Photoperiod Differentially Affects Immune Function and Reproduction in Collared Lemmings (*Dicrostonyx groenlandicus*)

Zachary M. Weil,[1] Lynn B. Martin II, and Randy J. Nelson
Departments of Psychology, Neuroscience, Evolution, Ecology, and Organismal Biology and Institute for Behavioral Medicine Research, Ohio State University, Columbus

Abstract Many nontropical rodent species experience predictable annual variation in resource availability and environmental conditions. Individuals of many animal species engage in energetically expensive processes such as breeding during the spring and summer but bias investment toward processes that promote survival such as immune function during the winter. Generally, the suite of responses associated with the changing seasons can be induced by manipulating day length (photoperiod). Collared lemmings (*Dicrostonyx groenlandicus*) are arvicoline rodents that inhabit parts of northern Canada and Greenland. Despite the extreme conditions of winter in their native habitat, these lemmings routinely breed during the winter. In the laboratory, collared lemmings have divergent responses to photoperiod relative to other seasonally breeding rodents; short day lengths can stimulate, rather than inhibit, the reproductive system. Male and female collared lemmings were maintained for 11 weeks in 1 of 3 photoperiods (LD 22:2, LD 16:8, or LD 8:16) that induce markedly different phenotypes. Following photoperiod treatment, cell-mediated immune function as assessed by delayed-type hypersensitivity reactions was elevated in lemmings housed in LD 16:8 and LD 8:16 relative to LD 22:2. However, antibody production to a novel antigen was unaffected by photoperiod. Exposure to LD 8:16 induced weight gain, molt to a winter pelage, and in contrast to previous studies, regression of the male, but not the female, reproductive tract. In conclusion, these data indicate that components of immune function among collared lemmings are responsive to changes in day length.

Key words collared lemming, photoperiod, seasonality, immune function, delayed-type hypersensitivity

Animals inhabiting nontropical habitats experience predictable annual variation in environmental conditions. For these individuals, winter represents an energetic bottleneck because reduced energy availability coincides with increased thermoregulatory demands (Bronson, 1989). Many rodent species confine breeding to the spring and summer when conditions are most conducive to reproductive success. Outside of the breeding season, investments are biased toward processes that promote survival, such

1. To whom all correspondence should be addressed: Zachary M. Weil, Department of Psychology, 1835 Neil Avenue Mall, Ohio State University, Columbus, OH 43210; e-mail: Weil.20@osu.edu.

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as fat storage and immune function (Bartness et al., 2002; Nelson, 2004). Generally, the suite of physiological, morphological, and behavioral changes that occur seasonally can be induced in the laboratory by manipulating day length (photoperiod) (Prendergast et al., 2002). In many rodent genera, short days inhibit reproductive function but enhance some components of immune function while suppressing others (Demas et al., 1996; Bilbo et al., 2002; Hadley et al., 2002; Demas, 2004; Pyter et al., 2005).

Collared lemmings (*Dicrostonyx groenlandicus*) inhabit northern Canada and Greenland; this species inhabits the highest latitude of any small mammal (Stenseth and Ims, 1993). Collared lemmings have been extensively studied to understand wildlife population cycles (Krebs, 1964; Krebs et al., 1995). They also display several characteristics that make them interesting candidates for studies of seasonality in physiology and behavior. In nature, wild lemmings breed predominantly in the spring, but they also breed opportunistically during winter (Millar, 2001). Different day lengths can stimulate or inhibit the reproductive system contingent on individuals' photoperiod history (Gower et al., 1997). For instance, prolonged exposure to long days (LD 22:2) induces gonadal regression in males (Gower et al., 1998). However, if LD 22:2 photoperiods are preceded by transient exposure to short days (LD 8:16), then gonadal growth is stimulated (Gower et al., 1997). Photoperiod also affects body mass and composition, bifid claw (a cornification of the toepad of the 3rd and 4th digits of the forepaws) morphology, and pelage color (Hansen, 1957; Maier and Feist, 1991; Gower et al., 1994b); however, directionality depends on photoperiod duration (Nagy et al., 1993a; Nagy et al., 1993b). Collared lemmings exhibit graded responses proportional to day length (Gower et al., 1994a); that is, body mass, pelage, and circulating prolactin concentrations differ among animals housed in LD 22:2 versus LD 16:8 versus LD 8:16 photoperiods (Gower et al., 1994a). In the arctic (69º N latitude), these photoperiods are experienced in early May and August (LD 22:2), early April and September (LD 16:8), and late January and mid-November (LD 8:16) (Gower et al., 1993). Individuals of this species present a unique opportunity to examine the effects of various day lengths on immune function of an arctic species.

