SEASONAL PATTERNS OF STRESS, IMMUNE FUNCTION, AND DISEASE

RANDY J. NELSON

The Ohio State University

GREGORY E. DEMAS

Indiana University

SABRA L. KLEIN

*Johns Hopkins School of
Public Health*

LANCE J. KRIEGSFELD

Columbia University

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Seasonality

1.1. Introduction

Life on Earth evolves in an environment with pronounced temporal fluctu-
ations. Rivers flow to the sea; the tides ebb and rise. Light availability and
temperature vary predictably throughout each day and across the seasons.
These fluctuations in environmental factors exert dramatic effects on biotic
activities. For example, the biochemical machinery of plants and animals un-
dergoes daily adjustments in production, performing some processes only at
night and others only during the day. Similarly, daily peaks in the metabolic
activity of homeothermic animals tend to coincide with the daily onset of
increased physical activity. Elevated activity alone does not drive metabolic
rates; rather, the general pattern of metabolic needs are anticipated by
reference to an internal biological clock. The ability to *anticipate* the onset
of the daily light and dark periods confers sufficient advantages that endoge-
nous, self-sustained circadian clocks are virtually ubiquitous among extant
organisms (Takahashi 1996; Menaker et al. 1997).

In addition to synchronizing biochemical, physiological, and behavioral
activities to the external environment, biological clocks are important to mul-
ticellular organisms in synchronizing internal processes. If a specific bio-
chemical process is most efficiently conducted in the dark, then individuals
that mobilize metabolic precursors, enzymes, and energy sources just prior
to the onset of dark would presumably have a selective advantage over indi-
viduals that organized their internal processes at random times. Thus, there
is a daily temporal chain, or phase relationship, to which all biochemical,
physiological, and behavioral processes are linked.

The scientific study of biological clocks and their associated rhythms is called chronobiology. This field borrows terms and concepts freely from engineering disciplines. For the purpose of this book, a biological clock is defined as a self-sustained oscillator (Pittendrigh 1960). A "rhythm" is a recurrent event that is characterized by its period, frequency, amplitude, and phase. "Period" is the length of time required to complete one cycle of the rhythm under study. "Frequency" is computed as the number of completed cycles per unit of time. The amount of change above and below the average value of a rhythm is called "amplitude." The "phase" represents a point on the rhythm relative to some objective temporal point associated with another cycle.

When animals, and virtually all plants, are placed in constant conditions, the period of their daily rhythms drifts slightly from 24 hours each succeeding day; these rhythms are called circadian (*circa* = about; *dies* = day [Halberg 1961]) rhythms. Individuals that are no longer synchronized (entrained) to the external factors are said to be free-running. The recurrence of free-running rhythms generated in the absence of external temporal cues is the most convincing evidence for an endogenous timekeeper. Additional compelling evidence that supports the contention that biological rhythms are generated from within the organism and not driven by unknown geophysical forces includes the observation that animals maintained in constant conditions aboard a spacecraft orbiting far above the planet (where geophysical cues are presumably absent) continue to display biological rhythms with periods similar to those observed on Earth. Other features of circadian biological rhythms are listed in Table 1.1.

The periods of some biological rhythms, including most central nervous system, cardiovascular, and respiratory rhythms, vary widely within the same

Table 1.1. *Features of Circadian Rhythms (from Pittendrigh 1960).*

 1. Free-running circadian rhythms only approximate the period of the daily rotation of the Earth.
 3. Circadian rhythms are endogenous.
 4. Circadian rhythms are typically self-sustained oscillators.
 5. Circadian rhythms are unlearned.
 6. Circadian rhythms are extant at all levels of physiological organization.
 7. Free-running circadian rhythms display little variance.
 8. Circadian rhythms compensate for temperature.
 9. Circadian rhythms are often affected by light intensity.
10. Circadian rhythms are usually unaffected by chemical perturbations.
11. Phase of circadian rhythms can be shifted by a single light (or temperature) signal.
12. Circadian rhythms can be synchronized by a limited set of environmental periodicities.

Table 1.2. *Relationship between Biological Rhythms and Environmental Cycles.*

Biological Rhythm	Environmental Cycle	Period Length	
		Entrained	Free-running
Circadian	Revolution of the Earth	24 hours	22–26 hours
Circatidal	Ebb and rise of tides	12.4 hours	11–14 hours
Circalunar	Phases of the moon	29.5 days	26–32 days
Circannual	Seasons of the year	365.25 days	330–400 days

individual. During physical exertion, for example, the period of heartbeats decreases (i.e., the frequency increases). Other biological rhythms, such as the ovarian rhythm, may remain relatively constant within the same individual, but vary significantly among different conspecific individuals. Four types of biological rhythms are typically coupled with geophysical cycles and generally do not vary under natural conditions. These relatively constant biological rhythms correspond to the periods of the geophysical cycles of night and day (circadian), the tides (circatidal), the phases of the moon (circalunar), and the seasons of the year (circannual) (Table 1.2). To appreciate the mechanisms underlying seasonal fluctuations in immune function and disease processes, the remainder of this chapter will emphasize the mechanisms of circadian and circannual rhythms as they relate to seasonality.

