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Invited Review

Effects of photoperiod and temperature on testicular function in amphibians

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Summary. Most amphibians present an annual testicular cycle characterized by a quiescent period (late autumn-winter) and a spermatogenic period (spring and summer). At the end of the period of spermatogenesis undifferentiated interstitial cells transform into steroid-secreting Leydig cells which regress in spring at the beginning of the new spermatogenetic cycle. The testicular cycle is controlled by the pituitary gonadotropin levels which are high in autumn and winter, low in spring and increase temporarily in the middle of summer.

Photoperiod and temperature seem to be the most important external factors involved in the regulation of this cycle in many amphibian species since the colder the geographic area, the longer the quiescent period and the shorter the spermatogenic period. This suggests the occurrence of a potentially continuous cycle in these species, in contrast with that which occurs in other species having an endogenous rhythm of testicular function which is much less sensitive to environmental factors.

Although the specific response to temperature can vary widely between species, the most frequent observation in amphibians with a potentially continuous cycle is that exposure to mild temperatures (15-20° C, according to the spring temperatures of the different geographic areas) stimulates spermatogenesis even during the period of testicular quiescence. If this mild temperature is combined with a long photoperiod, complete spermatogenesis is attained. Experiments performed during the period of germ-cell proliferation (development from spermatogonia to round spermatids) indicated that low temperatures (below 11° C) as well as short photoperiods (less than 8 h of light) hinder germ-cell proliferation. Moderately high temperatures (about 30° C) do not impair this proliferation. In the newt *Triturus marmoratus*, it has been shown that an excessively long photoperiod (over 16 h) has the same effect as a short photoperiod. In this species eyes are not required for the testicular photoperiodic response.

Photoperiod appears to have no effect on spermiogenesis (differentiation of round spermatids into spermatozoa), because once round spermatids are formed, spermiogenesis will occur even in total darkness. Mild temperatures seem to be necessary for spermiogenesis as well as for androgen biosynthesis because neither process will take place at extreme temperatures. Results on the effect of photoperiod in steroidogenesis differ between species.

Key words: Amphibians, Testis, Photoperiod, Temperature, Gonadotropins, Androgens

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1. Introduction

Baker (1938a) classified the factors that affect animal reproduction into two groups: a) ultimate factors, such as an abundant food supply, exert a selective pressure which ensures that a species breeds during a precise, optimal period of the year: and b) proximate factors, which are environmental factors directly affecting physiological processes including those involved in reproduction. Photoperiod, temperature, and rainfall are the major proximate factors. Animals inhabiting geographic areas with considerable seasonal changes in environmental conditions adapt to these variations and synchronize their reproductive activity to that period of the year when conditions are optimal for survival of the offspring. Thus, reproduction is limited to times with the most suitable climatic conditions and food supply (Lofts, 1975).

In tropical and ecuatorial regions, the annual variations in both temperature and photoperiod do not show important fluctuations. Tropical zones are characterized by a seasonal rhythm in the annual rainfall

and, consequently, seasonal rhythms in food supply. In these areas, many species show a clear relationship between reproduction and the seasonal distribution of rainfall (Gwinner, 1981; McArthur, 1981).

In cold and temperate regions, reproduction is usually concentrated in the favourable spring and summer seasons. With increasing latitude, the favourable conditions and, consequently, the reproductive period are shortened (Baker, 1938b; Saboureau and Outourne, 1981; Vivien-Roels and Pévet, 1983). A similar effect occurs with increasing altitude which reduces the favourable climatic period (Lofts, 1974; Callard and Ho, 1979).

Photoperiod is one of the most important exogenous factors that regulates reproduction. It is used by many species as a seasonal indicator because changes in photoperiod are constant throughout the year. A relationship between photoperiod and gonadal function has been shown in many mammalian species (Benoit and Assenmacher, 1970; Lofts, 1975). Experiments with poikilothermic vertebrates also revealed a reproductive dependency on photoperiod although the importance of the photoperiod varies in the different phylogenetic groups (Licht, 1972; Vlaming, 1975; Borg, 1982; Underwood and Hall, 1982; Fraile et al., 1988, 1989a,b).

The influence of photoperiod on reproduction is mediated by the pineal gland in many mammals (Fiske et al., 1960; Wurtman et al., 1968; Reiter, 1980). In poikilothermic vertebrates, the study of photoperiod effects is of particular interest since, in most of these animals, the pineal gland has a photoreceptive structure that can be stimulated by direct light and therefore, its function may be independent of ocular photoreception (Dodt, 1973; Oksche and Ueck, 1974; Fraile et al., 1988, 1989a).

Poikilothermic vertebrates are extremely sensitive to temperature changes because their metabolic activity depends on body temperature, and this in turn depends on environmental temperature. Seasonal changes in environmental temperature are consequently important in the regulation of reproductive cycles. The principal external factor which regulates the reproductive cycle in amphibians and reptiles seems to be temperature (Licht, 1972; Rastogi et al., 1978; Moll, 1979; Fraile et al., 1989c).

The present review evaluates the effects of photoperiod and temperature on spermatogenesis in amphibians and the possible role of the pineal gland in their response to photoperiod. The principal characteristics of the annual testicular cycles in these amphibians must be examined first.

2. Spermatogenesis in amphibians

2.1. Annual testicular cycles

Spermatogenetic development in amphibians occurs in coordinated clusters called cysts. Each cyst consists of a single type of germ cell plus its supporting follicular cells. In anurans, cysts are found inside seminiferous tubules (Fig. 1), whereas in urodeles the cysts are lodged within transitory cavities called lobules (Lofts, 1974) (Fig. 2). The germ-cells in the cysts undergo synchronous maturation from primordial germ cells to spermatozoa. The seminiferous tubules in anurans or the testicular lobules in urodeles are interspersed through an interstitial tissue composed of connective tissue, vascular capillaries and the steroid-secreting cells (interstitial cells or Leydig cells) (Lofts, 1974).

