

**Arabian oryx (*Oryx leucoryx*) respond to increased ambient temperatures
with a seasonal shift in the timing of their daily inactivity patterns**

Running title: Seasonal shifts in Arabian oryx activity patterns

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Abstract

The Arabian oryx inhabits an environment where summer ambient temperatures can exceed 40°C for extended periods of time. While the oryx employs a suite of adaptations that aid survival, the effects of this extreme environment on inactivity/sleep, where ambient temperatures often exceed mammalian thermoneutral zones, are unknown. To determine how the oryx manages inactivity/sleep seasonally we used fine and coarse-grain actigraphy, in 16 animals, to reveal when the animals were inactive/sleeping in relation to variations in ambient temperatures and light levels. We demonstrate that during the cooler winter months the oryx is inactive/sleeping during the cooler parts of day (pre-dawn hours), showing a diurnal activity pattern. In contrast, in the summer months, the oryx displayed a crepuscular activity pattern, with the major inactivity/sleep bouts occurring equally during both the coolest part of the night (pre-dawn hours) and the hottest part of the day (afternoon hours). Interestingly, the daily rhythm of the timing of changes in core body temperature did not vary seasonally, although the amplitude did change. The transition from winter diurnal activity to summer crepuscular activity occurred in May, while the reverse occurred in September. By having half of the major summer sleep bouts during the hottest part of the day, the oryx may take advantage of the thermoregulatory physiology of sleep to mitigate increases in body temperature. The seasonal summer desynchronization of circadian entrained daily rhythms (core body temperature and daily activity patterns) is suggestive of temperature acting to mask, or modify, output pathways from the suprachiasmatic nucleus.

Keywords: Arabian oryx; circadian rhythms; modifying factors; temperature; sleep; seasonality.

Introduction

The Arabian oryx is the smallest member of the genus *Oryx*, and is native to the gravel and hard sands of the deserts and steppes of the Arabian Peninsula. In the summer months peak ambient temperatures in the Arabian Desert regularly surpass 40°C for prolonged periods of time. In order to cope with this hot and dry environment the Arabian oryx employs a range of behavioural and morpho-physiological responses to high temperatures. During the summer, Arabian oryx have been observed to increase the time spent resting in the shade, where they appear to dig shallow depressions to rest in (Stanley Price, 1989; Seddon and Ismail, 2002), creating a cooler microclimate (Hetem et al., 2012a). In addition to these behavioural strategies, the Arabian oryx has morpho-physiological capacities that assist its survival, such as reduced fasting metabolic rates, lower total evaporative water loss rates, lower water influx/turnover rates compared to other similar sized artiodactyls (Williams et al., 2001), adaptive heterothermy (Ostrowski et al., 2003; Hetem et al., 2010) and selective brain cooling (Hetem et al., 2012b). This suite of behavioural and morpho-physiological features undoubtedly assists the Arabian oryx to survive in the desert of the Arabian Peninsula, although these features appear to be common to all species of oryx living in arid environments and are not specific adaptations of the Arabian oryx (Taylor, 1966; 1968, 1969, 1970a,b; Taylor and Lynn, 1972; King et al., 1975; King, 1979; Stanley Price, 1985).

Seddon and Ismail (2002) and Hetem et al. (2012a) indicate the possibility of an altered daily activity pattern between the cooler (diurnal activity) and hotter (nocturnal activity) months in the Arabian oryx, although this was not explored in detail in either of these studies. Amongst many mammals, the dominant, or primary, *zeitgeber* for

entraining circadian rhythms, including daily activity patterns, is ambient light (e.g. Daan and Aschoff, 1975; LeGates et al., 2014), but several secondary *zeitgebers* or modifiers of light entrained patterns (such as temperature, patterns of eating and drinking, social influences and physical activity) allow the entrainment of circadian rhythms in the absence of, or in concert with, the primary light *zeitgeber* (e.g. Erriksson et al., 1981; Tokura and Aschoff, 1983; Hastings et al., 1998; Pohl, 1998; Mistlberger and Skene, 2004; van Oort et al., 2007; Mircsof and Brown, 2013; El Allali et al., 2013). Intricately linked with the circadian entrained daily activity pattern is the timing of sleep, as sleep mostly occurs in the daily phase of general and prolonged inactivity (Monk, 1991). The central neural control of the timing of circadian rhythms and sleep, while mostly autonomous, are both located in the hypothalamus (McGinty and Szymusiak, 2005; Saper et al., 2005a,b), and both appear to be co-ordinated by the suprachiasmatic nucleus (Dubocovich 2007; Lu et al., 2010).

The Arabian oryx can undergo substantial seasonal changes in the variation of body temperature amplitude, although the timing of the daily rhythm of the body temperature does not appear to change seasonally (Hetem et al., 2010). Thus, the Arabian oryx may be able to alter its daily activity patterns depending on the season to mitigate the effect of temperature differentials, but it is unclear whether the timing of sleep changes in accordance with the potentially different seasonal daily activity patterns (Seddon and Ismail, 2002; Hetem et al., 2012a). In order to determine whether daily activity patterns and/or the timing of sleep change or remain the same seasonally in the Arabian oryx, we used actigraphy (Ancoli-Isreal et al., 2003) to explore the timing of

daily activity patterns and potential sleep periods during both summer and winter months. We also explored the potential modifying factors that may enable such timing shifts.

Materials and Methods

Animals used and experimental groups:

In the current study a total of 16 adult Arabian oryx (12 males; 4 females; mean body mass = 77.6 ± 11.6 kg) were used. These animals were divided into three groups: Group 1 – Nine adult males (mean body mass 79.8 ± 11.0 kg) being used for fine-grain actigraphy in the Mahazat as-Sayd Protected Area, northeast of Taif, Saudi Arabia ($22^{\circ}12'12''\text{N } 41^{\circ}49'36''\text{E}$) in February and July of 2014; Group 2 – Six adults (2 = male; 4 = female, mean body mass 72.7 ± 12.9 kg) being used for year-round coarse grain activity and core body temperature measurements in the Mahazat as-Sayd Protected Area from February 2014 through to January 2015; Group 3 – One adult male (85 kg body mass) being used for fine-grain actigraphy near Richmond in the Karoo region of South Africa ($31^{\circ}32'29''\text{S } 23^{\circ}41'34''\text{E}$) during February 2015. Ethical clearance for the current study was obtained from the University of the Witwatersrand Animal Ethics Committee (clearance certificate number 2014/53/D) and all animals were treated according to the guidelines of this committee, which parallel those of the National Institute of Health (NIH) for the care and use of animals in scientific experimentation.

Anaesthesia of the Arabian oryx

All animals were remote injected using a Dan-Inject dart gun (Daninject, Børkop, Denmark) with etorphine hydrochloride (CaptivonTM 98, Wildlife Pharmaceuticals Ltd., White River, South Africa; 19µg/kg; a semi-synthetic opioid that has an analgesic effect and is an opioid receptor antagonist), Ketamine (Ketaminol[®] Vet., MDS Animal Health, Intervet International B. V., Boxmeer, The Netherlands; 0.3mg/kg; a dissociative anaesthetic agent which is an NMDA receptor antagonist), Midazolam (Midazolam, Wildlife Pharmaceuticals Ltd., White River, South Africa; 0.13mg/kg; a benzodiazepine class anaesthetic agent that acts by enhancing the effect of GABA on GABA_A receptors), and Medetomidine (Zalopine 10 mg/ml, Orion Pharma, Espoo, Finland; 5µg/kg; a sedative analgesic that is an alpha-2 adrenergic agonist). All animals received oxytetracycline (Terramycin LA, Pfizer, Brazil, 20mg/kg IM; a broad spectrum antibiotic) and ketoprofen (Ketovet, Vetmedim Animal Health, Cantho City, Vietnam, 2mg/kg IM; a propionic acid class of nonsteroidal anti-inflammatory which acts to inhibit the production of prostaglandin). After the procedure, anaesthesia was reversed using naltrexone hydrochloride (Naltrexone, APL, Kungens Kurva, Sweden; 40mg IM; an opiate antagonist) and atipamezole hydrochloride (Antisedan, Orion Pharma; 2mg IM; a synthetic α 2 adrenergic receptor antagonist). After the recording period, the animals were again immobilized using a similar drug combination, and the implants were removed. Again, oxytetracycline and ketoprofen was administered and the animals were allowed to recover in a shaded pen prior to release to their normal environment.

Surgery for fine-grain actigraphy

Following recumbency, the animals were moved to a shaded area for surgery. Using aseptic techniques, a subcutaneous pocket was created on the left lateral aspect of both the neck and thigh, and an actiwatch embedded in an inert wax (SasolWax 1276, Sasol, Johannesburg, South Africa) was placed into each pocket. The actiwatch ambulatory device is commonly used for measuring sleep in humans. Within each actiwatch is a piezoaccelerometer device connected to a microchip that sums and records the number of acceleration events for each minute. The *Actiwatch Spectrum* (Philips Respironics, Philips) was used in the current study, which has a weight of 25 g and approximate dimensions of 35 x 35 x 12 mm. Each actiwatch, factory calibrated, was programmed (data acquisition rate set at 1 min intervals) with Philips Respironics Actiware 5 software (actiwatch communication and sleep analysis software), prior to implantation. The wristbands from the actiwatches were removed and the watches were insulated with standard electrical insulation tape and covered with 2 coats of biologically inert wax. The implantation sites of the actiwatches (i.e. the side of the neck and the hindleg) were shaved, washed with chlorhexidine, and disinfected with alcohol. Sterile drapes were taped in place over the respective surgical sites to isolate the disinfected area. A small incision (less than 50 mm in length) was made at each of the respective implantation sites and a subcutaneous pocket extending approximately 100 mm ventrally from the incision site was created. The actiwatch, sterilized within a container with formalin pellets for 48 hr prior to implantation, was inserted into the respective pockets and the incisions closed in an everting pattern with interrupted sutures, sterilized once again, and liberally sprayed with Necrospray (Bayer Animal Health). Following reversal

of the anaesthesia, the animals were then released into a 2 x 1 km fenced area of natural oryx habitat, and not provided with supplementary food or water for the entire recording period. For the single animal done in South Africa, a radio transmitter collar was attached to the neck of the animal and the animal released back to the wider game reserve (which measured 25 x 25 km) and not provided with supplementary food or water. After the recording period, the implanted animals were relocated, re-anaesthetized and the actiwatches were retrieved in the same manner as described above for implantation.

Data analysis for fine-grain actigraphy

Phillips Respiroics Actiware 5 was used to retrieve the recorded data from each of the implanted actiwatches. The raw data were exported to Microsoft Excel where they were manually scored and analysed. Data obtained from the neck and leg actigraphs were scored concurrently in 1 minute epochs as either active or inactive. For an epoch to be scored as active either the neck or leg actigraphs had to have an activity score greater than zero. Inactive epochs were scored when both the neck and the leg actigraphs had an activity score equal to zero. From the 1 minute scored data the modal state for 5 minutes was calculated and used to determine total active/inactive time, number of active/inactive episodes and the average duration of active/inactive episodes. The data presented in the results section is the grouped data for all individuals with the standard deviation provided in brackets following averages. No statistically significant differences were detected between males and females, therefore the data from all oryx, in each month, was grouped.