Not unexpectedly, circadian clocks have evolved in organisms that typically live for more than one day. Short-lived (i.e., <1 day) species, such as protists, do not exhibit true circadian rhythms. Similarly, biological clocks that measure yearly intervals are present only in animals that, on average, live longer than one year. During the course of a year, conditions can change dramatically. Seasonal fluctuations in energy availability create thermoregulatory and other physiological demands that serve as potent selective forces in the evolution of life. At high latitudes and altitudes, the seasonal fluctuations in temperature between winter and summer are substantial, and individuals that inhabit these challenging conditions have evolved mechanisms to cope with the wide alterations in ambient conditions. Animals that withstand severe seasonal fluctuations undergo such striking seasonal adaptations, that summer- and winter-captured individuals of the same species have often been mistaken for different species (Figure 1.1). Development of these seasonal adaptations permits plants and animals to exploit specific spatial niches that vary in quality over time.

Nontropical animals display many distinct behavioral adaptations to cope with the challenge of winter survival (Moffatt et al. 1993; Bronson &

Fig. 1.1. Siberian hamsters in summer and winter conditions. These animals undergo significant seasonal changes in physical appearance. In addition to the season change in coat color, which helps them blend into the background environment of summer (dark) or winter (white), many other adaptations have evolved to combat the energetic demands of winter. Some of these winter adaptations include changes in food intake and body mass, torpor, foraging, nest-building, reductions of social interactions including sexual interactions. Some animals such as collared lemmings, develop extra "digging claws" during short day exposure. These claws help these lemmings did through the winter snow to find food. (Photo by Aaron Jasnow.)

Heideman 1994). Behaviorally, individuals may hibernate, estivate, or migrate during specific energetically challenging seasons of the year. Social organization may change; highly territorial individuals that are aggressive during the breeding season may form social groups during the winter to reap the thermoregulatory benefits of huddling (McShea 1990; Ancel et al. 1997). Similarly, small mammals may join communal nests during the winter where warmth and humidity are conserved (Madison 1984). The foraging and feeding behaviors of animals may also change seasonally. For example, territorial birds may join large winter foraging flocks, or individuals may change from a nocturnal activity pattern during the summer to a diurnal activity pattern during the winter to conserve thermogenic energy while foraging

(Horton 1984). Animals may hoard food in the autumn to ensure sufficient provisions until spring (Bartness 1995). Many individuals build large, insulative nests during the winter (Dark & Zucker 1983).

Cessation of breeding during winter is perhaps the central seasonal adjustment used by animals to conserve energy. Presumably, the benefits associated with winter breeding rarely outweigh the costs. To prepare in advance for seasonal changes in ambient conditions, animals must be able to determine the season of the year. Seasonal adaptations often require significant time to develop so the ability to ascertain the time of year to *anticipate* energetically demanding conditions is critical for survival. Thus, the development of winter adaptations must begin prior to the onset of the challenging winter conditions, not in response to them. Several mechanisms have evolved that provide seasonal information. In some species, an annual clock, analogous to the circadian clock, has evolved; in other species, circadian clocks are used to determine day lengths (i.e., photoperiod), and day length information is transduced into seasonally appropriate responses (Gwinner 1986; Bartness et al. 1993). In any case, many species of animals have evolved biological clocks that time a wide variety of seasonal biological rhythms.

Post-technology humans are somewhat buffered from temporal perturbations in the environment. Despite the failure to identify adaptive benefits of seasonal breeding in humans, seasonal fluctuations in human reproduction have been reported (Roenneberg & Aschoff 1990; Bronson 1995). Although the factors driving seasonal breeding (e.g., decreased food availability, low ambient temperatures) in industrialized humans are now reduced in importance, there are no obvious forces selecting *against* seasonality. Therefore, seasonality in human reproduction persists (reviewed in Bronson 1995), and the timing mechanisms that measure yearly intervals presumably remain extant among humans (Nelson 1990; Czeisler 1995).

In addition to seasonal changes in behavior and reproductive function, several other physiological and morphological adaptations have also evolved to help individuals cope with winter energy shortages. For example, winter-evoked changes in basal metabolic rate, nonshivering thermogenesis, body mass, pelage development, gut efficiency, and endocrine function are common among small mammals (Moffatt et al. 1993). Gonadotropin and prolactin concentrations decline, sex steroid hormone production wanes, and reproductive activities stop prior to winter. Virtually all of these seasonal adaptations are initiated among small mammals in nature during autumn or in the laboratory by exposure to short (<12-hour light/day) day lengths. Taken together, these seasonal adaptations have evolved to maintain a positive energy

balance during winter energy shortages, that is, when food availability is low and thermoregulatory demands are high.