Most of amphibian species living in temperate or cold regions present an annual spermatogenic cycle with the highest testicular activity in spring and summer and a quiescent period in late autumn and winter. There have been numerous studies on the testicular cycle of many anuran species including *Rana iberica* (Crespo and Cei, 1971, 1973); *R. temporaria* (Witschi, 1924; Koskela et al., 1979; Arrayago and Bea, 1986); *R. catesbeiana* (Swingle, 1921); *R. esculenta* (Lofts, 1964); *R. nigromaculata;* (Satoh, 1971); *R. perezi* (Delgado et al., 1989); *Alytes obstetricans* (Crespo, 1982); and *Discoglossus pictus* (Champy, 1913) (Fig. 1). The testicular cycle has also been studied in many urodele species such as *Notophthalmus viridescens* (Adams, 1940); *Taricha torosa* (Miller and

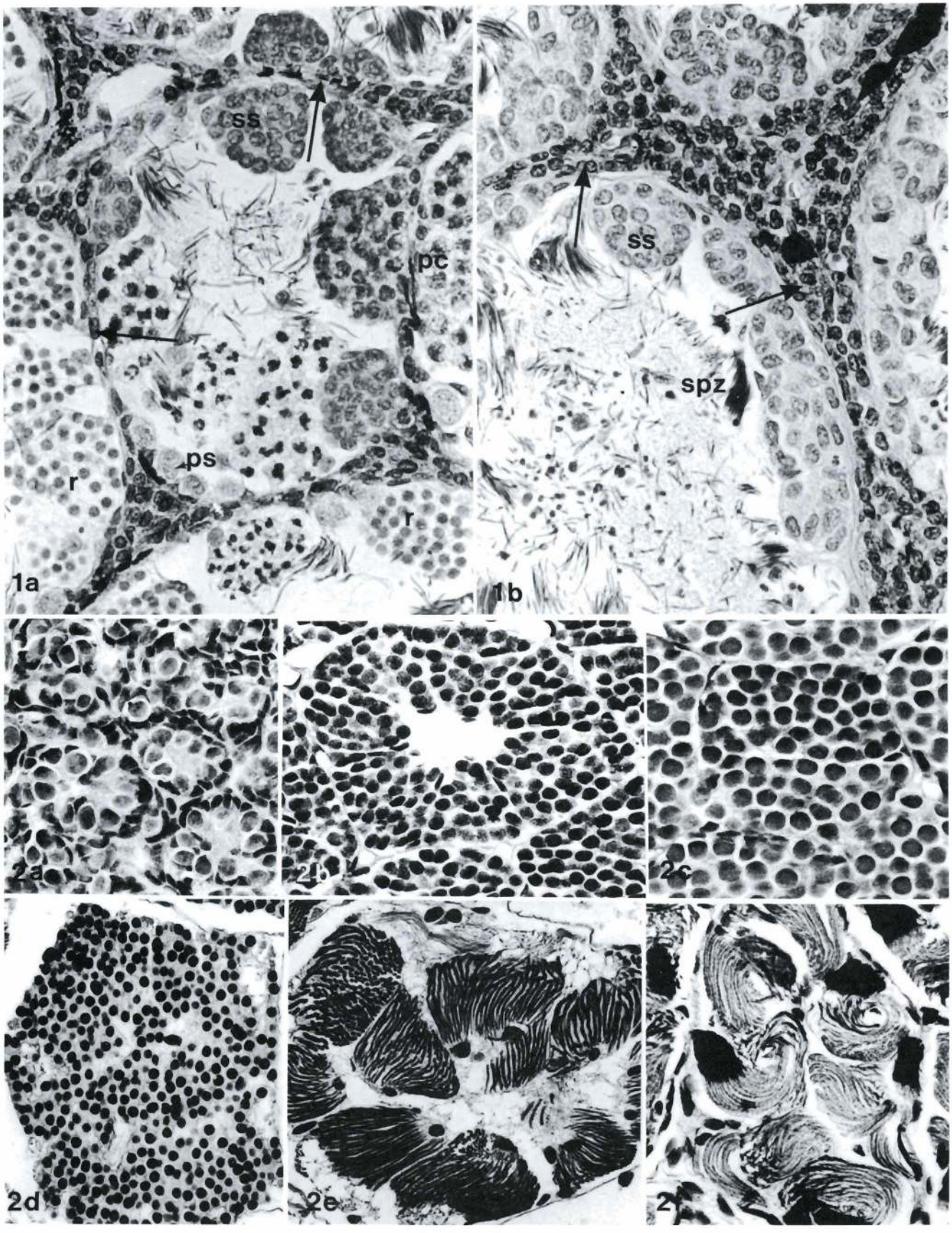
Fig. 1. Testis of *Bufo bufo gredosicola* in two different phases of the annual cycle. **a**: In July the seminiferous tubules show spermatogenic development up to the round spermatid stage. The spermatozoa in the tubular lumen are formed in the preceding cycle. **b**: In April only secondary spermatogonia and spermatozoa formed in the preceding cycle are seen. ps: primary spermatogonia; ss: secondary spermatogonia; pc: primary spermatocytes; r: round spermatids; spz: spermatozoa; arrow: interstitial cells. H & E. × 450

Fig. 2. Germ-cell progression in the testis of the marbled newt, *Triturus marmoratus marmoratus* **a**: primary spermatogonia; **b**: secondary spermatogonia; **c**: primary spermatocytes; **d**: round spermatids; **e**: elongated spermatids; and **f**: spermatozoon bundles.

Fig. 3. Testicular lobes from marbled newts *T. marmoratus* in three different phases of the annual cycle. **a**: In June at the end of the period of germ-cell proliferation from spermatogonia to the round spermatid stage. **b**: In September, at the end of the spermiogenesis period (differentiation of round spermatids into spermatozoa). **c**: In January, in the period of quiescence. Besides lobules with primary and secondary spermatogonia, spermatozoa formed in the preceding cycle and glandular tissue can be seen. ps: primary spermatogonia; ss: secondary spermatogonia; pc: primary spermatocytes; r: round spermatids; e: elongated spermatids; spz: spermatozoon bundles; and gt: glandular tissue. H & E. × 30

Fig. 4. Testicular lobes from marbled newts maintained for 3 months at 30° C with a natural photoperiod. **a**: newt killed in December showing germ-cell development up to the round spermatid stage. The spermatozoa and glandular tissue were formed in the preceding cycle. H & E. × 18. **b**: newt killed in September showing germ-cell development up to the round spermatid stage. No spermiogenesis occurred. H & E. × 30. ps: primary spermatonia; ss: secondary spermatogonia; pc: primary spermatocytes; r: round spermatids; spz: spermatozoon bundles; and gt: glandular tissue.