Surgery for coarse-grain actigraphy and body temperature recording

Following recumbency, the animals were moved to a shaded area for surgery. Using aseptic techniques as described above, a data logger that measured both activity and temperature was inserted intra-abdominally (AIC001: Abdominal implant, Africa Wildlife Tracking, South Africa). For activity the data were analyzed as a percentage of maximal activity reading (see below), and for the body temperature the device was calibrated against an accurate thermometer in an insulated water bath, providing an accuracy of better than 0.06°C. A small incision (less than 80 mm in length) was made through the skin at the implantation site and the muscle layers of the abdominal wall were separated using blunt dissection. The implant, coated in inert wax and sterilized within a container with formalin pellets for 48 hr prior to implantation, was inserted untethered into the abdomen, the incisions closed, and liberally sprayed with Necrospray. A satellite collar with a GPS unit was attached to the neck of each animal (iridium satellite collar, Africa Wildlife Tracking, South Africa). Following reversal of the anaesthesia, the animals were monitored in holding pens with access to food and water for 10 days, before being released into the wider Mahazat as-Sayd Protected Area (2200 km²) for the year-long recording period. The animals were not recaptured following the recording period, the collars and implants being left *in situ*.

Data analysis for coarse-grain actigraphy

The data obtained from the abdominal implant were downloaded into Microsoft Excel files for processing. The six animals were analysed individually and the data then pooled. Activity counts from each 10 minute recording period were summed across the

first 28 days of each month, averaged, and converted to a percentage of the maximum activity reading for that month to allow for inter-device variability between animals and between months. The averaged data from each animal were then pooled to create an overall average level of activity (as percent of maximum for each ten minutes) and depicted graphically. The core body temperatures were treated in a similar way, apart from conversion to a percentage, as the output was absolute.

Recording of weather conditions

A permanent weather station within the 1 x 2 km pen at the Mahazat as-Sayd Protected Area recorded weather parameters throughout the year in Saudi Arabia. This weather station provided a reading of ground temperature, air pressure, air temperature, humidity, wind speed, wind direction, rainfall and solar radiation every 15 minutes throughout all recording periods. The date and time stamps on the data were used to match the different recordings. Data for each month is provided in Table 1. In Richmond, South Africa, a portable, solar powered, weather station (Envirodata, www.envirodata.com.au/easidatamark4), which recorded, every 10 minutes, air temperature, black globe temperature, relative humidity, wind speed, wind direction, rainfall, solar radiation, and illuminance (lux), was placed within the game reserve during the recording period. Data for this month of recording are provided in Table 1. Sunrise and sunset times were obtained from various freely accessible databases.

Table 1: Weather parameters measured during the recording period from February 2014 through to January 2015 in Saudi Arabia, plus that of the recording made in South Africa in February 2015. The months of May and September (gray background) appear to be transition months between different activity pattern types.

Location	Month/Year	Highest temperature recorded (°C)	Average high temperature (°C)	Average temperature (°C)	Average low temperature (°C)	Lowest temperature recorded (°C)	Rainfall (mm)	Sunrise	Sunset	% of 24h daylight
Mahazat	Feb, 2014	32.0	26.1	19.3	11.6	5.7	0	06.49	18.15	47.7
Mahazat	Mar, 2014	34.9	30.4	23.5	16.1	7.6	0	06.26	18.27	50.1
Mahazat	Apr, 2014	37.8	35.0	28.5	20.8	11.0	3.3	05.58	18.37	52.7
Mahazat	May, 2014	41.7	37.2	30.6	22.9	17.5	8.4	05.40	18.49	54.8
Mahazat	Jun, 2014	41.2	39.8	33.4	25.4	21.6	0	05.36	19.01	55.9
Mahazat	Jul, 2014	41.6	39.7	33.1	25.7	21.1	0	05.45	19.03	55.4
Mahazat	Aug, 2014	41.8	40.6	33.9	25.9	23.5	0	05.56	18.48	53.6
Mahazat	Sep, 2014	40.3	38.4	32.0	24.6	18.9	0	06.05	18.21	51.1
Mahazat	Oct, 2014	36.5	34.2	27.2	18.8	14.7	0	06.14	17.54	48.6
Mahazat	Nov, 2014	33.6	28.2	21.4	14.3	9.3	4.8	06.29	17.36	46.4
Mahazat	Dec, 2014	29.8	26.2	19.1	12.1	9.4	1.5	06.48	17.39	45.2
Mahazat	Jan, 2015	33.3	25.0	16.7	8.3	2.1	0	06.58	17.57	45.8
Richmond	Feb, 2015	31.4	25.4	21.6	17.8	14.4	12.6	05.39	18.46	54.6

Results

The current report outlines our findings regarding seasonal effects on daily activity patterns and timing of inactivity/sleep in Arabian oryx studied in two different ways from two different environments. Initially, we used fine-grain actigraphy (1 minute resolution, 2 devices/animal, subcutaneous neck and hindleg) for 28 days in both a cold month (February) and a hot month (July) in the Mahazat as-Sayd Protected Area, northeast of Taif, Saudi Arabia, to reveal that there was a seasonal shift in the daily activity pattern from strictly diurnal in the cold month to crepuscular in the hot month. We then used a coarse-grain actigraphy method (10 minute resolution, 1 device/animal, intra-abdominal) in the Mahazat as-Sayd Protected Area, Saudi Arabia, to examine year round (365 days) patterns of activity to determine that the seasonal shift in daily activity patterns occurred in May and September. Lastly, to determine the potential modifying factors driving the seasonal shift, we examined an oryx with fine-grain actigraphy in the hot month of February (hot for South Africa) from the Karoo, South Africa (28 days) to disentangle the potential for ambient light or ambient temperature to be the modifying factor, demonstrating that the animal from South Africa, despite the increased day length, showed a very similar pattern of inactivity/sleep to the animals recorded in the Saudi Arabian winter.

Seasonal variance in the daily activity pattern and timing of sleep:

Nine male oryx (mean body mass 79.8 ± 11.0 kg), four in February 2014 (winter) and five in July 2014 (summer), were used for this portion of the study (see Fig. 1 and

Table 1 for environmental data for each of these months). During the winter recording period ($n = 4$, period = 28 days, February, 2014) the average daily inactivity/sleep measured was 5.01 h (± 1.71 h). The animals displayed polyphasic inactivity/sleep with an average of 18 (± 4) bouts of inactivity/sleep per day. The average daily onset of the main inactive/sleep bout (that being the longest period of inactivity) occurred between 03:30 and 04:00, with this bout having an average length of 127 min, just over 2 h (± 63.5 min). Thus, during the winter recording period, the oryx displayed a diurnal activity pattern, with the vast majority (94%, $\pm 7.25\%$) of inactivity/sleep, occurring during the night (Figs. 2, 3).

During the summer recording period ($n = 5$, period = 28 days, July, 2014) the average daily inactivity measured was 6.62 h (6h 37 min, ± 1.93 h). The animals displayed polyphasic inactivity/sleep with an average of 27 (± 6) bouts of inactivity/sleep per day. In this summer recording session, the main sleep bout was found to have an onset between 01:00 and 01:30 or 13:30 and 14:00. Thus, prolonged periods of inactivity/sleep were observed at two different times during the 24-hour period (Fig. 4). These main inactivity/sleep bouts had an average length of 137 min, just over 2 h (± 73.4 h). Thus, when compared to winter, during the summer the daily activity and sleep pattern of the oryx changed dramatically, showing a crepuscular activity pattern and having an extra 1 h and 37 min of inactivity/sleep (Mann-Whitney, non-parametric test, $P = 1.25 \times 10^{-12}$). The additional inactivity/sleep occurred mostly during the afternoon, but was also present in the pre-dawn hours (Figs. 2, 3, 4). During the summer, 68% ($\pm 24.6\%$) of inactivity/sleep occurred during the daylight period compared to 6% in the winter (statistically significant difference, Mann-Whitney, non-parametric test, $P = 2.49 \times 10^{-30}$),

while only 32% ($\pm 24.6\%$) of inactivity/sleep occurred during the dark period in summer compared to 94% in the winter (statistically significant difference, Mann-Whitney, non-parametric test, $P = 4.21 \times 10^{-8}$). Despite this seasonal variance in the inactivity/sleep pattern, we could find no seasonal change in the timing of the daily body temperature rhythm, although the amplitude did change (Fig. 3), confirming the results of an earlier study (Hetem et al., 2010).

Year round activity patterns and the timing of the pattern shift:

In order to determine at what times during the year the daily activity/sleep timing patterns switched from the typical winter diurnal pattern to the typical summer crepuscular pattern, we employed a coarser actigraphy method by the implantation of an abdominal activity meter into 6 Arabian oryx (2 = male; 4 = female, mean body mass 72.7 ± 12.9 kg). By pooling the results of the data obtained from the 6 animals (as done by others previously, Hetem et al., 2012a) we were able to identify five specific indicators of activity that relate to the seasonal variation in the daily activity/sleep timing patterns (Fig. 5; Table 2). The first of these indicators was a pre-dawn dip in activity, the second a matutinal (morning) activity peak, the third an afternoon dip in activity, the fourth a vespertine (evening) activity peak, and the fifth being the post-sunset activity level.

The pre-dawn activity dip, scored as being prominent when average activity was less than 10% of maximum for a period of at least 1 hr, moderate when between 10-15% of average activity for a period of at least 1 hr, and indistinct when activity was greater

than 15% for a period of at least 1 hr. The pre-dawn activity dip was a prominent feature of the activity record of all animals from February to April, became an indistinct feature from June to August, was moderately expressed in May, September and October, and again became a prominent feature from November to January (Fig. 5; Table 2). The matutinal activity peak was scored as being a prominent feature when average activity was greater than 30% of maximum for a period of at least 1 hr, and moderate when it was between 10 and 30% of maximum for a period of at least 1 hr. This was a prominent feature of the activity record of all animals from February to May, was only moderately expressed from June to September, but again became a prominent feature from October to January (Fig. 5; Table 2). The afternoon activity dip was scored as being moderate when the average activity was less than 10% of maximum for a period of at least 1 hr, and prominent when average activity was less than 10% for greater than 1hr, often exceeding 2 hr. This afternoon activity dip was only moderately expressed from February to May, but became a prominent feature of the activity record between June and October, before again only being moderately expressed from November to January (Fig. 5; Table 2). The vespertine activity peak was scored as being moderately expressed when the average activity was greater than 15% of maximum for a period of at least 1 hr, and absent when the activity was lower than 15% for a period of at least 1 hr. The vespertine activity peak was moderately expressed from February to May, was absent in the record from June to August, and then was moderately expressed from September to January (Fig. 5; Table 2). Post-sunset activity levels were scored as low when average activity levels were less than 15% of maximum for a period of 2 hr or more and moderate when average activity levels were greater than 15% for a period of 2 hr or more. Post-sunset

activity was scored as low from February to March, moderate from April to November, and low in December and January (Fig. 5; Table 2).

Table 2: Summary of the qualitative data of daily activity patterns across a full year for 6 Arabian oryx recorded in the Mahazat as-Sayd Nature Reserve, Saudi Arabia. The months of May and September (gray background) appear to be transition months between winter and summer type activity patterns.