Maintaining a positive energy balance is required for survival and reproductive success. The vast majority of studies of seasonal phenomena have focused on energetic adaptations, especially reproductive adaptations. However, other threats to survival must also be met in order for individuals to survive and increase their fitness. Individuals must avoid predators, they must avoid potentially dangerous attacks by conspecific competitors, and they must avoid succumbing to pathogens or parasites. In some cases, a marginal energetic balance can weaken animals to the extent that they are very susceptible to disease (Berczi 1986). Immunological defense against invading organisms requires cascades of mitotic processes that presumably demand substantial energy (Demas et al. 1997; Spurlock 1997). The energetic costs associated with immunity may be a critical factor in seasonal fluctuations in immune function. Also, environmental factors that can interrupt breeding (e.g., a flood or a late blizzard), or other conditions perceived as stressful, can compromise immune function and promote opportunistic pathogens and parasites to an extent that leads to premature death (Berczi 1986; Ader & Cohen 1993). Factors that interrupt breeding are often unpredictable (Wingfield & Kenagy 1991), but many potential stressful conditions, such as low ambient temperatures, reduced food availability, migration, overcrowding, lack of cover, or increased predator pressure, can recur on a somewhat predictable, seasonal basis potentially leading to seasonal changes in population-wide immune function and death (Fänge & Silverin 1985; Lee & McDonald 1985; McDonald et al. 1988; John 1994; Lochmiller et al. 1994; Sinclair & Lochmiller 2000). Thus, in addition to the well-studied seasonal cycles of mating and birth, there are also dramatic, albeit not as well studied, seasonal cycles of illness and death among human and nonhuman animal populations (Descôteaux & Mihok 1986; Lochmiller et al. 1986; Lochmiller & Deerenberg 2000; Sinclair & Lochmiller 2000) (Figure 1.2).

Seasonal fluctuations in immune function and survivorship may not be observed every year or in every population. Some winters may not be perceived as stressful, either because of mild ambient conditions or because energetic coping adaptations succeed to buffer individuals from harsh conditions. Consequently, populations in nature may exhibit compromised, enhanced, or static immune indices during the winter, although we suspect that the literature will be biased in favor of reporting *changes* in immune function. The literature on seasonal fluctuations in immune function is summarized in later chapters.

The working hypothesis of this book is that some individuals have evolved mechanisms to predict seasonal stressor-induced reductions in immune
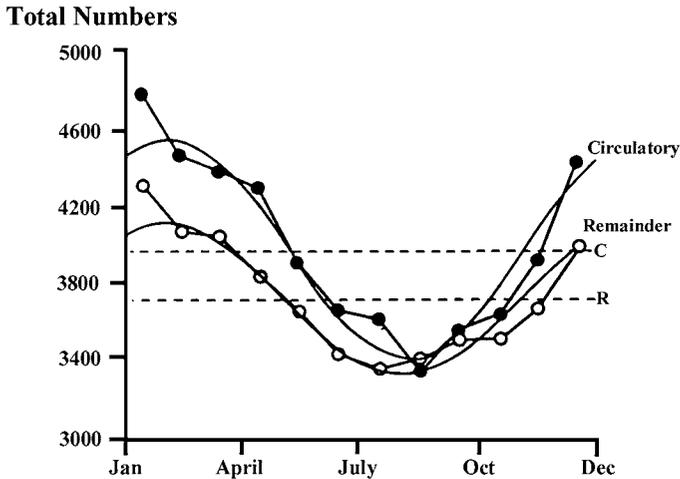
Total Numbers

Fig. 1.2. Seasonal cycle of death. These data show the number of human deaths over the course of 1 year in Aberdeen, Scotland. Human mortality from circulatory and other causes also shows a similar seasonal fluctuation with voles, with peak number of deaths observed during the winter and relatively low mortality during the summer. C, circulatory; R, remainder. (Data redrawn from Douglas et al. 1991.)

function and make appropriate adjustments in anticipation of challenging conditions, as a temporal adaptation to promote winter survival. From an adaptive functional perspective, we propose that individuals "optimize" immune function so that they can tolerate minor infections if the energetic costs of mounting an immune response outweigh the benefits. Available energy is partitioned into competing functions where most needed for survival. Thus, when energetic requirements are high (e.g., during migration, pregnancy, territory defense, lactation, or winter), we predict that immune function should be reduced. During energetically challenging winters, energy is used for thermoregulation and maximal immune function possible, rather than growth, reproduction, or other nonessential processes. Recent evidence from bighorn sheep suggests an energetic trade-off between the costs of immune and reproductive functions (Festa-Bianchet 1989); lactating ewes exhibit higher parasitic infections, compared with nonlactating ewes. Presumably, the energetic costs of lactation reduce the amount of energy that can be allocated to immune function.

We hypothesize that exposure to short day lengths enhances immune function. Many field and clinical studies are consistent with this hypothesis (i.e., lymphatic tissue size or immune function is elevated during the winter). All laboratory studies are consistent with this hypothesis: for example, immune

function is enhanced in animals housed in short days, compared with animals maintained in long day length conditions (reviewed in Nelson & Demas 1996; cf. Yellon et al. 1999). These results suggest that mechanisms have evolved to allow animals to anticipate immunologically challenging conditions by monitoring photoperiod. Presumably, this ability permits individuals to cope with these health-threatening seasonal conditions by bolstering lymphatic tissue development and immune function directly.

