Annual Cycles of Fat Bodies and Gonads in the Toad *Bufo bufo bufo (L.)*,

Compared with Cycles in other Temperate Zone Anurans

By C. BARKER JØRGENSEN
LIS OLESEN LARSEN *and* BRIAN LOFTS

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Synopsis

The *annual cycles* in nutritional condition, as evaluated from the weight of the *fat bodies,* and in *gonadal function* were studied in populations of *toads* from northern suburbs of Copenhagen, supplemented with samples of toads collected in other parts of Denmark. The results were compared with data from other species in order to assess the patterns in annual cycles of *nutritional states* and *reproduction* in *temperate zone anurans.*

After *hibernation* and breeding in spring most toads have small fat bodies, whereas medium to large fat bodies predominate during summer and early fall. Females mostly develop larger fat bodies than males. Fat body size tend to decrease again during late fall, indicating that the toads start to draw upon their energy stores already before hibernation.

After breeding the *ovaries* enter a resting period, lasting 2-3 months, before the next *ovarian cycle* begins. The initiation and maintenance of an ovarian cycle require secretion of *gonadotropin* from the pituitary gland at high levels. The activation of gonadotropin secretion seems to depend upon the restoration of an appropriate nutritional condition of the organism.

Ovarian cycles are not being initiated during late summer or in the fall. A mechanism presumably exists which prevents activation of gonadotropin secretion so late in the season that vitellogenetic oocytes cannot complete growth before hibernation. Female toads therefore breed annually or biennially depending upon whether they reach a suitable nutritional condition within a few months after breeding or not. In Denmark most females breed annually.

Male toads seem to breed every year. The *spermatogenetic activity* starts shortly after the spawning period, and it reaches a maximum during summer. Concomitant with the build-up of spermatogenetic activity the *interstitial tissue* regresses, as well as the *secondary sex characters,* the *thumbpads.* During fall and winter the interstitial tissue recovers, and the thumbpads develop again.

The pattern of annual cycles in nutritional state and gonadal function and their relationships as observed in the toad seem to be typical of temperate zone anurans.

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Contents

Material and Methods

An extensive literature deals with gonadal cycles, reproductive patterns, and energy balances in amphibians, especially in species inhabiting the temperate zone. The literature is, however, scattered, and most studies are incomplete or fragmentary. Detailed studies on cycles in wild populations of amphibians are therefore greatly needed, both because of their intrinsic value, and because they constitute the basis for an understanding of behavioural and physiological adaptations of the species to their habitats.

This paper describes the annual cycles of fat bodies and gonads in Danish populations of the toad *Bufo bufo bufo*. The cycle in size of fat bodies reflects the cycle in nutritional state of the organism.

Part of the material has been published previously in preliminary form (Jørgensen, 1973a).

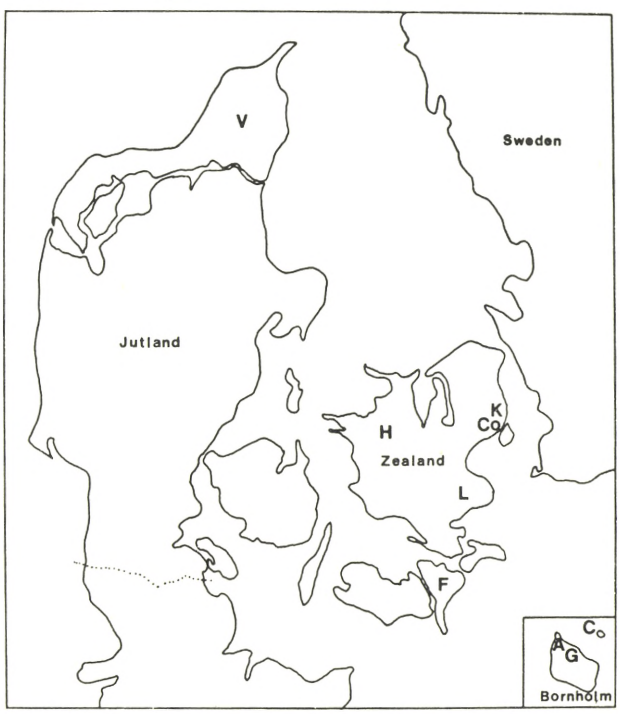
A systematic study was performed of annual cycles of gonads, oviducts, thumbpads, and fat bodies in female toads collected during the years 1965-66 and in male toads collected during the years 1966-67 in woods and parks located in northern suburbs of Copenhagen. The material includes 68 sexually mature, 41 immature female toads, and 71 sexually mature male toads. The material has been supplemented with toads, especially females, obtained over the years 1963-72 from different localities in Denmark (Fig. 1).

The toads were killed, by decapitation, within one to some few days after sampling. Toads that were not autopsied at capture were kept outdoors until sacrifice. Specimens examined during winter and early spring were collected during October. They hibernated until sacrifice in outdoor quarters in moss and leaves.

At sacrifice the various organs were immediately dissected out and fixed in Bouin's fluid. The gonads, oviducts, and fat bodies were weighed before fixation.

The functional state of the ovaries was determined by counting the numbers of large vitellogenetic oocytes under a dissecting microscope, and by determining the size-frequency-distribution of the oocytes. Also atretic oocytes were counted (Jørgensen, 1973b).

Fig. 1.
Map of Denmark showing localities for collection of toads *Bufo bufo bufo*. A, Allinge; C, Christiansø; Co, Copenhagen; F, Falster; G, Gudhjem; H, Hallebyore; K, Klampenborg; L, Lellinge; V, Vendsyssel.



The functional state of the testes was determined by histological and histochemical analysis. A portion of the Bouin fixed testicular tissue and a thumbpad were wax-embedded and sectioned at 4μm and 6μm, respectively. Testicular sections were stained with iron haematoxylin and orange G for investigation of the spermatogenetic condition. The degree of spermatogenetic activity was estimated by counting all the germinal cysts in different spermatogenetic stages in 20 cross sections of testis tubules. The mean for each spermatogenetic stage was then estimated and used as representative of the condition in that particular specimen. The different stages were represented as follows:

0 = cell nest with one primary spermatogonium
I = cell nest with secondary spermatogonia
II = cell nest with primary spermatocytes
III = cell nest with secondary spermatocytes
IV = cell nest with spermatids
V = Sperm bundles attached to a Sertoli cell
S = arbitrary indication of the amount of free spermatozoa in the tubule lumen

The sectioned thumbpad material was stained with haematoxylin and eosin.

Additional testicular material was fixed in formal-saline fluid and, after post-chroming, embedded in gelatin. Thin frozen sections, 4μm thick, were coloured with Sudan Black B and carmalum to reveal the lipoidal condition. Thicker sections (10μm to 15μm) were subjected to the Schultz test for cholesterol and unsaturated sterols.

Results

Annual life cycle

Gonadal and reproductive cycles in populations of toads living at higher latitudes are closely correlated with the alternating periods of hibernation and non-hibernation.

The duration of the hibernating and non-hibernating states varies geographically, as well as with local annual climatic variations. In Denmark, sexually mature *Bufo bufo bufo* usually emerge from hibernation underground and migrate to the breeding ponds in April. Observations over 20 years, from 1957 to 1978, showed that in 9 of the years the toads gathered at the breeding ponds during the first half of April, in 10 of the years during the second half of April, and only in one year as late as early in May (Werner Nielsen, personal communication).

The factors that regulate the duration of the hibernating state have not been studied systematically, but apparently temperature and precipitation are of primary importance. In cold and dry springs the toads will emerge later from hibernation than in warm and wet springs. Also internal factors may be of importance in determining when toads emerge from hibernation. In late winter higher temperatures are needed to make toads emerge than in spring. Thus, it was observed that male toads hibernating in earth in a wooden box remained buried for at least a week when exposed to a temperature of about 10°C in early March. But later in the season, in April, toads have been observed in amplexus at temperatures close to zero.

Emergence from hibernation presumably takes place several days, or even weeks, before the sexually mature toads start their migration to the spawning grounds, but little is known about the behaviour between termination of hibernation and initiation of the synchronous breeding migration of populations of toads. Breeding migrations include the entire population of sexually mature toads of a specific geographical area. Migrations seem to be triggered or favoured by a particular constellation of climatic factors, including afternoon rain combined with high temperature during the period following discontinuation of hibernation. Such specific combinations of climatic conditions may act to synchronize the migrations to and gatherings at the spawning grounds within populations of toads (Knud-Erik Hede, personal communication; Heusser 1968c).

The sexually mature toads do not feed until after spawning, and not before the temperature has reached about 11-12°C (Heusser, 1968b; Hanne Gyde Poulsen, personal communication). Moreover, toads do not seem to spend much time on feeding during the month following spawning. Very few catches of toads have been recorded from May. Toads are most easily caught from July to September, presumably because this is the period of the year when they are most actively feeding.

It is not known which factors determine when the toads enter the hibernating state, but probably both internal and external factors are involved, as in the case of emergence from hibernation.

The last observations of toads active above ground during the years from 1967 to 1973 have varied between early October and early November, during an exceptionally mild autumn.

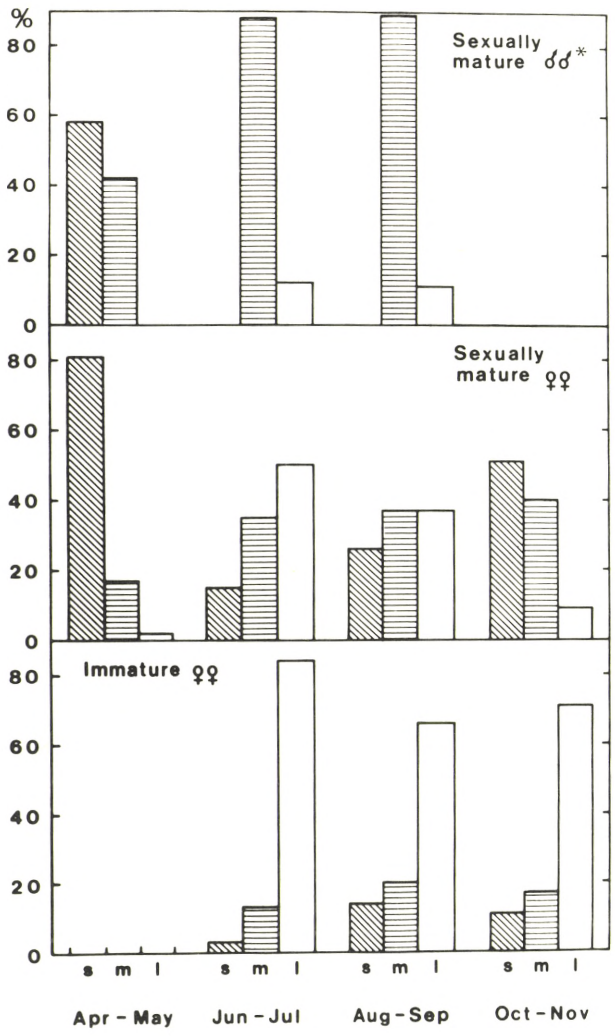


Fig. 2.
Annual cycle in size of fat bodies of the toad *Bufo bufo bufo*, expressed as a percentage of the body weight. s, ≤ 0.1%; m, 0.2-1%; l, > 1%.* Early June values included in April-May group. The material includes 131 males, 355 mature females, and 116 adult-sized females (>50 g b.w.) with ovaries lacking large oocytes. Ordinate: percentage of toads in the various groups.

Fat body cycle

The size of the fat bodies reflects the nutritional state of the toads. The annual waxing and waning in mass of the fat bodies therefore indicate variations in the nutritional state. When food is amply available and the climate is optimal the fat bodies grow due to fat deposition. When food is scarce or when temperatures are too low for feeding, fat is mobilized, and the fat bodies diminish in size.

In temperate zone amphibians and reptiles fat body size is correlated with gonadal development. The annual variation in fat body mass was therefore followed separately in sexually mature and immature females, and in sexually mature males (Fig. 2).

The data have been grouped according to the following periods: around and immediately after breeding (April-May), early summer, (June-July), late summer (August-September), and autumn (October-November). The fat bodies of mass up to 0.1% of the body weight were classified as small, fat bodies amounting to 0.2-1% of the body weight as medium-sized, and more than 1% as large. Completely reduced fat bodies, deprived of the fat stores, weigh about 0.01% of the body weight. Maximum weights recorded amount to about 5% of the body weight.

It may be seen from Fig. 2, that data from spring for immature females are lacking. This is because sexually immature toads emerge later from hibernation than the sexually mature, and therefore are not easily available for sampling at this time of the year. During all the subsequent sampling periods large fat bodies predominated in immature females.

Most females caught during or shortly after the breeding season had small fat bodies. During summer the proportion of sexually mature females with large fat bodies increased and decreased again during autumn.

Also in sexually mature males, small fat bodies prevailed around the breeding season. In early and late summer medium-sized fat bodies predominated. Unfortunately, no males were caught during late autumn. It is, however, indicated that also in the males the fat bodies tend to decrease in size before hibernation. It may be seen from Fig. 3 that fat body weights reach their maximum values in July-August. It may also be seen that fat body weights tend to decrease after breeding and reach their minimum values in early June.

The most striking difference in fat body mass is between mature and immature females, i.e. females with and without large oocytes in the ovaries. It is noteworthy that in the males, fat bodies mostly do not reach large sizes during the annual cycle. This is not due to an inherent difference in capacity for fat deposition between males and females. When males are maintained well fed in the laboratory, they develop fat bodies amounting to several percent of the body weight as do female toads (unpublished data).

