

**Photoperiodic effects on the male gonads of the Namibian gerbil,
Gerbilliscus cf. leucogaster from central Namibia**

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Abstract

Photoperiodism has been shown to be an important synchronizer of seasonal reproduction in many rodent species in the wild; it is a reliable cue as in the southern hemisphere it coincides with the onset of rainfall and hence the availability of food resources for maximal reproductive success. The photoperiodic effect on the reproductive status of the Namibian gerbil, *Gerbilliscus cf. leucogaster* from central Namibia was investigated. Twenty adult males were exposed to a long-day length (16L:8D), while further 20 adult males were subjected to a short-day length (8L:16D); all for a period of 3 months. Testicular mass per gram body mass, testicular volume and seminiferous tubule diameters were used to assess the effect of photoperiod on gonadal development. Body mass did not significantly differ between the two photoperiodic regimes. The testicular mass per gram of body mass was significantly heavier for the males maintained on a long photoperiod compared to those on a short photoperiod. Similarly, testicular volume and seminiferous tubule diameter were greater in males maintained on a long-day cycle compared to those on the short-day cycle. These findings suggest that *G. cf. leucogaster* is photoresponsive to day length changes. Photoperiodic changes in the semi-arid habitats can be used to herald the onset of reproduction as it often acts in concert with other proximate cues in desert rodents, but is a constant environmental cue that does not change from year to year, unlike rainfall patterns.

Keywords: Photoperiod; Namibian gerbil; *Gerbilliscus cf. leucogaster*, spermatogenesis; testicular development

Introduction

Photoperiod, rainfall and the sudden flush of green vegetation are important factors for the onset of reproduction in rodents inhabiting arid and semi-arid habitats. These cues do not act separately, but in concert with one another for the onset and maintenance of reproduction and maximizing reproductive success (Ben-Zaken et al. 2013). Photoperiod plays a very important role as a proximate factor, triggering reproductive events in terrestrial organisms and in particular those occurring at higher latitudes (Nelson et al. 1992; Rani and Kumar 2014). Rainfall, although sporadic in the semi-arid region of central Namibia, occurs during the spring and summer months when day length is longer. *Gerbilliscus cf. leucogaster*, is studied for the first time to determine the effects that photoperiod has on the reproductive system in relation to the other well-documented species in the region.

Changes in day length are perceived by the brain through changes in the melatonin signal that is secreted by the pineal gland (Prendergast et al. 2009; Rani and Kumar 2014). The effect of photoperiod in adults is manifested by the recrudescence and subsequent maintenance of reproductive function on long photoperiods and regression of reproductive structures and cessation of reproduction on short photoperiods in long-day animals and the opposite effects in short-day organisms (Yu et al. 1993). The pineal gland and its hormone melatonin have been implicated in the photoperiodic regulation of reproduction in all mammals studied to date (Yu et al. 1993; Rani and Kumar 2014).

Mammals that are responsive to long-day length cease to breed as the day length becomes shorter towards the end of summer and with the onset of autumn (Muteka et al. 2006; Prendergast et al. 2001). Effects of photoperiod on reproduction have been

investigated in several mammalian species such as the golden spiny mouse, *Acomys russatus* (Ben-Zaken et al. 2013), the California vole, *Microtus californicus* (Nelson et al. 1983), the vole, *Microtus agrestis* (Spears and Clarke 1986), black rat, *Rattus norvegicus* (Heideman and Sylvester 1997; Heidemann et al. 1998), the California vole, the four-striped field mouse, *Rhabdomys pumilio* (Jackson and Bernard 1999), and the white-footed mouse, *Peromyscus leucopus* (Young et al. 2000). Increasing day length coincides with the beginning of spring and reproductive recrudescence in seasonally breeding animals begins during this period to maximize the survival of the offspring (Jameson 1988; Flowerdew 1987). Spring and autumn are crucial to the survival of many animals in the natural environment as these periods are characterized by the onset of food resources that result from rainfall (Flowerdew 1987; Meheretu et al. 2015). Thus, these periods maximize survival and growth rates of juveniles with the available nutritional supplies (Meheretu et al. 2015; Mutze 2007; Nilsson 2001; Tinney et al. 2001), and favourable ambient temperatures (Benson and Morris 1971; Bronson and Prayor 1983; Bronson 1989), conditions which are associated with spring and summer months of the year.