The current study was designed to answer the following questions: 1) Do changes in photoperiod affect the immune system of these arctic rodents? 2) If so, are changes in the immune system proportional to day length or do immune alterations occur in an "all-or-none" fashion? For example, if short day lengths are associated with enhanced immune function, and this enhancement can occur in a continuous fashion, then we predict that the amplitude of the immune responses would be negatively related to day length. However, if photoperiod-mediated alterations in immune function occur in all-or-none fashion, then immune responses in photoperiods sufficiently short to enhance immune function will be similar. 3) Do photoperiod-mediated changes in the immune system occur under the same photoperiod conditions as alterations in reproductive and morphological traits? 4) Do photoperiod-mediated alterations in the immune system occur to the same extent in male and female lemmings? To answer these questions, lemmings were maintained in either LD 22:2, LD 16:8, or LD 8:16 photoperiods for 11 weeks (Maier and Feist, 1991), then T cell–mediated and B cell–mediated immune activity were assessed. Finally, reproductive and immunological tissue masses, as well as circulating steroid concentrations, were measured.

MATERIALS AND METHODS

Animals

Collared lemmings used in this study were bred in our colony at Ohio State University. The progenitors of the colony were trapped on Igloolik Island, Northwest Territories, Canada (69°23′N, 81°50′W) in 1985. The breeding colony was maintained on an LD (light-dark) 16:8 cycle (lights-off at 1500 h Eastern Standard Time [EST]). Experimental lemmings were weaned at 21 days, then housed in same sex groups of 3 to 5 in polypropylene cages (27.8 cm × 7.5 cm × 13 cm) with cedar shavings bedding and ad libitum access to food (Teklad 8630 Rabbit Diet, Harlan Teklad, Indianapolis, IN) and filtered tap water. Colony rooms were maintained at a temperature of 20 ± 4 °C and a relative humidity of 50% ± 10%. All procedures were conducted with approval of the Ohio State University Institutional Animal Care and Use Committee and in compliance with all US federal animal welfare requirements.

At approximately 60 days of age, male and female lemmings were transferred into 1 of 3 photoperiod treatments (LD 22:2, LD 16:8, or LD 8:16), with lights-off at 1500 h EST in all cases. Lemmings were housed with same sex siblings (3-5 per cage) for the duration of the experiment. A small patch was shaved into the fur on the back to identify individual lemmings.

Immunological testing began after 11 weeks of photoperiod treatment.

Three cohorts of lemmings were used in this study. All groups were represented in each cohort. Additionally, the immune measurements were separate for each cohort; that is, an individual lemming was used for testing delayed-type hypersensitivity (DTH) responses or anti-KLH (keyhole limpet hemocyanin) antibody production but not both. DTH responses were assessed on lemmings in LD 22:2 (n = 4 females, 9 males), LD 16:8 (n = 6 females, 5 males), and LD 8:16 (n = 4 females, 7 males). Humoral immune assays were conducted on LD 22:2 (n = 4 females, 7 males), LD 16:8 (n = 8 females, 8 males), and LD 8:16 (n = 3 females, 7 males). The organ masses and blood for hormone assays represent data from across all 3 cohorts, and both radioimmunoassays (RIAs) were run in a single assay.

Somatic and Reproductive Responses

Following the conclusion of immunological testing, lemmings were deeply anesthetized with isoflurane vapors, weighed, and then decapitated. Trunk blood was collected, allowed to clot for 30 min, the clot removed, and then centrifuged at 3000 rpm for 30 min at 4 °C. The resulting sera were stored at –70 °C for RIA of corticosterone and testosterone. Reproductive tissues, adrenal glands, spleens, and thymuses were excised, cleaned of connective tissue, and weighed. Pelage color was assessed by eye on a 5-point scale with 1 = gray summer pelage and 5 = white winter pelage (Maier and Feist, 1991).

