Reproductive photoresponsiveness in male spiny mice from South Africa

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Abstract

Many seasonally breeding mammals use changes in photoperiod as a reliable cue to time reproduction. Photoperiodic timing assists an animal in predicting annual environmental changes in its habitat and therefore, enables it to accurately time reproductive events to the most favourable conditions. Changes in day length are more pronounced in the temperate regions and photoperiod is used as a cue for reproduction by most mammals above 30° latitude, however, a number of sub-tropical species also use this proximate factor to regulate their reproductive cycle. We investigated the reproductive photoresponsiveness of 14 male spiny mice (*Acomys spinosissimus*) from southern Africa to short-day (SD; 8 hrs light:16 hrs dark) and long-day (LD; 16L:8D) photoperiods. Testicular mass and volume, seminiferous tubule diameter and plasma testosterone concentrations significantly increased in animals subjected to LD and they were regressed when the males were kept under SD. Body mass of the males was not significantly affected by the photoperiodic conditions. Although male *A. spinosissimus* appear to use photoperiod as a proximate factor to regulate reproduction seasonally, other environmental factors, such as rainfall, food quantity and quality as well as temperature, may regulate reproduction in *A. spinosissimus* in concert with photoperiod. In conclusion, the present study demonstrates the significance of photoperiodic time-measuring systems in the regulation of seasonal reproduction in a sub-tropical rodent.

Keywords: *Acomys spinosissimus*, photoperiod, environmental factors, seasonal reproduction, testosterone, gonadal development, sub-tropics, southern hemisphere

Introduction

Many mammals occur in habitats where seasonal changes in environmental parameters are predictable throughout the year; however, it is crucial for an animal's survival and reproductive success to be able to anticipate these changes accurately. The absolute day length as well as the direction of day length change (photoperiod) is used by a large number of mammal species as a proximate cue to time seasonal changes in reproduction as well as other changes in physiology and behaviour (Goldman, 2001). Changes in photoperiod are most pronounced at higher latitudes and it has been proposed that this proximate factor is primarily used by species occurring at latitudes above 30° where the photoperiodic signal is strongest and most reliable (Bradshaw & Holzapfel, 2007). For example, more northerly populations of the white-footed mouse (*Peromyscus leucopus*) and the deer mouse (*Peromyscus maniculatus*) were observed to be more reproductively photoresponsive than their southerly populations (Dark *et al.*, 1983; Lynch, Heath & Johnston, 1981). In many small and often short-lived mammals from temperate regions, a continuum of photoresponsive to non-photoresponsive individuals can be found in a single population which allows for a more plastic response to environmental changes and enables the non-photoresponsive individuals to breed opportunistically (Prendergast, Kriegsfeld & Nelson, 2001).

In the tropics and sub-tropics, where photoperiodic changes are less pronounced, most mammals do not use photoperiod as a proximate cue (Bernard & Hall, 1995; Nunes *et al.*, 2002) and some even appear to have abandoned any photoperiodic time-measuring systems (Bronson & Heideman, 1992). However, a few rodent species from sub-tropical Africa (Muteka, Chimimba & Bennett, 2006) and an Asian shrew species from the sub-tropics (Wayne & Rissman, 1990) have been

found to be reproductively photoresponsive. The climate at the lower latitudes, especially in Africa, is often characterised by the occurrence of one or two rainy seasons which have a major effect on vegetation growth and therefore, rainfall with (or without) a concomitant increase in food quality and quantity has been suggested to be the main factor influencing seasonal reproduction in mammals throughout most of Africa (Neal, 1986). In the pouched mouse (*Saccostomus campestris*) and the four-striped field mouse (*Rhabdomys pumilio*) from South Africa, it has been demonstrated that food quantity affects reproduction, however, an associated influence of low ambient temperature on reproductive decline was also suggested (Jackson & Bernard, 2001; Tinney, Bernard & White, 2001).