Fig. 5. Testicular lobes from marbled newts exposed to long photoperiods (16 h of light per day) with a constant temperature of 20° C for 3 months. **a**: newt killed in December showing germ-cell development up to the round spermatid stage. **b**: blinded newt killed in September showing complete spermatogenesis. ps: primary spermatogonia; ss: secondary spermatogonia; pc: primary spermatocytes; r: round spermatids; e: elongated spermatids; and spz: spermatozoon bundles. H & E. × 30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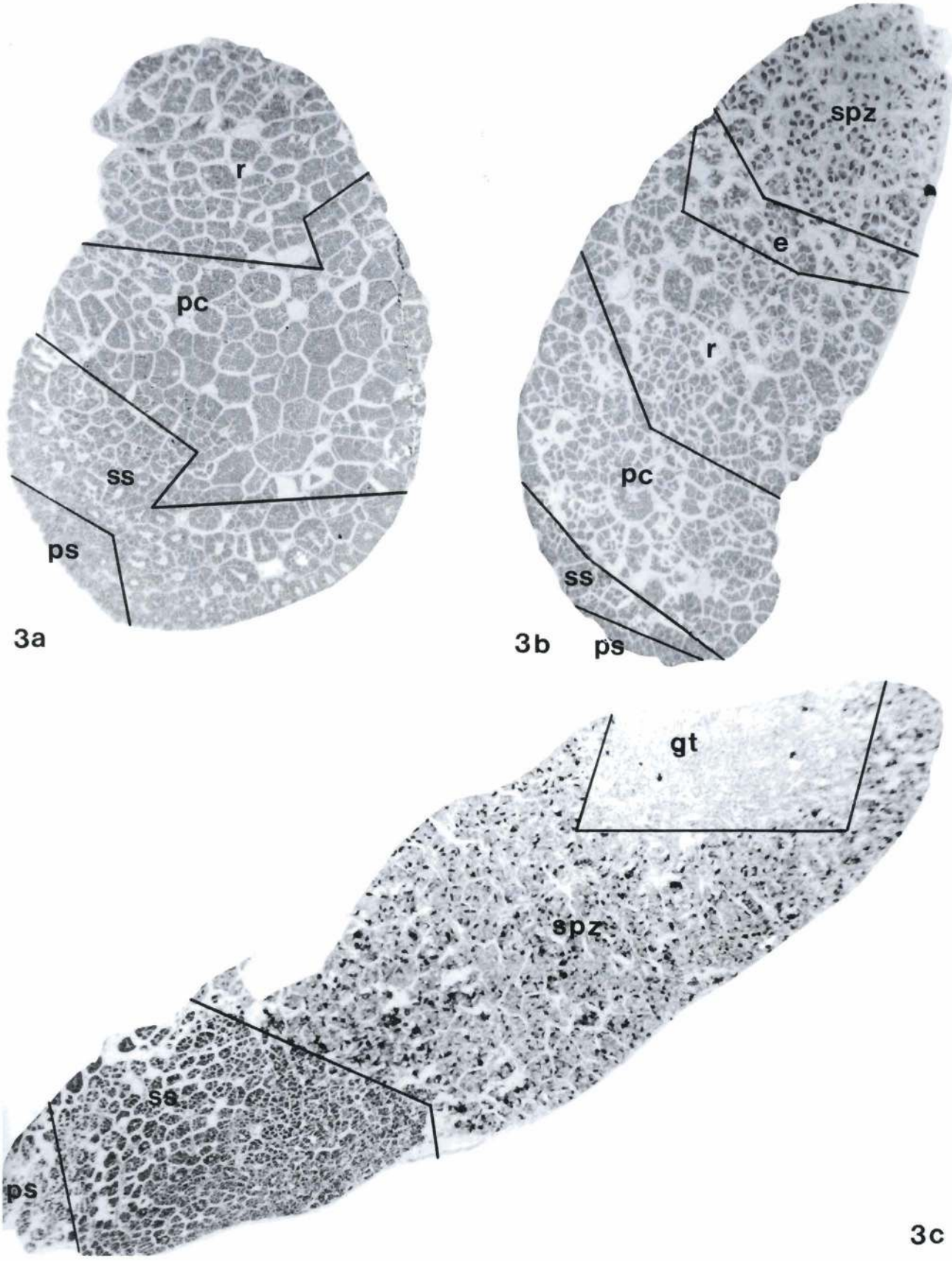
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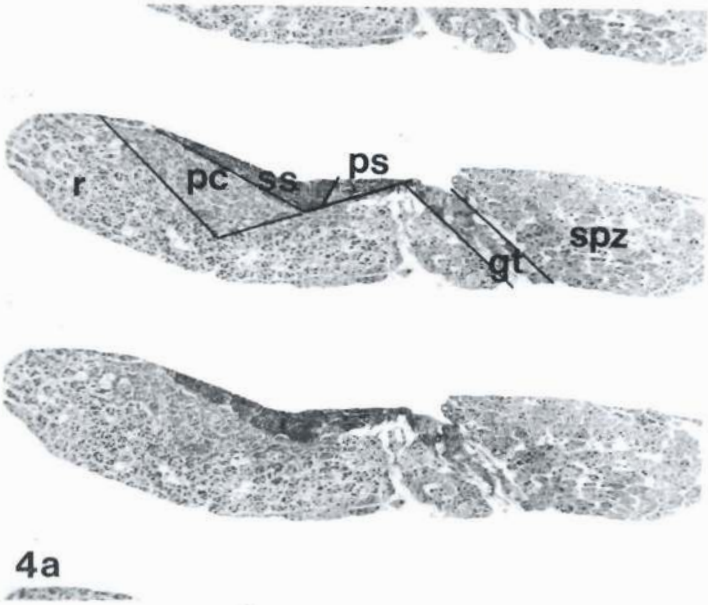

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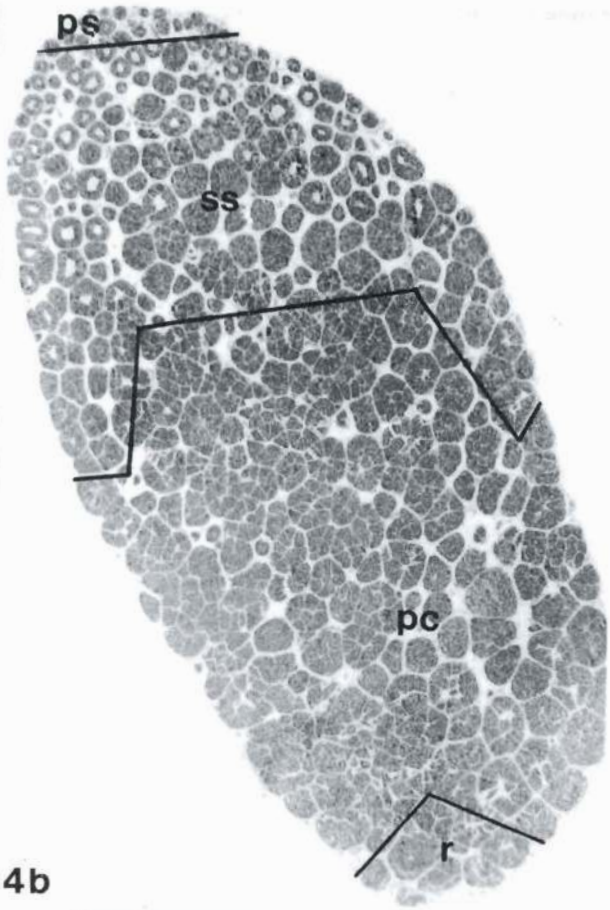