In natural ecosystems, populations of wild rodents and some domesticated animals rely on photoperiod as a major proximate cue to control reproduction (Muteka et al. 2006; Bronson 2009). However, some studies have shown that photoresponsive animals do not rely on photoperiod alone, but also on other changes in environmental conditions (Nelson et al. 1983; Sicard et al. 1993; Prendergast et al. 2001; Anand et al. 2002; Mutze 2007; Meheretu et al. 2015; Fabio-Braga and Klein 2018). These may include ultimate factors such as food availability and social factors (Anand et al. 2002; Meheretu et al. 2015). Some rodents such as the California vole, may opportunistically stimulate

gonadal development or prevent gonadal regression on short days when food is adequate (Nelson et al. 1983; Hamid et al. 2012), in which case, the nutritional effects override the photoperiodic impacts on reproduction. Other environmental variables such as low humidity have also been found to stimulate gonadal development in Kusu rats, *Arvicanthis niloticus*, but have minimal or no effects in other rodent species (Nelson et al. 1983; Sicard et al. 1993). Thus, photoperiod is a major controlling factor in many terrestrial mammals, but may act in conjunction with other environmental factors (Muteka et al. 2006).

Some rodent species such as the pouched mouse, *Saccostomus campestris*, mainly breed during summer when reproduction is controlled by photoperiod (Bernard and Hall 1995). Reproduction during winter, however, appears to be inhibited by reduced food availability (Tinney et al. 2001). Similar observations have also been reported in the four-striped mouse, where the inhibition of reproduction during winter is prevented by factors other than photoperiod (Jackson and Bernard 1999). These studies confirm that although photoperiod is a major cue controlling reproduction, additional factors may be simultaneously influential, together with day length (Bronson 1989, 2009). Given that essential changes in environmental conditions vary widely, it is however, likely that their predictability may be less reliable than photoperiod (Muteka et al. 2006).

In arid and semi-arid regions, there is an urgent need to investigate the reproductive aspects of small mammal species, since the parameters controlling reproductive onset are not well-documented as is the case in temperate regions (Bronson 2009). Consequently, this study is the first aimed to determine the effect of photoperiod on male gonads of the Namibian gerbil, *G. cf. leucogaster*, from the Otjozondjupa and

Khomas regions of central Namibia, under controlled laboratory conditions. This study has been undertaken to discern if photoperiod plays a role in the onset of reproductive activation in the male of this gerbil. Morphological and histological methods were used to investigate the reproductive changes in males *G. cf. leucogaster*. We, therefore, hypothesised that the Namibian gerbil, *G. cf. leucogaster* is a seasonal breeder that is responsive to day length; or that the reproduction in the Namibian gerbils, is not confined to a specific season and is non responsive to changes in day length.

Material and Methods

Study areas

A total of 40 adult male specimens of *G.cf. leucogaster* were sampled from Otjinakwi Farm, in the Otjozondjupa region ($-20^{\circ} 45' 3.81''$ $+17^{\circ} 1' 21.80''$) at 1440.24 m above sea level (a.s.l.) and Neudam Farm, in the Khomas Region ($-22^{\circ} 30' 16''$ $+17^{\circ} 22' 9''$) at 1656 m a.s.l. Both regions are situated in the central part of Namibia and are characterized by a semi-arid climate with an average annual temperature of 19.47° C (Goddard Institute, 1957–1987; Namibia Meteorological Service, 2014).

The winter months (June-August), usually experience little or no rain and minimum temperatures range between -5° C and 18° C. Nights are usually cool and very cold before dawn (Goddard Institute, 1957-1987). Days are usually warm to hot, ranging from a maximum of 20° C in July to 31° C in January with mean annual rainfall around 360 mm (Goddard Institute, 1957-1987). The natural vegetation of the area is scrub and

steppe (savannah woodland) (Goddard Institute, 1957-1987; Namibia Meteorological Service, 2014).