This book addresses the physiological mechanisms underlying the detection of and the response to seasonal environmental factors that affect immune function and disease processes. In this first chapter, the geophysical factors leading to seasonality are reviewed. We also describe the proximate mechanisms underlying seasonality. The literature on seasonal breeding is used as a departure point to understand the mechanisms of seasonality as it pertains to immune function. Although circadian rhythms in immune function have become recognized as important factors in disease and immunotherapy, this topic is beyond the scope of the present book. Interested readers are referred to several recent papers that have carefully reviewed this field (e.g., Hrushesky 1991; Petrovsky & Harrison 1998; Cardinali et al. 1999).

In Chapter 2, basic immunology is reviewed. Reports of seasonal fluctuations in disease prevalence are described in Chapter 3, followed by a review in Chapter 4 of the literature reporting seasonal fluctuations in lymphatic organ size and structure, as well as immune function. Possible interactions between lymphatic organ morphology and function and recurrent environmental stressors are also discussed in this chapter. Most research in seasonality has focused on the role of photoperiod in providing temporal information for breeding (Reiter 1993). In Chapter 5, the physiological sequelae by which photoperiod mediates the seasonal changes in immune function are assessed. The energetics of immune function will be discussed in Chapter 6. Among small mammals, short photoperiods reduce blood concentrations of sex steroid hormones (in long-day breeders) and prolactin, as well as alter the temporal pattern of pineal melatonin secretion (Goldman & Nelson 1993). The effects of these hormones on immune function and opportunistic diseases, including cancer, will be explored in Chapter 7. Melatonin will receive particular attention for its effects on immune function in Chapter 8 because this hormone has been reported to affect immune function and tumorigenesis in several model animal systems. The clinical relevance of these seasonal fluctuations in lymphatic tissue size and immune function for humans and nonhuman animals will be presented in Chapter 9. Our goal for this book is to present a comprehensive description of the field, laboratory, and clinical data on seasonal immunity and disease processes from an adaptive functional perspective.

1.2. Geophysical Factors Leading to Seasonality

To understand biological rhythms, it is helpful to understand the geophysical fluctuations that have shaped these rhythms. In reference to the stars, the Earth rotates on its axis every 23 hours, 56 minutes, and 4 seconds, a period of time termed a "sidereal day" (U.S. Naval Observatory 1999). The daily rotation (e.g., from noon to noon) of the Earth is 24 hours, 3 minutes, and 57 seconds when measured in reference to a closer object, namely the sun; this period of time is called the mean "solar day." The length of the solar day is not constant throughout the year because of the elliptical orbit of the Earth around the sun. The rate of orbit tends to increase slightly when a planet is near the sun (perihelion) and tends to decrease slightly when a planet is further from the sun (Kepler's Second Law) (Brosche & Sündermann 1990). The actual solar day is nearly 16 minutes shorter than the mean solar day on or about 3 November each year. The solar day length matches the mean solar day length on only four dates: 15 April, 14 June, 1 September, and 25 December. Although the astronomical measures of day length reveal substantial variation in the timing of the daily rotation of the Earth, for practical purposes, 24 hours is a reasonable approximation of the period of the Earth's daily rotation (Saunders 1977). Although day length, as measured by the position of the sun on each horizon, is very precise, the amount of twilight varies substantially and rarely is calculated into the amount of total day light received each day. Despite sounding sudden, "the break of day" or "night fall" really reflects gradual processes in changing light during dawn or dusk. The vast majority of laboratory studies of photoperiodism do not use gradual onsets or offsets of illumination (cf. Gorman et al. 1997); rather, they use timers that turn lights on and off in one step. Notably, the light sensitivity of the photoreceptors that measure day length is not characterized for most species, and the importance of dim light in photoperiodic time measurement remains unspecified.

