Temporal organization of activity in the brown bear (*Ursus arctos*): roles of circadian rhythms, light, and food entrainment

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**Ware JV, Nelson OL, Robbins CT, Jansen HT.** Temporal organization of activity in the brown bear (*Ursus arctos*): roles of circadian rhythms, light, and food entrainment. Am J Physiol Regul Integr Comp Physiol 303: R890–R902, 2012. First published September 12, 2012; doi:10.1152/ajpregu.00313.2012.—Seasonal cycles of reproduction, migration, and hibernation are often synchronized to changes in daylength (photoperiod). Ecological and evolutionary pressures have resulted in physiological specializations enabling animals to occupy a particular temporal niche within the diel cycle leading to characteristic activity patterns. In this study, we characterized the annual locomotor activity of captive brown bears (*Ursus arctos*). Locomotor activity was observed in 18 bears of varying ages and sexes during the active (Mar-Oct) and hibernating (Nov-Feb) seasons. All bears exhibited either crepuscular or diurnal activity patterns. Estimates of activity duration (α) and synchronization to the daily light:dark cycle (phase angles) indirectly measured photoresponsiveness. α increased as daylength increased but diverged near the autumnal equinox. Phase angles varied widely between active and hibernating seasons and exhibited a clear annual rhythm. To directly test the role of photoperiod, bears were exposed to controlled photoperiod alterations. Bears failed to alter their daily activity patterns (entrain) to experimental photoperiods during the active season. In contrast, photic entrainment was evident during hibernation when the daily photocycle was shifted and when bears were exposed to a skeleton (11:1:11:1) photoperiod. To test whether entrainment to nonphotic cues superseded photic entrainment during the active season, bears were exposed to a reversed feeding regimen (dark-fed) under a natural photocycle. Activity shifted entirely to a nocturnal pattern. Thus daily activity in brown bears is highly modifiable by photoperiod and food availability in a stereotypic seasonal fashion.

**ACTIVITY PATTERNS** are a useful behavioral measure to inform ecological and conservation studies. By examining these patterns, the impact of habitat destruction, food availability, and conspecific interactions can be revealed. Furthermore, a large body of literature has revealed that rather than merely being stimulated or suppressed by light and dark, many activity patterns are generated by a clock-like mechanism (17, 60). This biological clock is highly conserved because it enables animals to accurately and reliably perform functions integral to survival due to the predictable nature of the earth’s rotation and seasonal changes in daylength (5). The biological clock thus serves as both a timekeeper and calendar.

Considerable research has characterized activity patterns of wild brown (*Ursus arctos*) and black bears (*Ursus americanus*), although much less is known about the physiological mechanisms that generate them. Both brown and black bears are capable of exhibiting a wide variety of activity patterns depending on season, geographic location, food availability, and human influences, just to name a few (22, 27, 34, 41, 49, 54, 77). Bears exhibit distinct seasonal timing of activities such as reproduction and hyperphagia prior to entrance into hibernation (8, 9). For example, brown bears feeding at salmon streams in Alaska exhibit both diurnal and crepuscular patterns (54), whereas brown bears in Europe exhibit predominantly nocturnal activity (34). Similar to brown bears, black bears in various locations in North America are diurnal and crepuscular (2, 22). Sympatric black and brown bears also exhibit widely different activity patterns, presumably as a means for the smaller black bear to avoid brown bear encounters (77). Anthropogenic influences on bear activity patterns vary on both a daily and an annual basis, often in relation to food accessibility (26, 43, 50, 54, 55, 84). While these studies have contributed greatly to our understanding of the putative influences on bear activity patterns, a major gap remains in our knowledge of the basic physiological mechanisms responsible for the generation of daily and seasonal activity cycles in bears.

Annual changes in daylength (photoperiod) associated with the tilt of earth’s axis are reliable geophysical phenomena that are often anticipated by animals to synchronize important biological events, such as reproduction, migration, and hibernation, with specific times of the sidereal year (5, 6). Moreover, there are accompanying changes in the daily light-to-dark cycle that are transformed into a photoperiod-dependent hormonal signal in the form of melatonin secretion by the pineal gland (25). In mammals, this photoperiod transduction requires, in part, the integration of photic information from the retina with an endogenous pacemaker, or circadian clock, in the brain to organize the daily cycle of melatonin (31, 56).

This pacemaker, or circadian (about 24 h) clock, resides in the suprachiasmatic nucleus (SCN) of the hypothalamus and generates an endogenous oscillation with a period slightly different from 24 h via a molecular transcription-translation
feedback loop (17, 64). The rhythm of such an oscillation can be described by its period, amplitude, and phase. Period (or T) refers to the duration of each rhythm or distance between peaks in the rhythm (64). Amplitude is defined as the “extent of an oscillatory movement, measured from mean to extreme value” (15). Finally, the phase, or phase-angle, of a rhythm can be envisioned as the relationship between a periodic quantity, such as locomotor activity, and a reference point of the environmental cycle, such as dawn (15, 64).