A large number of other factors, besides photoperiod, rainfall and temperature, such as social cues (Demas & Nelson, 1998), green vegetation (Reichman & van de Graaff, 1975) as well as secondary plant compounds (Sanders *et al.*, 1981; Wube, Haim & Fares, 2009), have been found to directly or indirectly influence reproduction in temperate as well as tropical and sub-tropical mammal species. It should be noted that none of the factors mentioned above is mutually exclusive in the regulation of reproductive function and both proximate as well as ultimate factors may commonly be used in combination to facilitate the best reproductive response to the environmental conditions experienced by a species, population or even single individual (Bronson, 1998).

Reproductive responsiveness to photoperiod has been studied in only a few small mammals from southern Africa, such as *S. campestris* and *R. pumilio* but in both species, photoperiod was found not to be the primary factor regulating reproduction (Bernard & Hall, 1995; Jackson & Bernard, 1999). In contrast, the

strongly seasonally reproducing Namaqua rock mouse (*Micaelamys namaquensis* – formerly *Aethomys namaquensis*) and the Tete veld rat (*Aethomys ineptus*) from South Africa have been found to be reproductively photoresponsive (Muteka *et al.*, 2006). In order to gain further insights into the mechanisms shaping seasonality of reproduction in southern African rodents, this study aims to investigate the reproductive photoresponsiveness in male spiny mice (*Acomys spinosissimus*) from South Africa by comparing the development of the testes and concentrations of plasma testosterone between males subjected to either short-day (SD) or long-day (LD) photoperiods.

Acomys spinosissimus is relatively widespread in Africa, south of the equator, and in the southern African subregion it occurs in Mozambique, Botswana and north-eastern South Africa (Skinner & Chimimba, 2005). The habitat of *A. spinosissimus* in South Africa is characterized by one rainy season which spans from September until April and *A. spinosissimus* has been found to reproduce seasonally (Medger, 2010; Medger, Chimimba & Bennett, 2010). The breeding season was found to coincide with the warm and wet spring and summer months of the southern hemisphere, while breeding ceased during the cold and dry autumn and winter months. It has, therefore, been previously suggested that rainfall, resulting in an increase in food quantity and quality, is the ultimate cause for reproductive seasonality in *A. spinosissimus* (Medger *et al.*, 2010).

We now hypothesize that male *A. spinosissimus* from a population from less than 30°S are reproductively responsive to changing photoperiods because other rodent species, which co-occur with *A. spinosissimus*, were also found to be reproductively photoresponsive (see Muteka *et al.*, 2006). In addition, males of the

golden spiny mouse (*A. russatus*) were observed to reduce spermatogenesis under SD photoperiods although the common spiny mouse (*A. cahirinus*) was found to be reproductively non-responsive to photoperiod (Wube, Haim & Fares, 2008). We predicted that the testes of *A. spinosissimus* would regress and the plasma testosterone concentration would be lower under SD compared to LD photoperiods.

Materials and Methods

A total of 14 male *A. spinosissimus* were collected at the end of the breeding season and during the non-breeding season, namely between January and March 2008, and in June 2009. The males were caught in a private game reserve in the Soutpansberg region, Limpopo Province, South Africa (22°58'S, 22°57'S; 29°25'E, 29°24'E) under permits (CPM-333-00002, CPM-002-00002) issued by the CITES and Permit Management Office, Department of Environmental Affairs, Polokwane, Limpopo Province, South Africa. The animals were caught over-night along the rocky outcrops of the reserve with Sherman live traps (H. B. Sherman Traps, Inc. Tallahassee, Florida, U.S.A.) baited with a mixture of peanut butter, oats and fish. Male spiny mice were housed in standard polyurethane cages which were embedded with wood shavings and paper towelling was provided as shelter. They were fed daily with apples and carrots and mouse chow pellets (Die Klein Kooperasie, Pretoria, Gauteng, South Africa) and water was provided *ad libitum* during the entire experiment.

In the laboratory, animals were housed in climate-controlled rooms which allowed a constant temperature of 25 °C throughout the experiment. The males were weighed to the nearest 0.001 g with a digital balance (Scout Pro SPU123, Ohaus Corporation, Pine Brook, New Jersey, U.S.A.) before the onset of the experiments.