The Earth completes about 365.25 (365 days, 6 hours, 9 minutes, and 10 seconds) rotations during each revolution around the sun, a period of time called the sidereal year (Brosche & Sündermann 1990). Unlike the condition on the moon, where one side is always facing away from the sun, the daily rotation of the Earth allows most of the surface to bask in sunlight regularly. The shape of the elliptical revolution of the Earth around the sun explains why winters are generally milder in the Southern Hemisphere, compared with the Northern Hemisphere, but does not explain the basis of seasons. The phenomenon of seasons results from the tilt of the planet's axis of rotation (approximately 23.5°) with respect to its plane of revolution around the sun (Figure 1.3). Thus, as the Earth revolves around the sun, the proportion of
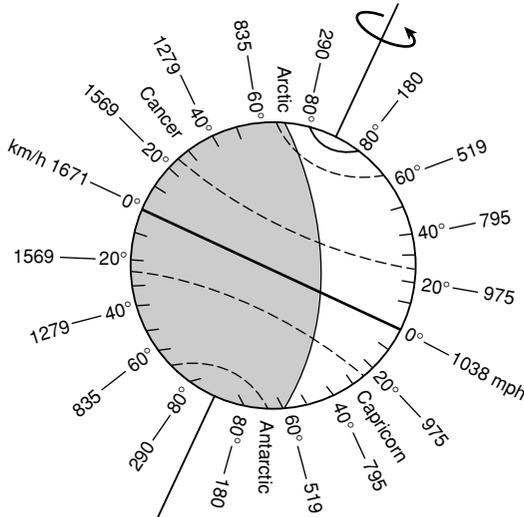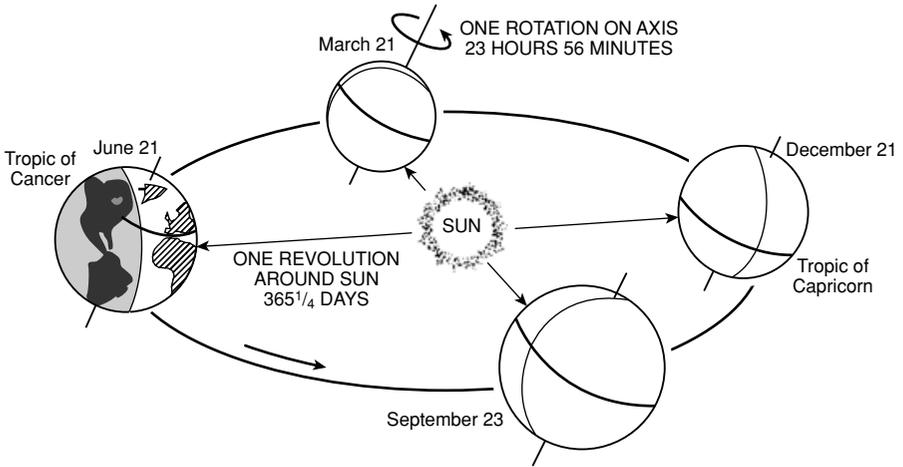
Fig. 1.3. The plane of revolution of Earth around the sun accounts for the seasons. (Reprinted from M. C. Moore-Ede, F. M. Sulzman, & C. A. Fuller, *The Clocks That Time Us*, copyright © 1982, p. 2.)

the planet exposed to the radiating energy changes (Officer & Page 1993). During the Northern Hemisphere winter, the Earth is furthest from the sun, and the planet is tilted away from the sun.

When viewed from Earth, the sun moves across the sky each day from east to west. There is also a yearly pattern of the sun's movement from day

to day. The sun reaches its most northern point in the sky (i.e., declination 23.5°N) about 21–22, June; this is the date of the summer solstice in the Northern Hemisphere and winter solstice in the Southern Hemisphere (Brosche & Sündermann 1990). The sun reaches its most southern point in the sky (i.e., declination 23.5°S) about 21–22, December; this is the date of the winter solstice in the Northern Hemisphere and summer solstice in the Southern Hemisphere. The equinoxes, which occur twice a year, are the points at which the Earth's celestial equator intersects its ecliptic (Figure 1.3). In the Northern Hemisphere, the autumnal equinox is about 21–22 September, and the vernal equinox is about 21–22 March.

One very important feature of this annual progression of the sun's movement is that day length (i.e., the amount of light/day) varies. Except at the equator, day lengths during the winter are shorter than day lengths during the summer. The exact value for day length depends on latitude (Figure 1.4), with higher variation between winter and summer day lengths at high latitudes and lower variation between winter and summer day lengths closer to the equator (Brosche & Sündermann 1990). At the two equinoxes, day length is exactly twelve hours at all points on the globe. Thus, with just two pieces of information – (1) the day length and (2) if day lengths are increasing or decreasing – the exact time of year can be determined. Plants and animals have evolved mechanisms to determine the precise time of year by using day length (photoperiod) information (Sadleir 1969; Goldman & Nelson 1993; Goldman 2001).