Environmental light serves as the primary effector of synchronization between the biological clock and the external photocycle; this function is mediated by the SCN via a diverse set of SCN neural projections (31, 64). The endogenous clock synchronizes to the external clock by being reset (or phase-shifted) each day to match the 24-h photocycle. This process of synchronization, referred to as entrainment, is integral to an organism’s optimum functioning, as it allows reliable predictions for behaviors such as feeding, migrating, and sleeping-waking based on the 24-h daily rhythm (1, 12). Entainment of the circadian clock combined with the direct masking effects of light and dark are therefore thought to be major determinants of activity patterns in animals (10, 65, 71). Together, these effects are thought to produce an optimal temporal niche occupied by an organism and one that is considered a relatively fixed feature of a species via selection (38). Unfortunately, most research examining entrainment has used prey species (primarily rodents) housed in artificial laboratory conditions. Much less is known about the physiological mechanisms determining activity patterns of predators, although a high degree of flexibility is evident.

The endogenous nature of the biological clock can be observed in the absence of external light-to-dark cycles (e.g., constant light or dark), where it free runs with a period significantly different from 24 h (33, 64). Intriguingly, these constant conditions are experienced normally by some species living at extreme latitudes and by those hibernating in burrows. Yet, the role of the circadian clock under these conditions is controversial. For example, arctic dwellers, including reindeer (Rangifer sp.) and Svalbard ptarmigans (Lagopus mutus hyperboreus) exhibit a complete absence of circadian rhythmicity during polar summers and winters (66, 82). In hibernating species, including those that show torpor, a role for the SCN is supported by lesion studies in golden-mantled ground squirrels (Citellus lateralis), Djungarian hamsters (Phodopus sungorus sungorus), and Syrian hamsters (Mesocricetus auratus) (13, 73–75, 87), but this may not be true for all species (68). In addition, entry into torpor is rhythmic while arousal from torpor does not exhibit circadian rhythmicity (72, 85). Bears enter a state of hibernation during the colder months when food is scarce. Bear hibernation is characterized and distinct from smaller rodent hibernators, in that body temperature decreases by only 3–5°C and bears do not undergo periodic arousals (19, 53). However, similar to smaller hibernators, there is a large decrease in metabolism, heart rate, digestive activity, and locomotor activity (19, 53). In black bears, midday cycles of body temperature have been recorded, but circadian rhythmicity may be absent (29, 81). Collectively, it appears that the role of the circadian system is more important during hibernation in those species that arouse periodically compared with those that do not undergo periodic arousal and torpor bouts (37), but this remains to be determined.

Considering the stereotypical seasonal behaviors of brown bears, including profound annual changes in body weight (52), annual cycles of hibernation (19) and reproduction (8, 18), together with a wide geographic distribution in northern latitudes (44), we hypothesized that photoperiod would serve as an important proximal cue requiring a functional circadian timing system. Photoperiodic time measurement is dependent on the circadian system (25, 56, 58). Furthermore, given the absolute necessity of bears to gain sufficient body fat to survive a prolonged fast of several months (18), we hypothesized that other nonphotic cues could serve as ultimate factors to entrain the bears’ daily activity patterns. This prediction is based on the evidence that bears must engage in feeding activities for extended periods (53) to increase their total energy uptake despite exposure to decreasing daylengths.

METHODS

Animals. Male (n = 6) and female (n = 12) brown bears (1–21 yr old) were housed at the Washington State University Bear Education, Conservation, and Research Center (WSU Bear Center, 46° 43’ 53” N/117° 10’ 43” W). The animals were maintained according to the Bear Care and Colony Health Standard Operating Procedures with all procedures approved by the WSU Institutional Animal Care and Use Committee (IACUC). Bears were housed in pairs in dens (3 m × 3 m × 2.5 m) with access to an adjacent outdoor pen (3 m × 5 m × 5 m) and an adjacent 0.56 ha outdoor enclosure. For characterization of seasonal and daily behavioral rhythms, animals were exposed to natural photoperiod and temperature fluctuations. During all photoperiod experiments (excluding hibernation) and the nighttime feeding experiment, bears were allowed daily access to the outdoor enclosures. Normal feedings during the active time of the year occurred twice daily at 0700 ± 1 h and 1600 ± 1 h (standard time). Bears were fed at, or slightly above, maintenance levels from April to early August. Then, because of the bears’ increased appetite between August until mid-October, feeding amounts were significantly increased to well above maintenance levels. During the active time of the year, bears are also expected to forage for grasses and clover in the irritated 0.56 ha exercise yard to supplement commercial foods fed twice daily (70). The commercial foods include a dry chow (25.3% protein, 16.2% fat, 51.7% carbohydrate, and 2.0% crude fiber; Hill’s Pet Nutrition, Topka, KS), apples, and small amounts of meat and pastries. In the fall, based on the bears’ appetite, food was gradually reduced in early to mid-October until completely withdrawn in late October. Hibernation began when all food was withdrawn (October 24 ± 7 days). Water was available ad libitum during all seasons. Hibernation ended based on subjective evaluation of general increases in activity at which time feeding was restored (March 1 ± 16 days).