All males were initially subjected to a photoperiod of eight hrs of light and 16 hrs of darkness (8L:16D; SD) for 40 days to attain a similar reproductive status for all the males before the start of the actual experimental treatments. Subsequently, male spiny mice were subjected to either a photoperiod of 8L:16D or 16 hrs of light and eight hrs of darkness (16L:8D; LD). Seven males were randomly assigned to each experimental treatment. After 30 days on either the LD or SD treatments, the male spiny mice were weighed again and subsequently euthanized with an overdose of halothane. Blood was taken from the heart of all the males by exsanguination and then centrifuged at 500 *g* for 15 min. The blood plasma was separated from the blood cells and frozen at -35 °C until analysis for testosterone concentration. The testes were dissected out and fixed in Bouin's fluid for approximately 20 hrs after which they were stored in 70 % ethanol. Seminal vesicles were dissected out and immediately weighed to the nearest 0.001 g. All experimental procedures were approved by the animal ethics committee of the University of Pretoria (ethics clearance number: A003-07).

Histology

All excess tissues were removed from the fixed testes before they were weighed separately to the nearest 0.0001 g using a high precision scale (Ohaus Corp. Pine Brook, N.Y., U.S.A.). The testes length and width (mm) were measured with a pair of digital callipers (Sylvac Opto RS 232, Ultra Praezision Messzeuge GmbH, Germany) to the nearest 0.01 mm and then utilized to calculate testicular volume (mm^3) by using the formula for the volume of an ellipsoid: $V = 4/3\ \pi ab^2$ where a represents half the maximum length and b half the maximum width (Woodall & Skinner, 1989). The average of mass (mg) and volume were calculated for both testes per male. The tissues were dehydrated by a series of ethanol baths of increasing concentrations

before being embedded in paraffin wax. The testes were then serially sectioned in 7 µm thick sections with a rotary microtome (820 Spencer, American Optical, Scientific Instrument Division, Buffalo, N.Y., U.S.A.) and mounted on microscope slides with gelatine. After approximately 48 hrs of drying in an oven, the sections were sequentially stained in Ehrlich's haematoxylin and eosin as described by Drury and Wallington (1967). Round seminiferous tubules were photographed at ×10 magnification with a digital camera (Moticam 1000 1.3 M Pixel USB 2.0, Motic China Group, LTD., Xiamen, P.R. China) attached to a light microscope (Diaplan, Ernst Leitz Wetzlar GmbH, Germany). Subsequently, the diameters of 50 randomly selected seminiferous tubules (µm) per testis per animal were measured with the computer program Motic Images Plus 2.0ML (Motic China Group, LTD., Xiamen, P.R. China) and the average of all 100 diameters per individual was calculated.

Testosterone analysis

Plasma testosterone concentrations were measured for all males with a coat-a-count hormone kit (Siemens Medical Solutions Diagnostics, Los Angeles, U.S.A.) according to the guidelines provided by the manufacturer. The assay was validated for *A. spinosissimus* by comparing a serial dilution curve with the calibration curve using an analysis of covariance (ANCOVA) and no significant difference between the two curves ($F_{1,3}$ = 5.87; n = 3; P = 0.09) was found. The intra-assay coefficients of variation were 3.5 % and 7.8 % and the inter-assay coefficient was 5.0 %.The minimum detectable amount of testosterone for the assay was 1.39 nmol/L.

Data analysis

A repeated measures analysis of variance (ANOVA) was performed to compare body mass before and after the experiment during which photoperiodic treatment (LD and

SD) was used as an in-between factor. ANCOVAs, with body mass measured at the end of the experiment as a covariate, were carried out to compare testicular mass and volume, seminiferous tubule diameter and plasma testosterone concentration between the two photoperiodic treatments. The analysis of plasma testosterone concentration was based on log-transformed data because the original data were non-parametric. *Statistical Package for the Social Sciences* (SPSS) Statistics version 17.0 (Polar Engineering and Consulting 1993-2007) was used for all statistical analyses. All values are given as mean ± 1 standard deviation and results were found to be significant at $P < 0.05$.