The lengthening days of summer are associated with rising ambient temperatures, increased biotic growth, and changes in humidity (Officer & Page 1993). Similarly, the daily cycles of day and night evoke the familiar daily cycles of temperature, humidity, and photosynthesis. Life on Earth has evolved in such a manner to exploit these daily and annual cycles in energy availability. Plants and animals have evolved to fill *temporal*, as well as spatial, niches. Because so many biochemical processes are affected by light, it is relatively straightforward to imagine how circadian rhythms may have arisen early in the evolution of life. Many protists do not display circadian rhythms. This observation may reflect that many protists do not live more than a few hours, so these organisms have no reason to track daily time; alternatively, this observation may simply reflect that protists have too limited DNA stores to encode a biological clock. The ability to measure photoperiod (i.e., photoperiodism) to determine time of year, likely evolved after organisms began to survive for longer than one year. The next section describes some of the environmental cues that can be monitored by animals in conjunction with, or in addition to, photoperiod to provide information about time of year.
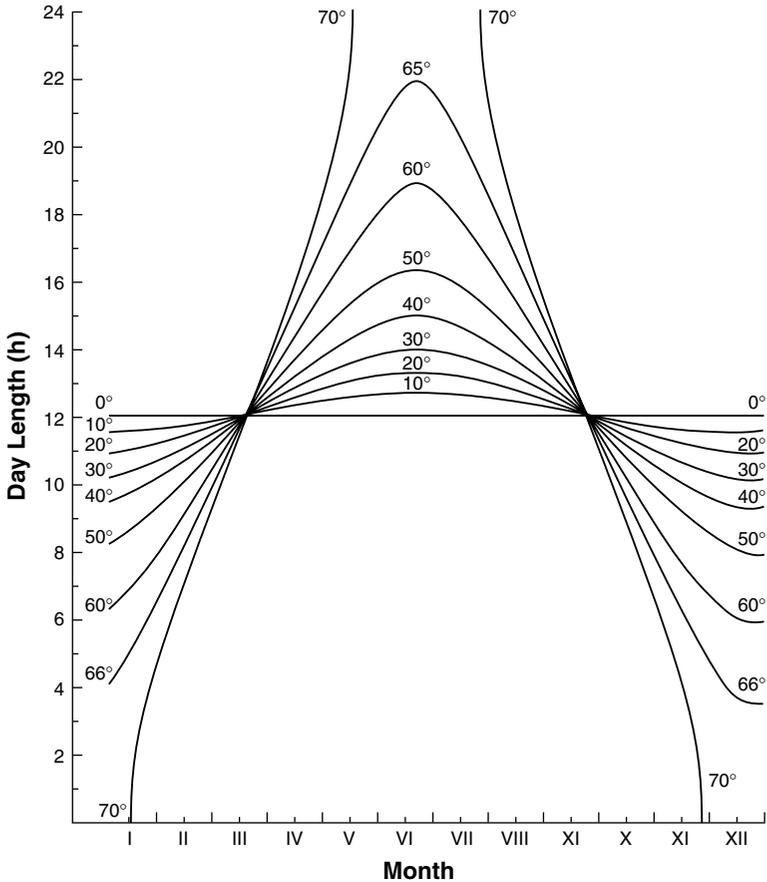
Fig. 1.4. Annual change in day length at different latitudes. Note that the variation in annual day length is highest at high latitudes, and this variation is reduced at low latitudes. Because the seasonal climatic changes are often more pronounced at high versus low latitudes, photoperiodism is more common among high versus low latitude species.

1.3. Potential Environmental Cues for Synchronization of Seasonal Cycles

Individuals inhabiting nontropical habitats experience seasonal deterioration and renewal of environmental energy resources. High thermoregulatory requirements for small mammals and birds during the winter typically coincide with low environmental food availability (Blank & Ruf 1992; Wunder 1992; Wingfield & Farner 1993). The energetic "bottleneck" created by high energy demands during times of low energy availability has led to the evolution of many adaptations that allow individuals to cope with winter. Breeding is

energetically expensive, and cessation of breeding activities is central among the suite of winter-coping adaptations observed in boreal and temperate zone species (Bronson & Heideman 1994). Tactics have evolved that permit individuals to maximize the length of the breeding season without jeopardizing survival vis-à-vis energy use. Because seasonal adaptations often require time to develop, the precise timing of behavioral and physiological adaptations necessary to cope with energy shortages is a critical feature of individual reproductive success and subsequent fitness. Mechanisms have evolved to ascertain the time of year precisely to phase territorial defense, breeding, molt, migration, and other energetically expensive activities to coincide with peak energy availability and other local conditions that promote survival (Bronson 1989; Moffatt et al. 1993; Wingfield & Farner 1993; Goldman 2001).

In some cases, physiological and behavioral changes that have an obvious and immediate adaptive function may occur in direct response to environmental factors. For example, food and water may be available only during certain times of the year, and a decrease in the amount of procurable nutrients can lead to reproductive inhibition (Nelson 1987; Wingfield & Kenagy 1991; Bronson & Heideman 1994). These types of environmental factors have been termed the "ultimate factors" underlying seasonality (Baker 1938). Many animals need to forecast the onset or offset of these ultimate factors to initiate time-consuming seasonal adaptations. Therefore, seasonally breeding animals frequently detect and respond to environmental cues that accurately signal, well in advance, the arrival or departure of seasons favoring reproductive success. The cues, or "proximate" factors (Baker 1938), used to predict environmental change may or may not have direct survival value (e.g., photoperiod), and may or may not be the same as the ultimate factor (e.g., food availability).

Several environmental factors that vary across seasons could potentially serve as temporal cues for animals to discern the time of year. For example, ambient temperature, food quality and quantity, water availability, relative humidity, precipitation, and sunlight (i.e., diurnal variation in visible and ultraviolet radiation, day length, etc.) all vary on an annual cycle in most habitats. Individuals may respond to a single cue or to numerous environmental factors. For example, California voles (*Microtus californicus*) breed during the winter months along the West Coast of the United States and Mexico. Their breeding season, from November to May, coincides with the onset of the California rainy season, the subsequent availability of green food, short day lengths, and reduced ambient temperature (Lidicker 1973). California voles regress their reproductive systems in the summer when exposed to long days, high ambient temperatures, reduced green vegetation, and reduced free-standing water (Lidicker 1973). To sort out the environmental factors responsible for timing