Cell-Mediated Immune Function

One cohort of lemmings was assessed for delayed-type hypersensitivity responses to the chemical antigen 2, 4-dinitro-1-fluorobenzene (DNFB; Sigma, St. Louis, MO). Lemmings were anesthetized with isoflurane vapors, and a 2 × 3-cm patch was shaved on the dorsum. Twenty-five microliters of DNFB (0.5% [w/v] in 4:1, acetone/olive oil vehicle) was applied to the dorsal skin on 2 consecutive days, a dose previously observed to induce measurable swelling responses (Weil, Martin, and Nelson, unpublished observations). Seven days later, lemmings were again anesthetized, and the thickness of both hind footpads was assessed with a constant loading dial micrometer (Long Island Indicator Service Inc., Hauppauge, NY). Immediately following baseline footpad measurements, 20 µL of DNFB (0.3% in 4:1 acetone/olive oil) was applied to the pad of the left hind paw. A vehicle solution was applied to the right hind paw. Footpad thickness was measured every 24 h for the next 5 days. For purposes of statistical analyses, footpad thickness was expressed as a percentage of baseline thickness. All measurements were performed between 1300 and 1430 h EST, and all measurements were conducted by the same experimenter.

Humoral Immune Function

To assess humoral immune function, lemmings were injected with 150 µL of KLH suspended in sterile saline. KLH is a respiratory protein from the giant keyhole limpet (*Megathura crenulata*) that induces a robust antigenic response but does not produce fever or prolonged inflammation (Dixon et al., 1966). Although this dose of KLH has not been used previously in this species, the dose employed here yielded similar patterns of serum immunoglobulin G (IgG) as have been observed in other arvicoline rodents (e.g., Klein and Nelson, 1999; Demas et al., 2003). Under isoflurane anesthesia, blood (75 µL) was drawn from the retroorbital sinus on the day of injection and 3, 5, 7, 14, and 21 days postinoculation. Lemmings were injected with sterile saline following the blood draw to prevent dehydration. Following the blood sample on day 21, lemmings were injected with an additional 30 µg of KLH to induce a secondary response. Lemmings were then bled over the subsequent 4, 7, and 10 days (days 25, 28, and 31, respectively). All blood samples were allowed to clot for 30 min, and the clot was removed and then centrifuged at 4 °C for 30 min at 3000 rpm.

KLH ELISA

Serum concentrations of anti-KLH IgG were determined using an ELISA (enzyme-linked immunosorbent assay), as previously described (Demas et al., 1997). Serum was diluted 1:80 with PBS+Tween, and 150 µL of each sample were added in duplicate 96-well plates coated with the KLH antigen. Positive control (pooled serum from lemmings previously determined to have high levels of anti-KLH antibodies) and negative control (pooled serum from lemmings injected with sterile saline vehicle) samples were also added in duplicate to each plate. The plates were sealed, incubated, and washed before addition of secondary antibody (alkaline phosphatase-conjugated

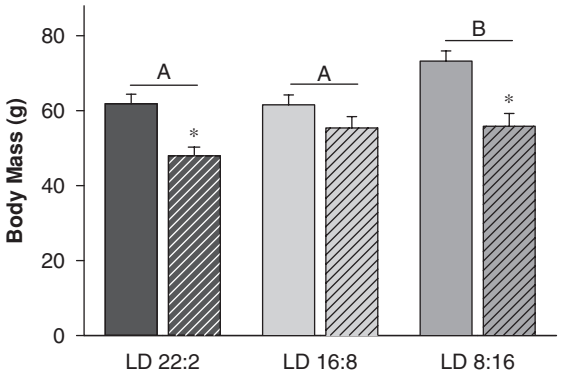

Figure 1. Lemmings housed in short day lengths (LD 8:16) were significantly heavier than those in other photoperiods. Data are presented as mean ± SEM. Groups with different letters are significantly different from each other. *Significantly lighter than males of the same photoperiod. Solid bars represent males, and hatched bars represent females.

anti-mouse IgG). Plates were again incubated and washed and then treated with the enzyme substrate (*p*-nitrophenyl phosphate). After 20 min, the optical density (OD) of each well was determined using a plate reader with a 405-nm wavelength filter. Average OD for duplicate wells was expressed as a percentage of its plate-positive control OD value for statistical analyses (Drazen et al., 2000).

RIA Procedures

Serum corticosterone (MP Biomedicals, Costa Mesa, CA) and testosterone (Diagnostic Systems Laboratories, Webster, TX) concentrations were assayed using double-antibody ^{125}I kits. Serum for RIA was collected in a terminal trunk bleed. Blood was collected within 2 min of touching the cage, which allows collection of blood to determine basal corticosterone concentrations (Riley, 1981). The assays were conducted following the guidelines set by the manufacturer. Serum was diluted 1:20 for corticosterone and undiluted for testosterone. These kits are highly specific, and cross-reactivity with other steroids was <1% for corticosterone and <3.5% for testosterone. Intra-assay variance was <10% for both assays, and minimum detection thresholds were 5 ng/mL for corticosterone and 0.19 ng/mL for testosterone.