Trapping and handling of animals

A mixture of peanut butter, syrup, oat meal, and fish oil was used as bait. The gerbils were trapped using Sherman live traps that were set prior to twilight and subsequently re-checked at dawn. Live animals were sampled and processed as approved by the Animal Ethics (Ethics clearance number EC066-17) Committee of the University of Pretoria, Pretoria, South Africa, with the necessary field collection permit issued by the Ministry of Environment and Tourism, Windhoek, Namibia. The animals were trapped during mid-January; and were subjected to photoperiodic treatment within 24 hours after field capture to avoid acclimatization. The animals were kept in polyurethane cages with wood shavings provided as bedding. Forty male *G. cf. leucogaster* were selected based on body mass (grams) to discern the adults from the juveniles; of which 20 were subjected to a long-day (16L: 8D), and twenty to a short-day (8L: 16D) lighting scheme, all for a period of three months. Mice pellets and water were provided *ad libitum* for the duration of the photoperiodic treatments.

The animals were exposed to the two lighting regimes, under constant environment conditions in a laboratory. The long-day length (LD) was set at 16 hours light and 8 hours darkness (16L: 8D), with lights on at 06h00 and off at 22h00. Short-day length (SD) was set at 8 hours of light and 16 hours darkness (8L: 16D), with lights on at 07h00 and off at 15h00. The photoperiodic lengths were selected to simulate the day lengths experienced during natural summer and winter periods in the Khomas and the Otjozondjupa regions of central Namibia. Temperature was controlled at $26 \pm 3^\circ \text{C}$ ($n =$

980) with relative humidity levels being maintained at $42 \pm 5\%$. The light intensities in the rooms were 552 ± 37 lumen/m² (Canon light meter).

Processing of samples

After 90 days, the animals were sacrificed using an overdose of halothane and body mass was obtained using a Mettler digital balance (Ohaus Corp. Pine Brook, N.Y. U.S.A.). The testes were removed and weighed, the length and width of each testes was measured using a pair of digital callipers (Sylvac Opto RS 232, Ultra Praezision Messzeuge GmbH, Germany). Testicular volume was calculated using the formula for the volume of an ellipsoid described by Woodall and Skinner (1989) as follows:

$$V = 4/3 \pi ab^2$$

Where: $a = 1/2$ maximum length and, $b = 1/2$ maximum breadth.

Gonads were then placed into Bouin's fluid for a minimum of 24 hours before being rinsed and stored in 70% alcohol. Voucher specimens were deposited in the Natural History Museum, Windhoek, Namibia.

Mitochondrial typing

DNA was extracted from a subset of tissue samples using the Roche High Pure Template Preparation kit (Roche). The cytochrome *b* gene region was amplified and sequenced as described by Bastos et al., (2011). The resulting sequences (deposited under accession numbers KT029850-KT029851) were used in nucleotide Blast searches against the Genbank database (www.ncbi.nlm.nih.gov/blast) to identify

reference sequences with the highest levels of sequence identity to the gerbils sampled from central Namibia.

Histology

All the testes were sectioned, mounted, and stained following the guidelines of Ross, Romrell and Kaye (1995) and Leeson, Leeson and Paparo (1985). Seminiferous tubule diameters were determined by selecting 100 testicular sections with circular tubules and photographs taken at 40x magnification using a DMX 1200 Nikon digital camera and Image Tools software version 3.0 (Melville, New York, USA). Following Ross et al. (1995), all testicular sections were also examined for signs of spermatogenesis, spermatozoa and stages of testicular development.

Data Analysis

Analysis of variance (ANOVA; McCullagh and Nelda, 1989) was used to test for differences in body mass between males on long and short photoperiods as well as to test for differences in seminiferous tubule diameter, testicular mass and testicular volume. The data were analysed using algorithms in Microsoft Excel version 14 (McCullagh and Nelda, 1989) and Statistical Application Software (SAS) version 9.1 (McDonald, 2014a, 2014b).

Results

Molecular Typing

Nucleotide searches against the Genbank database, using full length cytochrome *b* gene sequences generated for specimens from both sampling sites in central Namibia confirmed that the two closest matches in this public database (AM40989-90) corresponded to *Gerbilliscus leucogaster* sampled from South Africa (92.6% sequence identity). In light of relatively high level of sequence divergence (7.4%), *G. cf. leucogaster* is used to denote the species evaluated in this study.

Body mass

There was no statistically significant difference between the initial body mass of the *G. cf. leucogaster* males maintained on a long day length (ANOVA: body mass (LD) = 70.0 ± 2.1 g; $n = 20$) and the initial body mass of the animals on a short day length (ANOVA: body mass (SD) = 69.1 ± 2.6 g; $n = 20$) (*t*-test: $n = 20$; $P = 0.43$) at the start of the experiment (Fig. 1a).