the onset and termination of breeding in this species, animals were moved into the laboratory and housed individually in either long (Light:Dark [LD] 14:10; 14 hours of light and 10 hours of dark/day) or short (LD 8:16) days, and provided with *ad libitum* food and water (Nelson et al. 1983). Voles in short days display significant regression of reproductive function, compared with their long-day counterparts; these data suggested that if these voles only responded to photoperiod, then their reproductive function would be 180° out of phase with their breeding season. Presumably, some other factor in nature masked the short-day gonadal inhibition. Because the appearance of green vegetation coincides with the onset of breeding in this species, other short-day California voles were fed fresh spinach in the laboratory. The supplemental green food blocked gonadal inhibition in short day lengths (Figure 1.5).
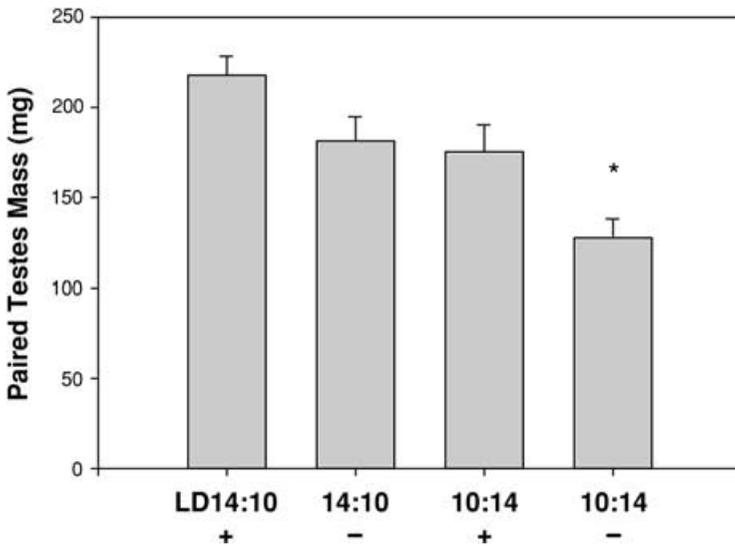

Fig. 1.5. Reproductive function in California voles exposed to different environmental factors. Mean (±SEM) paired testes size of California voles (*Microtus californicus*) in long (LD 14:10) or short (LD 10:14) photoperiods. Voles that received fresh spinach thrice weekly are noted by the "+" designation. Note that testes size (and function) was reduced in short days, but that exposure to greens prevented the short-day gonadal regression. Restricted access to water in either photoperiod suppressed testes mass. Taken together, these data suggest that the onset of the breeding season of California voles is mediated by the availability of fresh green vegetation during the California winter; cessation of breeding occurs during the annual summer drought. (Reprinted from the *Journal of Reproduction & Fertility*, Vol. 69, R. J. Nelson, J. Dark, & I. Zucker, "Influence of photoperiod, nutrition and water availability on reproduction of male California voles (*Microtus californicus*)," pp. 673–7, copyright © 1983.)

During the annual summer drought in the California and Baja Peninsula habitat, free-standing water is scarce, and California voles and other animals appear to obtain water by licking dew from plants. When water bottles were provided to laboratory-housed voles only during the 2 hours that dew was available on plants outside, the reproductive system regressed, despite exposure to long day lengths.

In sum, the breeding season of California voles is activated by the presence of green vegetation that overrides the inhibitory effects of short days on reproductive function (Nelson et al. 1983). Reproductive regression is observed during the summer in field-caught voles, and this appears to be induced by water shortages. Provision of voles with water extends breeding throughout the annual summer drought in nature (Lidicker 1973). Thus, seasonal adaptations, including reproductive inhibition, are often regulated by complex interactions among several extrinsic factors that affect survival and reproductive success. Because these extrinsic factors tend to covary in the field, laboratory studies are usually informative in teasing apart the contribution of various extrinsic factors used in the timing of seasonal adaptations.

1.3.1. Food Quality

The quality of food varies across seasons. Several components of food, including trace elements, display seasonal variation in abundance (Bronson 1989). One well-studied plant compound, 6-methoxy-2-benzoxazolinone (6-MBOA), has been implicated in the timing of rodent seasonal breeding. The precursor of 6-MBOA, 2,4-dihydroxy-7-methoxy-2-11,4-benzoxzin-3(4H)-one (DIMBOA), is abundant as the primary glucoside in young seedlings and vegetatively growing plants (Berger et al. 1981). Plant injury coincident with grazing evokes the release of enzymes that rapidly convert DIMBOA to 6-MBOA. Thus, detection of 6-MBOA in the diet is a reliable signal that the growing season has begun and that food is immediately available. 6-MBOA induces midwinter breeding in natural populations of arvicoline rodents (Berger et al. 1981). For example, two nonbreeding winter populations of montane voles (*Microtus montanus*) received supplemental feedings of rolled oats, with or without a coating of 6-MBOA. The first study was conducted in November to December and was replicated in January to February. In both cases, reproduction began after 3 weeks for 6-MBOA-supplemented voles; testicular weights doubled in the males receiving the compound. The incidence of pregnancy was 60% in the supplemented populations, compared with 0% in the unsupplemented animals (Berger et al. 1981). In laboratory studies, 6-MBOA overrides inhibitory photoperiodic information, and

stimulates ovarian and uterine development in several species (Epstein et al.
1986; Korn & Taitt 1987; Schadler et al. 1988). Rodents that feed mainly on
monocotyledons have been hypothesized to respond to 6-MBOA, whereas an-
imals that feed on dicotyledons have been predicted to be unresponsive to the
compound (Negus & Berger 1987). The phenomenology of a plant compound
overriding photoperiodic information is fascinating, but many questions re-
garding the mechanisms of action for 6-MBOA remain unanswered (Nelson
& Blom 1993). Although 6-MBOA has been reported to be structurally simi-
lar to melatonin (Sanders et al. 1981; Anderson et al. 1988), the interaction of
6-MBOA with melatonin receptors has not been established. The importance
of any number of other trace substances in the normal seasonal timing of
breeding is vastly understudied (e.g., Martinet & Meunier 1969; Bronson
1989).