Data Analyses

Reproductive tissue weights were analyzed among photoperiods with a 1-way analysis of variance (ANOVA) separately for each sex. Serum testosterone was log-transformed prior to analysis. Thymus, spleen, and adrenal mass; final body mass; pelage score; and serum corticosterone were analyzed with 2-factor ANOVAs (sex × photoperiod). All tissue weights are reported as estimated marginal means with body mass as a covariate. Unequal variances were detected for testosterone, testes, ovarian, and uterine masses, and as such, these values were log-transformed. Delayed-type hypersensitivity and anti-KLH antibody concentrations were analyzed with 2-way repeated measures (photoperiod × sex). Following a significant *F* score, Tukey tests were conducted for multiple comparisons. The humoral immune experiment was conducted in 2 iterations with all groups represented in both cohorts. All comparisons were considered statistically significant if $p < 0.05$.

RESULTS

Reproductive and Somatic Responses

Photoperiod significantly altered final body mass ($F_{2,83} = 4.972$, $p < 0.01$; Fig. 1) such that LD 8:16 lemmings were significantly heavier than all other groups. Males were also significantly heavier than females ($F_{1,83} = 26.051$, $p < 0.0001$; Fig. 1), but the photoperiod effect on body mass was not different between the sexes ($p > 0.05$). Lemmings housed in LD 8:16 underwent a molt to the winter pelage color ($F_{2,87} = 174.530$, $p < 0.0001$; Table 1) regardless of sex.

Short day lengths induced regression of the reproductive tract of male lemmings. Paired testis mass ($F_{2,51} = 3.504$, $p < 0.05$; Fig. 2A), seminal vesicles mass ($F_{2,44} = 5.160$, $p < 0.05$; Fig. 2B), and circulating testosterone concentrations ($F_{2,51} = 3.652$, $p < 0.05$; Fig. 2C) were all reduced in LD 8:16 lemmings relative to the other photoperiods. Epididymal fat mass was not affected by photoperiod ($p < 0.05$; data not shown). There was not strong evidence for regression of the reproductive system in females. Uterine ($p > 0.05$; Table 1) and ovarian mass ($p > 0.05$; Table 1) were not altered by photoperiod treatment. However, ovarian fat pad mass was higher in LD 22:2 lemmings ($F_{2,31} = 4.850$, $p < 0.05$; Table 1) relative to other treatment groups.

Circulating corticosterone (the primary glucocorticoid in this species [Gower et al., 1992]) did not vary among photoperiod groups or between the sexes. Adrenal mass was also not different among groups ($p > 0.05$ for both; see Table 1).