At the end of the experiment, the animals that were subjected to the long photoperiod (LD: 72.9 ± 1.01 g; $F_{(2,20)} = 0.42$; $n = 20$; $P = 0.02$) significantly gained 2.9g of body mass, whereas those maintained on a short-day length (SD: 65.2 ± 1.5 g; $F_{(2,20)} = 1.16$; $n = 20$; $P = 0.001$) showed a significant drop of 3.9g in body mass (SD: (Fig. 1a).

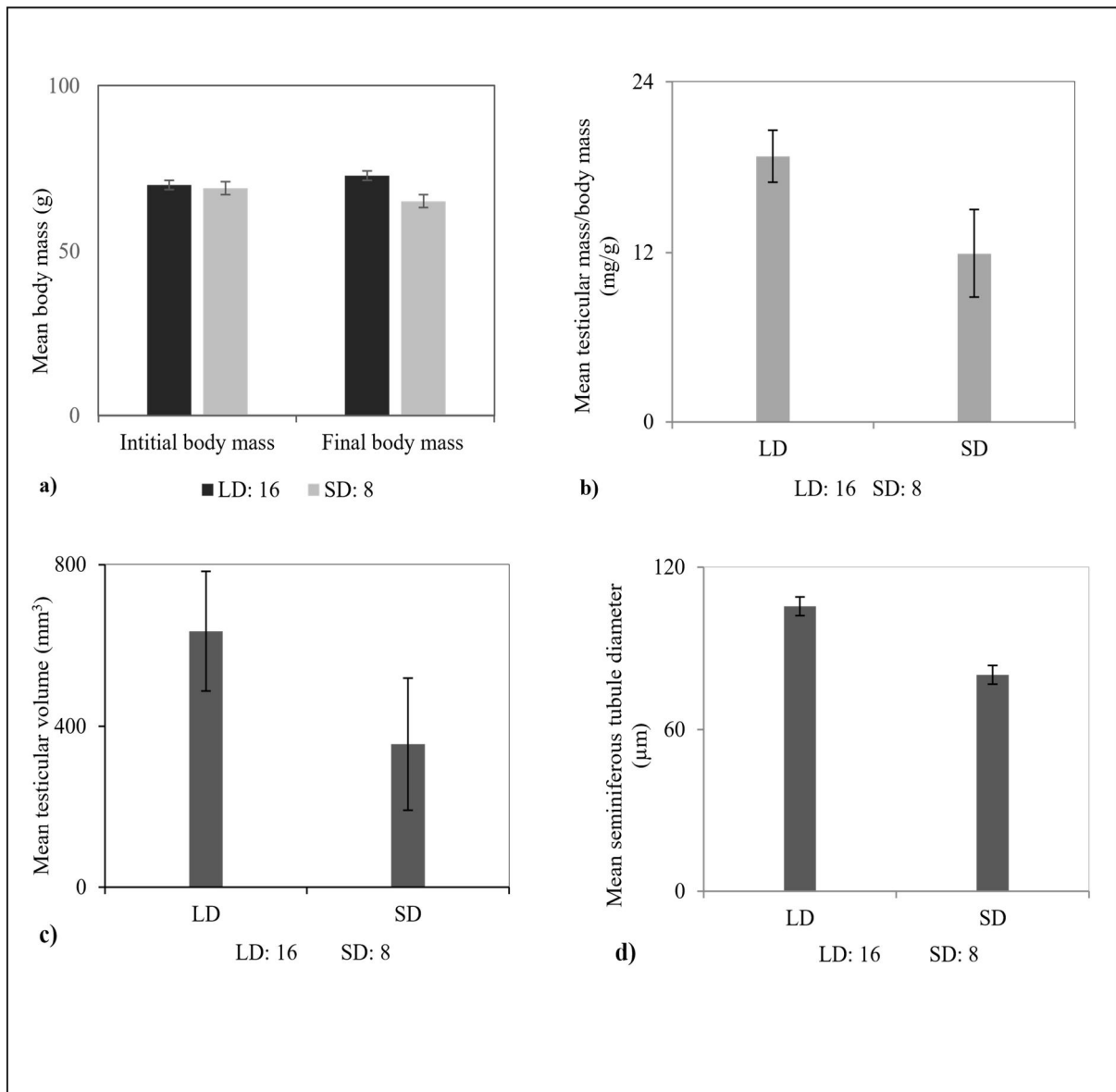


Fig. 1. The mean \pm 1 standard error (SE) of male Namibia gerbils, *Gerbilliscus cf. leucogaster* from the Otjozondjupa and Khomas Regions of central Namibia showing: a) initial and final body mass; b) specific testicular mass (mg/g); c) testicular volume (mm³); and d) seminiferous tubule diameter (μ m) after being subjected to long (LD) and short (SD) photoperiodic conditions.