1.3.2. Food Quantity

As noted previously, food availability is probably the ultimate factor driving
seasonal breeding (Immelmann 1973; Bronson 1979). In many cases, food
availability can also serve as a proximate factor in the timing of seasonal
breeding (Bronson 1989). Severe, chronic food restriction stops reproductive
activities in both male and female mammals. Most studies of the physiological
effects of restricted food intake on reproduction have been conducted in the
laboratory using rats. These studies generally indicate that the hypothalamic
gonadotropin-releasing hormone (GnRH) pulse generator of food-deprived
animals produces fewer bursts of GnRH release (i.e., reduced GnRH pulse
frequency) and less GnRH per pulse (i.e., reduced GnRH pulse amplitude)
(Campbell et al. 1977). It is likely that the hypothalamic GnRH system is the
final common pathway through which all extrinsic factors operate on breeding
(reviewed in Bronson & Heideman 1994).

   The gonads are compartmentalized into two component functions: (1) ga-
metogenesis and (2) steroidogenesis. In males, pituitary luteinizing hormone
(LH), released in response to GnRH, stimulates the Leydig cells of the testes
to synthesize androgens (Campbell et al. 1977). Androgens act on the hy-
pothalamus and anterior pituitary gland to reduce secretion of GnRH and
LH via a classic negative feedback loop (Figure 1.6). Prolactin is critical for
testicular steroidogenesis because, among other things, it promotes LH re-
ceptor formation in the Leydig cells. Spermatogenesis is mediated by follicle-
stimulating hormone (FSH) from the anterior pituitary and testosterone from
the testicular Leydig cells. FSH stimulates the epithelium of the seminiferous
tubules; androgens stimulate spermatogenesis. Thus, LH indirectly affects
spermatogenesis because of its effects on androgen production. The regulation
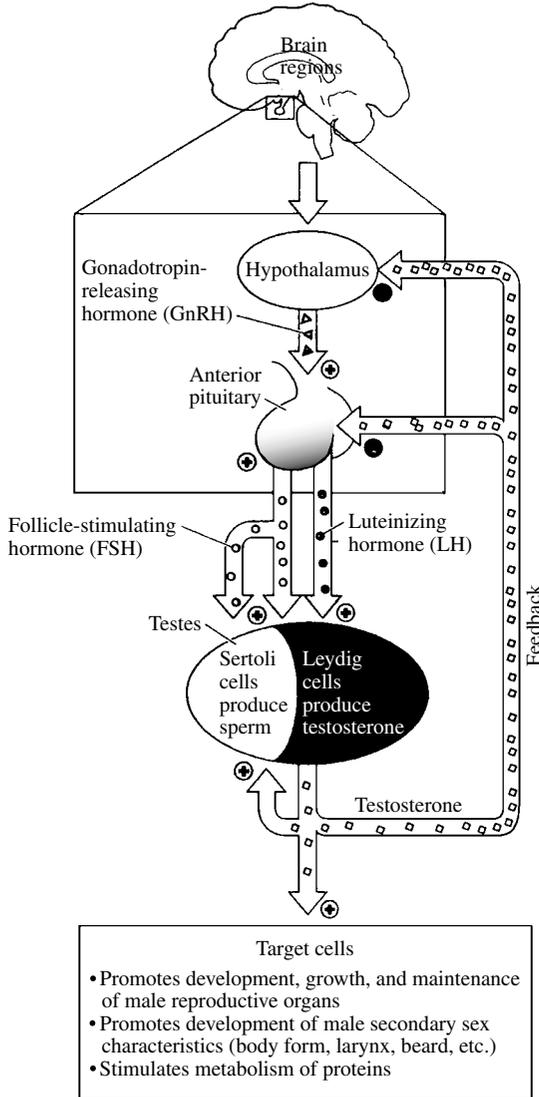
Fig. 1.6. Reproductive hormones and negative feedback. GnRH is released in a pulsatile manner from the hypothalamus. GnRH stimulates the pulsatile release of the gonadotropins (LH and FSH) from the anterior pituitary gland. LH stimulates steroid production from the gonads. A complex negative feedback relationship exists among the hypothalamus, anterior pituitary, and testes. The steroid hormones from the testes feedback to inhibit secretory activity in both the hypothalamus and pituitary gland. In parallel, increasing LH and FSH blood concentrations feedback to slow down their secretion from the anterior pituitary, as well as GnRH from the hypothalamus. Likewise, as GnRH is secreted, the hypothalamus responds to increasing concentrations of this hormone by reducing its secretion.