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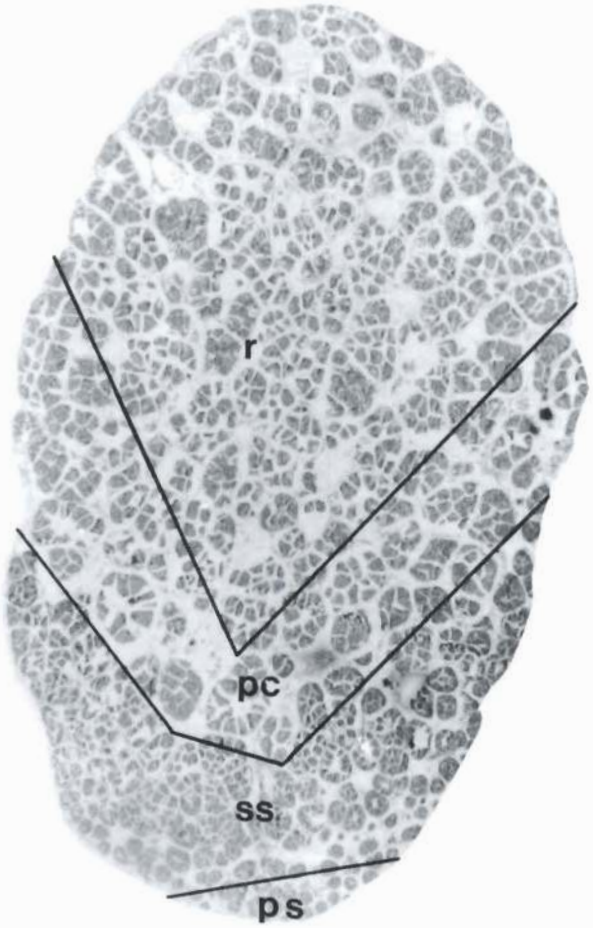

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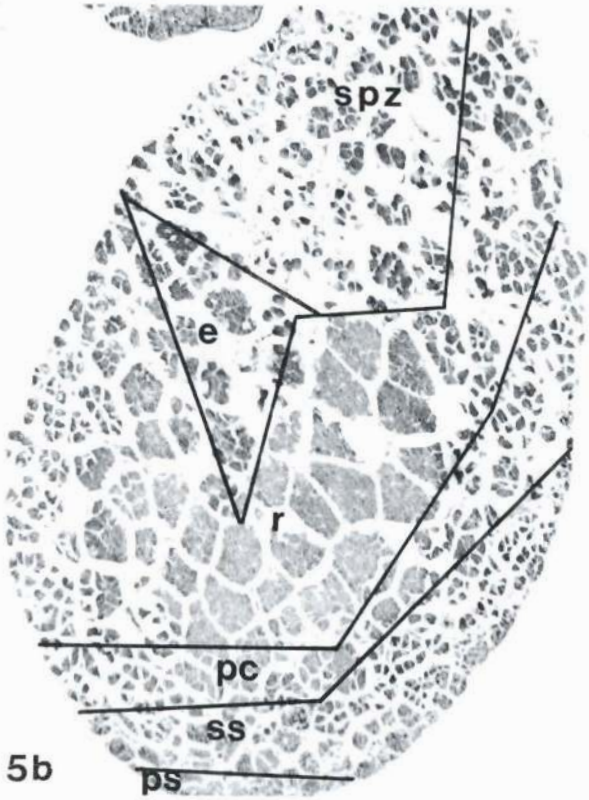

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Robbins, 1954); *T. granulosa* (Specker and Moore, 1980); *Cynops pyrrhogaster* (Tanaka and Iwasawa, 1979); *Ambystoma mexicanum* (Carrick, 1934); *A. tigrinum* (Norris et al., 1985); *Trituroides hongkongensis* (Tso and Lofts, 1977); *Euproctus asper* (Rouy, 1972); *Necturus maculosus* (Pudney et al., 1983); *Pleurodeles waltlii* (Patisson, 1963); *Triturus alpestris* (Cei, 1942); *T. marmoratus* (Sáez et al., 1990); and *Salamandra salamandra* (Joly, 1971).

As an example of an annual testicular cycle, we describe the cycle of *T. marmoratus* (Sáez et al., 1990). The testis consists of 2 or 3 identically organized lobes. Each lobe shows a progressive development of germ-cells from the anterior (primordial germ-cells and primary spermatogonia) to the posterior pole (spermatozoon bundles). Only in the anterior pole of the lobe do the two types of germ-cells primordial germ cells and primary spermatogonia, appear intermingled. In the rest of the testicular lobe, each lobule contains only one germ-cell type. The testicular cycle comprises 3 well-defined periods.

i) Period of germ-cell proliferation (Fig. 3a). In May, most of the testis is occupied by spermatozoon bundles and glandular tissue cells (developed Leydig cells), both of which are formed in the preceding cycle. In the anterior testicular pole there are primordial germ cells and primary spermatogonia that begin to proliferate giving rise to lobules with secondary spermatogonia, primary spermatocytes, secondary spermatocytes and round spermatids. All these germ-cell types can be observed in June. Testicular size increases markedly during this period.

ii) Period of spermiogenesis (Fig. 3b). From July to September, the testicular enlargement progresses. Spermatogonial proliferation is scarce but the meiotic process continues giving rise to numerous round spermatids. These cells undergo spermiogenesis and transform into spermatozoon bundles. The first spermatozoa can be observed in August. The mesenchymal-like, lobule boundary, interstitial cells transform into Leydig cells after spermiogenesis is achieved.

iii) Period of quiescence (Fig. 3c). In October, neither spermatocytes nor round spermatids are observed because all of them have developed into spermatozoa, except for a few elongated spermatids whose maturation is arrested until spring. Besides these elongated spermatids and the newly formed spermatozoa, a few primordial germ-cells and primary spermatogonia remain in the anterior testicular pole as stem cells for the following spermatogenic cycle. By winter the lobules have disappeared and the Leydig cells group together forming the glandular tissue which also contains abundant blood vessels and undifferentiated mesenchymal-like cells (Fraile et al., 1989d).

