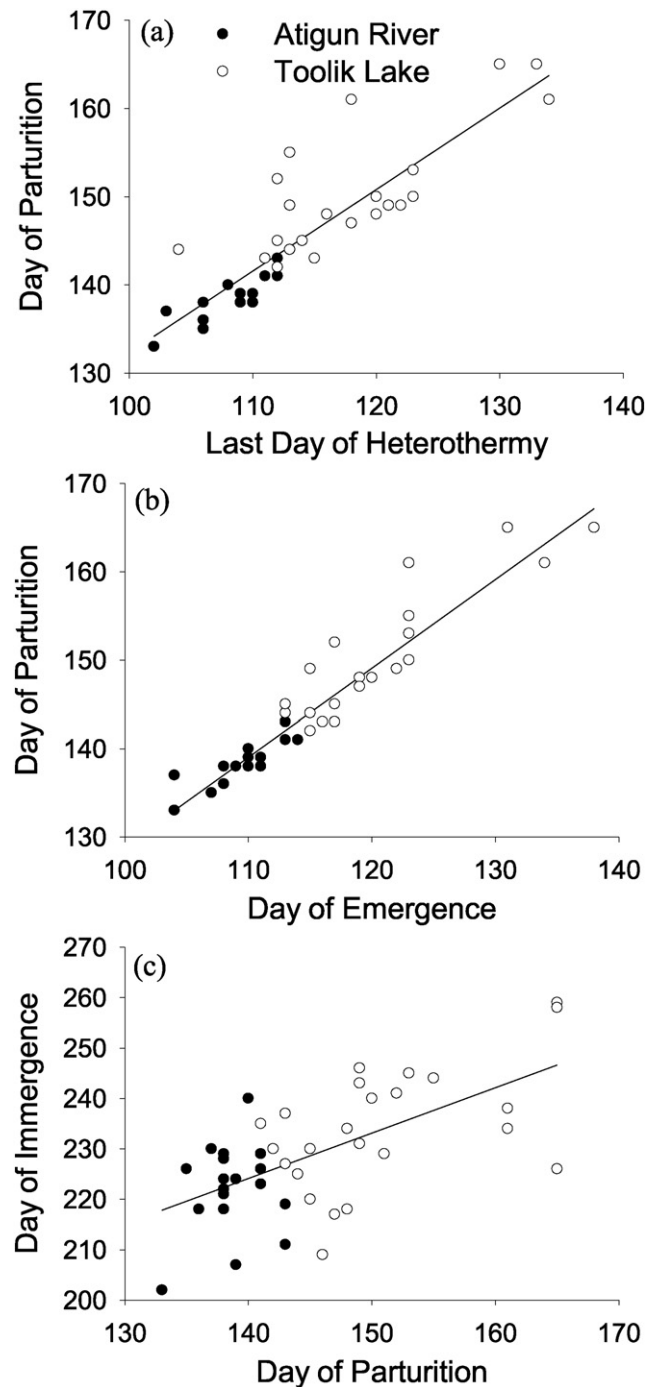
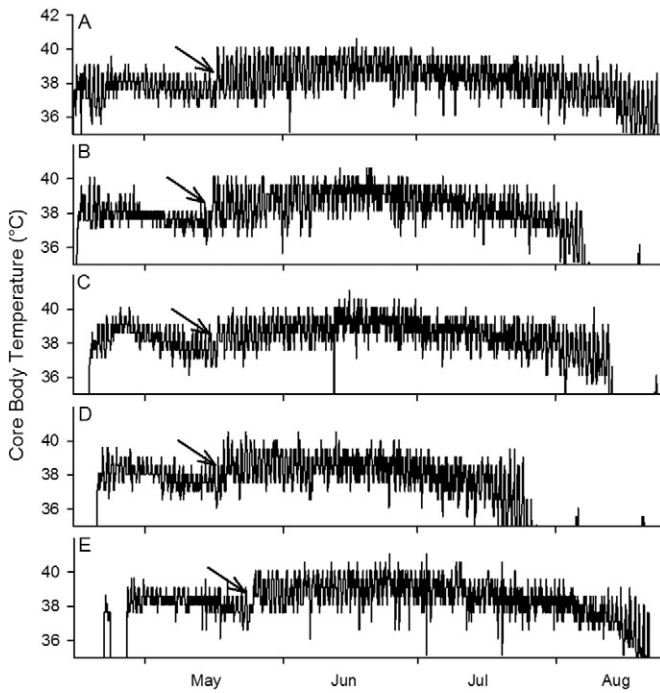


Fig. 2. The relationship between the timing of parturition and (a) last day of heterothermy, (b) date of emergence, and (c) date of subsequent immersion into the hibernacula for a single year in female arctic ground squirrels. Phenology data were acquired using abdominally-implanted Tb loggers. Snow melt occurs one month later at Toolik Lake (open circles) compared to Atigun River (closed circles). Figure from Williams et al. (2012c).

experience increases in precipitation (Kopec et al., 2016), and if this precipitation comes in the form of late spring snowstorms or results in higher winter snowpack it could result in delayed snow melt. Either of these scenarios could lead to delays in the termination of hibernation and onset of reproduction, which could potentially be manifest in lower juvenile survival and recruitment. Lane et al. (2012), for example, found that later snowmelt in the Rocky Mountains of Alberta led to delayed phenology and lower fitness for Columbian ground squirrels (*Urocyon columbianus*).