Testicular mass and volume, and seminiferous tubule diameter

Testicular mass expressed as a function of a gram of body mass in *G. cf. leucogaster* was significantly higher for males maintained on LD (19.0 ± 2.0 mg/g; $n = 20$) than those males on SD (SD: 12.0 ± 3.00 mg/g; $n = 20$) (two-way ANOVA: $F_{(2, 20)} = 6.98$;

$n = 20$; $P = 0.01$) (Fig. 1b). Similarly, testicular volume was significantly greater for animals on LD ($LD = 635 \pm 83.36 \text{ mm}^3$; $n = 20$) than those on SD ($356.0 \pm 51.12 \text{ mm}^3$; $n = 20$) (two-way ANOVA: $F_{(2,20)} = 2.68$; $n = 20$; $P = 0.01$) (Fig. 1c). Mean seminiferous tubule diameter was significantly larger in males on LD ($105.6 \pm 4.6 \mu\text{m}$; $n = 20$) than on SD ($81.24 \pm 3.4 \mu\text{m}$; $n = 20$) (two-way ANOVA: $F_{(2,20)} = 5.93$; $n = 20$; $P < 0.05$) (Fig. 1d).

Discussion

A number of studies have shown that photoperiod is a major environmental cue controlling reproduction in the wild (Ims, 1990; Sangeeta and Vinod, 2014). The findings in the present study demonstrate that individual male *G. cf. leucogaster* respond to a long-day photoperiod with variable reproductive responses. From a physiological perspective, the obtained data indicate that long days serve as an environmental signal stimulating reproduction in the *G. cf. leucogaster* from central Namibia. Research has shown that as a result of unpredictable climatic conditions in deserts, reproduction in desert species may not be successful in any given year, such that desert rodents breed opportunistically depending on relatively short-term climatic and nutritional conditions relying more on nutritional resource base other than photoperiod (Kenagy and Bartholomew, 1985, Rani and Kumar 2014).

Seasonal changes in the environment can affect a wide range of physiological and behavioural processes in most tropical and sub-tropical mammalian species. Food availability, rainfall, temperature, and day length (i.e., photoperiod) are among the environmental factors that affect the time of onset and the duration of the reproductive

period in mammals (Nilsson, 2001). . A variety of other seasonal physiological responses often accompany the photoperiodically-induced alterations in reproductive status and may include changes in pelage colouration or thickness (Duncan and Goldman, 1984, Tavoraro et al. 2015), body fat deposition or utilization (Bartness and Wade, 1985), and thermogenesis (Heldmaier et al., 1981). These seasonal responses also are typically regulated by changes in photoperiod (Bartness and Goldman, 1989)). The cessation of all breeding activity for several months during autumn and winter therefore, represents a significant period in the life of an individual small mammal during which all opportunity for reproduction ceases (Muteka et al., 2006; Sangeeta and Vinod, 2014) .

Bronson, (1985) and Forger; Zucker, (1985) and Tavoraro et al. (2015) suggested that long photoperiod may promote somatic growth. . The present study found animals on long day length to have gained body mass whereas those on short day-length displayed a significant dropped in body mass. This observation confirms that photoperiod does have effects on somatic growth as has been reported in *Rattus norvegicus* retrospectively by (Bronson, 1985; Forger and Zucker, 1985; Heideman et al., 1998). On the other hand, somatic effects may be more apparent in growing juveniles than that in adults, as all the animals used in the present study were adults.