The finding that, especially in sexually mature females, mean fat body weights decreased significantly during late fall indicates that fat reserves accumulated during the summer season begin to be drawn upon already in the prehibernating period. The question, therefore, arises as to the importance of fat bodies and other energy depots in covering the toads' metabolic requirements before as well as during and after hibernation. The pattern of utilization of energy depots varies greatly both between individuals within a population of toads and between populations inhabiting various localities. It also varies from year to year, probably depending upon prevailing feeding conditions. This large variation in patterns of annual fat body cycles is illustrated by the following examples.

Small fat bodies in sexually mature females ready to hibernate. Out of 10 female toads collected at Hallebyore, Zealand (Fig. 1), at the end of October, when hibernation has usually started, 9 were sexually mature. They all prossessed fat bodies practically depleted of fat (mean weight 0.03% of the body weight, with a range from 0.01 to 0.06%). The ovaries contained normal batches of full-sized oocytes in all 9 toads. One adult female, which had not produced a batch of large eggs that season, had large fat bodies, amounting to 2.1% of the body weight.

Reduction in fat body size during hibernation as a function of fat body size. (1). A group of female toads caught during October-November at Allinge,

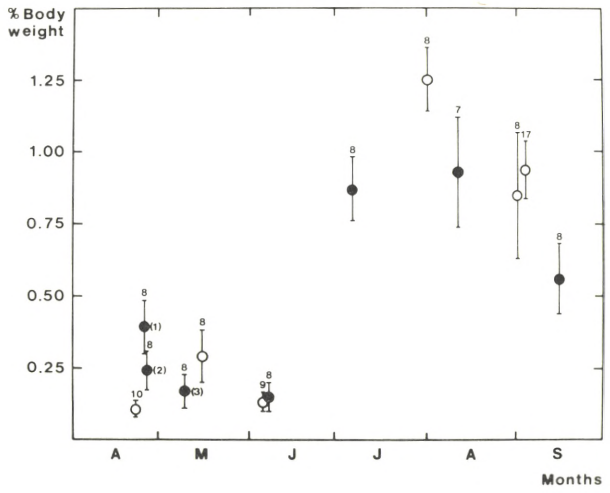


Fig. 3.
Annual cycle in size of fat bodies, expressed as a percentage of the body weight, in male toads, *Bufo bufo bufo*, collected north of Copenhagen. Open circles indicate toads collected in the years 1963-1965; filled circles in the year 1966. Vertical lines indicate standard errors of the means and superscript figures numbers of toads in the groups. (1) Toads collected during migration to the breeding ground; (2), toads in amplexus at capture; (3), toads collected in amplexus about 2 weeks previously. Ordinate: weight of fat bodies as a percentage of body weight.

Bornholm (Fig. 1), included exceptionally many specimens that had not developed large eggs, but had large fat bodies. The fat body mass was measured in groups of such females kept in a refrigerator at about 4°C. The initial mean fat body weight in a sample of 17 toads was 1.7 ± 0.4% of the body weight. After 1 month in the refrigerator the mean fat body weight was 3.2 ± 0.7% (18 toads), after 3 months it was 2.7 ± 0.5% (9 toads). No significant mobilization of the fat body depots could, therefore, be demonstrated after 3 months of simulated hibernation.

(2). A group of male toads was caught in late September and stored in the refrigerator, at 5-6°C, until the end of October when part of the toads was placed in an outdoor enclosure, where they buried themselves in the ground. The other toads remained in the refrigerator. Samples of 5 animals were autopsied monthly from each of the hibernation sites. The weights of the fat bodies are shown in

Table 1. Effect of low temperature and hibernation on fat body size in the toad[a]

	December	January	February	March	April
Refrigerator	1.2(0-2.4)	0.2(0-1.0)	0.3(0-1.3)	0	0
Outdoors	0.3(0.2-0.5)	0.8(0.1-1.7)	0.7(0-1.6)	0.5(0-1.3)	0.3(0-1.2)

[a] Figures indicate mean and range of fat body weights expressed as a percentage of the body weight. Each sample includes 5 male toads. 0 indicates that fat bodies weighed less than 0.1% of the body weight.

Table 1. It can be seen that in the December-February samples the fat bodies ranged from small to large both among the toads from the refrigerator and among those hibernating outdoors. However, the mean weight of the fat bodies in the refrigerator toads decreased, and all toads sacrificed in March and April had completely emptied fat bodies. The mean fat body weights did not change significantly in the toads hibernating outdoors. It is noteworthy, however, that whereas no toads had completely reduced fat bodies in the outdoor samples from December and January, such reduced fat bodies were observed i each of the samples from February to April.

(3). In a group of 8 female toads freshly caught in September the weight of the fat bodies was $0.18 \pm 0.04\%$ of the body weight. Further 7 toads of the same batch were kept hibernating outdoors until sacrifice at the end of March. At this time the fat bodies were practically emptied in all toads, the mean weight being 0.02% of the body weight. The batches of large eggs in the ovaries were normal with no or insignificant atresia among the eggs.

It is thus indicated that fat bodies that are small or medium-sized at the beginning of hibernation may be completely depleted of their fat at the end of hibernation, whereas large fat bodies mostly remain large also at the end of the hibernation period. It is, moreover, indicated that storage in the refrigerator at 5°C may cause faster depletion of fat from the fat bodies than does hibernation outdoors under conditions that more closely simulate hibernation in wild toads.

Ovary cycle

The reproductive capacity of female toads is expressed as the biomass of the eggs deposited during breeding. This biomass is determined by (1) the number of oocytes recruited to the final vitellogenetic growth, (2) number of oocytes that degenerate before ovulation or do not ovulate during spawning, and (3) the size of the full grown eggs. In the following, the ovarian cycle is analyzed in terms of ovarian weight, oocyte growth (vitellogenesis), number of large oocytes, and incidence of atresia among the large occytes.

Ovary weight

Fig. 4 shows the annual cycle in weight of ovaries that contain oocytes in the final vitellogenetic growth phase. Weights are also shown of newly spawned ovaries before a new batch of small oocytes has been recruited to the final growth phase. The figure includes samples of toads caught at a number of different localities. The open circles represent the samples collected north of Copenhagen in the years 1965/66. It may be seen that prior to spawning the ovaries constitute about 18-19% of the body weight. Immediately after spawning their weight drops to about 2.5%.

Toads are most easily caught when they gather on the spawning grounds. This is reflected by the relatively large number of toads studied immediately before and after spawning. In the following months both number of samples and of toads in the samples are few, partly because of difficulties in finding toads during the months of May to July and

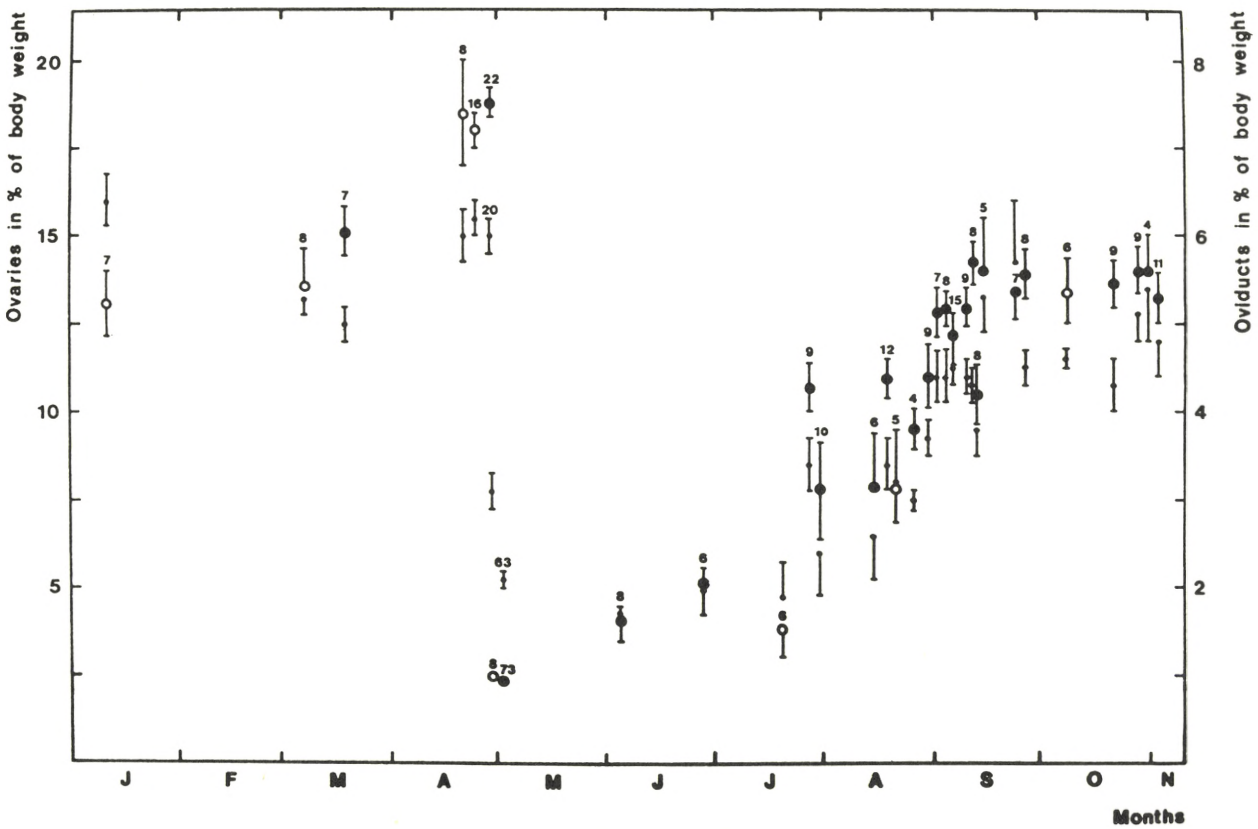


Fig. 4.
Annual cycle in weight of ovaries and oviducts, expressed as a percentage of the body weight, in female toads, *Bufo bufo bufo*. Circles indicate ovaries, points oviducts. Open circles indicate toads collected in the region north of Copenhagen during the years 1965 - 1966, filled circles toads collected from various parts of Denmark during the years 1963 - 1972 (see Fig. 1). Vertical lines indicate standard errors of the means, and superscript figures numbers of toads in the groups.

partly because at this time of the year a large proportion of toads has not yet started a new ovarian cycle (see below). Fig. 4 shows that ovarian weights are still low in sexually mature toads caught in June and the greater part of July. By the end of July and during August there is a great increase in ovarian weight. By the end of September the ovarian weight amounts to about 14% of the body weight. It remains constant during hibernation. However, shortly before spawning the relative weight increases. There was no significant difference in ovarian weight in toads newly emerged from hibernation or collected on their way to the spawning grounds and in toads during amplexus, before ovulation had commenced.

The increase in weight of the ovaries prior to spawning was significant. In a sample of 57 mature female toads sacrificed just before and during hibernation, from October to early March, the mean ovarian weight was $13.7 \pm 2.2\%$ of the body weight and in a sample of 46 toads collected shortly before spawning the weight was $18.4 \pm 2.5\%$ ($P < 0.001$).

As the toads ranged in weight from 39 to 139g the relations between ovarian weight and body weight were analyzed by linear regression. The following relations were found between ovarian weights and body weights in the two groups of toads:

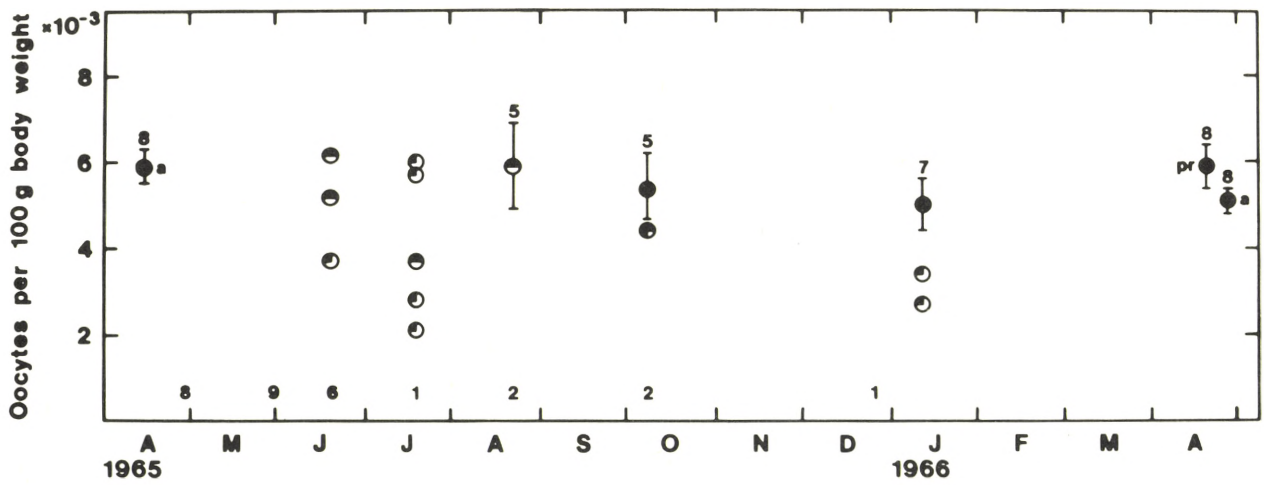


Fig. 5.
Annual ovarian cycle in female toads, *Bufo bufo bufo*, collected in a region north of Copenhagen during the years 1965 - 1966. Ordinate: number of large oocytes in ovaries, expressed per 100 g body weight. , Oocytes full-grown; , oocytes in late growth phase; , oocytes in middle growth phase; , oocytes in early growth phase. Vertical lines indicate standard errors of the means and superscript figures number of toads in the groups. Figures below indicate number of adult-sized toads (>50 g b. wt.) with ovaries lacking large oocytes. a, Toads in amplexus at capture; pr, prespawning toads.