**Fig. 3.** Core body temperature (Tb) of 5 females throughout their active season in 2014. Although females remained euthermic throughout the active season, Tb is dynamic and exhibits 24 h daily rhythms with an amplitude of 2–3 °C. The abrupt increase in Tb indicated by the arrows delineates when females gave birth (Williams et al., 2011). Tb begins to decline well before the onset of hibernation; the decline begins ~45 days before first entering torpor in late summer (Sheriff et al., 2012). The length of the active season is affected by the reproductive status of the females; female D, which had a much shorter active season, lost her litter in early lactation. On average, females that give birth earlier will subsequently enter hibernation significantly earlier (Williams et al., 2012c).

Given the widespread range distribution of AGS (patchily distributed throughout NE Siberia in Eurasia and from Alaska to Hudson Bay in North America) and their broad ecological niche (from arctic tundra to boreal forest), it seems likely that the effects of climate change on phenology will be highly variable and tied to impacts on localized climate/weather and other stressors. Nevertheless, in regions subject to later snow melt or increases in spring snowstorm events, our data suggests that females are more phenologically plastic and are therefore likely to be buffered from late springs to a greater degree.

Because the endocrine initiation of gonadal maturation triggers the earlier termination of torpor in sexually maturing males (Barnes, 1996), only females and non-reproductive males appear to be capable of re-entering torpor if spring foraging conditions deteriorate. Such a sex-specific response could have implications for demography, including population sex-ratios, if male survival decreases as a consequence of increased snow cover and lower resource availability following the termination of hibernation. However, male survival is also likely to be influenced by the size and quality of their food caches, conditions during fall fattening, and male density during the mating season. Overall, phenology of AGS will probably be strongly influenced by climate change, but the effects are likely to vary widely across their range and predicting the consequences of these phenological responses is not yet possible.

### 3.3. Circadian rhythms

The question as to whether circadian rhythms persist throughout the year in polar environments, which seasonally lack a light:dark cycle, has long been of interest to chronobiologists (reviewed in Williams et al., 2015). Evidence to date suggests that polar vertebrates, including year-round residents and seasonal migrants, exhibit diverse behavioral and physiological responses to the relatively continuous

lighting conditions that occur during summers and winters at high latitudes. Differences among species appear to reflect variance in the ecological niches they occupy, as well as differences in their evolutionary histories (Williams et al., 2015). ABIs have played an important role in assessing the persistence and period of rhythms in free living vertebrates. For example, accelerometers have shown that circadian rhythms of activity are lost in free-living Svalbard reindeer during the polar day and polar night (van Oort et al., 2005). Radio-transmitters have demonstrated that substantial diversity exists in the persistence of daily activity rhythms in shorebirds depending on species, sex and breeding stage; remarkably, in some species a free-running behavioral rhythm that is synchronized between pair mates has been detected (Steiger et al., 2013). However, measuring behavioral data alone is potentially problematic because rhythms may be produced as animals respond directly to external cues rather than being generated by an endogenous clock, a phenomenon known as “masking”. The continued development of ABIs that can measure a variety of physiological and behavioral variables, in combination with experimental field manipulations, should shed light on whether observed rhythms are driven by endogenous clocks or are the result of masking.

In addition to assessing the timing of seasonally recurring life-cycle events, Tb data are also very useful in examining questions associated with circadian timing. In fact, while most studies investigating circadian rhythmicity in free-living polar organisms rely on activity data or hormone data, we argue that Tb is a better metric. The advantage of Tb over activity data stems from the fact that, in mammals, the master circadian clock in the SCN drives circadian rhythms of Tb which subsequently entrain peripheral oscillators found throughout the body (Buhr et al., 2010). Because the SCN controls these Tb rhythms, they are likely to be less affected by masking. Although melatonin rhythms are also generated by the SCN, obtaining continuous measures of hormones in free-living animals is not currently possible.