Male *G. cf. leucogaster* showed a significant gonadal and somatic regression when subjected to short photoperiods in this study. Similar findings were obtained in adult Siberian hamsters, which exhibited gonadal regression on short day lengths in both sexes (Schlatt et. al., 1993, Wade and Bartness, 1984; Alibhai 1986). The increase in the values of testicular mass, volume and seminiferous tubule diameters between males

maintained under long photoperiod and those on short photoperiods suggests a positive response to the effects of long photoperiod and gonadal regression on short photoperiods. Comparing males of similar age and gonadal sizes in the natural population, males did not exhibit gonadal regression when exposed to a short photoperiod as observed in some mammals such as Syrian hamsters in which individuals subjected to short photoperiod had regressed testes relative to animals exposed to long photoperiods (Bartness and Wade, 1985, Tavolaro et al.2015).

A number of rodents have been reported to exhibit testicular regression during a short photoperiod to avoid adverse environmental effects on their offspring (Schlatt et. al., 1993, Tavolaro et al. 2015). In the grasshopper mouse, *Onychomys leucogaster*, gonadal recrudescence is inhibited on a photoperiod of 10L: 14D, but stimulated on a photoperiod of 14L: 10D (Frost and Zucker, 1983). A similar study on the desert pocket mouse, *Perognathus formosus*, showed that testicular development and recrudescence is stimulated when subjected to a 16L: 8D photoperiod, but inhibited when subjected to 8L: 16D day-length (Kenagy and Bartholomew, 1981). Muteka et al., (2006) showed that the Tete veld rat, *Aethomys ineptus* and the Namaqua rock mouse, *Micaelamys namaquensis* both utilize photoperiod to time the appropriate reproductive period in the wild.

In the Tete veld rat, the failure of male animals to undergo gonadal regression on a short day length has been proposed to be adaptive as this enables reproductively mature males the potential to continue their reproductive efforts during winter (Kriegsfeld et al., 1999). This observation is further supported by the presence of spermatozoa in the epididymis and minimal spermatogenic activity in the seminiferous tubules of the males

of *G. cf. leucogaster* during winter months. Although the offspring live under harsh conditions during winter, the possibility exists that a litter may be successfully reared if conditions for survival, such as adequate food resources or favourable microclimatic conditions such as temperature are suitable. The possibility of winter breeding in the Namibian gerbil could be due to one of several reasons: Firstly, gerbils may have a photoperiodic system much like that of typical LD-breeding rodents, but the potential inhibitory effect of SDs is overridden by other environmental factors such as ambient temperature, rainfall, humidity, or food availability; Secondly, the gerbils may be reproductively nonresponsive to the photoperiod and instead use other predictors to time their reproduction; Thirdly, seasonality in these animals may be a consequence of an opportunistic strategy in which reproduction is restricted to the times when climatic and nutritional conditions are optimal without the use of any environmental predictors.

The significantly higher testicular mass, volume, and seminiferous tubule diameter in animals maintained on a long day compared to those on a short day, implies an important role for photoperiodism in the recrudescence and regression of reproductive activity in *G. cf. leucogaster* in central Namibia. It's reported that animals exposed to a short day length, their gonads do not completely regress. Rather there is a cessation of spermatogenic activity on a short day length, as has been reported in the Egyptian spiny mouse, *Acomys cahirinus*, Anderson's gerbil, *Gerbillus andersoni* (El-Bakry et al., 1983; Kerbeshian et al., 1994; Kerbeshian and Bronson, 1996), the Deer mouse, *Peromyscus maniculatus* (Scheffer, 1924; Whitesett and Miller, 1982); the pouched mouse, *Saccostomus campestris* (Bernard and Hall, 1995), and the Siberian hamster, *Phodopus sungorus* (Hoffmann 1973). In young male F344 rats, Tavolaro Heidman et

al., (1998) reported a inhibition of testicular regression on day length of 16L: 8D, partial regression on 14L: 10D, and a total regression on a photoperiod of less or equal to 10 hours light/day In *G. cf. leucogaster*, it is possible that there is critical photoperiodic threshold required for complete testicular regression other than the two photoperiodic regimes to which the animals were exposed.

The present study demonstrates that *G. cf. leucogaster* is responsive to changes in photoperiod. It should however, be mentioned that other environmental variables may act synergistically to promote breeding at a particular season. Although it was demonstrated that reproductive activity in male *G. cf. leucogaster* is heavily influenced by exposure to different photoperiods, other environmental factors such as rainfall, food availability, and ambient temperature may also play a subtle role in the timing of reproduction in these species within their environments. However, photoperiod is clearly an important proximate cue for controlling the onset of reproductive parameters in *G. cf. leucogaster* from central Namibia.

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