Similar cycles have been reported in the urodele species *T. granulosa* (Specker and Moore, 1980), *E. asper* (Rouy, 1972) and *C. pyrrhogaster* (Tanaka and Iwasawa, 1979), as well as in the anuran *R. iberica* (Crespo and Cei, 1971).

This pattern of the cycle differs not only from one species to another but also within the same species, according to both latitude and altitude. In *R. temporaria* the quiescent period is 3 months in the Netherlands and Northern Spain (van Oordt, 1956; Arrayago and Bea, 1986), and 7 months in Finland (Koskela et al., 1979). In the urodele *E. asper*, spermatogenesis develops later in the animals which live at high altitudes than in those at low altitudes (Clergue-Gazeau, 1971; Rouy, 1972).

In warmer regions, the spermatogenic period is more prolonged. This has been shown in the urodeles *T. hongkongensis* (Tso and Lofts, 1977), *A. tigrinum* (Norris et al., 1985) and *P. waltlii* (Garnier, 1985), and in the anuran *R. temporaria* (Arrayago and Bea, 1986). On the other hand, in cold areas, the spermatogenic period shortens, as has been reported in *N. maculosus* (Pudney et al., 1983) and *N. viridescens* (Adams, 1940; Ifft, 1942).

In contrast to all the above-mentioned amphibian species which have a discontinuous cycle, that is, a cycle characterized by a well-defined quiescent period in which germ cell proliferaton is arrested, there are other amphibians that show a continuous cycle in which spermatogenesis takes place throughout the whole year (Lofts, 1974). This cycle may be observed in species such as the anuran *A. obstetricans* and *A. cisternasii* (Crespo, 1982), and the urodele *S. salamandra* (Joly, 1960) that inhabit tropical or subtropical areas where environmental temperatures are subject to hardly any fluctuations.

2.2. Hormonal regulation

Endocrine regulation of testicular function in amphibians is still a controversial subject due to the discrepant and even contradictory results reported. These differences might be attributed to the wide variety of species investigated, the use of hormone preparations obtained from varying sources, and differing experimental designs. Nevertheless, some aspects of hormonal regulation have been clarified.

The close relationship between pituitary activity and testicular function was soon established in different anuran (Skowler, 1925; Masselin, 1940) and urodele (Aplington, 1942; Miller and Robbins, 1955) species. Removal of the adenohypophysis causes regression of the reproductives organs in many species (Burgos, 1949; van Oordt, 1956; Lofts, 1961; Woronzova and Blancher, 1930). Administration of drugs with antigonadotropic effects in *R. esculenta* also causes gonadal atrophy (Rastogi and Chieffi, 1972; Rastogi et al., 1972, 1976). Amphibian testes are sensitive to administration of mammalian gonadotropins. Administration of mammalian FSH stimulates spermatogenesis in different anuran (Andreozzi, 1952; Burgos and Ruffino, 1953) and urodele (Miller and Robbins, 1954) species. In several anuran species, mammalian LH induces spermiation and adrogen synthesis whereas mammalian FSH causes spermatogenesis in hypophysectomized frogs (Burgos

Table 1. Effects of temperature on testicular cycle in urodeles of cold temperate areas with a potentially continuous cycle and exposed to long photoperiods (12-16 h light per day).

SPECIES	PERIOD OF THE CYCLE											
	QUIESCENCE (late autumn-winter)				GERM-CELL PROLIFERATION UP TO THE ROUND SPERMATID STAGE (spring-summer)				SPERMIOGENESIS (summer-early autumn)			
	4 °C	10 °C	20 °C	30 °C	4 °C	10 °C	20 °C	30 °C	4 °C	10 °C	20 °C	30 °C
Triturus marmoratus (Fraile et al. 1988, 1989a,b,c)	Spg		RSpt +few Spz	RSpt +few Spz	Spg		RSpt +few Spz	RSpt +few Spz	RSpt[1]		Spz	RSpt
Plethodon cinereus (Werner 1969)		Spc	Spz			Spc	Spz					
Triturus cristatus (Steinborn 1984)		Spc[2]	RSpt +few Spz							RSpt[2]	Spz	
Notophthalmus viridiscens (Ifft 1942)	Spg[3,5] +few Spc		Spc[4,5]		Spg[3,5]		Spz			RSpt +few Spz[5]	Spz	

Spg: primary and secondary spermatogonia; Spc: primary spermatocytes; RSpt: round spermatids; Spz: elongated spermatids and spermatozoa. 1: degenerating cells. 2: 11 °C. 3: 5-8 °C. 4: 14-16 °C. 5: constant light.

Table 2. Effects of photoperiod on testicular cycle in urodeles of cold temperate areas with a potentially continuous cycle and maintained at mild temperatures (20 °C)

SPECIES	PERIOD OF THE CYCLE											
	QUIESCENCE (late autumn-winter)				GERM-CELL PROLIFERATION UP TO THE ROUND SPERMATID STAGE (spring-summer)				SPERMIOGENESIS (summer-early autumn)			
	OL:24D	8L:16D	16L:8D	24L:0D	OL:24D	8L:16D	16L:8D	24L:0D	OL:24D	8L:16D	16L:8D	24L:0D
Triturus marmoratus* (Fraile et al. 1988, 1989a,b,c)	Spc	Spc	RSpt +few Spz	few RSpt	Spc	Spc	RSpt +few Spz	few RSpt	Spz	Spz	Spz	Spz
Plethodon cinereus (Werner 1969)		Spc	Spz			Spz	Spz					
Triturus cristatus (Steinborn 1984)		RSpt +few Spz	RSpt +few Spz						Spz	Spz	Spz	
Notophthalmus viridiscens (Ifft 1942)	Spc			Spc**			Spz		Spz		Spz	Spz*

Spg: primary and secondary spermatogonia; Spc: primary spermatocytes; RSpt: round spermatids; Spz: elongated spermatids and spermatozoa. *: no differences between blinded and non-blinded newts were observed. **: 15 °C.

and Ladman, 1957; Lofts, 1961; de Kort, 1967). Similar findings have been observed in the urodele *P. waltlii* (Andrieux et al., 1973). It is believed that the adenohypophysis of amphibians, like that of mammals, is under hypothalamic control, because administration of mammalian gonadotropin-releasing hormone (GnRH) to amphibians stimulates their gonadotropin secretion (Ball, 1981; Jackson, 1981).