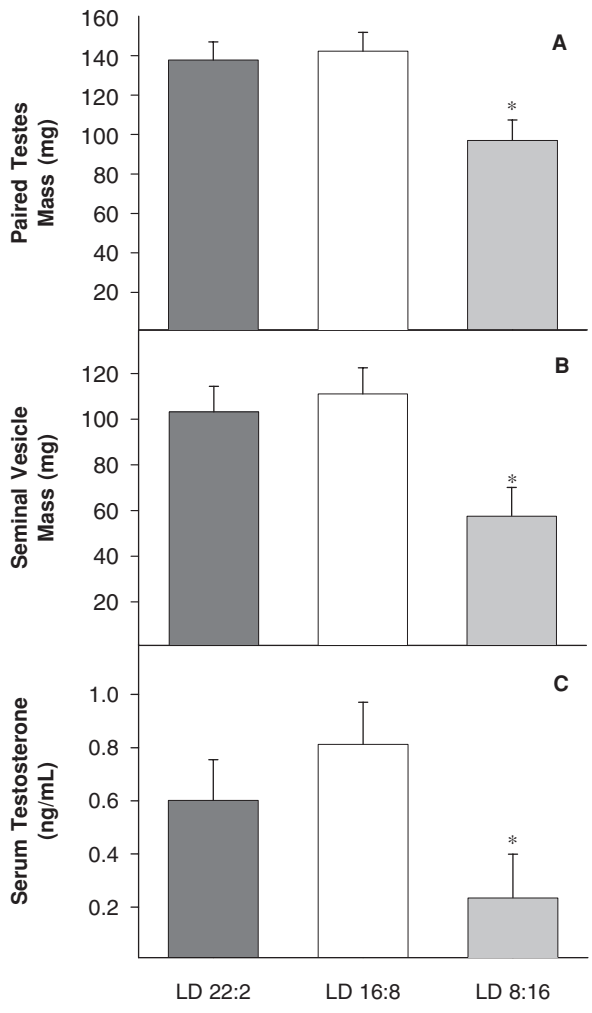

Figure 2. Exposure to short days (LD 8:16) resulted in suppression of the reproductive system of male lemmings. Lemmings in short days had smaller testes (A), smaller seminal vesicles (B), and lower concentrations of circulating testosterone (C). Data are presented as mean ± SEM. *Significantly lower than all other photoperiods.

Immune Responses

All lemmings exhibited robust footpad swelling responses over the measurement period ($F_{5,24} = 42.879$, $p < 0.0001$; Fig. 3). Photoperiod significantly altered DTH responses ($F_{10,50} = 2.491$, $p < 0.05$), such that lemmings housed in LD 22:2 had significantly smaller swelling responses than those in the other 2 conditions. Female lemmings had stronger swelling responses than males ($F_{5,24} = 6.949$, $p < 0.0001$); however, the interaction between the 2 variables was not significant ($p > 0.05$). Among males, the lemmings in LD 22:2 displayed significantly less swelling than males in the other 2 photoperiods. Female DTH responses were not strongly affected by photoperiod ($p > 0.05$ in all comparisons).

The thymuses of lemmings housed in LD 8:16 were significantly larger ($F_{2,80} = 4.094$, $p < 0.05$; Fig. 4) than lemmings in the other 2 experimental photoperiods. No sex differences in the size of the thymus ($p > 0.05$) or interaction between sex and photoperiod ($p > 0.05$) were observed. Spleen mass also did not vary among photoperiod treatments ($p > 0.05$; Table 1).

Anti-KLH IgG was significantly elevated in all groups following inoculation ($F_{6,23} = 92.118$, $p < 0.0001$; Fig. 5), but neither sex nor photoperiod affected anti-KLH IgG production over either the primary or secondary responses ($p > 0.05$ in all cases).

DISCUSSION

Photoperiod influenced immune responses and reproductive and somatic parameters in collared lemmings. Specifically, exposure to long days (LD 22:2) reduced delayed-type hypersensitivity responses in male lemmings relative to intermediate (LD 16:8) and short-day (LD 8:16) groups. Female responses to photoperiod were less robust than male responses. Humoral immune function, as measured by antibody production to a novel antigen, was not strongly affected by photoperiod in either sex. However, lemmings housed in LD 8:16 possessed larger thymuses compared to animals in other photoperiod treatments regardless of sex. In terms of reproduction, short days evoked reduced organ size and low testosterone concentrations in males. Female ovarian fat pads were largest in LD 22:2, but uterine mass did not vary significantly among groups. Finally, exposure to LD 8:16 induced winter pelage molt and significantly increased body mass in both sexes.

In contrast to previous studies in collared lemmings, short days suppressed reproductive function in male lemmings (Hasler et al., 1976; Maier and Feist, 1991; Nagy et al., 1993a). This is somewhat surprising given that most previous studies of reproductive changes in response to photoperiod have reported the opposite outcome. Indeed, it has been suggested that this species is not reproductively responsive to changes in day length (Nagy et al., 1993a). However, the observations that reproductive output is negatively affected by short day lengths (Hasler and Banks, 1975) and decreasing photoperiods can delay the onset of puberty in juvenile male lemmings (Gower et al., 1992) suggest that individuals of this species

Table 1. Somatic and Reproductive Responses to Day Length

	LD	*Males*	*Females*
Pelage score	**22:2**	4.95 ± 0.06	5 ± 0.0
	16:8	5 ± 0.0	5 ± 0.0
	8:16[a]	2.74 ± 0.19	2.45 ± 0.39
Spleen mass (mg)	**22:2**	62.13 ± 6.90	63.70 ± 10.61
	16:8	44.36 ± 7.34	67.04 ± 8.32
	8:16	48.43 ± 8.05	68.09 ± 9.41
Adrenal mass (mg)	**22:2**	11.72 ± 1.11	14.46 ± 1.65
	16:8	13.66 ± 1.14	13.66 ± 1.30
	8:16	13.52 ± 1.28	14.21 ± 1.48
Serum corticosterone (ng/mL)	**22:2**	305.76 ± 42.99	182.46 ± 64.48
	16:8	233.30 ± 41.82	214.27 ± 50.58
	8:16	252.90 ± 44.23	144.77 ± 60.79
Paired ovary mass (mg)	**22:2**	—	12.41 ± 2.11
	16:8	—	10.13 ± 1.64
	8:16	—	11.40 ± 2.14
Uterine mass (mg)	**22:2**	—	70.85 ± 8.27
	16:8	—	48.22 ± 6.53
	8:16	—	51.05 ± 7.46
Ovarian fat pad mass (mg)	**22:2**	—	177.12 ± 32.02
	16:8	—	79.84 ± 25.28[b]
	8:16	—	41.17 ± 28.88[b]