Activity determinations. For baseline activity, monitored bears were housed as described above and hibernated in pairs in their indoor and outdoor dens. For studies directly examining photic responsiveness during hibernation, bears (n = 5) were moved to an off-site location consisting of a light- and temperature-controlled building. For these studies the bears were housed individually in culvert-type bear enclosures (2.44 m × 1.22 m × 1.22 m, Teton Welding, Choteau, MT) fitted with automatic waterers. Lighting was supplied by overhead fluorescent lights and supplemented with halogen lamps to increase light intensity when necessary.

Activity measurements were made (n = 18) during the winters of 2003–2006, June to December of 2008, entire year of 2009–2010, excluding times when experiments were being conducted. Behavior was characterized using digital video recording (OpenEye Digital Video Security Solutions, Spokane, WA). Indoor and outdoor bear pens were fitted with infrared-sensitive cameras and infrared light sources to facilitate the monitoring of activity during the day and night. Subsequent studies used actical activity physical activity mon-
itors (Minimitter, Bend, OR) housed in protective aluminum cases that were glued to bears’ fur on the neck just cranial to the shoulder. Acticals were programmed to collect incidence of movement and movement velocity data via an omnidirectional accelerometer in 1-min epochs for a maximum recording duration of 45 days. Application and removal of the Acticals was performed on nonanesthetized bears \( (n = 4) \) that had been trained to remain still for a food reward. For nontrained bears \( (n = 6) \), application and removal of Acticals was facilitated by sedation with \( 1–1.5 \) mg/kg of tiletamine HCl and zolazepam HCl (Telazol, Pfizer Animal Health, New York, NY) and \( 0.04–0.08 \) mg/kg of medetomidine HCL (Dormosedan, Pfizer Animal Health). Intravenous or intramuscular administration of atipamezole HCl (Antisedan, Pfizer Animal Health) was used to reverse the anesthesia. Activity data were downloaded into Actical 2.12 software (Respironics, Murraysville, PA) and subsequently exported for further analysis (see below).

For both video and Actical-based measurements, activity onset and offset were recorded each day. Activity onset based on video records was defined as the time when the bear was standing, sitting, or exhibiting alert head movements (looking side to side or up and down) for at least 5 consecutive minutes. Activity offset was defined as the time when animals failed to exhibit any bouts of standing, sitting, or head movement activity for greater than 5 consecutive minutes. Activity onsets and offsets derived from Actical records were determined using Clocklab software (Actimetrics, Wilmette, IL). Comparison of the two methods confirmed a high concordance (Pearson \( r = 0.93 \), data not shown). We also determined the time difference between activity onset/offset and sunrise/sunset (phase angle) for each day of video recording in addition to the duration of activity (\( \alpha \)) by subtracting the time of activity onset from activity offset. Finally, when possible, estimates of \( \tau \) were made using ClockLab software.

**Photoperiod experiments.** Photoperiod was manipulated for \( \geq 2 \) wk at four times of year: spring (April-May, \( n = 7 \); avg. ambient photoperiod = 14:50:9.5), summer (June, \( n = 8 \); avg. ambient photoperiod = 15:33:8.67), fall (October, \( n = 6 \); avg. ambient photoperiod = 10:76:13.24), and winter (November-December, \( n = 4 \); avg. ambient photoperiod = 8:68:15.32). During the times of year when the bears were active, the photoperiod was extended by adding additional lighting to the indoor and outdoor dens. Photoperiodic contractions were not possible during the active season because ambient light levels could not be sufficiently reduced to mimic nighttime conditions. We therefore performed photoperiodic contraction only during the inactive period (winter) when bears were hibernated individually in enclosures (65) (described above) in an environmentally controlled facility. The length of each photoperiod extension was \( \geq 4 \) h longer (+2 h morning, +2 h evening) than either the prevailing photoperiod or the animals’ baseline \( \alpha \) during that time of year, whichever was longer. For photoperiodic contractions, the daylength was reduced by \( 4 \) h compared with the prevailing photoperiod. Thus the light-to-dark cycles were used the following: 18L:6D, 20L:4D, 18L:6D, and 4L:20D for spring, summer, fall, and winter photoperiod experiments, respectively.

For the spring, summer, and fall photoperiod extensions, two 500-watt halogen lights (Cooper Lighting, Houston, TX) were positioned to face the indoor den and three 500-watt halogen lights were placed 1.8 m above the outdoor den of each bear. Illumination levels at the approximate level of the bear’s head measured at five different locations within the indoor dens averaged 193.3 \( \pm 24.8 \) lux during lights on, with no ambient light filtering in from their outdoor den. Outdoor light levels measured at four locations in the outdoor dens averaged 151.7 \( \pm 24.3 \) lux after sunset. Daytime light levels were subject to normal daily fluctuations and weather conditions with noontime light levels on a sunny day measuring \( >10,000 \) lux. For the winter contraction photoperiod experiment lighting provided by one 500-watt halogen light placed 0.61 m from the end of the enclosure produced an average interior illumination of 1146.7 \( \pm 607.3 \) lux. During the hibernation photoperiod experiment, room temperature was maintained at \( 10 \pm 1.2^\circ \text{C} \) for the duration of the study.