A major point of controversy is whether amphibian gonadotropins are actually two hormones —follicle stimulating hormone (FSH) and luteinizing hormone (LH)— as is true in mammals, or only one. In different amphibian species, two different gonadotropins, chemically similar to mammalian FSH and LH, have been detected (Holmes and Ball, 1974; Licht and Papkoff, 1974; Licht et al., 1975, 1977; Farmer et al., 1977). The exact role of these gonadotropins has also been disputed. Using the *Rana*-FSH and LH gonadotropins purified by Papkoff and co-workers (1976), Muller (1977) found that *Rana*-LH induces steroid secretion in the frog testes, whereas *Rana*-FSH does not. These results suggest that the role of gonadotropin in amphibians is similar to that observed in mammals.

Nevertheless, the results of studies performed in other species contradict the assumption that there are two different gonadotropins with distinct effects on amphibian spermiogenesis. In the anuran *X. laevis* and in the urodele *A. tigrinum*, both FSH and LH obtained from mammals have a similar stimulatory effect on spermiation and synthesis of 3ß-hydroxysteroid dehydrogenase (3ß-HSD) (an enzyme involved in steroid biosynthesis) (Moore, 1975). Likewise, several authors found evidence for only one gonadotropin (Doer-Schott, 1976; van Oordt, 1974), and some of the investigators who had isolated two gonadotropins observed, however, that the hormones had different effects in different species. Muller and Licht (1980) investigated testicular steroid biosynthesis after *Rana*-FSH and LH-administration to different amphibian species and concluded that: 1) in some species, the two hormones stimulate steroid secretion to a similar degree; 2) in other species, LH is more potent that FSH; and 3) in still other species, FSH is more potent than LH. These marked interspecific variations in the response of each species to *Rana*-FSH and LH do not support the hypothesis of LH specificity for testicular androgen secretion in amphibians.

Tanaka et al. (1980,1981) studied the gonadotropin levels during the annual cycle in the pituitary of *C. pyrrhogaster*. These authors found that both FSH and LH levels were high in atumn-winter and low in spring, with an increase in July followed by a decrease in August, and a new increase in September. Their results support the van Oordt and de Kort (1969) hypothesis. These authors assume that there is only one pituitary gonadotropin in amphibians, and that the seasonal changes in testicular activity are due to seasonal variations in the sensitivity of both germ-cells and interstitial cells to gonadotropin. These seasonal sensitivity variations would be principally regulated by temperature.

The role of androgens in spermatogenesis control is not well understood either. Many investigators' results suggest that androgens inhibit spermatogenesis through inhibition of hypophyseal gonadotropin secretion (Cei et al., 1955; van Oordt and Basu, 1960; van Oordt and Shouten, 1961; Basu, 1962a,b, 1968). According to Lofts (1974), this effect only occurs during the first stages of spermatogonial proliferation, and androgens do not have negative effects on meiosis and spermiogenesis. Other authors failed to observe any effects of exogenous androgens on spermatogenesis in some amphibian species (Puckett, 1939; Galgano, 1942). In contrast, Blair (1946) and Penhos (1953) found a stimulatory effect of androgens on spermatogenesis. In the frog *R. esculenta*, Rastogi et al. (1976) reported that androgens stimulate spermatid formation. Recently, an androgen receptor has been found in testicular extracts from the urodele *N. maculosus* (Singh and Callard, 1988). This receptor is more abundant in the testicular extreme containing spermatogonia and follicular cells than in the portion with Leydig cells. In addition, an androgen-binding protein (ABP) has also been found in the testicular portion with abundant follicular cells (Singh and Callard, 1989). This suggests that this ABP is synthesized by the follicular cells and is equivalent to the mammalian ABP synthesized by the Sertoli cells. A possible role for amphibian ABP could be the capture of androgens in order to maintain the primordial germ-cells during the period of testicular quiescence as well as to trigger the beginning of a new spermatogenic wave in spring.

Mention should be made of a reference to the possible regulatory role on amphibian testicular function of melatonin and other methoxyindoles together with environmental factors, principally photoperiod. Studies performed in non-mammalian vertebrates indicate that, as in mammals, melatonin is involved in gonadal function (Vivien-Roels and Pévet, 1983; Vivien-Roels, 1985). In the anuran amphibian *Rana ridibunda*, melatonin injections cause ovarian regression, even in animals exposed to a long photoperiod and mild temperature (Delgado et al., 1983). Recently, it has been suggested that, besides photoperiod, temperature is also involved in the production of indolic derivatives by the pineal gland in some non-mammalian vertebrates (Vivien-Roels and Pévet, 1983). Removal of the pineal gland *R. esculenta* hinders spermatogenesis stimulation by photoperiod and temperature (Rastogi et al., 1976). Recent studies in anurans suggest that most serum melatonin levels are attributable to the melatonin synthesized by the retina and not by the pineal gland (Delgado and Vivien-Roels, 1989). However, in the urodele *T. marmoratus* photoperiodic sensitivity is similar in blinded and sighted animals (Fraile et al., 1988, 1989a). This suggests: a) the existence of an extraretinal light receptor: and b) that hormones or other susbstances synthesized by the retina are unnecessary for spermatogenic development in the marbled newt *T. marmoratus*.

3. Effects of temperature on testicular function

Temperature is the best studied external controlling factor for spermatogenesis in amphibians. The relationship between annual changes in temperature and spermatogenesis is obvious in amphibians that inhabit cold/temperate areas. In the first studies on the gonadal cycle in anuran (Galgano, 1932, 1934, 1936; Galgano and Lanza, 1951) and urodele (Galgano and Flachetti, 1940; Ifft, 1942) species, temperature was assumed to be the principal environmental factor regulating spermatogenesis. The development of discontinuous cycles was attributed to the occurrence of a cold season during which most activities, including spermatogenesis, were arrested.

Temperature effects seem to be mediated by the hypothalamus which controls pituitary gonadotropin secretion (Mazzi, 1970; Ball, 1981). Recently, it has been postulated that environmental temperature is also involved in the regulation of indolic derivatives synthesized by the pineal gland (de Vlaming and Olcese, 1981; Vivien-Roels and Pévet, 1983). Experiments by Rastogi et al. (1976) indicate that pinealectomy hinders temperature stimulation of the testis in *R. esculenta*.