NOTE: Data are presented as mean ± SEM.
a. Pelage scores significantly lighter than lemmings in other photoperiods.
b. Significantly smaller than LD 22:2.

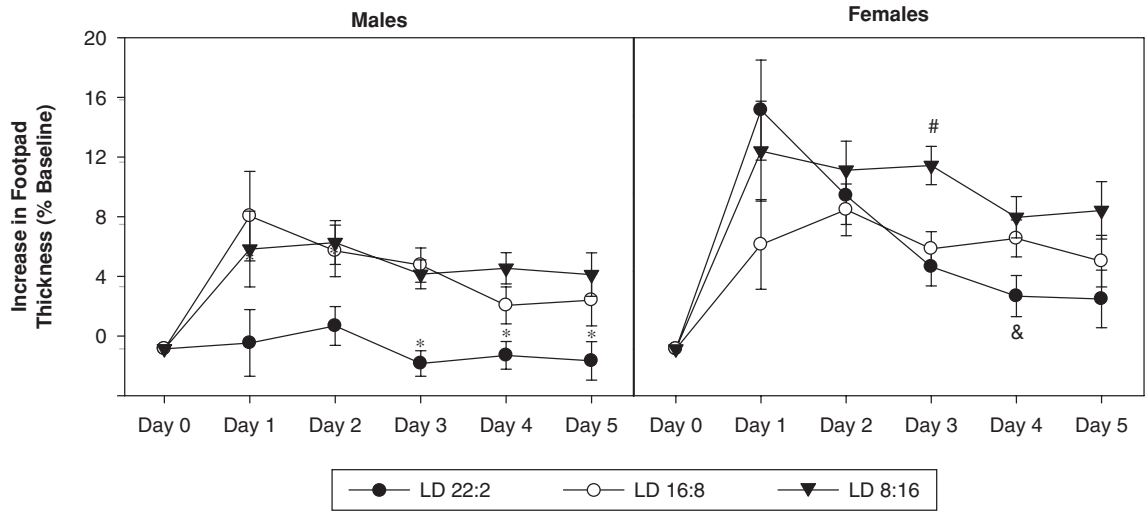

Figure 3. Photoperiod altered cell-mediated immune function. Delayed-type hypersensitivity (DTH) responses were higher in lemmings housed in LD 8:16 and 16:8 than those housed in LD 22:2. Females had stronger footpad DTH responses than males. Data are presented as mean percent of baseline footpad thickness ± SEM. *Significantly lower than all other photoperiod groups of the same sex. #Significantly higher than all other photoperiod groups of the same sex. &Significantly lower than other photoperiod groups of the same sex.

can process and respond reproductively to short days. Many previous studies bred lemmings in the same photoperiod they experienced as adults or transferred them to different photoperiods at birth or weaning. In the present experiment, lemmings were born and maintained in an intermediate day length (LD 16:8) until approximately 55 days of age. This prolonged exposure to a photoperiod regimen that is neither unambiguously stimulatory nor suppressive may have altered the way day-length information

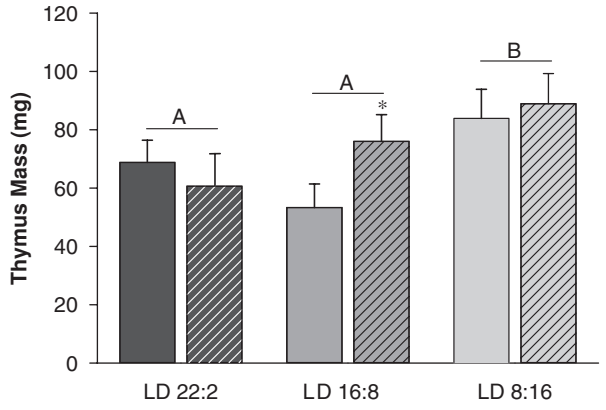

Figure 4. Exposure to short photoperiods resulted in increased thymic mass in both male and female lemmings. Data are presented as mean ± SEM. Bars with different letters are significantly different from each other. *Significantly different from LD 16:8 males. Solid bars represent males, and hatched bars represent females.

was processed later in life (Shaw and Goldman, 1995). Future studies are planned to reconcile the reproductive responses in our laboratory to those in previous reports.