(1) October-March sample: $y = -1.76 + 0.16x$
$(n = 48; r^2 = 0.84)$
(2) April (pre-breeding) sample: $y = -1.13 + 0.20x$
$(n = 45; r^2 = 0.78)$.

Calculated for a body weight (x) of 100g, the ovarian weights (y) are 14.2g and 18.9g, respectively.

The mean body weight of the October-March samples was 85g and the mean ovary weight was 11.8 = 13.9g/100g body weight. The mean body weight of the April sample was 89g and the mean ovary weight was 16.7g = 18.8g/100g body weight. The mean values of the samples thus differed only insignificantly from the values obtained by the linear regression, supporting that the weight of the full grown ovaries is closely correlated with the body weight.

Number of oocytes and stage in vitellogenetic growth

Fig. 5 shows the number of large vitellogenetic oocytes present in the ovaries of the females collected north of Copenhagen 1965/66. The vitellogenetic growth is staged as early, middle, late, and finished growth.

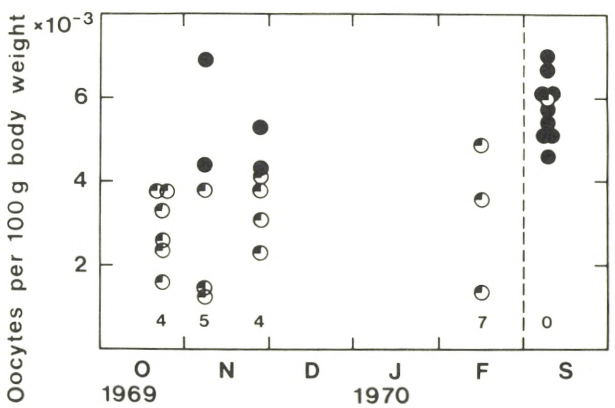
At the time of breeding the ovaries contained about 5,000-7,000 large oocytes per 100g body weight. The ovaries of adult females caught in late April and May had not yet recruited oocytes to the final vitellogenetic growth phase. In June, vitellogenetic growth had commenced in 3 out of 9 adult females, and full numbers of final growth phase oocytes had been reached in the 2 toads with ovaries in the middle stage of growth. It can be seen that full numbers of oocytes can be present even in ovaries in the early growth stage. It is thus indicated that recruitment of oocytes to the final growth phase is complete early in the ovarian cycle. In the August sample the middle growth phase predominated. From October onwards the large oocytes had finished growth. In Danish populations of *B. bufo*, adult females thus predominantly recruit oocytes to the final growth phase early in the summer, and vitellogenetic growth is fastest later in summer (Jørgensen, 1973a).

Most large females captured during late summer and autumn do possess ovaries containing oocytes in

the final growth phase. However, non-typical females can be encountered. Thus, only a few large females, of body weight well above 100g, collected at Allinge, Bornholm (Fig. 1), during the month of October, contained ovaries with oocytes that had finished growth (see p. 9). Mostly, the ovaries contained no final growth phase oocytes, or the oocytes were in a very early stage of growth. Some of the toads were kept artificially hibernating in a refrigerator at about 4°C. There was no clear change in the developmental status of the ovaries during hibernation. Toads collected from the same locality in October the following year exhibited normal ovarian patterns (Fig. 6).

The numbers of large oocytes have been counted in the ovaries of toads sampled from different localities. In some instances the dominant size of the full grown oocytes was also determined. The mean numbers in the samples varied from about 4,000 to about 7,000 per 100g body weight (Fig. 7). There was no correlation between body weight and number of oocytes/100g body weight. There were statistically highly significant differences in mean numbers of oocytes, e.g. in toads from Christiansø and Hallebyore on one hand and from Falster and

Fig. 6.
Example on an atypical pattern of ovarian function in adult-sized female toads, *Bufo bufo bufo*, collected at Allinge, Bornholm (see Fig. 1), in two subsequent autumns. Further explanation, see legend to Fig. 5. Toads from October 1969 and September 1970 are freshly collected. Toads autopsied in November and February belonged to the batch collected in October 1969.



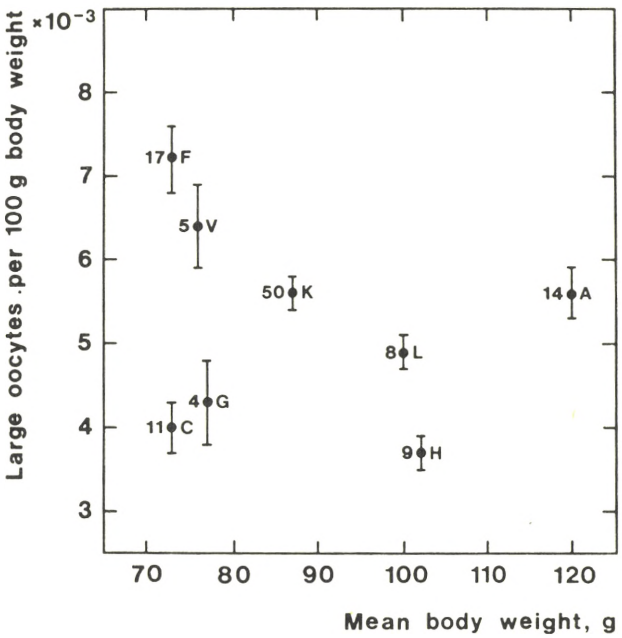


Fig. 7.
Numbers of large oocytes in ovaries of toads collected during autumn at different localities in Denmark. Figures to the left of the mean values indicate number of toads in the groups and vertical lines standard errors of the means. Letters to the right of the mean values refer to localities identified on Fig. 1. Abscissa: mean body weight of the toads; ordinate: number of oocytes expressed per 100 g b. wt. Differences in body weights were highly significant between samples of toads from F and A, and C and A ($P < 0.001$). Differences in numbers of oocytes per 100 g b. wt. were highly significant between samples of toads from F and H, F and C, F and L, F and K, and C and A ($P < 0.001$).

Vendsyssel on the other. This difference in oocyte numbers did not show any clear geographical trend.

The size of the full grown oocytes varied inversely with the number of oocytes (Fig. 8). An inverse relationship between number and size of full grown oocytes was expected because no significant differences have been observed in mean ovary weights expressed per 100g body weight in samples obtained from the different localities in Denmark (Fig. 4). Apparently the same amount of yolk stored in the ovaries may be distributed among varying numbers of oocytes. It remains to be seen whether the differences observed in mean numbers of oocytes in

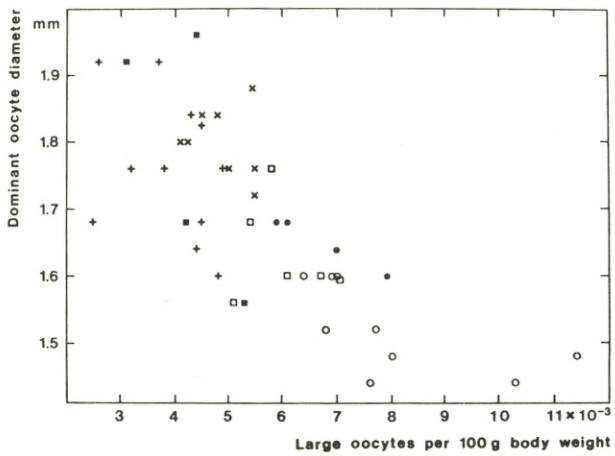


Fig. 8.
Relationship between number and predominant size of full-grown oocytes in ovaries of populations of toads from various Danish localities. Abscissa: number of large oocytes per 100 g b. wt. Ordinate: predominant oocyte size, in mm. , Vendsyssel; x, Lellinge; , Falster; +, Christiansø; , Allinge; ■, Gudhjem (see Fig. 1).

samples obtained from different localities are representative for the populations sampled, and thus may be genetically determined, or whether the differences are caused by varying local factors in the environment. Fig. 7 shows that the mean body weights of the samples of toads also varied, from about 75g to 120g, the differences being statistically highly significant. Again it remains to be seen whether the differences between body sizes of sexually mature females in the various populations are genetically determined or not.

Atresia of large oocytes

Atretic large oocytes are commonly observed in the ovaries of adult female toads. The atretic oocytes are easily recognized by their content of black pigment, which is the last component of the oocyte to become degraded and reabsorbed. It is not well known how long it takes a large oocyte that has become atretic to disappear completely, but it lasts many months. No systematic studies are available on the frequency of atresia among the large oocytes of wild toads. Atretic oocytes were therefore counted in the ovaries of samples of toads collected at various localities in autumn. The result is shown in Table 2. It can be seen that atresia was absent in 8 out of the 38 toads included in the study. Slight to moderate atresia, up to 30% of the number of intact large oocytes, was observed in more than half the toads. Mostly the atretic oocytes were small, presumably mainly representing oocytes that did not ovulate during spawning the previous spring. However, also large, *i.e.* more recently degenerated oocytes were found regularly, indicating that atresia may occur as a sporadic phenomenon within a healthy population of oocytes. In one of the 38 toads the number of atretic oocytes equalled that of the healthy large oocytes, indicating that a complete batch of oocytes had become atretic.

Most instances in which batches of large oocytes became atretic seem to represent failing ovulation during the normal breeding season, presumably because the females for some reason did not reach

Table 2. Atresia among large oocytes in ovaries of wild Danish female toads. [a] Figures indicate number of toads.

Locality	Atretic oocytes, as percent of intact oocytes				
	0	1-10	11-30	31-60	>60
Vendsyssel	0	3	2	0	0
Falster	4	3	5	3	1
Lellinge	0	2	2	4	0
Allinge	4	2	3	0	0
Total	8	10	12	7	1

the spawning grounds (Knud-Erik Hede, personal communication). However, complete atresia of batches of large eggs may also occur at other times of the year. An exceptional example for Danish populations of *B. bufo* was provided by a sample of 12 sexually mature female toads freshly caught in Dyrehaven, north of Copenhagen. Six of the toads had ovaries with freshly atretic large oocytes. In 5 of these toads all large oocytes had become atretic, in one only part of the oocytes. The other 6 toads possessed normal ovaries devoid of fresh atresia among the large oocytes. The 6 toads with fresh atresia had all small fat bodies. The mean weight was 0.06% of the body weight, range 0.02-0.13%. Most toads with intact ovaries had substantial fat bodies, the mean weight was 0.81%, range 0.07 - 1.56%. It is thus suggested that in this group of toads whole batches of large oocytes had become atretic because of starvation.

Oviduct cycle

The annual cycle in oviduct weight in sexually mature female toads from various Danish localities is shown in Fig. 4. It can be seen that the cycle closely follows that of the ovary weight. During ovulation and oviposition the weight of the oviducts becomes strongly reduced, presumably due to the emptying of the gland cells producing the egg jelly. The oviducts remain small until the end of July when they increase rapidly in weight concurrently with the rapid growth of the ovaries. The full grown oviducts approach 5-6% of the body weight. There was no clear change in oviduct weight prior to ovulation, as in the case of the ovarian weight (Fig. 4).

Testis cycle

Testis weight

The annual cycle in testis weight of freshly collected sexually mature males from the region north of Copenhagen is shown in Fig. 9. It can be seen that testis weight reaches a minimum after spawning,

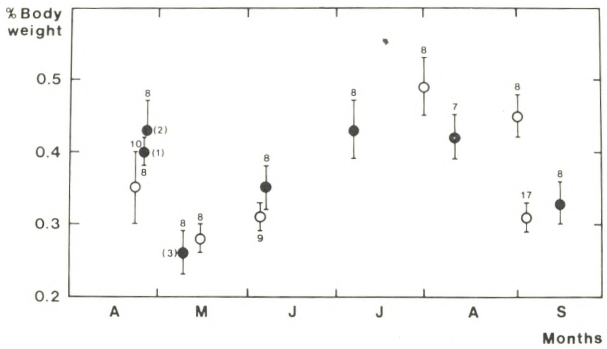


Fig. 9.
Annual cycle in weight of testes, as percent of the body weight, in toads collected north of Copenhagen. Open circles indicate toads collected in the years 1963 - 1965, filled circles in the year 1966. Vertical lines indicate standard errors of the means and superscript figures numbers of toads in the groups. (1), Toads migrating to the breeding ground singly; (2), toads migrating in amplexus; (3), toads collected in amplexus about 2 weeks previously.

presumably caused by spermiation (see below). Testis weight has increased again in the June samples and levels off during July-August. It is indicated that the testes undergo a second decrease in weight before reaching the weight at spawning. There was no difference in weight of testes from males migrating to the spawning grounds, singly or in amplexus (Fig. 9).