Results

Photoperiod affected reproductive development in male *A. spinosissimus*. Seminal vesicles were enlarged in males which were subjected to LD (0.11 ± 0.06 g; range: 0.04 - 0.24 g) and reduced under SD conditions where seminal vesicles were found to be too small to be weighed. There was no relationship between body mass and testicular mass, seminiferous tubule diameter or plasma testosterone concentration ($F_{1,11} < 4.53$; $P > 0.06$). Testicular volume was, however, significantly positively correlated with body mass ($F_{1,11} > 7.38$; $P < 0.02$). Testicular volume and mass were significantly larger in male spiny mice subjected to LD compared to SD photoperiods ($F_{1,11} > 12.73$; $P < 0.01$; Figs. 1a and 1b). The diameter of the seminiferous tubules was also significantly larger in males under LD than in males under SD ($F_{1,11} = 13.36$; $P < 0.01$; Fig. 1c) and significantly more plasma testosterone was recorded under LD compared to SD conditions ($F_{1,11} = 31.20$; $P < 0.001$; Fig. 1d).

Male spiny mice were significantly heavier at the end of the experiment (21.8 ± 3.5 g) than they were at the beginning (18.4 ± 2.5 g; $F_{1,12} = 28.14$; $P < 0.001$).

However, we did not detect an influence of photoperiod on male body mass because body mass was not significantly different between SD and LD photoperiods ($F_{1,12}$ = 0.57; P = 0.47) and also, there was no significant relationship between the photoperiodic treatments (SD and LD) and body mass measured at either the beginning or end of the experiment ($F_{1,12}$ = 3.02; P = 0.11; Fig. 2).

Discussion

In a previous study, it was demonstrated that *A. spinosissimus* is a seasonal breeder which reproduces during the warm and wet spring and summer months in the southern African sub-region but the factors which lead to this reproductive pattern were unknown (Medger, 2010; Medger *et al.*, 2010). The present study provides the first evidence that male *A. spinosissimus* are reproductively responsive to a change in photoperiod which may be used as a proximate factor to regulate seasonal reproduction in this species. Male *A. spinosissimus* responded to LD photoperiods with an increase in testicular mass and volume, seminiferous tubule diameter, mass of accessory glands (seminal vesicles) and testosterone concentrations in comparison to SD photoperiods. It is interesting to note that testes size and seminiferous tubule diameter of males subjected to LD photoperiods were similar to those previously found for wild-caught males in July (the start of breeding season in male *A. spinosissimus*; Medger, 2010) and not the size observed for males collected at the peak of the breeding season in September (e.g. testicular volume, LD: 99.6 ± 23.6 mm³, July: 100.0 ± 50.9 mm³, September: 230.6 ± 44.2 mm³; Medger, 2010). This may indicate that 30 days of LD conditions is an insufficient time for maximum growth of the testes and it may be speculated that males kept longer under LD conditions would show a larger increase in testes size and seminiferous tubule diameter than presently reported.

Our findings on the reproductive responsiveness of male *A. spinosissimus* are similar to those of Muteka *et al*. (2006) on the sympatric *M. namaquensis* and *A. ineptus* from South Africa. Both species showed significantly higher testicular mass and volume and larger seminiferous tubule diameters under LD than under SD photoperiodic conditions, however, plasma testosterone concentration was not different between the photoperiodic conditions in *A. ineptus* although it was higher under LD than SD in *M. namaquensis.* In contrast, day length did not affect either testes size or spermatogenesis in both *S. campestris* and *R. pumilio*, both of which seem to breed opportunistically in southern Africa (Bernard & Hall, 1995; Jackson & Bernard, 1999; Jackson & Bernard, 2006). In addition, Nunes *et al*. (2002) found that photoperiod does not influence plasma testosterone concentration, testes size and seminal vesicle mass in males of the seasonally breeding Nile grass rat (*Arvicanthis niloticus*) from an equatorial population. Other rodents, bats and shrews, which occur near the equator, have also been found to be non-responsive to photoperiod (Heideman & Bronson, 1990; O'Brien, Curlewis & Martin, 1993; Rissman *et al.*, 1987). These findings suggest that opportunistically breeding small mammals from tropical and sub-tropical regions do not use photoperiodic cues to regulate reproduction. However, sub-tropical rodents, which reproduce seasonally, appear to depend considerably on photoperiod as a proximate factor to anticipate environmental changes and shape reproduction as appears to be the case in *A. spinosissimus*, too. However, the day lengths chosen for the present study were much longer/shorter than the day lengths *A. spinosissimus* would experience in its natural environment. It should, therefore, be noted that the present study tested the reproductive responsiveness to photoperiod, but not the actual responses which may be observed in the habitat of *A. spinosissimus*. Future studies should also test if *A.*