For photoperiodic experiments during the active season twice-daily feeding was randomized to occur at least 2 h postsunrise and 2 h presupnet to remove any confounding influence of feeding cues with photic cues. Random feeding times within the specified interval (i.e., between 2 h postsunrise and 2 h presupnet) were obtained by using an online random number generator (www.random.org). However, because the online number generator exported the random times in ascending temporal order, the order of the randomly generated feeding times had to be rerandomized as follows. Briefly, the randomly generated feeding times were exported into an Excel spreadsheet. Then the “rand” function was applied to fill cells in the adjacent column to the feeding times. Finally, the two adjacent columns were sorted in ascending order according to the random numbers generated by Excel. The amount, placement, type, and approximate total energy content of food provided were similar to that during the twice-daily scheduled feeding. Bear center staff and visitors were not allowed in the facility before the first feeding of the day to reduce disturbance and possible entrainment by human presence. Bears were not eating during hibernation when photoperiodic contraction was being performed.

**Light entrainment and circadian rhythms.** We also determined whether activity: 1) could entrain to a 5-h shift (delay) of the light-to-dark cycle, 2) could be entrained to a minimal (so-called skeleton) photoperiod, and 3) would exhibit an endogenous circadian rhythm when held in constant conditions. These manipulations were only possible during the hibernating season (winter) when the duration and application of photoperiod could be closely controlled. Once in hibernation, bears \( (n = 4) \) were placed on a 4L:20D photoperiod for 17 days and then the photophase was delayed by 5 h. Bears were exposed to the shift for 21 days and then animals were transferred into constant light conditions (LL, 24L:0D) for 42 days to determine whether an endogenous circadian rhythm was expressed. Finally, the bears were exposed to a skeleton photoperiod consisting of 1-h dark pulses interposed between 11 h of light (11:1:1:1) for 3 wk. Dark pulses were applied at 1100 h and 2300 h. Further evaluations of circadian rhythmicity were made with two additional bears. One bear was held in constant dark for 33 days during hibernation while the other bear was held in constant dark during the active season for 20 days. Activity profiles based on Actical data were used to evaluate entrainment and circadian rhythmicity.

**Food entrainment.** The role of food to entrain activity patterns was examined as follows. After a period of standard twice-daily feeding, bears \( (n = 8) \) were exposed to a reversed feeding schedule for 18 days in the fall (September-October, 2011, average natural daylength during experiment 11.63 h). Average ambient temperatures during the experiment were 19.7°C and 5.7°C for maximum and minimum, respectively. In this case, the bears were fed twice nightly at 2100 h and 0300 h. Nighttime feeding and cleaning of the dens was facilitated with the aid of a red lamp producing \( <5 \) lux illumination in the dens. After feeding and cleaning, the red lamps were turned off leaving the animals exposed only to ambient moonlight. To further limit any disturbances and possible entrainment, daytime visitation to the WSU bear center was tightly restricted. During this time the bears were exposed to natural photoperiod and temperature conditions. As noted above, the location of the food, amount and type of food, and energy content was kept identical to the twice-daily scheduled feeding protocol described above. Under these conditions bears were exhibiting an annual cycle of body weight gain with an average weight gain of \( 4.8 \pm 0.93 \) kg during the experiment. Annually, the bears at the WSU center fluctuate an average of 77.2 kg in body mass.

As for the photoperiod experiments, activity onsets and offsets were calculated. To confirm that a temporal niche shift occurred, both phase angles and midpoint of the daily rhythm (acrophase) were also estimated. To provide additional evidence of true entrainment by food, we also quantified anticipatory activity occurring before feeding onset by dividing each bear’s hourly activity count by its total daily activity count to produce a normalized activity ratio. Activity ratios were computed for all bears during twice-daily timed daytime feeding.
twice-daily random daytime feeding, and nighttime feeding experiments. Means for activity ratios were calculated and compared between feeding conditions.

Statistical analysis. Data were analyzed using Graphpad Prism5 (La Jolla, CA) and SAS v9.0 statistical software (Cary, NC). One- and two-way ANOVAs were used for estimating seasonal variation in activity counts, phase angles, and activity profiles. Bonferroni posttests were used to evaluate differences among means for treatment groups.

Linear regression was used to evaluate activity onset/offsets against time, monthly averages against daylength, and activity onset/offsets against time during photoperiod extension experiments. Statistical comparisons of regression slopes were made using a Student’s t-test. Ability to phase shift and entrain to a skeleton photoperiod were tested by t-test comparing activity counts in the light and dark period for each experimental condition. Period determinations were made using Lomb-Scargle periodograms in Clocklab software. Nighttime feeding was evaluated by analyzing acrophases on the last day of the reversed feeding using circular statistics (Oriana, RockWare, Golden, CO). Differences between acrophases were evaluated using Watson’s U2 test (42). Effects were considered significant at $P < 0.05$.