Spermatogenesis, interstitial tissue, and thumb-pads

The annual testicular cycle can be sub-divided into four main phases:

Phase I. The breeding period (April). All of eight toads collected during their migration singly to the spawning grounds, and of eight autopsied during amplexus, possessed testes in a similar histological and histochemical condition (summarized in Fig. 10). The lumina of the seminiferous tubules were filled with free spermatozoa (Fig. 11). Compact sperm bundles were comparatively few in number, and those that were present showed a loosening of the spermatozoa as they became released into the tubule lumen. Interspersed with this seminal mass

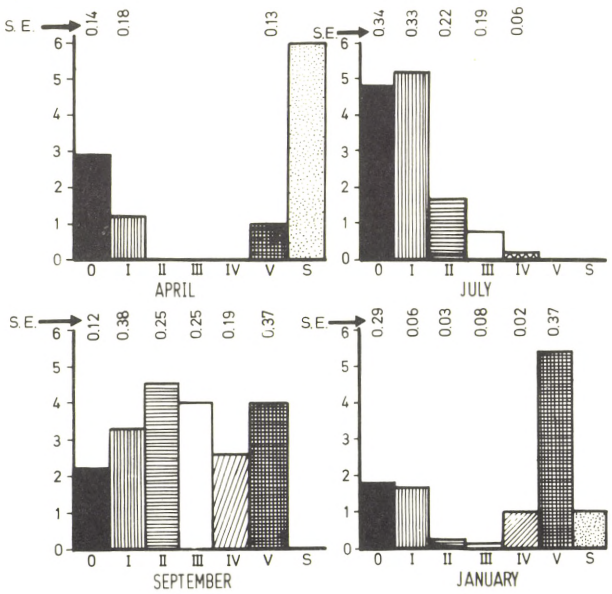


Fig. 10.
Spermatogenetic composition of the seminiferous tubules in *Bufo bufo* at different times of the year. For explanation of symbols see text, p. 6.

were detached Sertoli cells with a light scattering of sudanophilic droplets in the cell cytoplasm. Generally, however, there was very little intratubule lipid (Fig. 19).

The germinal epithelium showed little or no spermatogenetic activity. No mitotic figures could be detected in the residual primary spermatogonia, and only few small germinal cysts of secondary spermatogonia were present in some, but not all, of the sectioned tubules. In two of the amplectic males, many of the tubules had discharged their seminal content and were largely devoid of spermatozoa (Fig. 19). In none of the specimens were germinal cysts found in advance of spermatogonial stages.

Contrasting with the inactive germinal epithelium, the adjacent interstitial tissue was conspicuous and was obviously in a highly secretory condition. The cells were hypertrophied and possessed large, round nuclei with coarse clumped chromatin but no conspicuous nucleolus (Fig. 15). This has previously been described as a "secretory type" nucleus (Lofts, 1963). The interstitial cell cytoplasm contained a fine scattering of small, discrete lipid droplets (Fig. 19) which were negative to tests of cholesterol.

As might be expected with such an active interstitium, the thumbpads were maximally developed, with a thick epithelial layer whose outer surface was raised into sharp, keratinized spikes (Fig. 16). They were dark brown in colour.

This phase then, is marked by a period of low spermatogenetic activity, but intense spermiation resulting in the liberation of spermatozoa which become discharged over the eggs during oviposition. It is a period too, of high interstitial cell activity, and the high androgen levels maintain the androgen-dependent accessory sexual structures, such as the thumbpads, at the maximally developed level, and probably also stimulate the reproductive behavioural acitivites such as amplexus.

Phase II. The spermatogenetic period (May to July). Under natural conditions, there is generally an overlap between the previous period and the first few days of this period, as the amplectic phase dies out. All of the eight specimens autopsied in May had been caught in amplexus on 25th April and the pairs kept in a basin with water and sand until the males had left the females. These males were then killed and the testes removed and fixed on 9th May. The small testes (Fig. 9) contained regressed seminiferous tubules largely cleared of spermatozoa, although a residue of unevacuated seminal material remained in some tubules. The tubule lumina contained some germinal debris and detached Sertoli cells which appeared largely non-lipoidal, but which were seen to contain a few small lipoidal droplets when examined under high magnification. It is interesting to note that the heavily lipoidal and cholesterol-rich masses that occlude the lumen of the seminiferous tubules in *Rana temporaria* (Lofts *et al.*, 1972) and *R. esculenta* (Lofts, 1964) in the immediate post-nuptial period, do not occur in this species.

Spermatogenesis had begun as some of the primary spermatogonia were seen to be undergoing

Plates I-III

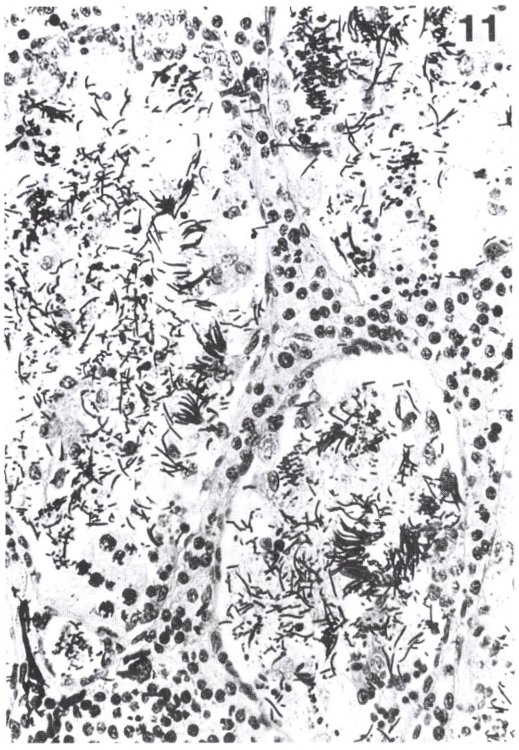
Figs. 11-14.
Bufo bufo testis material fixed in Bouin's fluid, wax embedded and stained with iron haematoxylin and orange G. X 300.

Fig. 11.
April. The seminiferous tubules contain large numbers of free spermatozoa in the tubule lumen, but only a few small spermatogonial cysts. The interstitium is hypertrophied and is in a highly secretory condition.

Fig. 12.
May. The spermatozoa have become largely discharged and the tubules are now becoming filled with a new generation of germinal cysts, which at this stage are mainly of secondary spermatogonia. The free spermatozoa in the lumen are the residue of the prevous generation. The interstitium is less conspicuous and non-secretory.

Fig. 13.
August. All stages of spermatogenesis can now be seen in the seminiferous tubules, and the more advanced stages are much more common. Some spermatids and young spermatozoa can be seen.

Fig. 14.
January. Most of the germinal cysts have now developed into spermatids and spermatozoa.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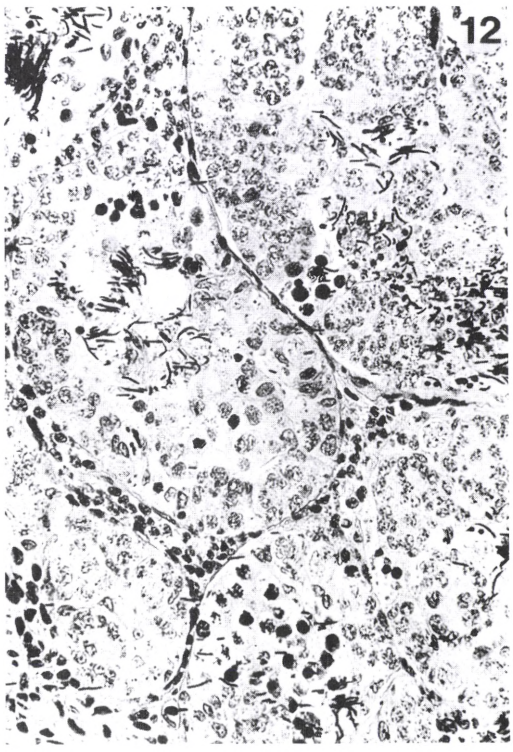
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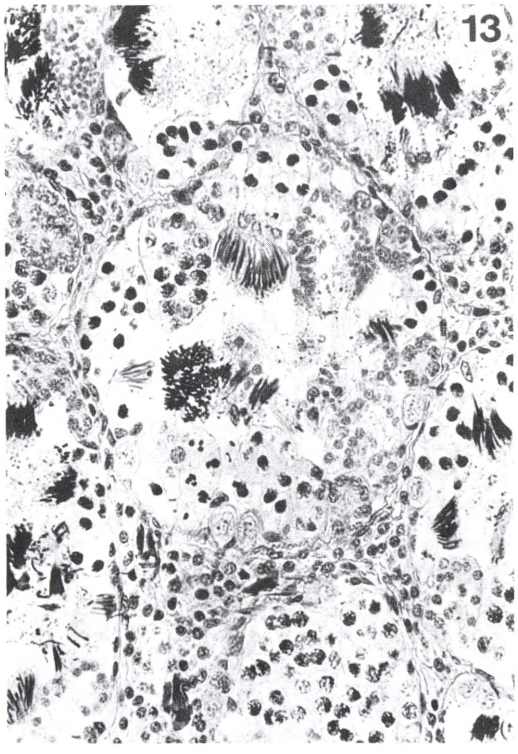
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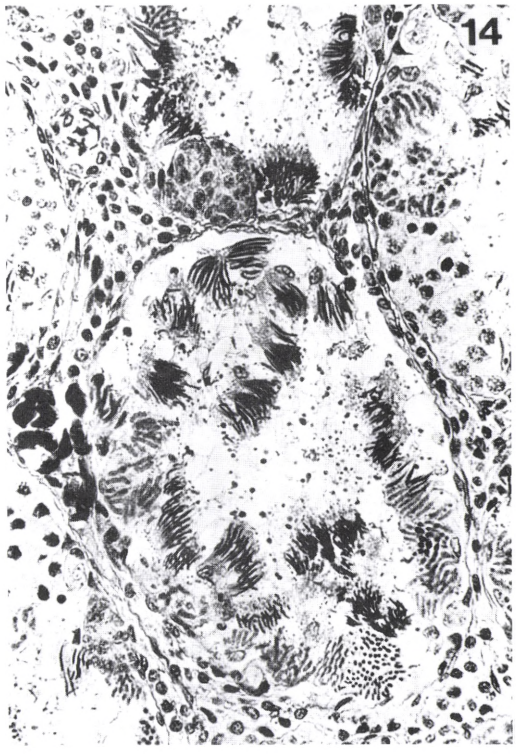
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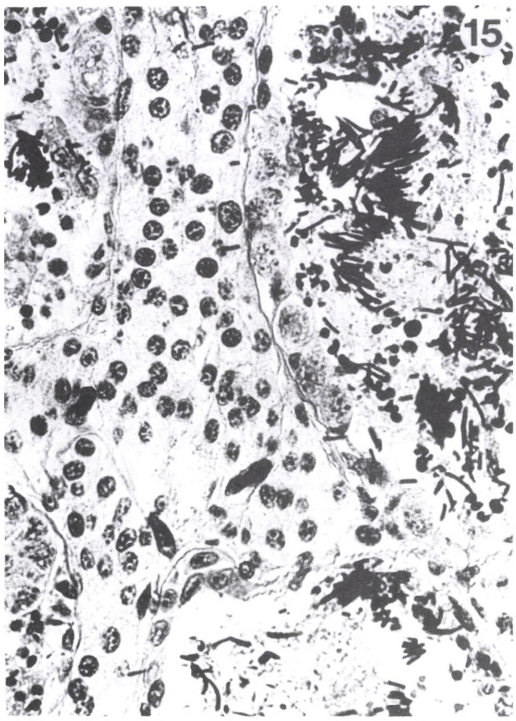
Fig. 15.
Shows the large rounded nuclei in the hypertrophied interstitial tissue of an April toad. Bouin; Iron haematoxylin and orange G. X 500.

Fig. 16.
Sectioned thumbpad of April toad showing the spiked and keratinized surface. Bouin; Haematoxylin and eosin. X 150.

Fig. 17.
Shows the regressed interstitial cells with shrunken wrinkled nuclei, of a May toad. Bouin; Iron haematoxylin and orange G. X 500.