spinosissimus is also responsive to photoperiods typical for populations near the equator. We, however, propose that male *A. spinosissimus* from the sub-tropics are able to respond to these photoperiods and, therefore, also use photoperiod to time reproduction in their natural habitat because an exclusion of any photoperiodic time-measuring systems would be expected otherwise.

Responses to photoperiod have been studied in a number of other *Acomys* species. Males of *A. russatus*, for example, were spermatogenically more active when kept under LD photoperiods than SD photoperiods, but females of the same species did not show any reproductive response to photoperiod (Wube *et al.*, 2008). Both male and female *A. cahirinus* were found to be reproductively non-responsive to changing photoperiods (Wube *et al.*, 2008). In another study, however, spermatogenic activity was found to be decreased in *A. cahirinus* subjected to SD photoperiods (El-Bakry, Zahran & Bartness, 1998). Photoperiod and other factors which influence seasonal reproduction have not been investigated in any other *Acomys* species. However, the Cape spiny mouse (*Acomys subspinosus*) from the Eastern Cape Province of South Africa may be reproductively non-photoresponsive because it is an opportunistic breeder (Fleming & Nicolson, 2002). Latitude does not seem to play a role in the responsiveness to changing day length within the genus *Acomys* and other environmental factors may play a more important role for seasonal reproduction in this genus. Trainor *et al*. (2006) found no distinct relationship between latitude of origin and photoperiodic responsiveness in five species of *Peromyscus* from different latitudes. They, therefore, suggested that, among closely-related species, photoperiodic effects on reproductive function may be mediated by different physiological mechanism (Trainor *et al.*, 2006).

Body mass increased during the course of the experiment which is likely a result of captivity as the animals were provided with a more protein-rich food source and were likely to be less active in the laboratory than in their natural environment. However, there was no effect of photoperiodic treatment on body mass and the males weighed the same under SD and LD photoperiods. It appears to be fairly common in rodents that body mass is not affected by different photoperiods. El-Bakry *et al*. (1998) observed no change in body mass with varying photoperiods in four desert rodents including *A. cahirinus*. In contrast, factors which indicate body condition were affected by photoperiod and for example, fat pad mass and carcass lipid content increased under SD photoperiodic exposure in male *A. cahirinus* (El-Bakry *et al.*, 1998). Although photoperiod appears to be used to time reproduction, it does not seem to be important in regulating body mass in male *A. spinosissimus*. However, moderate photoperiodic effects on body mass may have been masked by the high food availability in the laboratory and body condition of *A. spinosissimus* may be influenced by changing day length.

Acomys spinosissimus occurs in a highly seasonal habitat where the abundance of high quality food is mediated by seasonal rainfall. Besides photoperiod, food quantity and quality may regulate reproduction in the spiny mouse as described for other rodents. For example, food deprivation negatively affected reproductive organ growth in deer mice although not in house mice (*Mus musculus*) (Blank & Desjardins, 1984). Protein content of the diet of mammals fluctuates with seasons and it has been suggested that it may limit reproduction in tropical rodents (Field, 1975). In addition, the salt content of plants increases during the dry season in xeric environments due to evaporative water loss and thus, Wube *et al.* (2009) suggested that dietary salinity may be used as a proximate factor to predict the best

time for reproduction in *A. russatus*. In addition, low sodium and calcium content of seeds was found to cause lower reproductive success in the California vole (Batzli, 1986). The availability of food often interacts with photoperiod and food restriction has been found to enhance the effects of SD photoperiods (Nelson *et al.*, 1997) whereas availability of green food may counteract the inhibitory actions of SD (Nelson, Dark & Zucker, 1983).