RESULTS

Baseline activity. There were dramatic differences in amount and amplitude of activity among seasons, with a clear 24-h periodicity detected for all animals (Fig. 1A; Lomb-Scargle periodogram analysis, $\tau$, $P < 0.05$). Activity differed among seasons (one-way ANOVA, $P < 0.0001$, Bonferroni posttests, $P < 0.05$) with hibernation having the lowest activity ($25,567.3 \pm 1,914.0$ mean counts/day) and activity onsets/offsets during the fall transition season (287,319.0 mean counts, respectively) (Fig. 1B). Activity increased during the spring transition season and decreased during the fall transition season ($20,379.0$ mean counts/day, respectively) (Fig. 1B). Activity counts increased during the spring transition season and decreased during the fall transition season ($20,379.0$ mean counts/day, respectively) (Fig. 1B). Activity increased during the spring transition season and decreased during the fall transition season ($20,379.0$ mean counts/day, respectively) (Fig. 1B).

The phase angles shifted with the animals initiating activity before sunrise (positive values) and ceasing activity after sunset (negative values). This pattern continued until late September to mid-October when the phase angles spontaneously began to compress as the bears became anorectic in preparation for hibernation. During hibernation, the phase angles were completely inverted compared with the late summer/fall period with activity rising before sunrise and ending well before sunset (Fig. 1B). Activity duration ($\tau$) was strongly related to daylength ($r^2 = 0.80; P < 0.0001, F = 475.6 (1 122);$ Fig. 3A). $\tau$ varied significantly with season even when daylengths were similar ($P < 0.001$, unpaired t-test, $t = 1.65, df = 14$; Fig. 3B).

Photoperiod experiments. During the active season, bears were exposed to photoperiod extensions at three times: spring (18L:6D, April), summer (20L:4D, June), and fall (18L:6D, October). Activity did not expand with the extended daylength (Fig. 4, A and B). In both spring and summer, regression analysis failed to detect any differences in locomotor activity onsets or offsets compared with control periods ($P > 0.05$; Fig. 5A). By contrast, extension of the photoperiod in the fall caused activity to contract compared with control periods (Fig. 5B).

During hibernation (December), a shortened photoperiod (4L:20D) applied for 17 days caused activity onsets and offsets to quickly shift in apparent synchrony with the new light-to-
dark cycle. This apparent entrainment however was not stable and soon began to dissociate from the light:dark cycle after about 1 wk (Fig. 5B). Although nonlinear regression fits of activity onsets and offsets against time revealed significant differences between control and experimental periods ($P < 0.001$) during hibernation, $\alpha$ (estimated from last 5 days) did not differ significantly from the control period (Fig. 4B; Bonferroni posttest, $t = 3.16, P > 0.05$).

**Light entrainment and circadian rhythms.** After the 17-day exposure to 4L:20D photoperiod during hibernation, the photocycle was shifted (delayed) by 5 h (Fig. 6A). Examination of the last day of the initial photocycle revealed a clear aggregation of activity in the photophase compared with the scotophase (Fig. 6B1). When the photocycle was shifted by 5 h, a gradual shifting of activity with transients occurred with a corresponding accumulation of activity (on the last day) within the photophase (Fig. 6B2). Comparison of light versus dark counts following the shift revealed a significant difference (one-sample $t$-test, $P < 0.01$). Interestingly, little evidence of anticipatory activity was observed under either 4L:20D photocycle (compare Fig. 6, B1 and B2). Exposure to a skeleton photoperiod consisting of 22L:2D (11:1:11:1) resulted in activity concentrating between the later dark pulses (~1700 h; Fig. 6, A and B). Locomotor activity was significantly greater in the photophase between 1200 and 2300 h compared with the period between 2400 and 1100 h (one sample $t$-test, $P < 0.001$).

To explore whether or not an endogenous circadian rhythm could be expressed during hibernation and active seasons, we maintained animals in either constant darkness (DD) or in constant light (LL) in the active and hibernating seasons for approximately 4 wk. Only one animal remained on trial for the active season DD experiment with $\tau = 24.05$ h (Fig. 7A). The effects of hibernation coupled with the darkness, suppressed locomotor activity to such an extent that only one bear remained sufficiently active to allow an accurate assessment of the free-running period to be made (24.5 h, Fig. 7B). In constant light during hibernation, the free-running circadian rhythm ($n = 4$) was $24.3 \pm 0.17$ h (Fig. 7C). While exposed to LL and constant temperature, all bears exhibited a spontaneous increase in activity toward the end of hibernation (Fig. 7D).

**Food entrainment.** The general lack of responsiveness to photic manipulations during the active season led us to test food entrainment as a potentially more important modulator of bear activity patterns. After a period of standard twice-daily feeding, bears were exposed to a reversed feeding schedule

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**Fig. 2.** A: mean ± SE weekly phase angles of activity onset and offset relative to sunrise and sunset, respectively, across the year. Dashed line represents hours of daylight. Shaded areas indicate hibernation (winter) season. B: comparison of mean weekly (± SE) phase angles among seasons. Different letters indicated differences $P < 0.01$.

**Fig. 3.** A: mean ± SE monthly $\alpha$. Shaded area represents hibernation period. B: comparison of $\alpha$ for months with similar hours of daylight. *$P < 0.001$. 