Month, Year	Pre-Dawn activity dip	Matutinal activity peak	Afternoon activity dip	Vespertine activity peak	Post-sunset activity level	Activity pattern type
Feb, 2014	Prominent	Prominent	Moderate	Moderate	Low	Winter
Mar, 2014	Prominent	Prominent	Moderate	Moderate	Low	Winter
Apr, 2014	Prominent	Prominent	Moderate	Moderate	Low	Winter
May, 2014	Prominent	Prominent	Moderate	Low	Moderate	Mixed
Jun, 2014	Indistinct	Moderate	Prominent	Absent	Moderate	Summer
Jul, 2014	Indistinct	Moderate	Prominent	Absent	Moderate	Summer
Aug, 2014	Indistinct	Moderate	Prominent	Absent	Moderate	Summer
Sep, 2014	Moderate	Moderate	Prominent	Moderate	Moderate	Mixed
Oct, 2014	Prominent	Prominent	Prominent	Moderate	Moderate	Mixed/Winter
Nov, 2014	Prominent	Prominent	Moderate	Moderate	Moderate	Winter
Dec, 2014	Prominent	Prominent	Moderate	Moderate	Low	Winter
Jan, 2015	Prominent	Prominent	Moderate	Moderate	Low	Winter

By combining these five key indicators of the activity level, we were able to determine a specific winter-type activity pattern, a mixed pattern and a specific summer-type activity pattern (Table 2). The months of February, March, April, November, December and January displayed the winter-type activity pattern, with a prominent pre-dawn activity dip, a prominent matutinal activity peak, a moderate afternoon activity dip, a moderate vespertine activity peak and a low level of activity after sunset. The months of June, July and August evinced the typical summer-type activity pattern, with an indistinct pre-dawn activity dip, a moderate matutinal peak, a prominent afternoon activity dip, an absent vespertine activity peak and a moderate level of post-sunset activity. The months of May, September and October, displayed a mixed, or transitional, activity pattern. Thus, the transition from a winter-type diurnal activity pattern, to the summer-type crepuscular

activity pattern, appears to occur during May, while the return to the winter-type diurnal activity pattern appears to take place mostly during September, but possibly extending into October. The coarser records of the actigraphy technique used year round prevents us from being more accurate with the timing of activity pattern changes, but the transitional months determined do appear to be related to specific light and temperature conditions (Fig. 5; Table 1).

For the winter-type diurnal activity pattern months, the percentage of 24 hours occupied by daylight ranged from 45.8 to 52.7% (Fig. 1; Table 1). In the summer crepuscular months, this percentage rose to 53.6 – 55.9% of the 24-hour period. The transitional month September (51.1%) fell between this range, but the transitional month of May (54.8%) had slightly longer day lengths than those observed in the summer month of August (53.6%), but not June or July. Thus, no clear relationship between day length and the transitions in daily activity patterns was observed. In contrast, the average temperatures in the winter-type diurnal daily activity pattern months (average temperature range 16.7 – 20.8°C; average high temperature range 25.0 – 28.5°C; average low temperature range 8.3 – 20.8°C) were markedly lower than those observed in the summer-type crepuscular daily activity pattern months (average temperature range 33.1 – 33.9°C; average high temperature range 39.7 – 40.6°C; average low temperature range 21.1 – 23.5°C), with the transitional months falling between these (Fig. 1). No relationship to sporadic rainfall was observed (Table 1). Thus, the relationship between temperature and transitions in the daily activity patterns appears to be stronger than those associated with day length.

Is light or temperature the dominant modifier of activity rhythms?

In order to disentangle whether light or temperature is the modifying factor driving the seasonally switching patterns of the circadian rhythm of daily activity patterns and inactivity/sleep timing in the Arabian oryx recorded in Saudi Arabia, we examined a single adult male (body mass estimate = 85 kg) from a free-ranging population near Richmond, in the Karoo region of South Africa. The advantage of including this particular individual is that the weather in the Karoo, while hot in summer, does not reach the temperatures recorded in summer in Saudi Arabia, but matches the temperatures seen in the winter months in Saudi Arabia (Fig. 1, Table 1); however, the day lengths within South Africa during the summer are similar to those seen within Saudi Arabia during the summer (Fig. 1, Table 1). Thus, we have a natural experimental paradigm where temperature is controlled, but day length can vary.

With this individual animal we used fine-grain actigraphy recording and found that the Arabian oryx in South Africa displayed a clearly diurnal daily activity pattern (Fig. 6), very similar to that seen in the winter recordings of oryx in Saudi Arabia (Figs. 2, 3). During the summer recording period in South Africa (n = 1, period = 28 days, February, 2015) the average daily inactivity/sleep measured was 5.20 h (5 h 12 min, ± 1.7 h) (not significantly different to the inactivity of the oryx measured in the Saudi winter, Mann-Whitney, non-parametric test, $P = 0.052$, but significantly less than the oryx measured during the Saudi summer, $P = 6.04 \times 10^{-6}$). The animal displayed polyphasic inactivity/sleep with an average of 16 (± 4) bouts of inactivity/sleep per day. The average daily onset of the main inactive/sleep bout occurred between 03.30 and 04.00, with this bout having an average length of 131 min, just over 2 h (± 56 min). Thus, during the

South African summer recording period, the single oryx displayed a diurnal daily activity pattern, with the vast majority (81.6%, \pm 12%) of inactivity/sleep, occurring during the night (Fig. 6). The only noted difference between the South African animal and the Saudi Arabian winter animals was the slight increase in inactivity in the late afternoon in the South Africa animal compared to those of the Saudi Arabian winter animals (Figs. 2, 3, 6). This may reflect some effect of increased summer temperatures, but does not match the increase in summer afternoon inactivity levels observed in the Saudi Arabian summer animals.

Discussion

The current study reveals how the Arabian oryx manages daily activity patterns and inactivity/sleep in relation to the dramatic seasonal conditions of a hot and inhospitable environment. First, we have observed that the daily activity pattern of the Arabian oryx changes from a distinct diurnal pattern in the cooler months, to a characteristic crepuscular pattern during the hotter months. Second, the timing of the majority of inactivity/sleep also changes seasonally, from the coolest parts of the winter nights to the hottest parts of the summer days. Third, through comparison with a conspecific inhabiting a different environment, we propose that temperature is the dominant modifier driving these changes in daily activity and sleep timing patterns. Fourth, while some daily rhythms appear to change seasonally, others, such as core body temperature, do not change, indicating a desynchronization of circadian rhythms and the potential for the existence of a masking effect of temperature on the synchronization of

circadian rhythms, or, speculatively, more than one *zeitgeber* acting in the summer months for the entrainment of circadian rhythms in the Arabian oryx of Saudi Arabia.

Seasonal changes in the temporal niche of daily activity patterns

The fine-grain actigraphy analysis clearly revealed two strikingly different daily activity patterns in the Arabian oryx, from strictly diurnal in the cooler months to clearly crepuscular in the hotter months. Seddon and Ismail (2002) used focal 10 minute observations from 6 am to 6 pm, and Hetem et al. (2012a) used a coarse grain method of actigraphy, in their studies, but were not able to clearly show this variation due to the observations either not including the full 24 hour period (Seddon and Ismail, 2002), or the recordings not being made throughout the entire year, with the cooler months of December to March not being recorded (Hetem et al., 2012a). While many studies report seasonal variations of the daily activity patterns, termed temporal niche switching (reviewed in Refinetti, 2008 and Hut et al., 2012), from a range of mammalian species, only a few examples of this occurring under natural conditions have been reported. Of specific interest to the current study are those examples where increased seasonal temperatures appear to drive the switching of the temporal niche of daily activity, from diurnal to nocturnal in the degu (Vivanco et al., 2010), blind mole rat (Oster et al., 2002), and bat-eared fox (Lourens and Nel, 1990). While these examples are similar to what we observed in the Arabian oryx, the oryx switches its temporal niche from diurnal to crepuscular (rather than nocturnal), which does not appear to have been reported previously under natural conditions (Refinetti, 2008; Hut et al., 2012). It is also possible

that the dromedary camel, under experimental dehydration conditions, where it becomes an adaptive heterotherm (El Allali et al., 2013), may show similar changes in the daily activity patterns observed in the Arabian oryx under high ambient temperatures, but this has not yet been shown (El Allali et al., 2013; Bouaouda et al., 2014). Our year round analysis and the analysis of the Arabian oryx in the South African summer, suggests that temperature is the driving force behind this change, whereas day length and rainfall appear to have little or no role.

Seasonal changes in the timing of sleep

While we have not used polysomnography (PSG) to record sleep in the current study, the use of actigraphy is becoming an increasingly accepted and reliable method for determining when sleep occurs in human (but not for parcellating sleep into its different stages) (e.g. Sadeh and Acebo, 2002; Ancoli-Isreal et al. 2003; Hurelbrink et al., 2005; Chae et al., 2009; Sadeh, 2011), but to date has not been validated in other species. Despite this, if we focus on the timing of the main bouts of inactivity (those greater than 1 h), many of which lasted for almost 2 hours in both seasons in all the oryx studied in Saudi Arabia, it is reasonable to postulate that during these periods of inactivity the oryx were likely to be asleep for the majority of the time and that this period would be when the majority of sleep occurred. In this sense, during the winter/cooler months, the majority of sleep would have occurred between approximately 03.00 and 05.00, while during the summer/hotter months, the majority of sleep would have occurred in two periods during the day, between approximately 03.00 – 05.00 and 12.30 – 14.30. Thus,

the majority of sleep in the winter occurs during the coolest part of the day when the body temperature is at its lowest, while in summer it occurs during both the coolest and the hottest part of the day (when the body temperatures are at their highest) (Fig. 3), as the timing of the daily rhythm of body temperature variation does not appear to change across seasons (Hetem et al., 2010; confirmed in this study).

The pattern of polyphasic sleep, with the main sleep bout, occurring mostly during the night in the winter in the Arabian oryx, with an overall amount of inactivity/sleep being approximately five hours, represents a typical sleep pattern in terms of timing, phasing and duration for an artiodactyl of approximately 80 kg in body mass (e.g. Campbell and Tobler, 1984; Siegel, 2005). In contrast, the timing of sleep observed in the Arabian oryx in the summer months is unlike that observed in any artiodactyl studied to date. This dramatic change in the timing of summer sleep in the oryx, with the majority of sleep being moved to the hottest parts of the day, and a major increase in the amount of time spent asleep (an extra 1.5 hours per day, which contrasts with many other mammals, including humans, previously studied which sleep more in the winter than the summer, e.g. Erriksson et al., 1981; Everts et al., 2004; Lariviere and Messier, 2009; Yetish et al., 2015), may indicate that this is an adaptive response to increased ambient temperatures. Indeed, during slow wave sleep the body and brain temperature of all mammals studied to date drops significantly, while in REM (rapid eye movement) sleep the body temperature continues to drop, but brain temperature increases (reviewed in Kräuchi and Deboer, 2010). Thus, by shifting the majority of sleep, up to 4 hours, to the daytime period, specifically the hottest part of the day, the Arabian oryx may be taking

advantage of the thermoregulatory physiology of sleep to lessen the impact of the high summer ambient temperatures on its core body temperature.

Temperature as a dominant modifier of activity rhythms in the Arabian oryx during summer – a masking effect or primary zeitgeber?

Ambient light as the primary *zeitgeber* for circadian entrainment of a range of daily rhythms in mammals is undisputed (e.g. Erriksson et al., 1981; Tokura and Aschoff, 1983; Hastings et al., 1998; Pohl, 1998; Mistlberger and Skene, 2004; van Oort et al., 2007; Mircsof and Brown, 2013; El Allali et al., 2013; LeGates et al., 2014). The presence of several secondary *zeitgebers* (such as ambient temperature, patterns of eating and drinking, social influences and physical activity) that allow the entrainment of circadian rhythms in the absence of light is also well accepted (e.g. El Allali et al., 2013; LeGates et al., 2014). Despite the demonstration of the possibility for ambient temperature to act as a primary *zeitgeber* in the absence of light for the entrainment of a range of circadian rhythms in the camel (El Allali et al., 2013; Bouaouda et al., 2014), no specific demonstration of ambient temperature being the primary *zeitgeber* for the entrainment of circadian rhythms in the presence of light has yet been reported. Rather, current thinking indicates that temperature, or other modifiers of circadian rhythms, may act as masking agents, modifying the output pathway downstream from the suprachiasmatic nucleus, rather than affecting the entrainment of suprachiasmatic neurons by light (Hut et al., 2012; Pellman et al., 2015).