The natural environment of an animal is much more complex than the conditions provided in laboratory experiments (Bronson, 1998) which may explain some of the discrepancies between the present laboratory study and results on wild-caught animals. If the present results were compared with the results on wild-caught animals (Medger, 2010; Medger, *et al.* 2010), it appears that food quantity and possibly quality may interact with photoperiod to shape reproduction in *A. spinosissimus.* Testicular mass and volume and seminiferous tubule diameters of male *A. spinosissimus* were higher in males under SD (e.g., volume: 34.0 ± 28.0 mm^3) than in wild-caught individuals from the non-breeding season (e.g., volume (March): 12.8 ± 8.1 mm^3; Medger, 2010) which may have been the result of the large amount of high quality food in the laboratory (for effect on body mass see above). Moreover, fat pads around the testes appeared to be larger in individuals housed longer in captivity although time in captivity did not seem to have a significant effect on testes mass and volume, seminiferous tubule diameter and testosterone concentration (these non-significant results were not presented in the present study). As it is unlikely that 70 days under SD was too short for total regression of the testes and all males were caught during the non-breeding season when testes are regressed, we suggest that the higher food quantity and quality in the laboratory caused the difference of testicular size between laboratory and wild-caught animals.

In addition, ambient temperature may also have had an effect on the size of the testes as ambient temperature was higher during the entire experiment (25 °C) than in the natural habitat during winter (< 20 °C). Nelson *et al*. (1989) demonstrated that cold ambient temperatures further suppress gonadal size in male prairie voles under SD.

Furthermore, other factors may influence reproduction in female *A. spinosissimus*. It is unknown if the females of *A. spinosissimus* are reproductively photoresponsive and because reproduction of females of both *A. cahirinus* and *A. russatus* have been found to be independent of photoperiodic changes (Wube *et al.*, 2008), reproduction of female *A. spinosissimus* may not be influenced by photoperiod either. The onset of reproduction in female *A. spinosissimus* was previously found to coincide with the start of the rainy season (Medger *et al.*, 2010). Either rainfall or water availability in general may, therefore, be proximate but very likely ultimate factors regulating seasonal reproduction in female *A. spinosissimus.* In the California mouse (*Peromyscus californicus*), for example, water availability may regulate reproduction independent of either photoperiod or the availability of food (Nelson, Gubernick & Blom, 1995).

In conclusion, male *A. spinosissimus* are reproductively responsive to photoperiod with long-day lengths stimulating gonadal development. As a result, photoperiod may be the proximate factor which regulates seasonal reproduction in males of this species. A number of other environmental factors are also discussed and it is likely that either rainfall by, for example, influencing plant growth and composition, or temperature affect reproduction in *A. spinosissimus*. In addition, an interaction of several factors may be likely. The present results demonstrate that

photoperiod may possibly be an important factor in the regulation of seasonal reproduction not only in temperate but also in sub-tropical and perhaps even tropical rodents. Furthermore, the present study emphasizes the ecological importance for the precise and premature timing of reproductive events in seasonally breeding rodents from the sub-tropics.

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Figure legends

Fig. 1.

Standardized residual of testicular volume (mm^3) by body mass (g; a), testicular mass (mg; b), seminiferous tubule diameter (μm; c) and plasma testosterone concentration (nmol/L; d) of male spiny mice (*Acomys spinosissimus*) from South Africa subjected to either a photoperiod of 16hrs light and 8hrs darkness (LD; $n = 7$) or 8hrs light and 16hrs darkness (SD; $n = 7$). Values are presented as mean ± 1 standard deviation.

Fig. 2.