Fig. 18.
Sectioned thumbpad of May toad showing the regressed condition of the epithelium which has become smoother and non-keratinized.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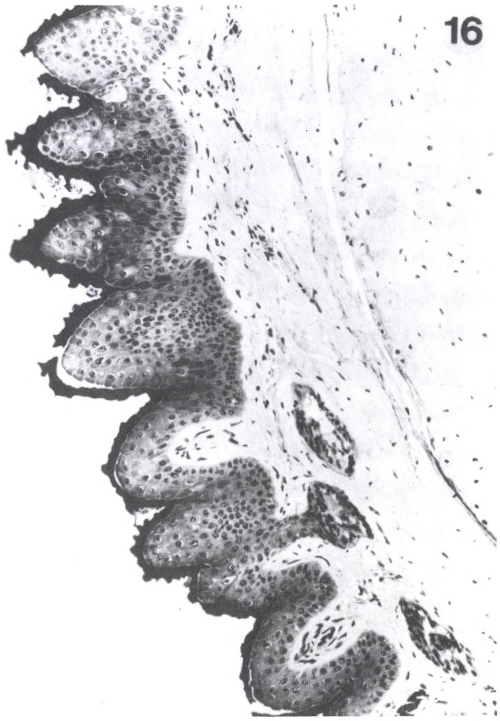
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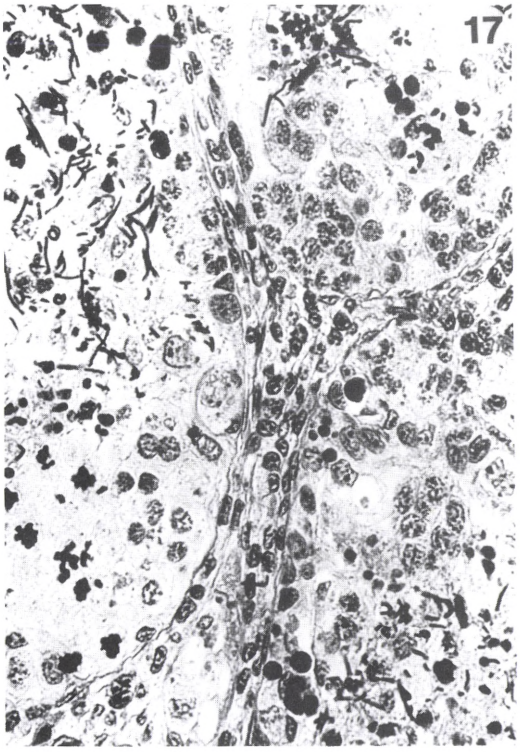
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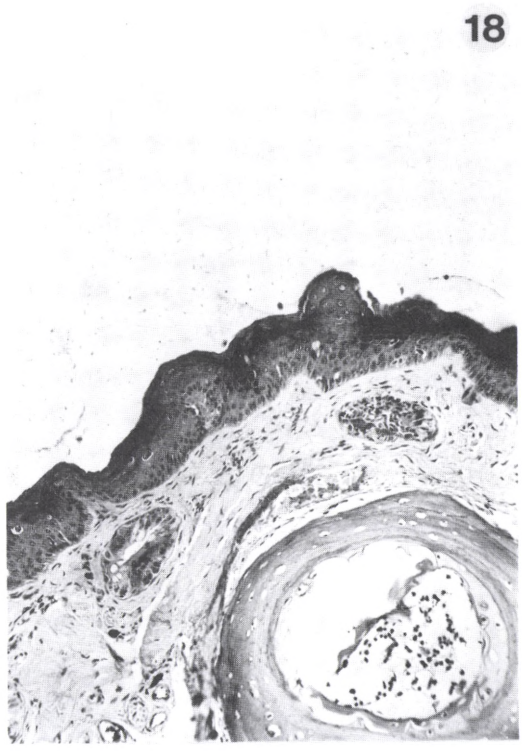
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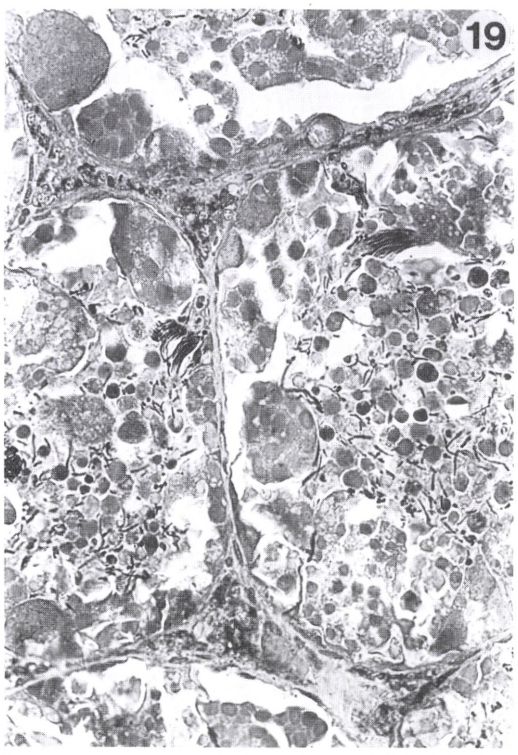
Figs. 19-22.
Testis material fixed in formal-saline, gelatine embedded and stained with Sudan Black B and carmalum.

Fig. 19.
April. Testis of a frog in amplexus in which most of the spermatozoa have become discharged. Detached Sertoli cells are accumulating in the lumen, but are not very sudanophilic. The interstitial cells contain a few small lipoidal droplets. X 500.

Fig. 20.
May. The regressed testis immediately after the breeding period showing the densely lipoidal interstitial cells. X 260.

Fig. 21.
August. The interstitial tissue is becoming less lipoidal at a time when spermatids and young spermatozoa are beginning to appear in the tubules. X 300.

Fig. 22.
February. The interstitial lipids have become depleted as the tissue becomes secretory and as the animal approaches the next breeding season. X 300.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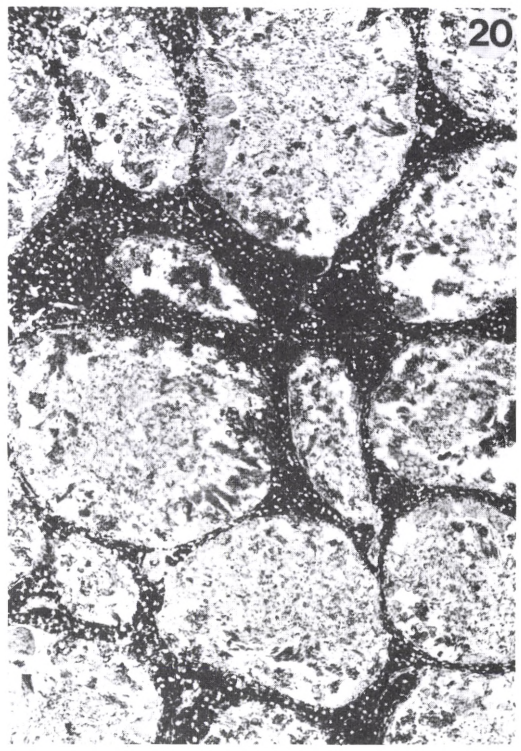
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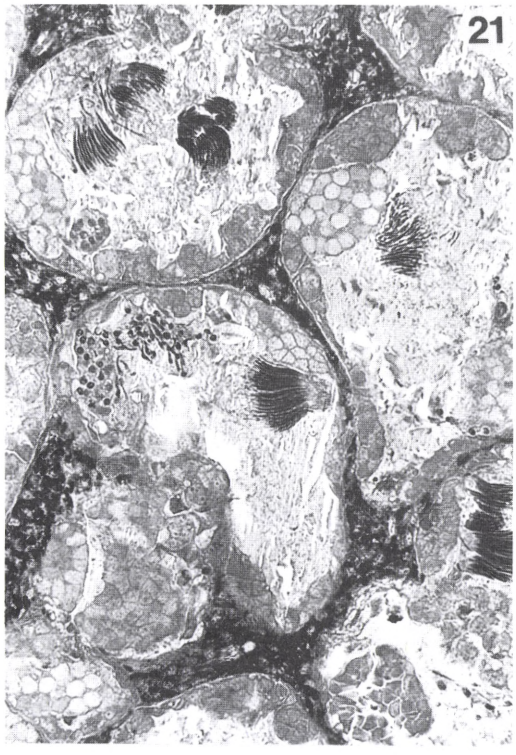
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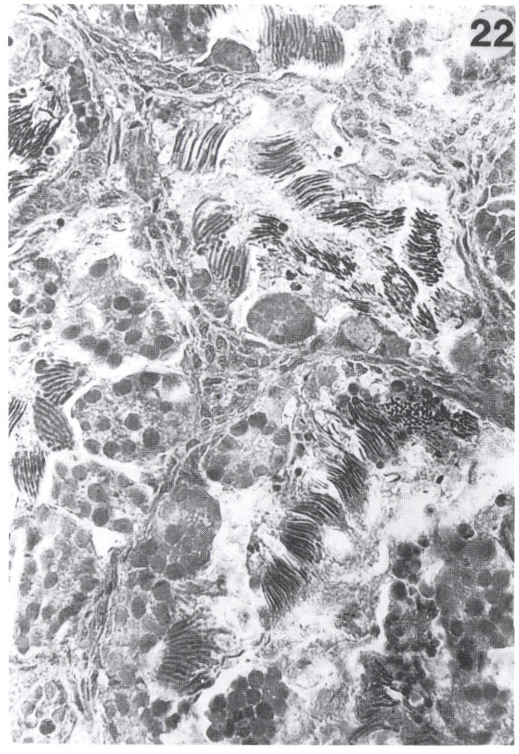
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mitosis. They were more numerous per cross sectioned tubule than during the first phase, as were also the secondary spermatogonial cysts. Germinal cysts were now more numerous and larger, and as the phase progressed the seminiferous tubules gradually became packed with developing cysts (Fig. 12). In the May sample these were almost exclusively spermatogonial cysts, but by the end of the phase, in July, primary spermatocytes were also represented but rarely spermatids (Fig. 10). There were occasional spermatozoa noted in isolated tubules of some of the June specimens, but these were old residual spermatozoa (Fig. 12) which were degenerating, and by July these had generally been cleared away.

Concomitant with the build-up of spermatogenetic activity, there was a regression of the interstitial tissue, whose cells became smaller and their nuclei shrunken and wrinkled in outline (Fig. 17). The cell cytoplasm became densely lipoidal (Fig. 20) and strongly cholesterol positive, indicating a rapid decline in androgen synthesis. This decline in sex hormone production was correlated with the rapid atrophy of the thumbpads. These structures sloughed off their keratinized layer and the epithelial zone become thinner and smooth (Fig. 18). In June animals they were light brown, but by the end of the phase they were colourless and almost completely absent.

Thus this phase is marked by a cessation of interstitial cell activity and a wave of spermatogenetic activity which results in the propagation of a new generation of germinal cysts packing the seminiferous tubules.

Phase III. The maturation period (August to January). There is an overlap between the beginning of this phase and the termination of the latter. Of the eight toads in the 8th August sample two were identical in testicular condition with the July sample of the previous phase, but in all the remainder the seminiferous tubules had reached a more advanced spermatogenetic condition. In addition to numerous spermatogonial and spermatocytic cysts, many specimens had developed some spermatids and even had isolated bundles of spermatozoa in some tubules (Fig. 10 and Fig. 13). Generally, however, the spermatogenetic activity was seen to decline during this period, and mitotic figures among the residual primary spermatogonia were no longer noticeable. Spermatogenesis probably did not cease altogether since at no stage was there a complete absence of small secondary spermatogonial cysts. However, their production was probably very slow and, more characteristically, the phase was characterized by a gradual maturation of the germinal cysts which had already been propagated during the highly spermatogenetically active period, and throughout the rest of the year spermatogonial and spermatocytic cysts became fewer as they matured into spermatids and, eventually, bundles of spermatozoa. By January the seminiferous tubules were found to contain numerous Sertoli cell-sperm bundle systems and spermatids, but only a few spermatogonia and primary and secondary spermatocytes (Fig. 14). This is the reverse of the situation noted at the start of the phase in August (Fig. 10).

The recovery of the interstitial tissue took place during this phase. In August the interstitial cells began to enlarge and the nuclei began to resume their "secretory type" structure, so that by September the interstitial tissue was once again well developed. This rehabilitation of the interstitium was accompanied by a depletion in the cytoplasmic lipid material (Fig. 21) and a decline in the intensity of the cholesterol reaction. The development of the interstitial tissue was paralleled by the development of the thumbpads, marked first by a thickening of the epithelium and then by the surface becoming papillate. By January the surface had become more spiky and had become keratinized, and the thumbpads appeared light brown.

This then is a phase marked by the decline of spermatogenesis concomitant with an acceleration of spermatohistogenesis resulting in numerous bundles of spermatozoa populating the tubules. It is also marked by a recrudescence of interstitial cell

activity and elevated androgen secretion which stimulates the development of the thumbpads.

Phase IV. The spermiation period. Late hibernation. This phase could be regarded as the culmination of the preceding phase and leads up to the next breeding period. The eight toads examined in March showed an increasing acceleration of the spermatohistogenetic processes. Bundles of spermiating spermatozoa filled all the tubules, and freed spermatozoa were accumulating in the tubule lumina. Apart from primary spermatogonia, only small isolated cysts of secondary spermatogonia remained in the germinal epithelium and spermatocytic cysts had disappeared.

Interstitial cell activity was high, and this tissue had once again assumed the appearance noted in the previous April with hypertrophied cells depleted of most of their lipid (Fig. 22), and rounded enlarged nuclei. The thumbpads were dark brown and fully developed.

Table 3. Annual cycle of fat body weight in some temperate zone anurans

			Mean weights of fat bodies as percent of body weight						
Species	Locality	Sex	Max.	Month	Min.	Month	Size at spawning	Month	References
Bufo bufo	Denmark	♂	1.0	Aug	0.15	Jun	0.3	Apr	Present work
		♀	ca 1	Aug	~ 0	May	~ 0	Apr	
	Peking,	♂	2.3	Aug	0.05	May	0.08	Apr	Ting and
	China,	♀	3.5	Aug	0.02	Jun	0.04	Apr	Boring, 1940
Rana temporaria	Besançon	♂	1.4	Oct	0.11	Apr	0.11	Apr	von Kennel, 1913
	France	♀	ca 3	Oct-Nov	0.06	May	0.08	Apr	
	Bonn,	♂	0.5	Jun-Jul	~ 0	Apr	0.05	Mar	Zepp, 1923
	Germany	♀	0.7	Aug	0.03	May	0.2	Mar	
	Shropshire, U. K.	♂ + ♀	0.8-0.9	Jul-Oct	0.02	May	0.04	Mar	Smith, 1950
	Cracow,	♂	1.0	Jul-Sep	0.02	Mar	0.02	Mar	Krawczyk, 1971
	Poland	♀	1.3	Jul	0.03	Mar	0.03	Mar	
	Haapavesi,	♂	0.7	Sep	ca 0.1	May	ca 0.1	May	Pasanen and
	N. Finland	♀	0.4	Sep	0.03	May	0.03	May	Koskela 1974
Rana esculenta	Zürich,	♂	2.0	Sep	0.12	Jun	0.12	Jun	Gaule, 1901
	Switzerland	♀	1.3	Aug-Sep	0.03	Jul	0.04	Jun	
	Bonn,	♂	1.2	Sep	0.13	May	0.13	May	Zepp, 1923
	Germany	♀	0.9	Aug	0.10	May	0.10	May	
	Cracow,	♂	2.3	Sep	0.08	May	0.08	May	Krawczyk, 1974
	Poland	♀	1.7	Oct	0.03	May	0.03	May	
Rana pipiens	U.S.A.	♂ + ♀	0.4	Aug	0.01	Jun	0.03	Apr	Mizell, 1965

Discussion and Comparisons

Fat body cycle

In anurans the most conspicous site of fat storage is the fat bodies, but fat is also stored in other organs, especially in the liver and as carcass fat (Gaule, 1901; Athanasiu and Dragoin, 1910; Barthélémy, 1930; Smith, 1950; Mazzocco, 1938; Bush, 1963; Mazur, 1967; Brenner, 1969; Krawczyk, 1971, 1974; Seymour, 1973; Pasanen and Koskela, 1974; Byrne and White, 1975; Fitzpatrick, 1976). In contrast to these other storage sites, the fat bodies are primarily, and perhaps exclusively, depot organs. The size of the fat bodies is therefore a convenient indicator of the nutritional state of the organism. It is of special interest to note the periods of the annual cycle during which the fat bodies are largest or smallest. This maximum and minimum indicate the general change in the food balance of the organism from positive to negative and *vice versa*. In Danish toads the fat bodies tend to be largest during late summer or early autumn. It thus seems that most toads have entered negative food balance before the period of hibernation. The fat bodies usually reach minimum size in May-June, some time after spawning. Feeding in the post-spawning period thus seems insufficient to increase the fat depots even in the absence of vitellogenetic growth of oocytes in the females. It is not known to which extent motivation for feeding, food availability or other external or internal factors are limiting the food uptake. We only know that toads are rarely observed.