The clock that sets the circadian entrainment of daily rhythms in mammals is located within the suprachiasmatic nucleus of the hypothalamus. Generally, within the neurons of this nucleus, oscillatory feedback loops involving the production and repression of several different genes in feedback loops that have a cycle of approximately 24 hours are present. These feedback loops are then, in general, synchronized to 24 hours by the input of naturally occurring ambient light *via* the projections from melanopsin containing retinal ganglion cells. Interestingly, in a short-day rodent model, several daily rhythms, usually entrained by the circadian clock, were shown to become desynchronized, where locomotor activity, the sleep wake cycle, and slow-wave sleep expressed two rhythms (one entrained and one free-running), and core body temperature and REM sleep showed only one free-running rhythm (Cambras et al., 2007). It has since been shown, in a forced desynchrony jet lag model, that REM sleep and core body temperature rhythms are controlled by neuronal oscillators in the dorsomedial suprachiasmatic nucleus (the shell of this nucleus), while other rhythms, such as daily activity patterns, appear to be controlled by the ventrolateral suprachiasmatic nucleus (the core of this nucleus) (Lee et al., 2009).

Our observations in the Arabian oryx indicate that in the summer months there is desynchrony of circadian rhythms compared to the winter months, where the core body temperature rhythm remains stable (putatively controlled by the dorsomedial suprachiasmatic nucleus), but the daily activity pattern changes (putatively controlled by the ventrolateral suprachiasmatic nucleus). How this desynchrony, or the switch in the temporal niche of the daily activity pattern and timing of sleep, in the Arabian oryx is brought about by the neural systems involved in the control of circadian rhythms is

unknown. It is possible that, throughout the year, light is the only *zeitgeber* entraining the neurons of the suprachiasmatic in the Arabian oryx; however, during the summer months, temperature (a non-photic input) may act as a dominant modifier of the circadian rhythms of activity and sleep timing. This non-photic temperature input would act on the downstream circuitry of circadian control, such as the ventral subparaventricular zone of the hypothalamus (Saper et al., 2005b), to mask the entrainment effect of the photic input (Hut et al., 2012). By masking, or modifying, the downstream effect of circadian entrainment by light, ambient temperature may be able to modify the circadian timing of activity and sleep in the Arabian oryx.

A second, albeit highly speculative, possibility is that during the summer months temperature may act as a primary *zeitgeber* for specific circadian rhythms in the Arabian oryx, even in the presence of a normal light-dark cycle. This second possibility is worth exploring as the Arabian oryx is one of the few large mammals exposed to ambient temperatures that are well above the thermoneutral zone for extended periods, a situation not normally encountered by other mammalian species. While the projections from the melanopsin light sensitive retinal ganglion cells are found throughout the entire suprachiasmatic nucleus (e.g. Hatter et al., 2006), the ventrolateral suprachiasmatic neurons contains a significantly higher proportion of warm sensitive neurons than the dorsomedial suprachiasmatic neurons (Derambure and Boulant, 1994). This distinction in the sensitivity to sensory inputs in the different regions of the suprachiasmatic nucleus may be related to the desynchronized rhythms and potential *zeitgebers* observed in the current study of the Arabian oryx. As mentioned above, the core body temperature rhythm of the Arabian oryx does not appear to change seasonally. As this rhythm is

controlled by the dorsomedial portion of the suprachiasmatic nucleus, it is reasonable to postulate that ambient light is the primary *zeitgeber*, entraining this rhythm throughout the year. In contrast, the two modified rhythms observed in the summer period of the Arabian oryx (daily activity pattern and sleep timing), may both be controlled by the ventrolateral portion of the suprachiasmatic nucleus. The ventrolateral portion of the suprachiasmatic nucleus has a higher proportion of warm sensitive neurons than other regions of the suprachiasmatic nucleus, and therefore has the potential for ambient temperature, which affects body temperature, to act as a *zeitgeber* to entrain these altered daily rhythms during the summer months. This possibility is consistent with our findings of the lack of effect of day length to alter the summer daily activity pattern in the South African animal studied where the temperatures were far cooler than the Saudi Arabian summer. Thus, it may be very speculatively proposed that two potential primary *zeitgebers* are entraining different daily rhythms in the Arabian oryx during the summer period in Saudi Arabia, especially given the salience of temperature for survival of the Arabian oryx. Thus, there are two potential explanations for the neural control of the altered and unaltered circadian rhythms observed in the Arabian oryx during the summer months. Further experimentation is required, especially under controlled conditions rather than the natural conditions in which the current study was undertaken, in order to determine which of these two potential explanations account for the currently reported observations made in the natural environment of the Arabian oryx.

Declaration of Interest Statement and Acknowledgments: All authors declare no competing interests. This project was funded by the National Plan for Science,

Technology and Innovation (MAARIFAH), King Abdulaziz City for Science and Technology, Kingdom of Saudi Arabia, Award Number 11-ENV1918-02 (A.N.A., O.B.M., M.F.B., N.C.B. and P.R.M.). The authors are extremely grateful to Prince Bander bin Saud Al-Saud, President of the Saudi Wildlife Authority (SWA) for his unlimited and enthusiastic support to undertake these studies on the Arabian oryx managed by the SWA. We are also grateful for the tremendous help provided by our colleagues at the National Wildlife Research Center in Taif, Saudi Arabia, especially Mr. Ahmed Boug, Dr. Saud Anagariyah, Mr. Ghazi Raziman, Mr. Raed Aljuaid and Mr. Ali Aljuaid. We also extend our sincerest thanks to Mr. Jan Pickard at Ratelfontein, South Africa who allowed us to use one of his Arabian oryx in South Africa.

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Figure Legends:

Figure 1: Graphs illustrating the variance in day length (**A**) and temperature (**B**) during the year-long recording period in the Mahazat as-Sayd, Saudi Arabia, as well as the month in Richmond, South Africa.

(**A**) This graph represents the average percentage of each day for the labelled months occupied by day light (light blue bars) and absence of day light (black bars) across the year for Saudi Arabia, and for the month in Richmond. Note the increase in day length for the months of June-August in Saudi Arabia, which match the day length for the month of February in South Africa. The months labelled with a grey background represent transitional months in the switching of daily activity and sleep timing patterns (see text and Figs. 2 – 6). (**B**) This graph represents the variation in temperatures across the year for Saudi Arabia, and for the month in Richmond. Dark red data represents the highest temperature recorded in that month, pale red the average of the daily highest temperatures recorded in that month, black the average daily temperature of that month, dark blue the average of the daily lowest temperatures recorded in that month and light blue the lowest temperature recorded in that month. Note that the highest temperatures were recorded between June and August. In addition, note that the temperatures recorded in Richmond are similar to the cooler monthly temperatures in Saudi Arabia (November to March).

Figure 2: Graphs illustrating the average count of inactivity for any given 5 min period scored over 28 days in the winter month (upper graph, February, 2014, Saudi Arabia, pooled from the 4 animals recorded) and the summer month (lower graph, July, 2014, Saudi Arabia, pooled from the 5 animals recorded). Note the clearly diurnal pattern of activity (inverse of inactivity) during the winter month, with very little inactivity occurring during the day light period. In contrast, in the summer month we observed a crepuscular activity pattern, with matutinal and vespertine peaks of activity (see also Fig. 5), and a great deal of inactivity during the day light period. The average ambient temperatures, recorded every 10 minutes, across the month of recording are overlain on the inactivity graphs (blue for winter, red for summer). Note the substantially higher average temperatures in the summer months (see also Fig. 1 and Table 1). The grey regions represent the period between sunset and sunrise. Note the longer day length in summer (see also Fig. 1 and Table 1).

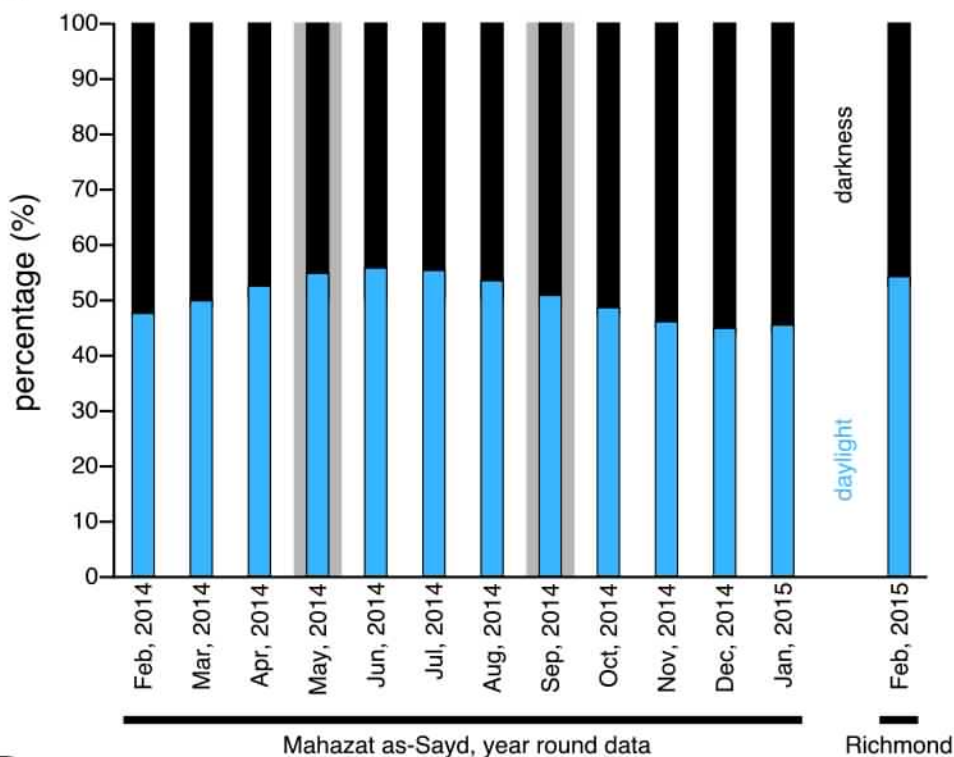
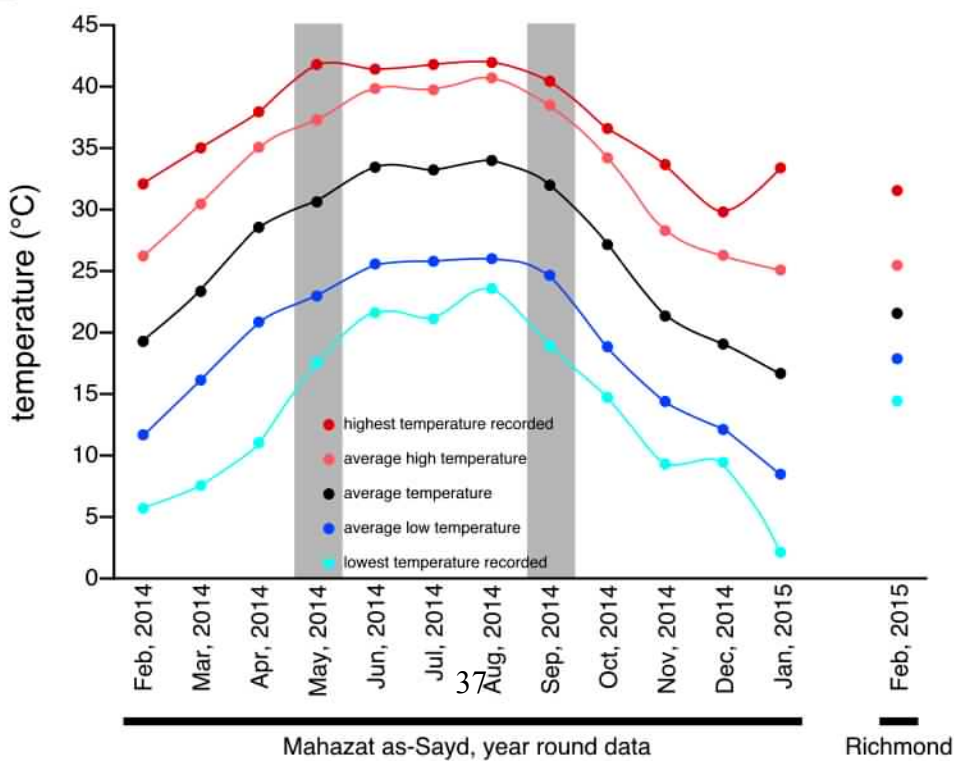
Figure 3: Similar graphs to the ones presented in Fig. 2, however, in this case the average body temperatures, recorded every 10 minutes, across the month of recording are overlain on the inactivity graphs (blue for winter, red for summer). Note that while the daily activity pattern changes from diurnal in winter to crepuscular in summer, and that the average body temperatures are slightly higher in summer than winter, the daily rhythm of core body temperature remains unaltered.