The study of amphibians with discontinuous testicular cycles led Witschi (1924) to postulate the existence of an internal mechanism that was responsible for changes in sensitivity to external factors involved in spermatogenesis. These cycles are characterized by insensitivity to the experimental increase in temperature during the period of testicular quiescence and contrast with the potentially continuous cycle in which a temperature rise during the quiescent period causes spermatogenic development. Specimens of *R. temporaria* moved from their own habitat to other colder or warmer habitats did not change their testicular cycles (Witschi, 1924). The probable sites of this mechanism involved in the control of temperature sensitivity are the adenohypophysis (Cei, 1944; Galgano and Lanza, 1951), and primary spermatogonia (van Oordt, 1956).

According to the first hypothesis, the adenohypophysis would have an internal cyclic rhythm which is insensitive to increased temperatures during the first part of the quiescent period, but not during the last part of this period. At this time, temperature would stimulate pituitary gonadotropin secretion which, in turn, would trigger the development of primary spermatogonia into spermatozoa. Experiments combining different doses of gonadotropins with different temperatures in both hypophysectomized and normal specimens of *R. temporaria* revealed that pituitary activity is under temperature control (van Oordt, 1956).

Testicular insensitivity to gonadotropins during the testicular phase of refractoriness to increased temperatures (the first part of the quiescent period) has been observed by van Oordt (1956) in *R. temporaria*. This observation led this author to suggest that the primary spermatogonia of *R. temporaria* are insensitive to temperature-mediated gonadotropin secretion during this period.

In addition, it has been observed that gonadotropins do not induce spermatogenesis while the testis still contains spermatozoon bundles (formed in the preceding cycle) and their transformed follicular cells. This gonadotropin insensitivity in the first period of testicular quiescence has been explained as follows: gonadotropins are necessary at this time to eliminate the spermatozoon bundles and follicular cells from the preceding cycle. Only when these have disappeared can the hormone stimulate primary spermatogonia (van Oordt and Lofts, 1963).

The urodele *Plethodon cinereus* also shows a similar insensitivity to temperature during early testicular quiescence (Table 1), and a mechanism similar to the one postulated for *R. temporaria* has been proposed for this urodele species (Werner, 1969).

Except for this refractory phase, spermatogenesis shows a pronounced dependence on temperature in amphibians with a true discontinuous cycle.

Below 12°C spermatogenesis is arrested in the urodele *Notophthalmus viridiscens*, and the spermatids and spermatocytes that are present degenerate (Ifft, 1942) (Table 1). Similar findings have been reported in *T. cristatus* (Steinborn, 1984) (Table 1). However, experiments with another urodele species, *Plethodon cinereus*, indicate that a temperature of 10° C permits primary spermatocyte proliferation during the quiescent period and that this temperature only causes some delay in spermatogenic development during the period of spermatogenesis (Werner, 1969) (Table 1). These differences between species suggest that the optimum temperature range for spermatogenesis varies from one species to another.

The influence of low temperature is not the same on every species or on every cell type in the testis. Low temperatures (below 10° C) enhance the proliferation of primary spermatogonia and hinder the development of secondary spermatogonia and primary spermatocytes in *R. esculenta* (Rastogi et al., 1976). Temperature under 4° C arrest the proliferation of spermatogonial type in *Bufo spinulosus* (Bustos-Obregón, 1979).

Mild temperatures affect spermatogenesis favourably even when the photoperiods are short. In Newts *(Notophthalmus viridescens)* maintained at 20° C with a short photoperiod (8-10 h of light daily) for three months during the phase of testicular quiescence develops spermatogenesis until it reaches the round spermatid stage (Ifft, 1942). Similar results have been obtained with the marbled newt (*T. marmoratus)* (Fraile et al., 1988). In similar photoperiod and temperature conditions, complete spermatogenesis was observed in *T. cristatus* with only 6 weeks of exposure during the quiescent period (Steinborn, 1984).

Moderately high temperatures affect the different phases of the cycle in *T. marmoratus* differently (Table 1). Newts maintained at 30° C for 3 months during the quiescent period with the naturally short photoperiod showed the same spermatogenic development as those maintained at 20° C (Fraile et al., 1989c) (Fig. 4a). If the animals were exposed to 30° C during the period of germ-cell proliferation (March-June) with a naturally long

photoperiod (12-14 h of light daily), spermatogenic development was normal, like that of the newts maintained in their natural environment or those kept in the laboratory at 20° C (Fraile et al., 1989c). However, when the newts were maintained at 30° C during the period of spermiogenesis (July-September), spermatogenesis was arrested at the round spermatid level (Fraile et al., 1989c) (Fig. 4b) (Table 1). Diminished spermiogenesis was also observed by Ifft (1942) in newts *(N. viridescens)* maintained at 25° C during the period of spermiogenesis (Table 1). Therefore, the effect of moderately high temperature is similar to that of mild temperatures during the phase of germ-cell proliferation and meiosis, and detrimental during the phase of spermiogenesis.

Rastogi (1980) and Rastogi et al. (1981) studied the effect of low (4° C), mild (15° C) and moderately high (24 °C) temperatures on the testis of *R. esculenta* maintained in a constant long photoperiod (12 h of light per day) for several months. The results of the experiment indicated that: 1) exposure to mild temperature did not modify the natural rhythm of the annual cycle; 2) maintenance of the frogs at low temperatures only arrested spermatogenesis after 120 days of exposure; 3) exposure to moderately high temperatures did not hinder the initial stages of spermatogenesis although gonadotropin secretion and spermatogenesis were finally arrested. These findings led these investigators to suggest that even in anurans with a potentially continuous cycle, like *R. esculenta*, there is an endogenous annual rhythm that is synchronized with temperature. Temperature would not therefore act as a determinant factor for the testicular cycle but as an indicator for the endogenous cycle. This hypothesis reduces, although it does not suppress, the importance of temperature in the control of spermatogenesis. It is probable that spermatogenic control is the result of an interaction between an endogenous rhythm and external factors like temperature.

Androgen biosynthesis is regulated by environmental temperature. Immunohistochemical detection of testerone in *T. marmoratus* revealed that a decrease in testosterone content in the glandular tissue cells occurs in the coldest months of the years (January-February), whereas in the months before (October-November) and after (March-April) this period which have mild temperatures, testosterone content is high. In the warmer months (June-September) no testosterone content was observed (Fraile et al., 1989d). In other urodeles, such as *C. pyrrhogaster* (Tanaka and Takikawa, 1983) and *Taricha granulosa* (Specker and Moore, 1980), measurements of serum androgen levels indicated two peaks in testosterone levels. These peaks coincide with the months of highest testosterone content in the glandular tissue cells of *T. marmoratus*.