Processing of day-length information into a physiological signal is achieved by nighttime secretion of the pineal indole, melatonin. Melatonin is the primary mediator of photoperiod-induced changes in the reproductive system and other nonreproductive traits of many seasonal breeders. Properly timed melatonin infusions of an appropriate duration can induce the winter phenotype in animals housed in long day lengths (Bartness et al., 1993). The immune system also responds to day-length information conveyed via changes in circulating melatonin (Nelson and Demas, 1997). Exogenous melatonin can induce the short-day enhancement of cell-mediated immune function in rodents (Demas and Nelson, 1998a). The melatonin rhythm is intact in collared lemmings (Reiter et al., 1990), and the period of melatonin rhythm is closely correlated with night length for each of the 3 photoperiods used in this study (Reiter et al., 1990; Gower et al., 1996). However, the amplitude and duration of the pineal melatonin rhythm were significantly blunted in lemmings housed in LD 22:2 (Gower et al., 1996). Collectively, these data suggest that the reduction in cell-mediated immune function in the lemmings housed in LD 22:2 may be mediated at least in part by a near absence of pineal-derived melatonin.

One of the most marked morphological responses to photoperiod in collared lemmings is the dramatic increase in body mass. Previous studies have reported a 50% increase in body mass following transfer from long to short days (Nagy et al., 1993b). Although mass gains in this study were more modest, lemmings housed in LD 8:16 were significantly heavier than those in other photoperiod conditions. Previous studies have indicated a link between fat stores and immune function. For instance, surgical removal of adipose tissue reduced humoral immune function

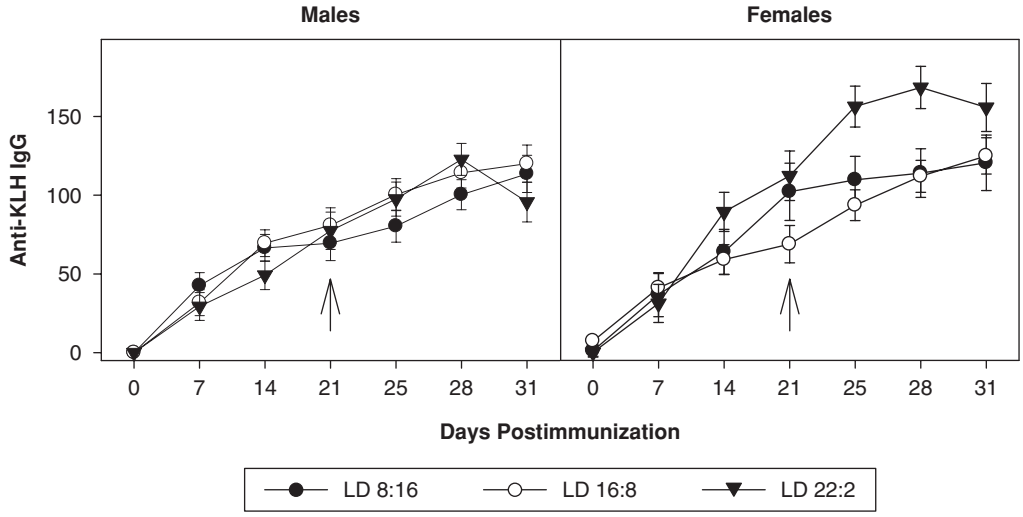

Figure 5. Primary and secondary anti-KLH IgG responses. Photoperiod did not strongly modulate the antibody production. Data are presented as mean ± SEM. Arrows indicate time of secondary immunization. KLH = keyhole limpet hemocyanin; IgG = immunoglobulin G.

(Demas et al., 2003). It is somewhat unexpected therefore that increased body mass was not specifically associated with enhanced immune function in the current study (i.e., body mass increases occurred only in LD 8:16 lemmings, but enhanced immune function occurred in both LD 8:16 and LD 16:8).