Reasonably complete data on annual cycles in fat body size are available for only a small number of anuran species (Table 3). Comparisons of the fat body cycles in these species, or in populations of the same species from various regions of its geographical range of distribution, are made difficult owing to a general lack of information on the length of time that lapsed between capture and autopsy of the animals, or on storage conditions. The recorded fat body sizes may therefore not be representative of populations of wild toads. In one instance, the fat body sizes recorded are obviously substantially lower than they were in the freshly captured frogs, because of the treatment of the animals (*Rana pipiens*, Mizell, 1965).

Table 3 shows that the fat bodies generally reach their smallest size at or after the spawning period, when fat body sizes less than 0.1% of the body weight predominate. Fat bodies of this size are practically depleted of fat (Roca *et al.*, 1970), and also the protein content of the fat bodies is being reduced (Victoroff, 1908). Other less complete studies on annual cycles in amphibian fat bodies support the view that the fat bodies in temperate zone anurans reach minimum size around the breeding time (*Bufo arenarum*, Argentine, Mazzocco, 1940a; *Bufo fowleri*, Georgia, U.S.A., Bush, 1963). These observations agree with the finding that anurans generally feed little or not at all until after breeding (Heusser, 1968b, 1969b; Itämies and Koskela, 1970; Jastrezebski, 1968; Juszczyk, 1950, Juszczyk *et al.*, 1966; Koskela and Pasanen, 1974; Mizell, 1965; Victoroff, 1908).

It is widely believed that the fat bodies in amphibians reach their maximum size in autumn, and that the fat bodies serve as an energy depot primarily during hibernation (e. g. Brown, 1968; Holmes, 1916). However, as shown in Table 3, the most complete studies suggest that also in populations of *B.*

bufo from China, as well as in other temperate zone anurans the fat bodies tend to reach their largest size well in advance of hibernation.

Only in the populations of *Rana temporaria* inhabiting the northernmost part of the range of distribution of this species the time of maximum fat body size coincides with the start of the hibernating period. This may be correlated with the short duration of the feeding period and early hibernation of the frogs at the high latitudes. Also the size reached by the fat bodies tends to be smaller in the frogs from northern Finland than in frog populations from other parts of Europe (Table 3).

Our data from *B. bufo* indicate that individual variation, local conditions, and annual variation strongly influence the size attained by the fat bodies. Similar variability has been recorded in other species. It was early noticed that in *Rana esculenta* fat body size varied greatly among individuals of the same population even when collected at the same time of the year (Victoroff, 1908). Regional or annual variation may be responsible for the different sizes recorded for the fat bodies in *Rana temporaria* in England. According to Smith (1950) maximum size in frogs collected in Shropshire amounted to 0.8-0.9% of the body weight, against 7-8% in frogs collected in Norwich (Morton and Rosen, 1949). Morton and Rosen also noticed that the fat body size varied with the climate, the fat bodies being especially large in a mild year. Also Kennel (1912) and Zepp (1923) assumed that differences in size of the fat bodies of *R. temporaria* and *R. esculenta* from one year to another reflected differences in feeding conditions, which again depended upon the climatic conditions.

It thus seems that adult temperate zone anurans usually begin to draw upon their energy reserves already late in summer or early autumn, in advance of the hibernating period, not to enter the phase of accumulation of energy as fat until after completed breeding.

Ovary cycle

Resting period

After spawning in April the ovaries of the toad enter a period of quiescence before the next ovarian cycle is initiated by recruitment of a batch of small oocytes to the final growth phase. The resting period normally lasts 2-3 months. Occasionally, however, in Danish populations of toads the next

Table 4. Resting period between spawning and next ovarian cycle in some temperate zone anurans

Species	Locality	Month of spawning	Duration of resting period, months	References
Bufo bufo	Denmark	Apr	2-3	Present work
	Peking, China	Apr	2-3	Ting and Boring, 1940
Rana temporaria	France	Mar	3-4	Barthélémy, 1930; Gallien, 1940
	Germany	Mar	3-4	Bleibtreu, 1911; Zepp, 1923
	Poland	Mar	3-4	Krawczyk, 1971
	England	Mar	3-4	Smith, 1950
	N. Finland	May	0	Koskela and Pasanen, 1975
Rana arvalis	Poland	Apr	2-3	Jastrzębski, 1968
Rana esculenta	Switzerland	May-Jun	1-2	Gaule, 1901
	Poland	May	2	Juszczyk and Zamachowski, 1973
Rana pipiens	NE. U.S.A.	Apr	1-2	Zahl, 1937
	Wisc., U.S.A.	Apr-May	2-3	Mizell, 1964
Rana catesbeiana	Wash., U.S.A.	Jun-Jul	0	Byrne and White, 1975
Rana nigromaculata	Peking, China	Apr	3-4	Ting and Boring, 1940

ovarian cycle may be postponed until the following year (Hede and Jørgensen, 1978; Jørgensen, 1973a). According to Heusser (1968a), who studied a population of toads near Zürich, Switzerland, biennial breeding was the rule in these female *B. bufo*.

Resting periods before the start of the next ovarian cycle have been described in several other temperate zone anurans. It may be seen from the examples shown in Table 4 that early spawning is correlated with long resting periods. This correlation is especially striking in *Rana temporaria*. In southern Europe, where this species may breed in February, the resting period lasts about 4 months, whereas the period is practically absent in northern Finland, where spawning normally occurs in May.

Resting periods are most easily identified within populations of anurans that exhibits short, early spawning periods, such as *B. bufo* and *R. temporaria*. The data available on species with protracted and late spawning periods, such as *Rana esculenta* and *Rana catesbeiana,* are sometimes difficult to interpret in terms of presence and duration of resting periods. Thus, Byrne and White (1975) record ovarian weights for *R. catesbeiana* that decline gradually during the breeding period from late June to late July, followed by a slow increase in weight. However, the values recorded during the breeding period seem to include ovarian weights of both unspawned and spawned frogs. This way of presentation of the data may therefore conceal resting periods in individual frogs, especially in those that ovulated early in the breeding period. A similar argument applies to Gaule's (1901) data for *R. esculenta*. Also in this species mean ovarian weights decrease gradually during the long breeding period. However, recalculation of the tabulated raw data shows that the weights of spawned ovaries did not change significantly from the beginning of the breeding period in May, until August, after termination of breeding early in July. From August to September the ovarian weights increased abruptly. It is thus suggested that early spawners among the females tended to exhibit longer resting periods than late spawners.

The condition of ovarian quiescence that succeeds the breeding period may be interpreted as an adaptive feature in ovarian function of temperate zone anurans securing that rapid vitellogenetic growth of a new batch of oocytes is postponed until the energy reserves of the organism are restored. This interpretation is supported by the finding that if freshly spawned female toads are taken into the laboratory and fed well they mostly resume the next ovarian cycle with little delay (Jørgensen, 1973b).

As mentioned, in the toad *B. bufo* biennial breeding may occur as an alternative to initiation of the next ovarian cycle within the year of breeding. Little is known about the frequency of biennial breeding in temperate zone female anurans. Examples are, however, known on biennial breeding in female urodeles. Thus, Eagleson (1976) observed that *Ambystoma gracile* inhabiting lakes situated at altitudes of 100, 830 and 1200 m in British Columbia, Canada, varied in pattern of breeding. At the lowest altitude the females were annual breeders, exhibiting ovarian resting periods of 2½ months' duration, whereas the females of the population inhabiting the lake at the highest altitude spawned biennially. A similar plasticity in ovarian cycles was observed in *Pseudotriton mantanus,* in South Carolina, which exhibited irregular cycles, annual or biennial (Bruce, 1975).

In *B. bufo*, and probably other anurans and urodeles, a mechanism that restricts the period of the year within which an ovarian cycle can be initiated seems to be an important factor in determining whether the next ovarian cycle follows within the year of spawning, or whether it is postponed until the following year. Thus, in *B. bufo* an ovarian cycle is normally not initiated later than July (Jørgensen, 1975).

Ovary weight

The ovaries of female toads, *Bufo bufo*, as well as of other annually breeding anurans, attain maximum weight shortly before spawning. Ovulation drastically reduces ovarian weight, which reaches minimum values shortly after spawning. The max-

imum and minimum ovarian weights vary between species, between populations of the same species as well as among individuals within a population. Table 5 shows that mean maximum ovarian weights range from about 10 to 20% of the body weight in four Eurasian species of anurans. These values are comparable to values of weights of mature ovaries in other species (*Rana pipiens*, Minn., U.S.A., 14%, Ott, 1924; *Bufo fowleri*, Georgia, U.S.A., 25%, Bush, 1963; *Xenopus laevis*, Capetown, S. Africa, 11%, Zwarenstein and Shapiro, 1933, Shapiro and Shapiro, 1934; Transvaal, S. Africa, 15-40%, Kalk, 1960).

It seems that in *Rana temporaria* the mature ovaries mostly weigh less than in the other species listed in Table 5. Typical mean weights in populations from central Europe range from 11 to 14% of the body weight. In England mean weights of the mature ovaries of *R. temporaria* reach 15-17%, in northern Finland only 10-11%. It is thus indicated that the annual reproductive potential of a species, as expressed by the mass of the eggs produced, varies with the environmental conditions, presumably especially with the length of the feeding period. This interpretation is supported by the finding that, within Polish populations of *R. temporaria* from varous localities, the mass of the spawn was lowest in the high altitude locality (Kozlowska, 1971).

It is noteworthy that the weight of the freshly ovulated ovaries varies substantially between species, from 1-2% in *Rana temporaria* and *Rana arvalis* to 4-5% in *Rana esculenta*. This variation results from differences in size and number of the small oocytes remaining in the ovary after ovulation. These oocytes reach sizes up to 1.3 mm in diameter in *R. esculenta* (Burkardt, 1912; Zepp, 1923), against only 0.8 mm in *R. temporaria* (Gaupp, 1904, Jørgensen, unpublished) and *Bufo bufo* (Jørgensen, 1973b). The lower weight of the ovaries in spawned *R. temporaria* as compared with *B. bufo* (Table 5) is correlated with smaller numbers of oocytes in the spawned ovaries of *R. temporaria* than in *B. bufo* (Jørgensen, unpublished).

Table 5 shows that in Danish populations of *B. bufo* and in *R. esculenta* the ovaries increase substantially in weight in the period between the beginning of hibernation and spawning, whereas less conspicuous increases have been observed in some populations of *R. temporaria*. Such increase in ovarian weight during or after hibernation has

Table 5. Annual cycle of ovary weight in some temperate zone anurans

Species	Locality	Mean weights of ovaries as percent of body weight, prior to spawning	after spawning	at hibernation	References
Bufo bufo	Denmark	19	2.5	14	Present work
	Peking, China		1	20	Ting and Boring, 1940
Rana temporaria	France	13	1	13	Barthélémy, 1930
	Germany	11-14	1-2	11-14	Bleibtreu, 1910, 1911; Zepp, 1923
	Poland	12-13	1	11	Juszczyk, 1959; Krawczyk, 1971
	England	15-17	1	15	Smith, 1950
	N. Finland	10-11	1.9	10	Koskela and Pasanen, 1975
Rana arvalis	Poland	16	0.9	14	Jastrzębski, 1968
Rana esculenta	Switzerland	20	3.5	15	Gaule, 1901, recalculated
	Germany	17	5	12	Zepp, 1923
	Poland	18	4.5	12	Juszczyzyk and Zamachowski, 1973

often been interpreted as being due to continued vitellogenetic growth of oocytes (Zepp, 1923; March, 1937; Mazzocco, 1940; Miller and Robbins, 1954). However, verification of this interpretation requires the demonstration that the amount of organic matter increases in the ovaries. The question therefore arises whether the increased ovarian weights observed during or after hibernation in the species listed in Table 5, as well as in other species, represent true growth of oocytes or not.

Bufo bufo. There is good evidence that in *B. bufo* vitellogenetic growth of the oocytes has been completed before the onset of hibernation (Su and Yulan, 1963a; Jørgensen, 1973a). Presumably vitellogenesis cannot proceed at the low temperatures of hibernation (Jørgensen *et al.*, 1978b). It is noteworthy that the weight of the ovaries, expressed as a percentage of the body weight, remains constant during the period of hibernation, because during this period the body weight normally increases by up to 30% due to salt accumulation and expansion of especially the extracellular space (Jørgensen *et al.*, 1978a). It therefore seems that the ovaries increase their fluid content at the same rate as the other parts of the body. The relative increase in ovarian weight that occurs shortly before ovulation presumably results from an extra increase in the fluid content of the ovaries.