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**Fig. 4.** A: mean weekly phase angles of activity onset and offset relative to sunrise and sunset, respectively, across the year. Dashed line represents hours of daylight. Shaded area represents hibernation period. B: comparison of mean weekly ($\pm$ SE) phase angles among seasons. Different letters indicated differences $P < 0.01$.
with feedings occurring during the scotophase at 2100 and 0300 for 18 days in the fall. Normally, when bears were fed twice daily at 0700/1100 and 1600/1100 (standard time), a crepuscular pattern of activity was evident (Fig. 8A). Coincident with this feeding pattern was clear anticipation of both feeding times evident as increases in activity before feeding. When feeding times were randomized during daylight hours, activity profiles changed to reveal two daily peaks in activity, with one occurring near the time of the previous feeding time of 0700 and the second peak occurring between 1200 and 1400, approximately 4 h earlier than the previous 1600 feeding time. Comparison of the two feeding schedules by two-way ANOVA revealed a significant interaction between time of feeding and type of feeding schedule ($F = 7.54$, $df = 69$, $P < 0.0001$, Bonferroni posttests $P < 0.01$ at 0700, 1200, 1300, 1400, and 1700). While there was a main effect of time on
Fig. 6. A: actograms of 4 bears during hibernation illustrating responses to a photic shift, constant light conditions, and skeleton photoperiod (11:1:11:1). B: daily activity profiles over the last 2 days of entrainment during 4L:20D photoperiod (1), following a 5-h phase shift (2) and during a skeleton photoperiod. Comparisons between light and dark periods from last 2 days of each photoperiodic condition are also presented. *P < 0.05.
activity, there was no main effect of feeding type ($F = 11.44$, $df = 23$, $P < 0.001$ and $F = 0.05$, $df = 3$, $P > 0.05$, respectively). Activity in both cases was constrained to the light portion of the photocycle.

When feeding was changed from scheduled daytime feeding to nighttime feeding, patterns of activity aligned with the nighttime feedings (Fig. 8B). Actograms reveal how bears quickly became nocturnal within several days of transients (Fig. 8A) suggesting true entrainment rather than a simple masking effect of food. Once nighttime feeding ended, the bears returned to their diurnal niche coincident with twice daily feedings (Fig. 9A). This was reflected in adjustments of the
phase angles until activity was stably entrained to the feeding schedule but not the photocycle (Fig. 9B). By the last day of nighttime feeding, clear anticipation of the evening meals was exhibited by all bears (Fig. 8B; two-way ANOVA time by feeding schedule interaction, $F = 6.31$, $df = 23$, $P < 0.0001$, Bonferroni posttests $P < 0.05$ at 0200, 0300, 0400, 0800, 0900, 1600, 1700, and 2000) further suggesting entrainment. Regression fits for the last 5 days of phase angle onsets and offsets under reversed feeding revealed no significant differences from zero, indicating stable entrainment (Fig. 9B, bottom; one-sample $t$-test, $P = 0.73$ and $P = 0.16$ for onset and offset phase angle regressions, respectively). Acrophases aligned near the midpoints of the respective daily feeding schedules [i.e., around midday for daytime feeding (1254 h) and nighttime feeding (0240 h)] (Watson’s $U^2$ test $P < 0.001$, Fig. 9C). Despite the complete inversion of activity patterns, nighttime feeding did not significantly affect total activity ($P = 0.26$), although the percentage of activity occurring in the light phase was significantly lower when animals were fed during the night (Fig. 9, D and E).

**DISCUSSION**

Activity patterns during both active and hibernating seasons in the captive bears varied significantly as a function of daylength and food availability in a time-of-year-dependent fashion. Manipulations of the light-to-dark and feeding schedule revealed that daily rhythms in bears are jointly regulated by light-entrainable and food-entrainable oscillators, permitting adaptive flexibility of activity patterns. Entrainment to light, and perhaps food, is likely to be mediated by a circadian clock that is expressed in brown bears during hibernation. We hypothesize that the inherent behavioral flexibility exhibited by brown bears is likely to have conferred a selective advantage to this apex predator.

Estimation of the activity phase angles and $\alpha$ allowed us to indirectly examine the influence of photoperiod on activity. One would expect if bears were entrained to environmental photoperiod that stable (statistically revealed as linear) relationships with those light cues would be evident. Contrary to our initial expectation the bears in this study exhibited quite variable, yet predictable, changes in phase angles. Unstable phase angles have been observed in animals residing at high latitudes when daylengths shorten to less than 5 h or increase to more than 19 h (11) likely due to the limits of entrainment being reached. In two extreme cases occurring at polar latitudes, reindeer (*Rangifer sp.*) and Svalbard ptarmigans (*Lagopus mutus hyperboreus*) exhibit a complete loss of circadian rhythmicity (i.e., activity becomes arrhythmic) during the continuous days and nights of the polar summer and winter, respectively (66, 82). Although the animals in our study never experienced such extreme daylengths except in one experiment (see below), the phase angles of entrainment varied. Reproductive hormones have previously been shown to influence phase angles of entrainment (11, 28, 86). Although we did not directly test the effect of reproductive hormones, male bears exhibit a clear breeding season with annual changes in circulating testosterone and testicular recrudescence beginning in hibernation with regression beginning in summer [(8), Jansen, unpublished observations]. In other hibernators, reproductive hormones were not responsible for circannual variation in entrainment of activity, but it should be cautioned that although other species that hibernate or exhibit torpor also undergo changes in activity duration and phase angles (20, 36, 39, 83), it may not be appropriate to compare these species with larger hibernators such as bears that do not undergo periodic torpor and arousals bouts.