Mean body mass (g) ± 1 standard deviation of male spiny mice (*Acomys spinosissimus*) from South Africa compared between long-day (LD; $n = 7$) and short-day (SD; $n = 7$) photoperiodic treatments and measured at the start (black bars) and end (grey bars) of the experiment.

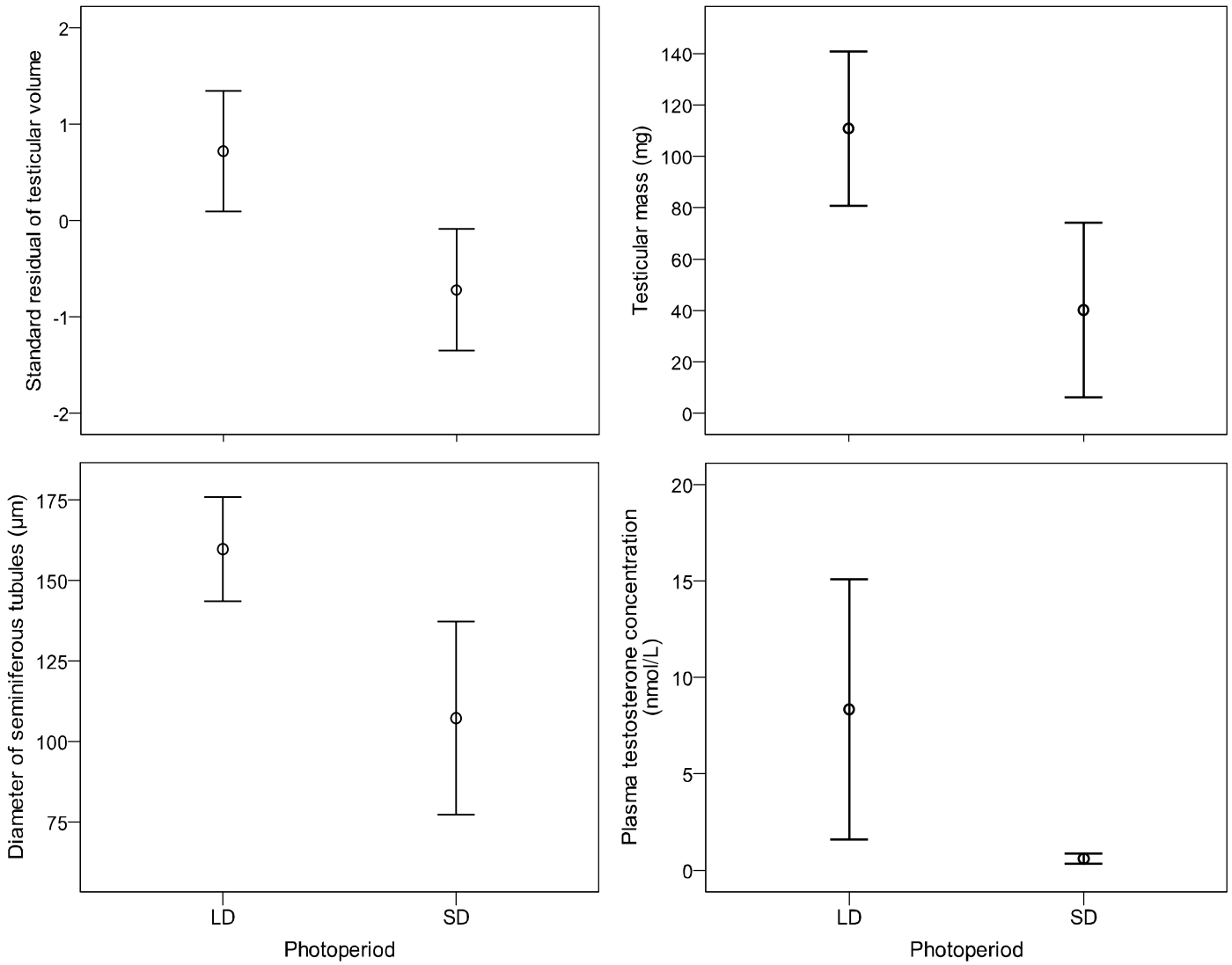
Standard residual of testicular volume
2
1
0
-1
-2
Testicular mass (mg)
140
120
100
80
60
40
20
0
Diameter of seminiferous tubules (µm)
175
150
125
100
75
Plasma testosterone concentration (nmol/L)
20
15
10
5
0
LD
SD
Photoperiod
LD
SD
Photoperiod