Female collared lemmings had stronger DTH responses and a trend toward greater antibody production as compared to males in this study. Sex differences in immune function have been reported for a number of species, with females generally having stronger responses than males (Klein, 2000). In the present study, male collared lemmings were more responsive to changes in photoperiod, both in terms of reproduction and immune function, than were females. Ultimately, it remains unclear why photoperiod-induced alterations in immune function should occur in males but not females. In any case, changes in thymus mass and pelage color in female lemmings suggest that the mechanism underlying photoperiodic time measurement in this species was functional in females despite the lack of alterations in the immune or reproductive systems. This result is consistent with studies in Siberian hamsters in which photoperiod-induced changes in DTH responses were apparent in males but not females (Bilbo and Nelson, 2003), although female Siberian hamsters undergo reproductive regression in short days (Weil et al., 2006). Future studies will address the proximate mechanisms underlying this sex difference in both immune function and immunological responsiveness to photoperiod.

Photoperiod differentially regulated reproductive, immunological, and somatic responses in collared lemmings. Cell-mediated immune function was stronger in animals housed in LD 16:8 and LD 8:16 as compared to LD 22:2. However, increased body mass, molt to a winter pelage, gonadal regression, and reduction in circulating testosterone occurred only in lemmings housed in LD 8:16. These patterns would suggest that there is not a direct link among the somatic, reproductive, and immunological responses to day length. That is, the enhanced DTH responses reported here are not simply secondary to reduced androgen concentrations or increased body mass. This outcome is consistent with previous reports that have suggested that photoperiod-mediated enhancement of immune function is independent of sex steroids (Demas and Nelson, 1998b; Prendergast et al., 2005). It is possible that a single chemical messenger is mediating photoperiod alterations in each of these processes (i.e., body mass, reproductive system, immune function), albeit with different thresholds for responses (Duncan et al., 1985; Nagy et al., 1993b). On the other hand, it remains possible that separate regulatory factors are controlling photoperiod-mediated alterations in reproductive, immunological, and morphological traits. For instance, prolonged exposure to LD 22:2 can induce gonadal regression in collared lemmings (Hasler et al., 1976; Gower et al., 1998). Because photoperiod history can alter the response to subsequent photoperiods, it will be interesting in the future to determine whether long-day exposure, in a pattern that induced gonadal regression, will alter the immunological adaptation to day length as well.

These data do not provide evidence that collared lemmings are less "immunocompetent" in LD 22:2 than in other photoperiods. Rather, these data indicate that the immune system of collared lemmings is capable of attending and responding to day-length cues. However, the DTH response does incorporate a number of immunological processes including immunological memory, cell proliferation, antigen presentation, leukocyte trafficking, and inflammatory processes into an integrative measure. DTH responses are associated with resistance to bacteria, fungi, and viruses (Dhabhar and McEwen, 1999). The ultimate causes of this phenomenon remain to be specified, and the use of static photoperiods may obscure ultimate causality in a rodent that experiences rapid changes in day length. However, it does appear that in concert with other photoperiodic rodents, the short days of winter are associated with enhanced immune function. Future research will be required to determine if short day lengths are enhancing cell-mediated immune function or if long day lengths are suppressing it. Finally, because of the dramatic multiyear population cycles that occur in the wild, future studies should investigate the role of population density in modulating photoperiod changes in the immune system.

These extremely high latitude–dwelling rodents display photoperiod-mediated variation in immune activity. In particular, exposure to short or intermediate day lengths enhanced DTH responses relative to lemmings housed in LD 22:2. This effect was mostly dependent on the nearly complete lack of DTH response in male lemmings housed in LD 22:2. There were no differences detected between lemmings housed in LD 8:16 and LD 16:8 in cell-mediated immune responses. This would suggest that photoperiod modulation of the immune response occurs in an "all-or-none" manner despite the evidence that other traits can vary in a manner proportional to day

length. To test this hypothesis further, however, requires testing multiple different lighting regimens at different points throughout development. Humoral immune function was not strongly regulated by photoperiod. This result is consistent with the general trend that cell-mediated immune function is more plastic than humoral immune function in a seasonal context (Demas et al., 1998a). (For a review of differential investment in immune function across the year, see Nelson, 2004). In any case, this model system will be informative for examining the interaction of ultimate and proximate factors in the evolution of photoperiod modulation of the immune system. One potential strategy to investigate these issues would be to use the naturally occurring properties of the lemming photoperiod system. Future studies will address both the mechanisms underlying these phenomena and the effects of early life photoperiods and photoperiod history on adult phenotype.

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