Alpha varied seasonally, but in a less-than-straightforward way. This was clearly evident when comparing months in which daylengths are similar but seasons differed (e.g., near the equinoxes). The circadian system can respond to photoperiods differently depending on prior photoperiod history. Specifically, although animals are exposed to the same photoperiods twice each year, the changes in daylength preceding these differ dramatically—in one the daylengths are increasing while in the other they are decreasing. Prior photoperiod history is deterministic to reproductive outcome in sheep (69) and birds and mammals living at high latitudes (11). A second possibility is that animals in the wild are acutely influenced by multiple environmental cues such as food availability and temperature, either of which can alter activity patterns (58). For providing predictive value, the first mechanism would have a clear long-term advantage compared with second.

Consistent with the variable phase angles expressed by bears in our first experiment, extending the photoperiod at three different times during the active season resulted in dramatic changes in phase angles but without significant effects on $\alpha$. Thus, although there appears to be a relative insensitivity to changes in photoperiod during the active season, an influence of light per se cannot be ruled out. Specifically, changes in light intensity, also known as continuous or parametric effects of light (3) or discrete effects of dawn and dusk (i.e., nonpara-
metric effects), may be involved. Continuous (parametric) effects of light refer to the relationship between light intensity and speed, or period, of the endogenous clock. Thus the increases and subsequent decreases in light intensity across the day serve as entraining signals for organisms (16). For example, diurnal European ground squirrels (Spermophilus citellus) that emerge from their burrows after dawn and return before dusk never are exposed to discrete dawn and dusk cues yet remain stably entrained to the ambient photocycle, indicating that the subtle changes in light intensity during the period in which the animal is out of the burrow are sufficient to entrain the circadian clock (10). Increases in light intensity have also been shown to affect 9270 and 9251 in degus (Octodon degus) housed in constant conditions (40). Thus, despite the additional lighting to extend the photoperiod in the current study, our bears still experienced sufficient natural changes in light intensity to potentially overcome the photoperiod extension. Alternatively, discrete effects of light on the circadian clock may have occurred but were masked by another entraining signal such as food (discussed below). These discrete (nonparametric) effects of light cause phase shifts of the endogenous clock sufficient to advance or delay the free-running period to match the external light cycle; under natural conditions, these light cues are the sunrise and sunset transitions (16). Nonparametric effects have been shown to be effective at entraining seasonal rhythms of reproduction in the European hamster but only at specific times.

Fig. 9. A: representative actograms during daytime and nighttime scheduled feeding. B: phase angles (PA) of activity onsets/offsets to sunrise/sunset, respectively, during daytime and nighttime scheduled feeding for entire experiment with the last 5 days replotted in inset (boxed area indicates replotted days) to illustrating stable entrainment occurring by end of the reversed (night) feeding experiment. C: acrophases of activity on the last 2 days of nighttime feeding compared with daytime feeding. Total activity counts (D) and percentage of activity (E) occurring during the light period during the last 2 days of daytime and nighttime feeding schedules. *P < 0.05.
of the year (summer and winter solstice) (48, 76). These findings are important in light of our finding that during the period surrounding the summer solstice, bears were unresponsive to increases in photoperiod. Because of our limitations for performing photoperiod contractions, we cannot rule out the possibility that bears may have been photosensitive to short days at that time. However, in the face of decreasing photoperiods, bears still remained unresponsive to photoperiod manipulations, suggesting that nonparametric effects of light do not exert powerful effects on activity patterns during the active season. Regardless of the precise reason(s), our observations are similar to those in ptarmigan where $\alpha$ increases while the birds are foraging and exposed to rapidly shortening day-lengths (67).

The apparent lack of responsiveness to photoperiod manipulations raised the possibility that the light entrainable circadian clock in bears is nonfunctional or was masked by another circadian oscillator. Arguing against this, bear activity rhythms began to free run with a $\tau$ differing significantly from 24 h when housed in constant light or darkness. These results provide strong support of a circadian clock in the brown bear operating during both active and inactive (hibernating) periods. Although body temperature measurements indicating level of torpor were not made in this study, activity and temperature are related. Furthermore, evaluations of decreased heart rate and metabolism have been characterized previously at this facility during hibernation, suggesting bears at our location were indeed in hibernation torpor during this study (51). In addition, the spontaneous changes in activity that occurred before entering hibernation combined with the spontaneous increases in activity toward the end of hibernation suggest an even longer duration endogenous cycle, i.e., a circannual rhythm in brown bears, although this remains to be determined directly. Similar spontaneous changes in length and depth of torpor bouts and seasonal sleep rhythms have been observed in other hibernators and species that exhibit bouts of torpor (21, 23, 59, 83).