Rana temporaria. March (1937) assumed that the ovaries of English frogs continued to grow during the hibernation period because the ratio ovary weight to weight of the other parts of the body increased from 0.16 (range 0.09-0.26) in October-November to 0.24 (range 0.18-0.33) in February-March. Smith (1950) observed that in a mild winter "hibernating" frogs ate throughout the winter, as judged from the consistent presence of water insects and larvae in the stomachs of the frogs. It therefore cannot be excluded that some vitellogenetic growth of oocytes may occur when frogs are not strictly hibernating. Krawczyk (1971), in a Polish population of *R. temporaria*, measured both wet weight and dry weight of the ovaries as a percentage of the body weight. He observed a slight increase in the mean dry weight of the ovaries during the first half of the hibernation period, from 4.8 to 5.4% of the body weight. It cannot be seen whether the increase is significant. There was no change in the dry weight percentage during the second half of the hibernation period, whereas the water content of the ovaries increased slightly, from 54.5% to 57.1%. Thus, convincing evidence is lacking for continued vitellogenetic growth of oocytes in truly hibernating *R. temporaria*. The moderate increases in relative weight of the ovaries that has been observed in many populations may therefore result from accumulation of fluid.

Rana esculenta. The ovarian weight remained constant during hibernation, at about 15% of the body weight in a Swiss population (Gaule, 1901) and at 12% in German and Polish populations (Zepp, 1923; Juszczyk and Zamachowski, 1973). No vitellogenetic growth of oocytes could therefore be demonstrated in the hibernating state. After hibernation there was a large, statisticially significant increase in the weight of the ovaries in all the three populations studied (Table 5). This post-hibernation increase in ovarian weight was interpreted as being due to growth of oocytes taking place when feeding is resumed after the hibernation period (Zepp, 1923; Juszczyk and Zamachowski, 1973). In the Swiss population of frogs the fat bodies continued to decrease in size until after spawning, which is consistent with the view that even this late-spawning species feeds little until after spawning (Victoroff, 1908; Juszczyk, 1950). It therefore remains uncertain to which extent the pre-spawning increase in ovarian weight in *R. esculenta* results form vitellogenetic growth of oocytes and to which extent it reflects accumulation of fluid (Milone *et al.*, 1978).

In conclusion, there seems to be no significant vitellogenetic oocyte growth after the onset of hibernation in temperate zone anurans that hibernate at low temperatures. In these species the large oocytes have normally reached full size well in advance of the hibernation period. This is contrary to species

that hibernate or become dormant at higher temperatures, e.g. species inhabiting xeric regions. In such species vitellogenesis may not be restricted to the period of feeding, but the large oocytes may continue to grow at the expense of energy reserves, including the fat bodies, during the non-feeding period of the year (*Xenopus laevis*, Capetown, S. Africa, Berk, 1938; *Bufo fowleri*, Georgia, U.S.A., Bush, 1963; *Scaphiopus* spp., Arizona, U.S.A., Seymour, 1973).

Size and number of eggs

Records of egg sizes in a great number of anurans show that most species produce eggs of sizes within the range from 1 to 2 mm in diameter (Ahl, 1930; Boulenger, 1897, 1920; Hempelmann, 1908; Morgan, 1904; Rugh, 1948; Terentjev, 1960). Few studies have been made to assess the variability in size or number of eggs within a species, or to elucidate controlling factors. Size and number of eggs in anuran species both exhibit geographic variation, and vary among individuals within the same population.

The geographic variation has especially been studied in *Rana pipiens,* in North America (Moore, 1949a,b, 1950) and in *Rana temporaria,* in Europe. In *R. pipiens,* Moore (1949a) noticed that within eastern USA-Canada mean values for egg size increased towards the north, and he ascribed an adaptive significance to this increase. However, studies of more populations of *R. pipiens* ranging from northern Canada to Panama, blurred the picture in showing no clear correlation between geographic distribution and egg size (Moore, 1950). It has been argued that the *Rana pipiens* complex consists of a number of species (Brown, 1973; Tucker, 1976). Moore's data on geographic variation in egg size of *R. pipiens* therefore need to be reconsidered in the light of the reevaluation of the systematics of the species.

In *Rana temporaria*, egg size tends to be large both in populations living at high latitudes (northern Finland, Koskela and Pasanen, 1975) and at high altitudes (Poland, Koslowska, 1971). Also in the frog *Pseudacris triseriata,* a population living at 5000 feet produced eggs of smaller mean diameter (0.80 mm) than did a population living at 9300 feet (1.15 mm). The difference in egg size is ascribed an adaptive significance, the larger eggs produced by the high altitude population facilitating more rapid development to metamorphosis (Pettus and Angleton, 1967).

It has been repeatedly noticed that the size may vary of eggs produced by females belonging to the same population (*Rana temporaria,* Chambers, 1908; Lieberkind, 1937; Juszczyk, 1959; Hönig, 1966; Koskela and Pasanen, 1975; *Rana esculenta,* Chambers 1908; *Rana sylvatica* and *Rana palustris,* Dickerson, 1931; *Rana pipiens,* McAlister, 1962; *Rana chalconota, Hing, 1959-61).* In many of these studies egg size was positively correlated with the body size of the female (Boulenger, 1897; Héron-Royer, 1883; Hönig, 1966; Koskela and Pasanen, 1975; McAlister, 1962; Pettus and Angleton, 1967).

In the present work, the mean size of full grown eggs in *B. bufo* females, sampled from various Danish localities, has been found to vary from about 1.4 mm to about 2.0 mm in diameter. The mean egg size was inversely correlated with numbers of eggs produced (Fig. 8), resulting in constant ovarian weights, as expressed per 100 g body weight. It thus seems that vitellogenetic growth of the ovaries is regulated to deposit a specific amount of yolk that may be partitioned between a larger number of small eggs or a smaller number of large eggs.

This type of relationship between size and number of eggs produced has also been found in *Rana temporaria*. Thus, within a Danish population of frogs the mean oocyte diameters (1.1 - 1.9 mm) varied inversely with the number of oocytes 9,000 - 2,500 per 100 g body weight (unpublished results).

The size of full grown eggs varies little in the ovaries of individual female toads and other anurans. The ultimate size varies, however, depending upon the conditions for vitellogenetic growth. Toads maintained at a high temperature in the fall grow larger eggs than do wild toads in which growth

of the oocytes continues only until hibernation begins (Jørgensen *et al.*, 1978b).

It is noteworthy that the variation observed in egg size among individuals belonging to the same population (*R. temporaria)*, or to different populations within a small geographical range with uniform climatic conditions (*Bufo bufo*), are of the same magnitude as the differences that various authors have ascribed adaptive significances (Kozlowska, 1971; Koskela and Pasanen, 1975; Moore, 1949a; Pettus and Angleton, 1967; Salthe and Duellman, 1973; Salthe and Mecham, 1974).

The variations observed in size and number of eggs in Danish populations of *Bufo bufo* and *Rana temporaria* seem to be chance.

Atresia

Most observations on atresia in the ovaries of anurans and other amphibians have been made on large, gonadotropin-dependent oocytes. Thus it is well established that the follicles that do not ovulate during the normal breeding season become atretic (Bühler, 1903; Burkardt, 1912; Gatenby, 1916; Cheng, 1932; Rugh, 1951; Hedeen, 1972; Jørgensen, 1973b).

Atresia among large oocytes during an ovarian cycle seems not to be obligatory and thus to form part of the normal ovarian function. However, more or less extensive atresia has been described among the oocytes undergoing vitellogenetic growth or among full sized eggs during the pre-spawning period (*Acris crepitans,* Fisher and Richards, 1950; *Bufo stomaticus*, Guraya, 1969). Atresia among large oocytes during the normal ovarian cycle presumably often reflects adverse environmental conditions, among which lack of food is probabaly the most important (Burkardt, 1912; Ott, 1924; Alexander and Bellerby, 1938; Penhos, 1952). In the present work complete atresia of large oocytes in freshly collected toads, *B. bufo*, could be correlated with lack of energy reserves, i.e. reduced fat bodies.

Atresia of larger oocytes may be looked upon as an adaptive response to adverse nutritional conditions favouring survival of the toad at the expense of its reproductive capacity. The response seems generally to be of minor importance in Danish populations of *Bufo bufo*. In another bufonid, *Bufo viridis,* higher incidences of complete atresia of pools of large oocytes has been observed among females from certain localities (unpublished observations).

Starvation-induced atresia of large vitellogenetic oocytes has also been described in other vertebrate classes, including birds (Nalbandov, 1976, p. 146).

Only scattered observations are available on the occurrence of atresia among the small gonadotropin-independent oocytes. Small atretic oocytes are recorded in the ovaries of various species of anurans (*Rana temporaria*, Gatenby, 1916; Cheng, 1932; *Bufo stomaticus,* Guraya, 1969; *Bufo bufo,* Anne-Grete Byskov, personal communication). Little is known, however, about the incidence of atresia among small oocytes and the factors that control atresia. Presumably, atresia among the small oocytes may exhibit large individual variation (Jørgensen *et al.*, 1978b).

Oocyte maturation and ovulation

It has long been recognized that the time of breeding of temperate zone anurans varies within their range of distribution (Héron-Royer, 1885). The different species tend to restrict the duration of the spawning season and to breed later the further to the north they live. Frogs and toads that breed only in spring in northern Europe may start breeding already in the fall in southern France. Héron-Royer stressed the importance of climate in determining the spawning time, and he ascribed the adaptability of the spawning season within the species to the faculty of the females to delay the spawning of their full grown eggs. Such delay may result from postponed maturation of the eggs or from delayed ovulation of mature eggs.

Maturation of the large follicles in the ovaries of temperate zone anurans, as in other amphibians, presumably implies that the follicles become more sensitive to the ovulatory effect of gonadotropin from the pituitary gland. It is therefore possible to

follow the progress in maturation of the follicles by testing the ovulatory response of the follicles towards exogenous gonadotropin. Tests of this type have shown that the amounts of homologous pituitary extracts needed to induce ovulation decrease during winter (Rostand, 1934; Houssay, 1947; Rugh, 1948). In the toad *Bufo bufo*, the amounts of human chorionic gonadotropin needed to induce ovulation of full grown follicles decreased with time in toads that were exposed to low, hibernating temperatures in the fall. Maximum sensitivity was reached after several months at low temperature (Jørgensen *et al.*, 1978b). Su and Yu-lan (1963a, b) assume that in the subspecies *Bufo b. asiaticus* from the Shanghai district, China, exposure to the low temperatures of hibernation (about 2-3°C) is indispensable for maturation of the follicles. However, hibernation and ovarian maturation are not necessarily coupled processes, and their relationships may vary between species, as well as within the geographic range of distribution of one species. Some examples will demonstrate this.

In late breeders, follicles may not mature until after the hibernation period. Rostand (1934) made the interesting observation that during hibernation, in November, the early breeder, *Rana temporaria*, ovulated after injection of 2-4 pituitary glands, whereas the late breeder, *Rana esculenta*, showed no or incomplete ovulation even after injection of up to 12 pituitary glands. There was no difference in response between the two species to injection of homologous or heterologous glands.

Bufo b. bufo from Denmark provides an example on an early breeder that may breed spontaneously in the absence of the normal hibernation period (Jørgensen *et al.*, 1978b). Thus, amplexus, spawning and development of tadpoles was observed in a group of toads that remained active and feeding during the winter at temperatures that did not fall below 13°C. It is noteworthy that the breeding behavior was initiated only a few weeks before breeding in nature.

It thus seems that the time course of maturation of the large follicles in temperate zone anurans varies both between species and, within a species, with the geographic location of the populations. Hibernation and local variations in temperature and other climatic factors may act as modulators of a largely endogenously determined time course of the maturation processes of the large follicles.

Ovulation. At ovulation of the large follicles the eggs are released into the body cavity and transferred by ciliary tracts into the oviducts. An extensive literature deals with the ovulatory mechanisms and their endocrine control in various amphibians. This literature shall not be reviewed, but reference is made to some recent papers on ovulatory mechanisms and hormonal regulation (Anderson and Yatvin, 1970; Snyder and Schuetz, 1973; Thibier-Fouchet *et al.*, 1976; Thornton and Evennett, 1973; Wasserman and Masui, 1976).

Several ovulatory and spawning types may be distinguished among anurans. In some species ovulation is induced by mating (amplexus), whereas other species may ovulate independently of amplexus, and ovulation has often taken place before pairing of the sexes (Spallanzani, 1786).

Amplexus-induced mating has been described in *Scaphiopus* spp. According to Bragg (1965), spadefoots and other species from xeric regions ovulate in response to amplexus. Anurans from xeric regions are typically opportunistic breeders that spawn at irregular intervals when rainfalls have made temporary pools of water available for spawning and development of tadpoles.

In temperate zone anurans from mesic regions amplexus independent ovulation may turn out to be the rule, contrary to common beliefs (Bragg, 1941; Heusser, 1963). Ovulation in the absence of males was recorded already by Héron-Royer (1883) in species of *Hyla, Rana, Discoglossus* and *Bombinator*. "Spontaneous" ovulation was later rediscovered e.g. in *Microhyla carolinensis* (Anderson, 1954), *Hyla crucifer* (Oplinger, 1966), and *Bufo bufo* (Heusser, 1963).

Amplexus-induced and amplexus-independent ovulation in anurans may be compared with reflex ovulation and spontaneous ovulation in mammals.