Experiments on the effects of temperature on the androgen levels in *R. esculenta* during the annual cycle revealed that: 1) in summer, the testis is refractory to androgen biosynthesis whatever the experimental temperature is; 2) in autumn, when environmental temperatures are still warm, a decrease in temperature increases androgen production; and 3) in winter, when androgen levels are low in natural environmental conditions, maintenance of frogs in mild temperatures stimulates androgen synthesis (Iela et al., 1980). The observations of a natural androgen synthesis cycle, together with the results of experiments studying the effects of different temperatures, suggest that, during the period in which the testis is not refractory to androgen synthesis (between September and April), this synthesis can be stimulated by mild temperatures and improved by either low or high temperatures.

4. Effects of photoperiod on testicular function

The role of photoperiod in the control of testicular function, as mediated by pineal gland-produced melatonin, is well known in mammalian species (Reiter and Sorrentino, 1970). In poikilothermic vertebrates, such as reptiles and amphibians, the regulatory role of photoperiod seems to be less important than that of temperature (Lofts, 1975).

The first study of photoperiod effects on testicular fuction in amphibians was performed by Ifft (1942) in *Notophthalmus viridescens*. This investigator only observed small differences between groups and concluded that photoperiod had no effect on testicular function (Table 2). Later experiments in other amphibians including anurans (Rastogi et al., 1976) and urodeles (Werner, 1969; Steinborn, 1984; Fraile et al., 1988, 1989a,b) disproved that notion. During the phase of testicular quiescence, in the urodeles *Plethodon cinereus* (Werner, 1969), *Triturus cristatus* (Steinborn, 1984) and *Triturus marmoratus* (Fraile et al., 1988), long diurnal photoperiods (12-16 h of light per day) stimulate spermatogenesis if temperature is maintained at 20° C (Fig. 5a). Short photoperiods (less than 8 h of light per day) fail to permit germ-cell proliferation, even when temperature is maintained at 20° C (Table 2).

Similar experiments performed during the phase of germ- cell proliferation from spermatogonia to round spermatids revealed that laboratory maintenance in long photoperiods at mild temperatures induces effects like those found in the natural long photoperiods and mild temperatures occurring in the natural habitat during this season of the year. However, shortening the photoperiod reduces germ-cell proliferation even when temperature is maintained at 20° C (Werner, 1969; Steinborn, 1984; Fraile et al., 1989a) (Table 2).

In the marbled newt, the most favourable photoperiod was shown to be between 12 and 16 h of light per day whereas the effect of exposure to constant light was like that of exposure to a short photoperiod (Fraile et al., 1988). This suggests that the marbled newt and, possibly, other urodele species, has an optimum photoperiod. Longer or shorter photoperiods discourage testicular function.

These findings might explain the absence of photoperiod effects found by Ifft (1942) during the phase of testicular quiescence. This author exposed the newts either to constant light or complete darkness (Table 2),

and both these photoperiods are outside the favourable photoperiod limits.

The results of experimental studies in newts during spermiogenesis concluded that photoperiod has no effect on testicular function during this phase of the cycle (Ifft, 1942; Steinborn, 1984; Fraile et al., 1989b) (Table 2), suggesting that although long photoperiods are necessary to stimulate spermatogenesis, once germ-cell differentiation has reached the round spermatids stage, photoperiod is not required for the differentiation of round spermatids into spermatozoa. In the anuran *R. esculenta* maintained at 17° C, spermiogenesis occurred in animals exposed to short photoperiods, as well as in blinded or pinealectomized frogs. However, in contrast with that which was observed in urodeles, spermiogenesis did not take place in the frogs exposed to long photoperiods (Rastogi et al., 1976). This suggests that photoperiod is not neutral in the development of spermatids to spermatozoa. It is possible that temperature is the limiting factor, because, in contrast with the above-mentioned urodeles which develop spermiogenesis in the high temperatures and long photoperiods of summer, *R. esculenta* spermiogenesis occurs in late autumn, when photoperiod is short and temperature is low.

Photoperiod seems to play an important role in the regulation of androgen synthesis by *R. esculenta*. In winter, androgen production can be induced with mild temperatures only when the frogs are not kept in total darkness (Iela et al., 1980). In this season, androgen production can also be stimulated by Gn-RH administration at either mild or low temperatures even in complete darkness. These findings suggest that the detrimental effect of photoperiod on androgen synthesis in this species might be mediated by the hypothalamus being impaired by prolonged maintenance in complete darkness (Pierantoni et al., 1985).

In mammals, ocular photoreception is transmitted to the hypothalamus through the optic nerve. The light-stimulated hypothalamus acts on the pineal gland which synthesizes melatonin and other methoxyindoles (Fiske et al., 1960; Wurtman et al., 1968; Reiter, 1981). The Harderian gland also seems to be involved in photoreception and stimulation of the pineal gland in the rat (Wetterberg et al., 1970).

In many non-mammalian vertebrates the pineal gland also possesses photoreceptors. In anurans, the pineal complex consists of a frontal organ located extracraneally beneath the skin, and an intracraneal pineal body connected to the brain (Dodt, 1973). The urodele species only have the intracraneal pineal organ (Oksche and Ueck, 1974). Both the anuran frontal organ and the urodele pineal organ possess sensory cells similar to retinian photoreceptors (Oksche and Ueck, 1974). In addition, the amphibian skin contains photoreceptors and melatonin (Noble, 1931). The occurrence of several photoreceptors and melatonin-production sites in amphibians suggests that eyes are not essential for the transmission of photo stimuli to the pineal gland, although this observation makes it difficult to identify the principal photoreceptor involved in melatonin synthesis.

Recently, it has been reported that there are no differences in the testicular response to different photoperiods (varying from total darkness to constant light) between blinded and non-blinded marbled newts *(T. marmoratus)* (Fig. 5b) (Table 2) (Fraile et al., 1988, 1989a). This corroborates the assertion of Ifft (1942) who held that eyes were not involved in the control of testicular function in the urodele *N. viridescens*. This observation cannot be extended to anuran species that synthesize melatonin in the retina and it has been suggested that the retina, and not the pineal gland, is the principal source of serum melatonin levels in the frog (Delgado and Vivien-Roels, 1989).

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