Fig. 1

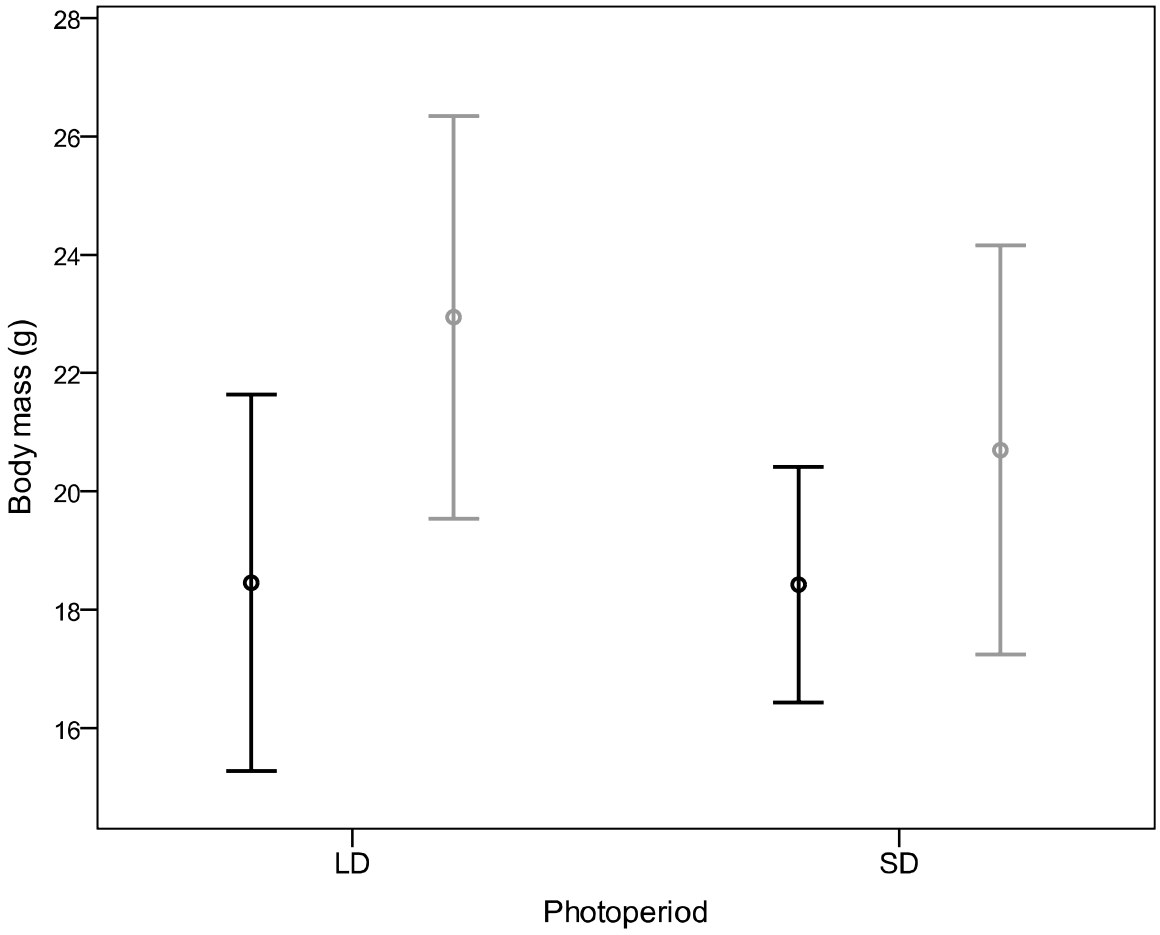

Fig. 2