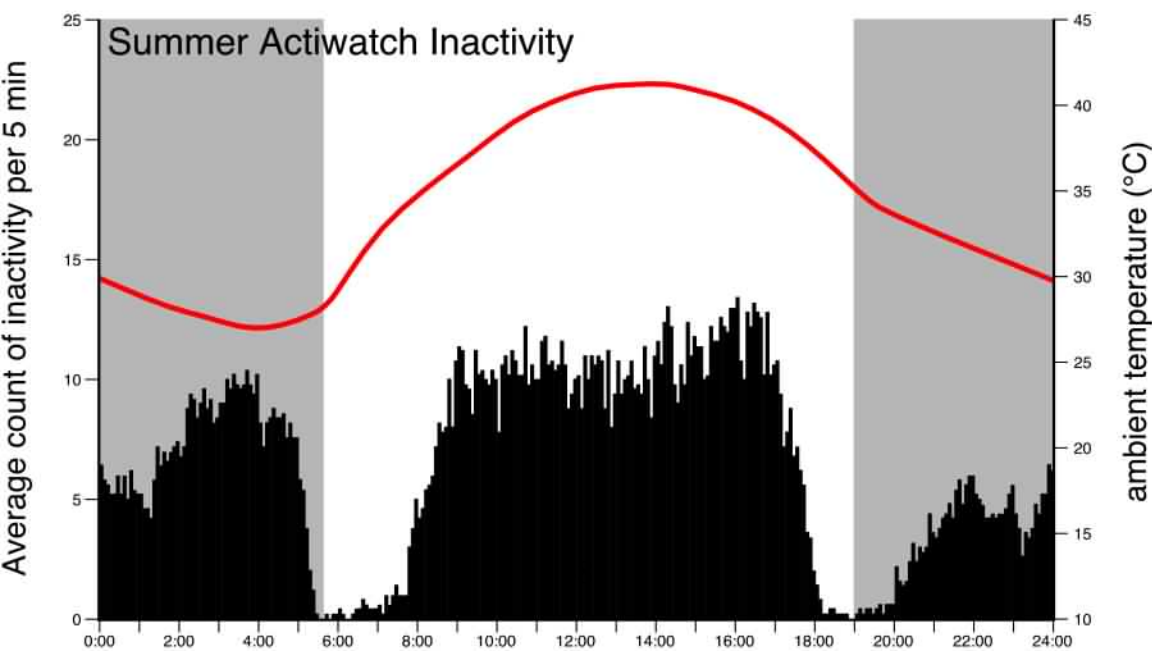
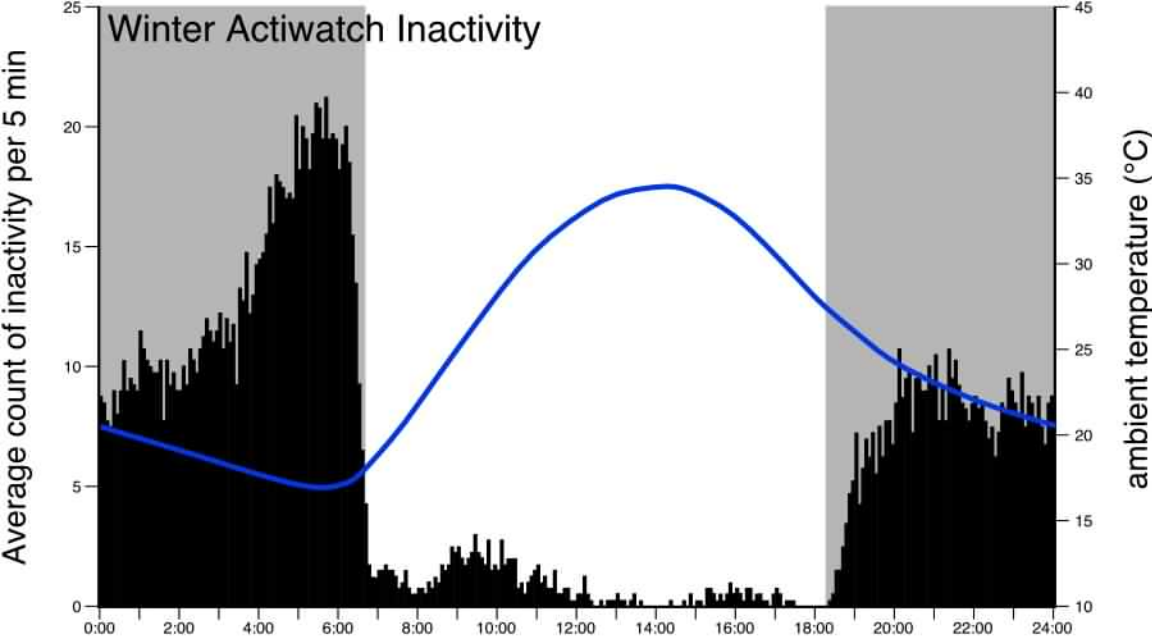
Figure 4: Frequency distribution graphs showing the number (in 30 min intervals) of inactive/sleep bouts of greater than 1 hr duration during the winter and summer recording periods for the Arabian oryx measured with fine-grain actigraphy in Saudi Arabia. Note that during the winter all bouts of inactivity/sleep greater than 1 hr occurred between sunset and sunrise (grey shaded regions) with the vast majority (97 of 111 bouts, 87.4%) occurring in the early morning hours between midnight and dawn. In contrast, in summer, the majority of inactive/sleep bouts (55 of 110 bouts, 50%) occurred during the daylight hours, with 42 of 110 bouts (38.2%) occurring between midnight and dawn. The number of inactive/sleep bouts occurring between sunset and midnight were similar between seasons (winter –14 bouts, 12.6%; summer – 13 bouts, 11.8%). These differences are statistically significant (day summer *vs* day winter, Mann-Whitney, non-parametric test, $P = 9.14 \times 10^{-5}$; night summer *vs* night winter, Mann-Whitney, non-parametric test, $P = 2.15 \times 10^{-5}$).

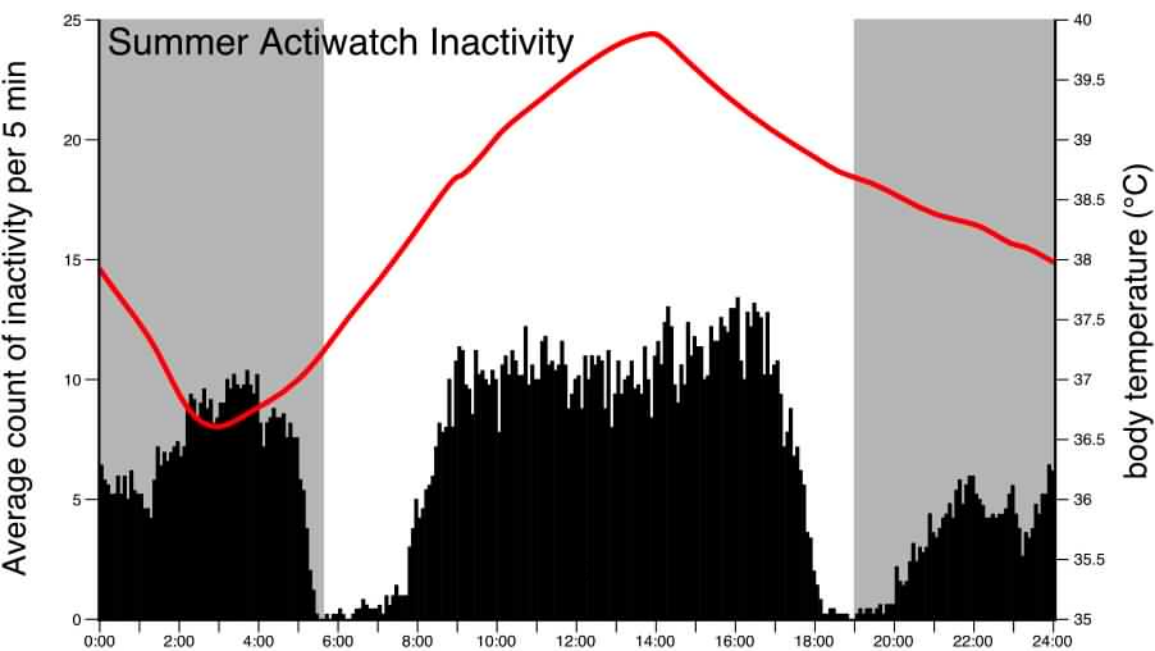
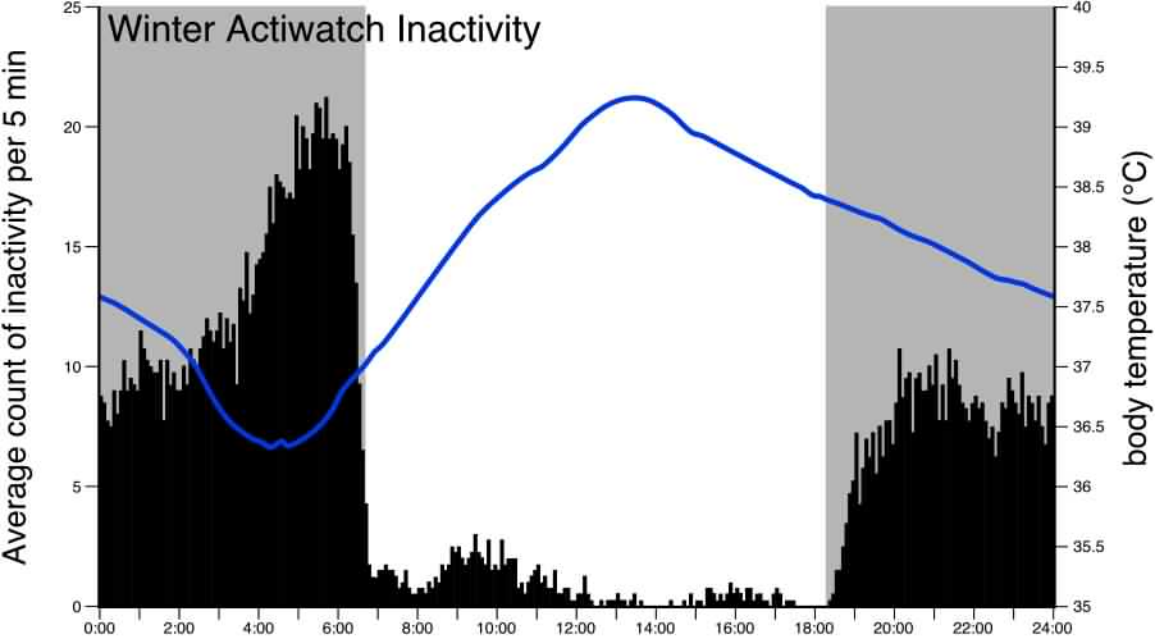
Figure 5: Graphs depicting the pooled and averaged monthly activity levels (every 10 minutes), for an entire year from six Arabian oryx. Note the seasonal variance in the pre-dawn dip in activity, the robustness of the matutinal activity peak, the afternoon dip in activity, the vespertine activity peak and the level of post-sunset to midnight activity (see text for full description of these notable activity features). The variations in these features correlate with the seasonal changes in activity patterns (see Figs. 2, 3), and indicate that May and September are the transitional months where changes between typical winter-type