In contrast to what was observed during the active season, hibernating bears responded to a daylength compression quickly (but not permanently). Two features of this experiment are worth noting: 1) Bears were not exposed to natural light cues, and 2) light intensity was approximately 10 times greater than what we could achieve in the active season. These results suggest that both parametric and nonparametric effects are involved. Nonparametric effects of light have been well characterized in many species and are manifested as large, predictable changes in the activity phase after application of discrete light pulses (61). Although a definitive conclusion regarding entrainment mechanism is not possible, based on the effects observed using a photoperiod contraction, clear evidence to support a nonparametric effect of light to entrain the circadian clock comes from the phase shift study. A 5-h delay of the photocycle resulted in all bears shifting their activity to the new light-to-dark cycle after several days of transients. However, a masking effect of light to stimulate activity (or suppress, in a nocturnal animal) is also possible (63, 65). Indeed, there was very little evidence of anticipatory activity occurring before light onset on the last day of the shifted photophase. Nevertheless, a more likely explanation is that the duration of exposure was simply not long enough to allow full entrainment of the circadian clock to occur. Also arguing against simple masking effects is the clear daily adjustment (delay) in activity that was observed for each bear following the phase shift.

The third approach used to examine light entrainment in the brown bear consisted of applying a minimal (skeleton) photoperiod comprising two 1-h dark pulses spaced 12 h apart on a background of bright light during hibernation. Skeleton photoperiods can supplant a complete photoperiod to maintain entrainment (62). Dark pulses rather than light pulses were used in the current study because the diurnal phenotype of our bears resulted (with rare exception, see Fig. 8B) in exceedingly low levels of activity when housed in constant darkness, thus making interpretation of endogenous rhythmicity or entrainment difficult. However, the application of discrete dark cues resulted in well-organized activity patterns, suggesting true entrainment had occurred. Interestingly, the consolidation of activity was greatest during the second portion (1200–2300 h) of the diel cycle compared with the first portion. We hypothesize that this was due to the relationship between the dark pulses, the individual bear’s circadian phase, and position on the phase response curve. The general form of the response curves is very similar between nocturnal and diurnal animals (62) and appeared opposite to that of light in our study, supporting similar findings in rodents (4, 80). In addition, responses to dark pulses appear to differ between nocturnal and diurnal species with nocturnal species generally being most responsive during the day and diurnal species during the night (7, 14, 24, 30, 32, 40, 45). Indeed, for half the bears, the first dark pulse appeared to fall on the presumed dead zone of the phase response curve (i.e., no effect) but phase advances or delays occurring in response to the second dark pulse, depending on whether the pulse fell late in the subjective day or early in the subjective night, respectively. The opposite was true for the other bears. These results indicate that the activity consolidation that occurred within the second photophase of the symmetrical light-to-dark skeleton photoperiod was interpreted by the bears as subjective day rather than subjective night.

The sensitivity to photic manipulations during hibernation and apparent lack thereof during the active season suggested that other nonphotic cues could be responsible for entrainment during the active season. Food is a powerful entraining signal sufficient to override the effects of light under certain experimental conditions (47). In support of the importance of food entrainment in bears, we observed robust activity changes in response to shifted feeding schedules. Thus, even with exposure to natural photoperiod and bright ambient light when bears were fed twice daily, their activity patterns changed within 4 days to a completely nocturnal activity pattern. Similar observations have been made in other species (35, 79). Much evidence suggests that an endogenous food entrainable oscillator (FEO) operating entirely independently of the light entrainable oscillator is involved (46). Much like light entrainment, that a true food oscillator was involved in this behavioral shift, rather than simple masking, is supported by three observations: 1) clear transients were expressed following the shift to nocturnal feeding, 2) a shift in anticipatory increases in activity corresponding to the new feeding times on the last day of the experiment was observed, and 3) a stable phase angle relationship between anticipation and feeding was established during the last week of the experiment.

The current findings suggest that light entrainable and food entrainable oscillators in bears may be operating in a mutually
exclusive or mutually suppressive fashion to modulate seasonal activity patterns. Indeed, such temporal flexibility would serve bears well because restricted food availability is common to virtually all bears in the wild (57). At its extreme, temporal niche switching is considered rare (38); however, it has been observed in other animals and in wild bears potentially as a mechanism to cope with food availability, predation, and temperature fluctuations (35, 65, 78, Fortin and Robbins, unpublished observations). Our current findings add to the body of evidence to suggest it may be a relatively common occurrence in brown bears.

**Perspectives and Significance**

Activity patterns in captive brown bears are modulated by a seasonally dependent shift in Zeitgeber strength with food entrainment predominating during the active season, whereas sensitivity to light-to-dark cues is uncovered during hibernation. The ability of brown bears to respond to nonphotic and photic cues in a seasonal manner may explain the extensive behavioral flexibility exhibited by these animals. This flexibility may be required for the massive gains in body mass necessary to survive a prolonged fast while also retaining the ability to emerge from hibernation at an advantageous time of year or before starvation. Implicit in this hypothesis is the existence of an underlying circannual rhythm entrained by these two cues. Regardless of the precise mechanisms involved, these adaptations may have evolved to allow brown bears to successfully navigate a human influenced landscape.

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**AUTHOR CONTRIBUTIONS**


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