We have shown that during the active season, daily rhythms of activity and Tb in AGS persist throughout the arctic summer, which includes 6 weeks of continuous daylight while the sun remains above the horizon at our study site (Long et al., 2005; Williams et al., 2012a, 2012b). How AGS maintain rhythmicity in the absence of a light–dark cycle remains unclear. Although AGS might be sensitive to daily rhythms in the intensity of light, we suspect that the spectral quality of light may be more important, as rhythms in the color temperature of light are relatively robust in the Arctic and are less sensitive to disruption by changing cloud cover compared with total light intensity (Ashley et al., 2013). A recent study demonstrating that the mammalian circadian clock can be entrained by changes in color also strengthens this hypothesis (Walmsley et al., 2015).

We have also shown that abdominal Tb is constant during deep torpor, remaining within the 0.2 °C resolution of the loggers we were then using (Williams et al., 2012a). In fact, Tb measured during torpor using much higher resolution loggers (0.02 °C) also lacks circadian organization (Fig. 1). In free-living males, Tb also remains arrhythmic during the 21–28 day intervals of below-ground euthermia that follow heterothermy; daily rhythms do not resume until exposure to light following emergence (Williams et al., 2012a, 2012b). Collectively, our results suggest that circadian clocks in AGS remain functional and entrained under the midnight sun, but are either arrested or disrupted during the constant darkness of hibernation. These results are consistent with the finding that clock gene expression is arrested during deep torpor in the European hamster, *Cricetus cricetus* (Revel et al., 2007).

### 3.4. Activity & energetics

Data generated from ABIs (Tb and light loggers) we have deployed on free-living AGS indicate that the timing of key seasonal events differs by gender and age and is plastic and adjusted depending on environmental conditions. Further, these data clearly demonstrate that AGS

maintain entrained daily rhythms of physiology and activity throughout the arctic summer. For the past several years we have also been using light loggers to examine how the timing and duration of daily above-ground activity are modified in response to changing weather conditions across the active season. We are also interested in understanding whether differences in reproductive requirements between males and females result in sex-differences in either the amount of time spent above ground each day or the plastic response to changing environmental conditions. Finally, we have begun examining whether plasma level of thyroid hormone, a potent mediator of metabolism, is correlated with daily and seasonal variation in activity.

Perhaps not surprisingly, we have found that the amount of time spent above ground each day is strongly influenced by environmental parameters that affect thermal exchange, including ambient temperature, precipitation, and solar radiation (Fig. 4; Williams et al., 2014a, 2014b). While we were not surprised to find an effect of weather conditions on the amount of time animals spent above ground, the size of the effect was surprisingly large; the duration of time above ground each day has a coefficient of variation between 26 and 34%, depending on site, and weather conditions explain more than 50% of the day-to-day variation (Williams et al., 2014a, 2014b; in review). Our finding that >50% of the day to day variation in above ground activity is affected by weather that influences thermal exchange conditions suggests that the effects of climate change on weather conditions during the active season could have important population-level implications. However, we currently lack data linking patterns of activity and energy expenditure to measures of fitness, such as fecundity and survival.

Our studies have also revealed that differences between males and females in how and when energy is allocated towards reproduction produce sex-differences in time spent above-ground. Light logger data revealed that females spend less time above ground during early lactation but slightly more time aboveground during late lactation (Williams et al., in review). This result appears to be at odds with earlier studies of ground squirrels which indicate that daily energy expenditure, as measured using the doubly-labeled water approach, is much higher in lactating females compared to males (Kenagy et al., 1989). However, time spent above ground does not necessarily reflect activity levels or efficiency in foraging.

To address this issue, we estimated daily movement-based energy expenditure by calculating ODBA from long-term (up to 60 ) deployments of accelerometers. We found that mean daily ODBA was positively correlated with time spent above ground, but females were more active per unit time spent above ground and had higher movement-based energy expenditure than males throughout gestation

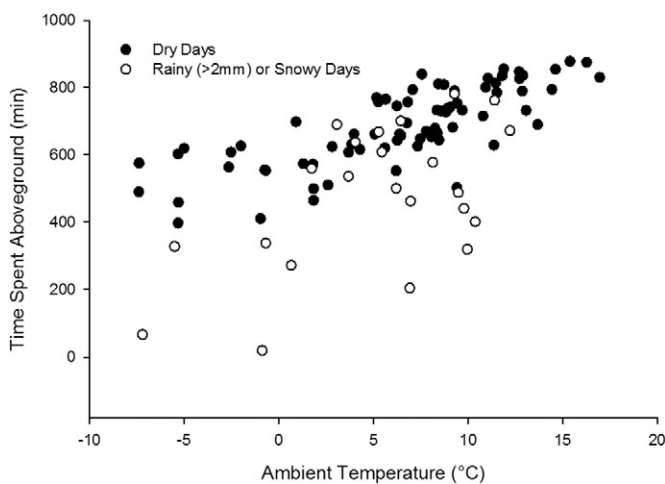


Fig. 4. The relationship between total time spent aboveground by AGS during the day (calculated based on light logging) and ambient temperature conditions in 2014 on days without precipitation (black circles) and days with >2 mm of precipitation (open circles).

and late lactation (Williams et al., in review). Similar to time spent above ground, ODBA varies substantially from day-to-day with more than 50% of this variation being attributable to variation in thermal exchange conditions. Interestingly, while Fletcher et al. (2012) recently showed that seasonal stage differences overwhelm environmental factors as determinants of energy expenditure in red squirrels, the opposite appears to be true in our system, at least in terms of movement-based energy expenditure.