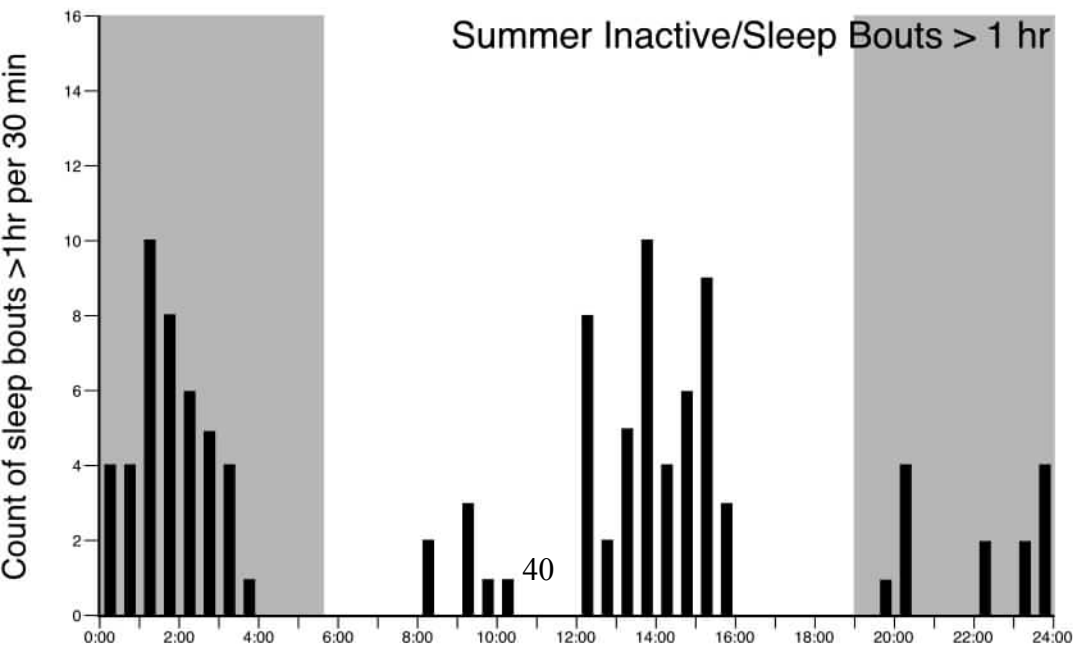
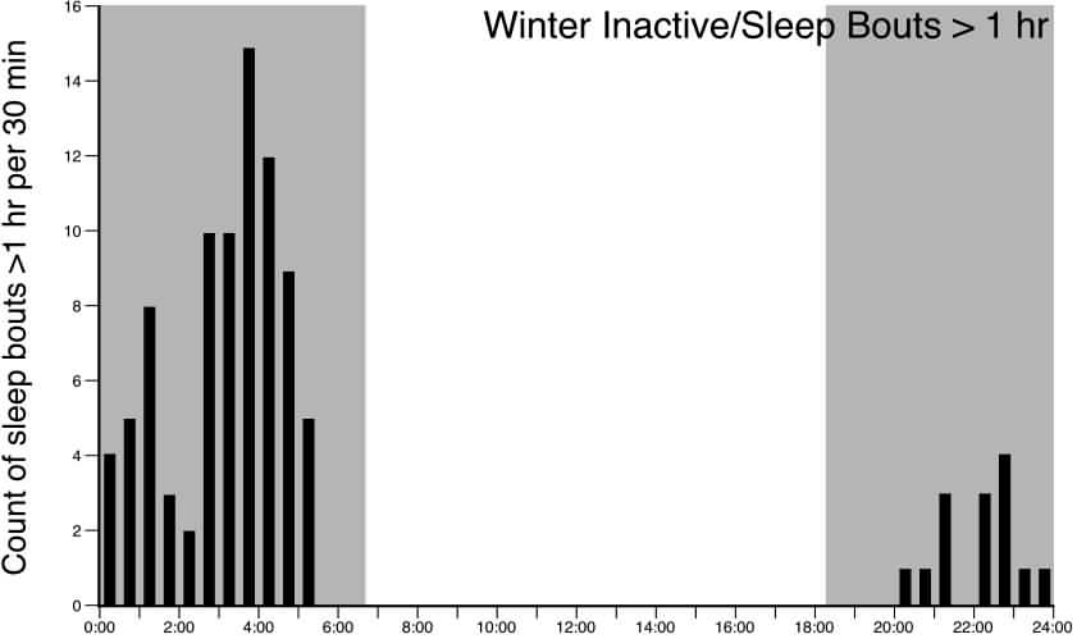
and summer-type daily activity and sleep timing patterns occur. The grey regions represent the period between sunset and sunrise. Note the longer day length in summer (see also Fig. 1 and Table 1).

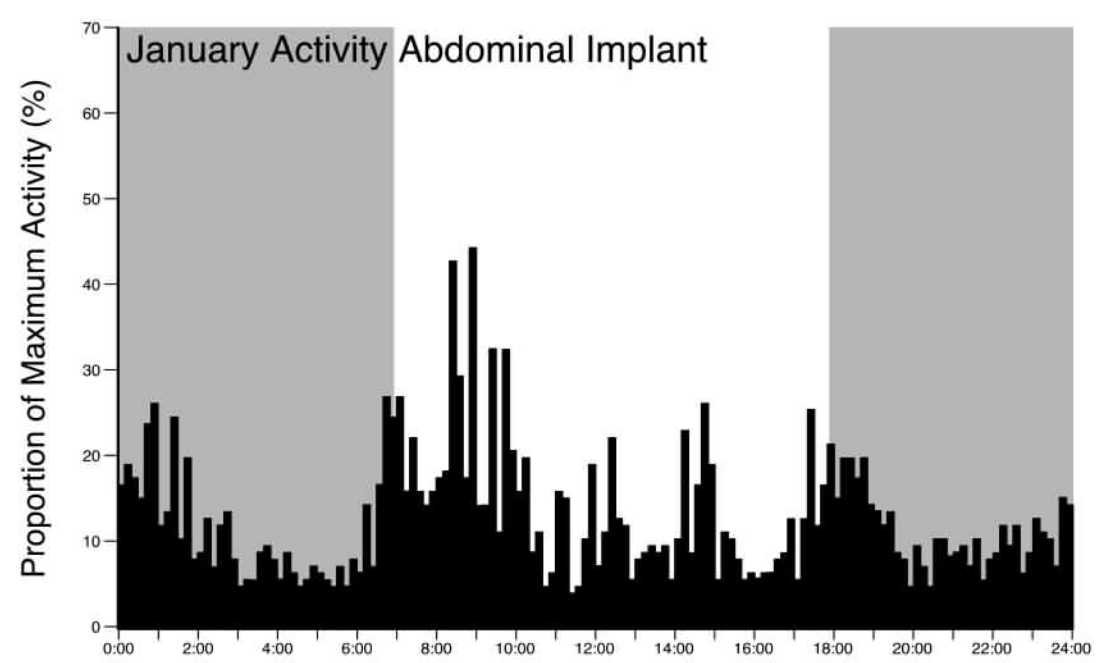
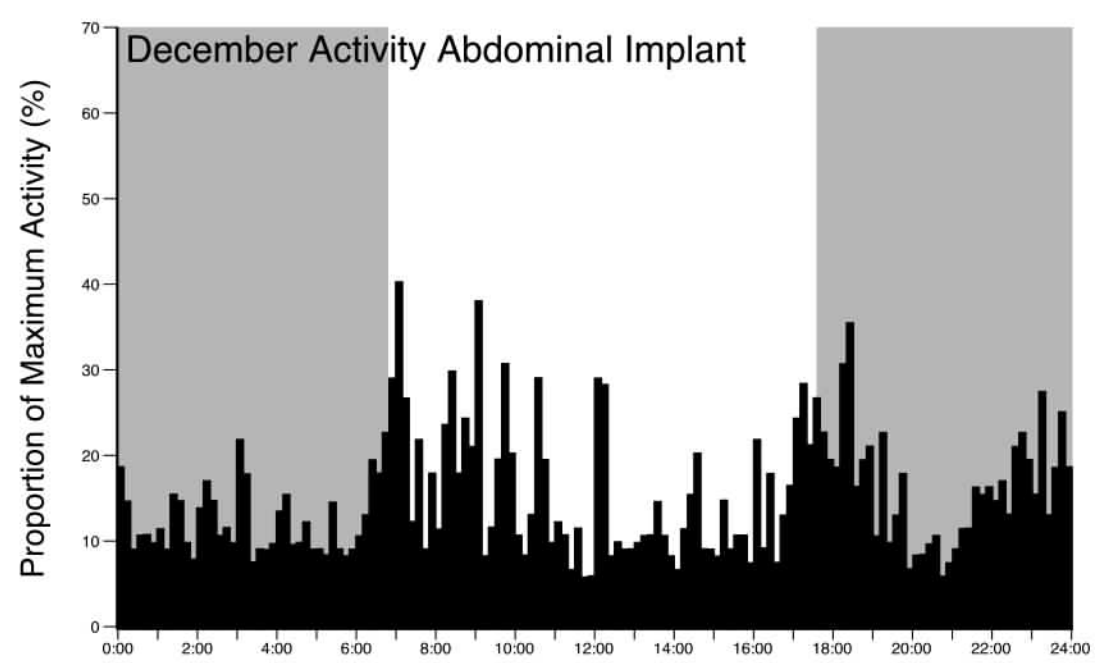
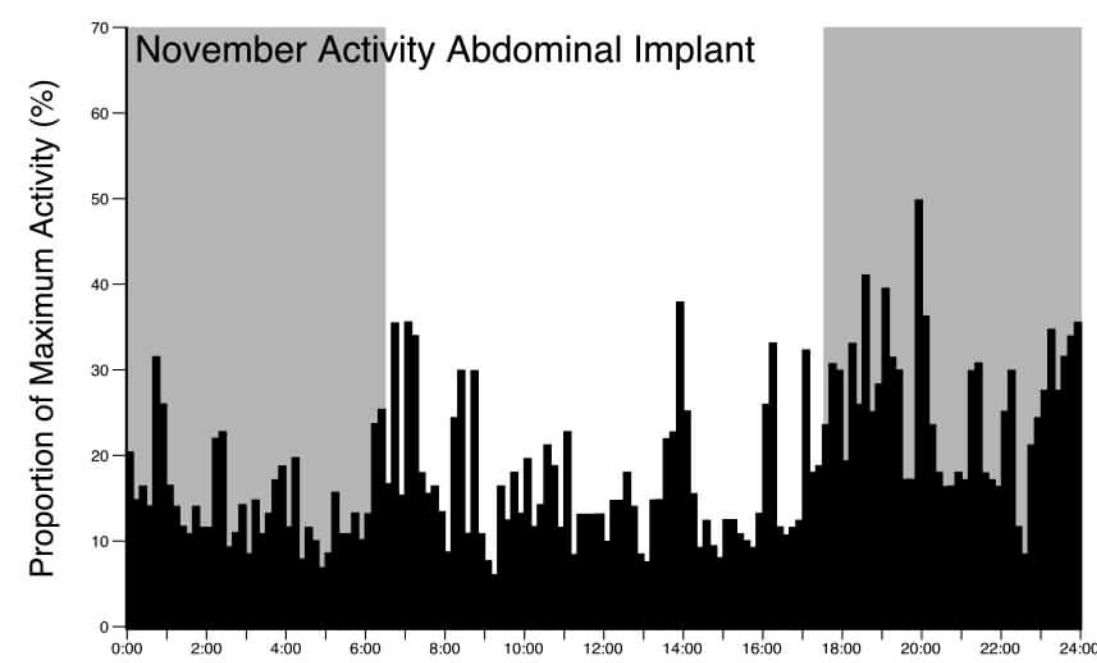
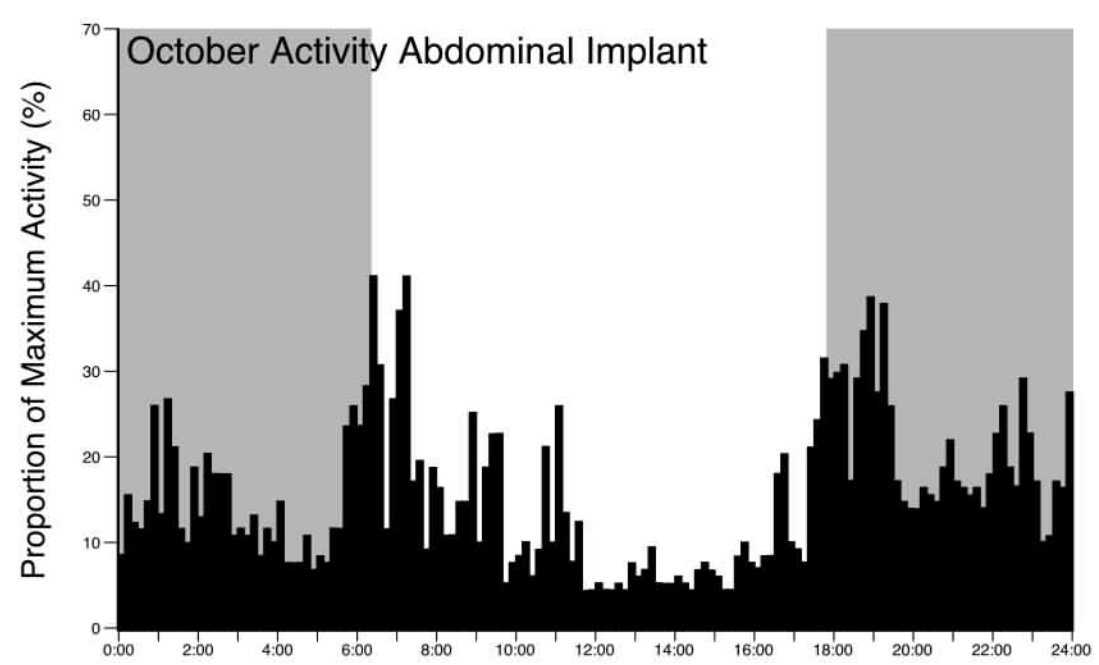
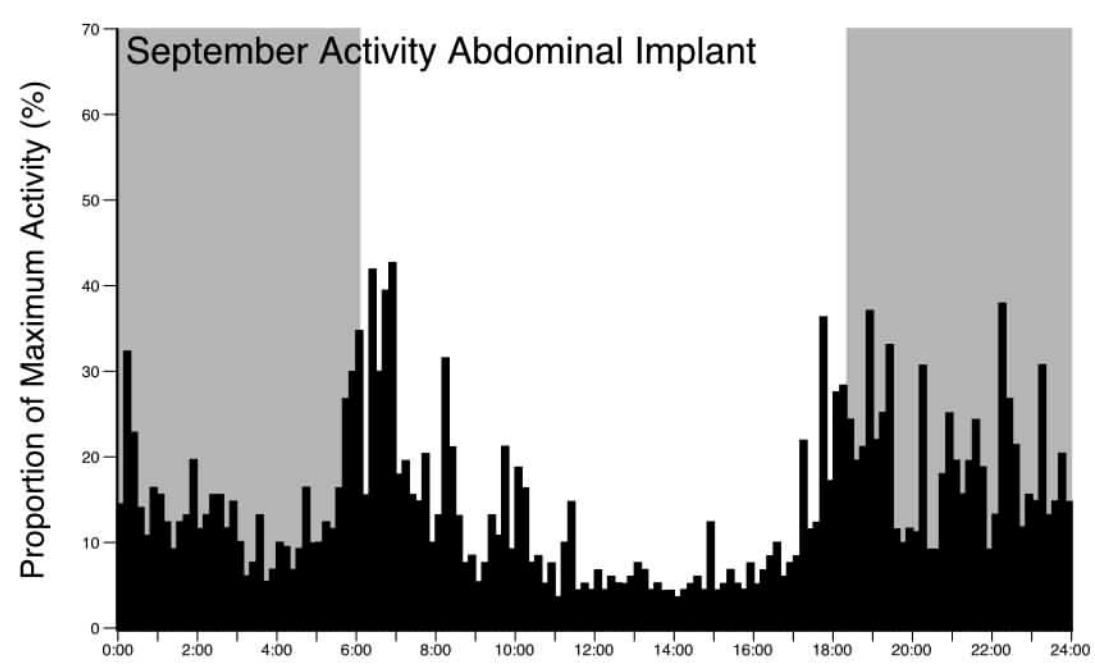
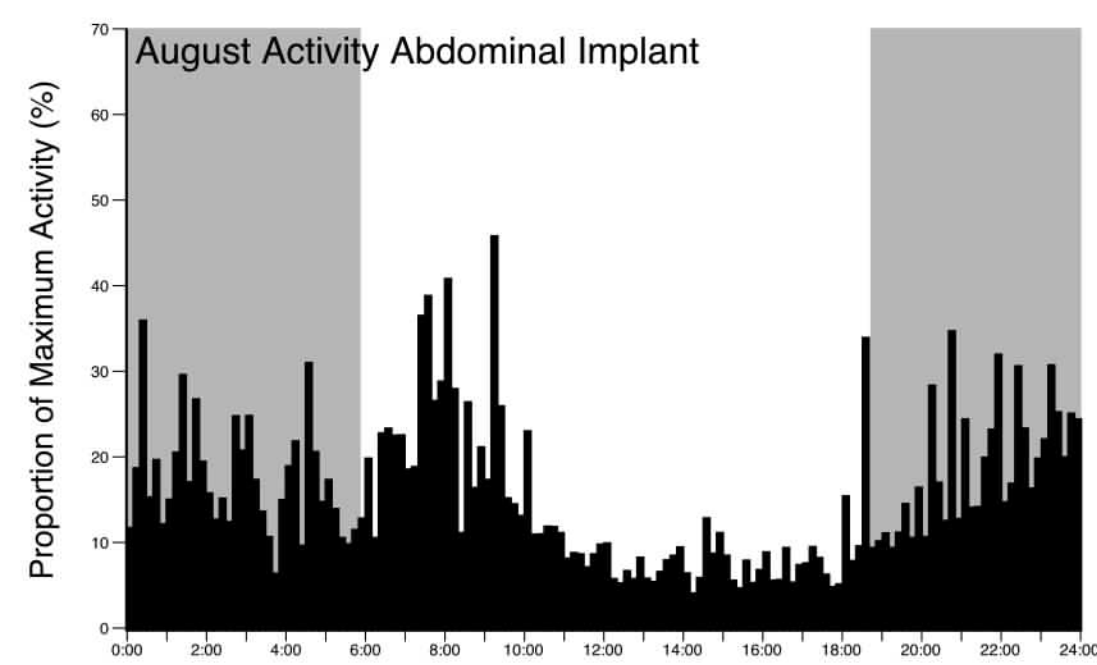
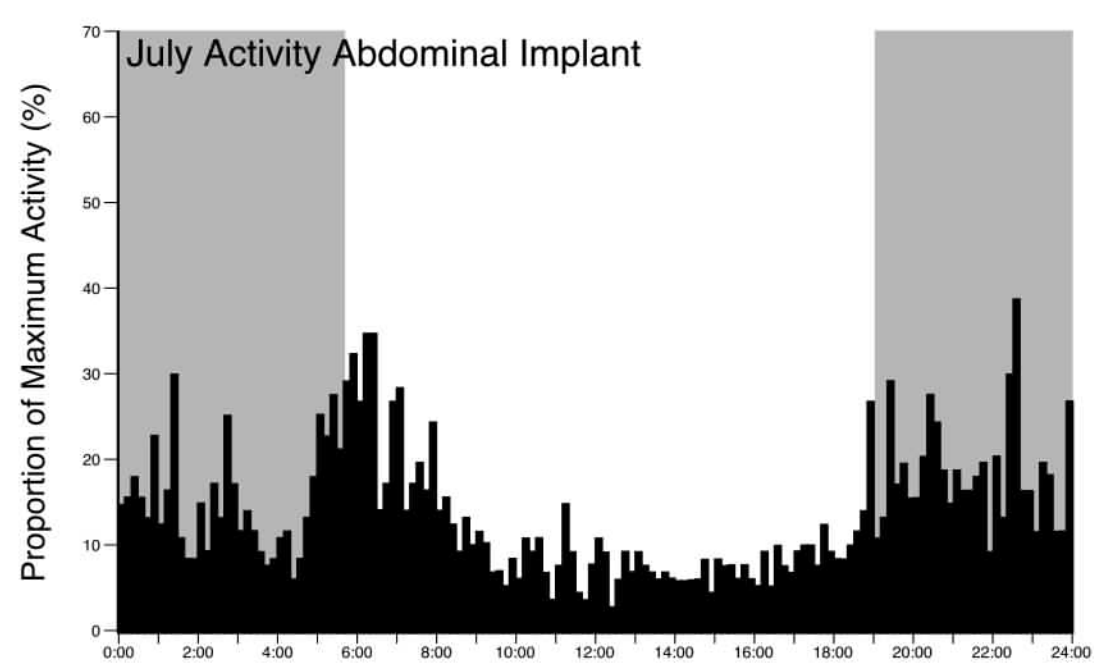
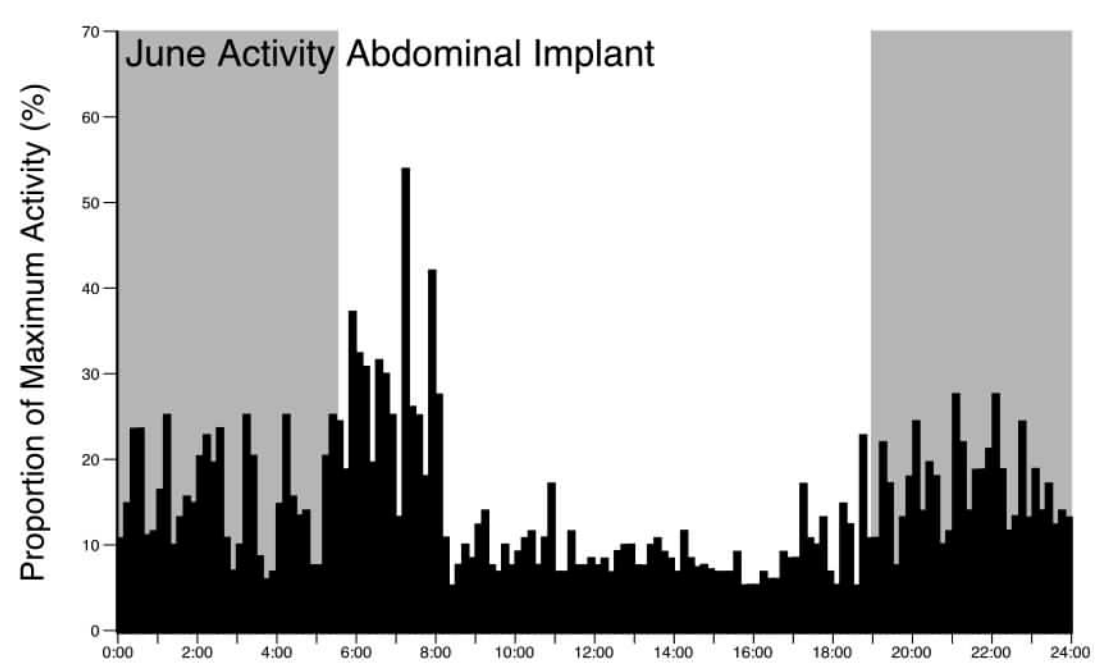
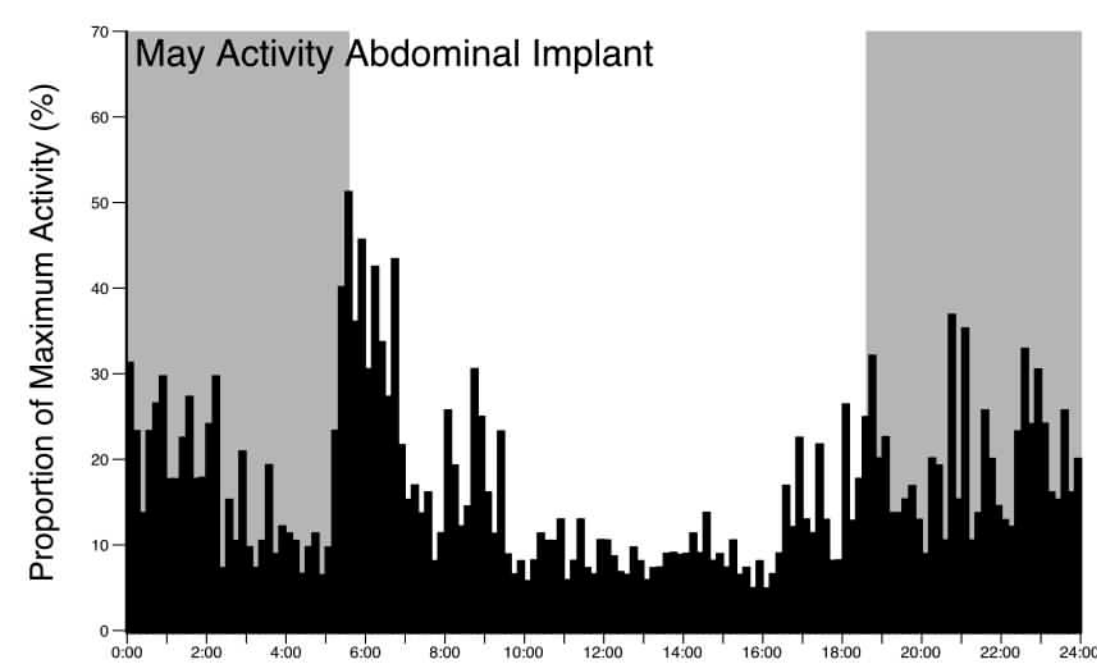
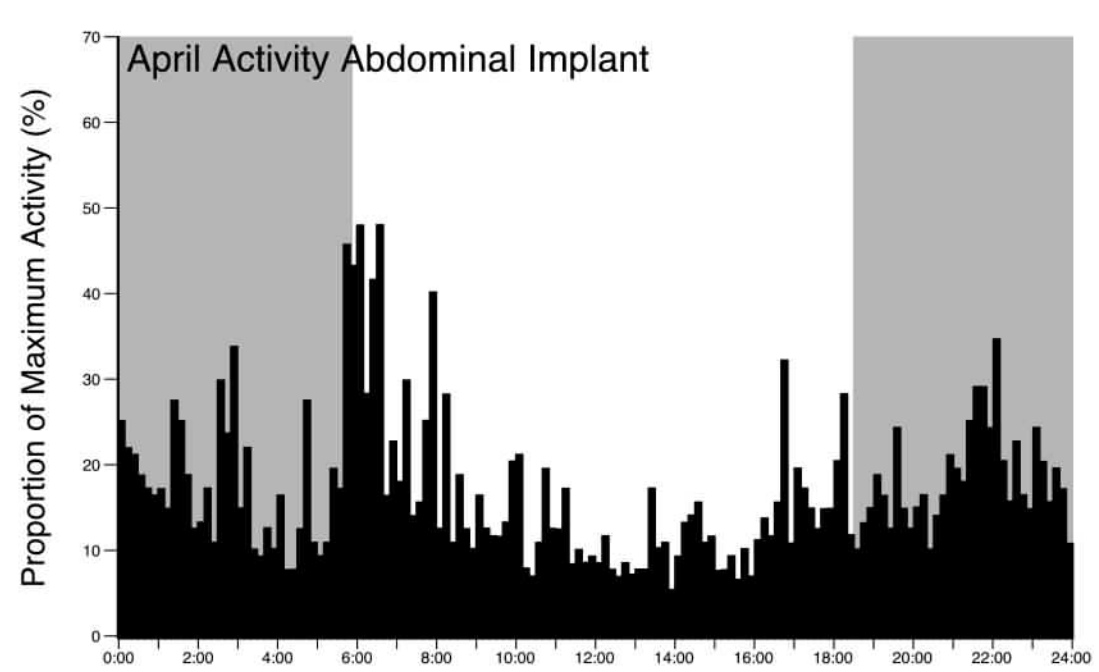
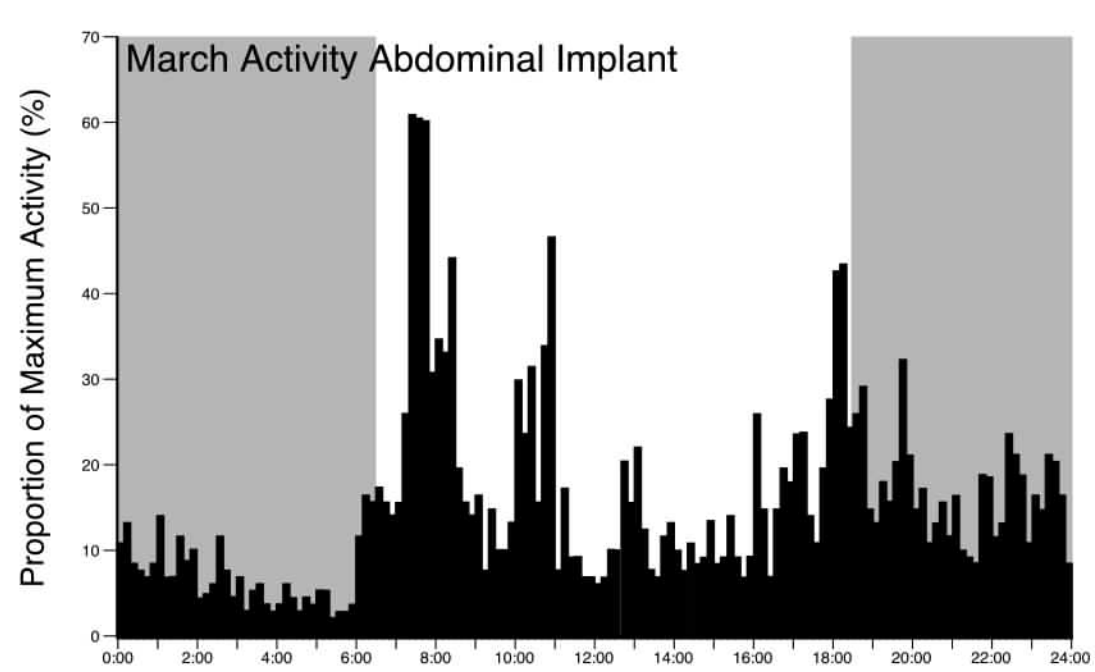
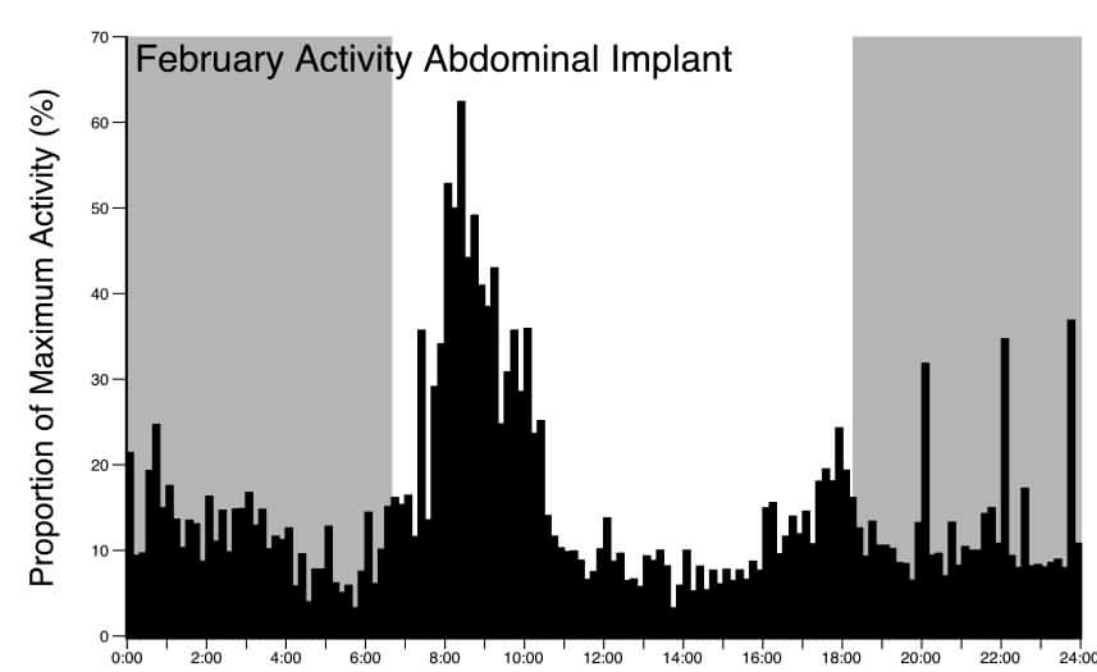
Figure 6: Graph illustrating the average count of inactivity for any given 5 min period scored over 28 days in the South African summer (February, 2015) for a single male Arabian oryx. Note the clearly diurnal pattern of daily activity (inverse of inactivity) during this summer month, with relatively little inactivity occurring during the day light period. This contrasts with the recordings made in the summer month in Saudi Arabia (see Fig. 2) where we observed a crepuscular daily activity pattern and a great deal of inactivity during the day light period. While the day length during this period was similar to the Saudi Arabian summer months, the temperatures were more like the colder months in Saudi Arabia (see also Fig. 1 and Table 1).

A**B**









Summer Actiwatch Inactivity, South Africa

Count of inactivity per 5 min