Total energy expenditure includes basal metabolism, movement-based energy expenditure, as well as energy allocated to other processes (e.g., thermoregulation, digestion, lactation, growth, etc.). Maintaining a high core Tb makes up a substantial portion of RMR, and in AGS, levels of core Tb vary substantially across the season (Fig. 3; Williams et al., 2011). Recently, we showed that circulating total triiodothyronine (T<sub>3</sub>; the active form of thyroid hormone), a potent modulator of basal metabolism, varies substantially across the active season in free-living AGS (Wilsterman et al., 2015). When we compare T<sub>3</sub> levels to abdominal Tb in free-living AGS, we see that they are positively correlated (Fig. 5) which suggests that T<sub>3</sub> may contribute to increases in RMR through modulation of core Tb. Perhaps more surprising than the associations between T<sub>3</sub> and Tb was our finding that both T<sub>3</sub> and thyroxine (T<sub>4</sub>; a precursor of T<sub>3</sub>) were positively correlated with time spent above ground each day, as measured using light loggers (Wilsterman et al., 2015). These data support the common assumption that basal metabolism, total energy expenditure, and activity levels of individuals are physiologically linked (Biro and Stamps, 2010). However, the relationship between thyroid hormones and time spent above ground was context dependent and only occurred during particular life-history stages (Wilsterman et al., 2015). Additionally, we now know that time spent above ground explains only ~40% of the variation in movement-based energy expenditure in female AGS (as measured by ODBA; Williams et al., in review). Nevertheless, these findings provide a framework for understanding thyroid hormone function in free-living animals and provide the first link between thyroid hormones and behavior in free-living mammals. The continued use of field endocrinology, in combination with ABIs, particularly those that can measure multiple physiological and behavioral parameters simultaneously (Wilson et al., 2008), will help shed light on the physiological links between endocrine drivers and their behavioral and physiological outputs.

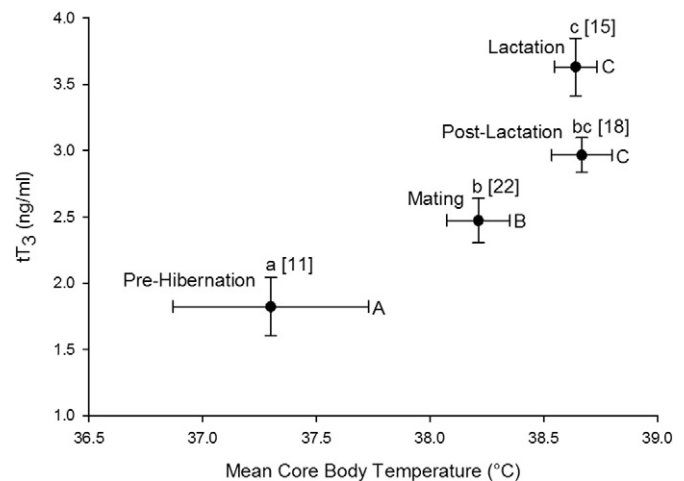


Fig. 5. Total triiodothyronine (tT<sub>3</sub>; mean ± SE) vs. mean abdominal body temperature (Tb; mean ± SE) of female arctic ground squirrels during four stages of the active season. Different lowercase letters indicate significant ( $p < 0.05$ ) differences in tT<sub>3</sub> between groups whereas different uppercase letters indicate significant differences in Tb between groups. Sample sizes for tT<sub>3</sub> data shown in brackets. Core Tb was measured in 6 females.

Thyroid hormone data from Wilsterman et al. (2015).



#### 4. Conclusions

The world is changing on global, regional, and local scales and understanding how individuals, populations, species, and communities will be affected requires integrative studies designed to measure behavior and physiology in free-living animals. Our studies on AGS illustrate that ABIs provide a powerful tool to address a wide variety of behavioral, physiological, and ecological questions, from understanding the role of circadian clocks in polar environments to assessing how heterotherms might cope with climate change. The development of small ABIs that can measure many parameters, store data on an onboard high capacity chip, and later be downloaded, reprogrammed, and charged remotely, will greatly enhance our ability to monitor behavior and physiology in free-ranging, undisturbed subjects, thereby advancing basic and applied biological research.

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