The comparison may, however, be misleading because at least in some anurans that ovulate independently of amplexus, other external stimuli may serve to trigger the ovulatory reflex. Thus, in the toad *Bufo bufo*, Heusser (1963) found that females sampled singly on their way to the breeding ponds, when placed in buckets of water would spawn simultaneously with pairs in amplexus in nature. Presumably, contact with water acted as a stimulus to induce ovulation. Also light seemed important, because darkness reduced spawning in isolated females in water to about 50% (Heusser, 1968c). Heusser, however, also observed that amplexus might serve as an auxilliary stimulus to ovulation. In *Hyla crucifer*, Oplinger (1966) speculates that the call of the males from the spawning grounds or climatological factors as rain may act as external stimuli to induce ovulation in the mature females.

In *Rana temporaria*, ovulation is correlated with an activity pattern that is remarkably independent of external factors, as observed in Poland (Juszczyk, 1959; Juszczyk and Zamachowski, 1965) and Finland (Koskela and Pasanen, 1975). Frogs emerge from the wintering quarters in the mud at the bottom of lakes or springs, and copulate and ovulate before any change in temperature of the water. Ovulation also occurs in frogs maintained at constant low temperature in a refrigerator. It has therefore been assumed that termination of hibernation and ovulation is governed by an internal rhythm that is adapted to the latitude and the altitude at which the populations of the species live (Juszczyk and Zamachowski, 1965; Koskela and Pasanen, 1975). These observations seem, however, to have been made on groups of frogs that included both sexes. They are therefore not conclusive concerning the role amplexus may play in inducing ovulation in *R. temporaria*.

Anurans may also be grouped according to the relationship between ovulation and oviposition. One group includes anurans in which oviposition is coupled with ovulation, so that ovulation and transfer of eggs to the oviducts automatically results in oviposition of the eggs. In the other group of anurans ovulation and oviposition are independent processes. In species belonging to this group the ovulated eggs accumulate in a specialized section of the oviduct, the uterus, from which they are later expelled in clumps.

Examples of anuran genera in which ovulation and oviposition are coupled processes are *Xenopus* (Waring *et al.*, 1941) and *Bufo* (Heusser, 1963), exaples of genera that store the ovulated eggs in an uterus are *Hyla* (Oplinger, 1966) and *Rana* (Waring *et al.*, 1941). In the latter type the question arises as to the importance of amplexus. Both *Hyla* and *Rana* have been observed to be able to deposit eggs in the absence of the males (Spallanzani, 1786; Héron-Royer, 1883; Oplinger, 1966). However, the observations seem not finally to have elucidated the role of the male in oviposition in these and other anuran species.

Control mechanisms

As mentioned, *B. bufo*, and other temperate zone anurans, typically exhibit one annual ovarian cycle, with a resting period interspaced between successive cycles. In interpreting normal ovarian function in terms of control mechanisms two states may therefore be distinguished, the ovarian cycle proper, including vitellogenetic growth, maturation and ovulation of follicles, and the resting period.

Vitellogenetic growth of oocytes depends upon secretion of gonadotropin at levels that require central nervous stimulation (van Dongen *et al.*, 1966; Dierickx, 1966; Jørgensen, 1970; Vijayakumar *et al.*, 1971). The start of the next ovarian cycle after spawning thus depends upon the secretion of gonadotropin at high levels and upon the presence in the ovaries of oocytes that can respond to gonadotropin by entering the final vitellogenetic growth phase. Oocytes can respond to gonadotropin only when the follicles have reached a certain stage in their development (Billeter and Jørgensen, 1976). The ovaries of sexually mature toads, however, seem always to contain a larger number of gonadotropin-sensitive oocytes than is normally recruited to

vitellogenetic growth during the annual ovarian cycle (Vijayakumar *et al.*, 1971). The ovaries of freshly spawned toads show a normal vitellogenetic response to exogenous gonadotropin (human chorionic gonadotropin) (Jørgensen, 1973b). Moreover, the response is little influenced by the nutritional condition of the toads, whether they are starved or well fed (unpublished results). It can therefore be concluded that in the toad the start of the next ovarian cycle after breeding results from increased secretion of gonadotropin from the pituitary gland under central nervous stimulation.

The question then arises which factors influence the secretion of gonadotropin. Probably the nutritional state of the organism is a primary factor. After spawning the organism is depleted of energy reserves, and it seems that the next ovarian cycle is postponed until an appropriate nutritional state has been reestablished, as judged e.g. from the increase in size of the fat bodies. The importance of the nutritional state is supported by the finding that toads taken into the laboratory immediately after spawning and fed will usually start a new ovarian cycle with little delay (Jørgensen, 1973b; Jørgensen *et al.*, 1978b).

Often the ovaries of adult-sized toads lack large vitellogenetic oocytes in the fall, even when the toads are in a good nutritional condition when caught. This lack of large oocytes appears to be centrally determined, because the ovaries show a normal vitellogenetic response to exogenous gonadotropin (Jørgensen, 1975). Presumably, a mechanism exists which prevents activation of gonadotropin secretion too late in the season for the oocytes to complete growth before hibernation. The mechanism may result in biennial breeding in toads that remain in a bad nutritional condition too long after breeding. The mechanism may also postpone an ovarian cycle until the following year in young toads that reach adult size late in summer or in the fall.

Completion of vitellogenetic growth and maintenance of full grown oocytes depend upon gonadotropin secretion remaining high. Starvation can lead to atresia of the large oocytes, probably due to reduced gonadotropin secretion. This is indicated by the finding that atresia can be prevented by treating starving *B. bufo* females with gonadotropin in the same doses as are needed to stimulate normal vitellogenesis (unpublished data).

Ovulation, which terminates an ovarian cycle, presumably requires an ovulatory surge of gonadotropin secretion, as has been demonstrated in other classes of gnathostomes. This assumption is supported by the finding that ovulation of mature follicles in the toad, as in other anurans, requires a much higher dosage of gonadotropin than needed to stimulate vitellogenetic growth and survival of oocytes (Kjær and Jørgensen, 1971; Jørgensen *et al.*, 1978b).

Breeding in temperate zone anurans typically results in the ovaries entering a resting period. As mentioned, the ovaries of spawned toads contain numerous oocytes capable of responding to gonadotropin when this is being secreted from the pituitary gland at a high rate. As gonadotropin secretion presumably is high up to and during spawning, it is indicated that breeding normally causes a fall in gonadotropin secretion which lasts until the environmental, and especially nutritional, conditions are favourable for the reactivation of gonadotropin secretion.

Oviduct cycle

In the annual reproductive cycle of the toad, and other amphibians, the growth of the oviduct is closely correlated with the growth of the ovaries. Presumably, the rate of growth of the oviducts is determined by the rate of secretion of an ovarian factor, because reduction in ovarian mass by subtotal ovariectomy in toads decreased the rate of oviduct growth (Jørgensen and Vijayakumar, 1970). Estrogens have been found capable of stimulating growth of oviducts in amphibians generally (for references, see Lee, 1965). In *B. bufo*, estradiol-17β stimulated oviduct growth when administered in a physiological dosage (Jørgensen and Vijayakumar, 1970).

From Table 6 it may be seen that the ratio

Table 6. Oviduct size and its relationship to ovary size in some temperate zone anurans

Species	Locality	Oviduct weight as percent of body weight and oviduct to ovary ratio: During hibernation %	Ratio	Prior to spawning %	Ratio	References
Bufo bufo	Denmark	6	0.4	6	0.3	Present work
	Peking, China	5	0.2			Ting and Boring, 1940
Rana temporaria	Germany			17	1.2	Zepp, 1923
	Poland	18		19		Juszczyk et al., 1972
		17	1.4	17	1.4	Juszczyk, 1959
	Finland	17	1.6	20	1.9	Koskela and Pasanen, 1975
Rana arvalis	Poland	10	0.7	13	0.8	Jastrzębski, 1968
Rana esculenta	Germany			5	0.4	Zepp, 1923
Rana catesbeiana	Wash., U.S.A.			ca. 7	1	Burne and White, 1975
Rana nigromaculata	Peking, China		0.3		0.4	Ting and Boring, 1940

oviduct weight to ovary weight is several times higher in *Rana temporaria* than in *R. esculenta* or *B. bufo*. The weight of the oviducts is mainly determined by the amount of secretory granules accumulated in the gland cells, i e. the amount of precursor material for the secretion of jelly that coates the ovulated eggs passing the oviducts. Already Zepp (1923) noticed that the relative size of the oviducts was correlated with the thickness of the jelly coat of the eggs, the oviducts being larger in *R. temporaria,* which produce thick coats of jelly, than in *R. esculenta,* whose eggs have thinner jelly coats.

Testis and thumbpad cycles

Testis weight and spermatogenesis

In the male toads the gonads did not exhibit a resting period after spawning, as did the ovaries in the females. The testes of toads collected in June had increased in weight, and spermatogenesis was in full progress. Also in French (Rey, 1939b) and German (Obert, 1971) populations of *B. bufo*, increased spermatogenesis has been observed immediately after spawning. A post-spawning gonadal resting period may be absent also in the males of other temperate zone amphibians, even in such species whose females may be biennial breeders. According to Bruce (1975) the absence of a gonadal resting period in the males is related to the lower reproductive costs in males than in females. However, the males of some temperate zone anurans do have resting periods comparable to those of the females. The best studied example is *Rana temporaria*, in which males as well as females exhibit gonadal resting periods, in central Europe and England lasting 2-3 months (Ploetz, 1890; Zepp, 1923; Smith, 1950; van Oordt, 1956; Lofts *et al.*, 1972).

The amplitude of annual cycles in testis weight seems to be correlated with the absence or presence of a resting period. Thus, in most temperate zone anurans the testes show only moderate annual variations in weight. Some examples are listed in Table 7. The testes weights tend to be lowest after spawning, but often a definite annual cycle in weight is lacking. In all instances the species belonging to this type have potentially continuous spermatogenetic cycles (van Oordt, 1960). Spermatogenesis in wild animals proceeds throughout the greater part of the year and may take place even during hibernation, the rate of spermatogenesis primarily being determined by the temperature and other environmental factors. In *Rana temporaria*, on the contrary, spermatogenesis is discontinuous, depending on an internal rhythm (van Oordt, 1960). When spermatogenesis is resumed after the resting period it proceeds at a high rate for a restricted period during which the testes may increase 10-30 times in weight (Table 7). Lofts *et al.*, (1972) found the dry weights

Table 7. Annual cycle of testes weight in some temperate zone anurans

Species	Locality	Mean weights of testes as percent of body weight: Maximum weight	Month	Minimum weight	Month	Ratio max. to min.	Weight prior to spawning	Month	References
Bufo bufo	Denmark	0.5	Jul-Aug	0.25	May	2.0	0.4	Apr	Present work
	Peking, China	0.4	Aug	0.17	Apr-May	2.4			Ting and Boring, 1940
Rana temporaria	Germany	3.5	Aug	0.25	Apr-Jun	14	0.9	Mar	Zepp, 1923
	Poland	1.6	Sep	0.18	May	9	0.9	Mar	Krawczyk, 1971
	Holland	2.2	Aug	0.16	May	14	0.8	Mar	van Oordt, 1956
		4.1	Aug	0.14	Jun	29	0.9	Mar	Lofts **et al.**, 1972
	England	6.0	Aug	0.3	Jun	20	1.2	Mar	Smith, 1950
R. esculenta	Switzerland	0.35	Aug	0.21	Jun	1.7	0.25	May	Gaule, 1901
	Germany	0.43	Jul	0.19	May	2.3	0.20	May	Zepp, 1923
	Holland	0.27	Aug	0.14	Jan	1.9	0.25	May	Lofts, 1964
R. pipiens	Wisc., U.S.A.	0.32	Oct	0.09	May	3.6	0.11	Mar	Mizell, 1964
R. nigromaculata	Peking, China	0.16	Sep	0.07	Jun-Jul	2.3	0.13	Apr	Ting and Boring, 1940

of the testes to increase about 20 times from the minimum value in June to the maximum value in August-September. During the subsequent months testes weights decreased again. Ploetz (1890) showed that the cycle in weight changes can be correlated with stages in spermatogenesis. Cell proliferation predominates from June to August, followed by a cell differentiation when testis mass is again reduced.

The striking differences observed between *R. esculenta* and *R. temporaria* in the annual cycles of testis weight and spermatogenesis have been correlated with their geographic range of distribution. *R. esculenta* is a southern species that, when living in the Mediterranean region, may remain active throughout the year also with respect to spermatogenesis. *R. temporaria* is a northern species that has become adapted to climates that restrict the duration of annual periods of activity, including spermatogenesis (Ploetz, 1890; Zepp, 1923).

Tubular histochemical cycle

It is of interest to note that in *Bufo bufo bufo*, after spermiation, the detached Sertoli cells which accumulated in the tubule lumen did not become intensely sudanophilic and cholesterol positive, as had been noted in *R. temporaria* (Lofts and Boswell, 1960) and, to a lesser extent, in *R. esculenta* (Lofts, 1964). In these latter two species the seminiferous elements become densely lipoidal as the tubule lumina become occluded with spent, heavily sudanophilic Sertoli cells. In *R. temporaria* the tubules remain in this condition for some weeks, and then the lipids and cholesterol disappear with the resumption of spermatogenetic activity. This intra-tubule lipid material mainly consists of cholesterol esters (Lofts *et al.*, 1972) which are rapidly utilized once spermatogenesis begins. In *Bufo*, in which the spermatogenetic wave begins immediately after breeding, and some low level of activity also continues in winter months, it is possible that an immediate large demand after the breeding season, and a continuing demand at fairly low tonic levels throughout the remainder of the year, prevents the accumulation of these cholesterol esters in any significant amounts. This is in contrast to *R. temporaria*, in which species the postponement of the next spermatogenetic wave for several weeks after breeding is correlated with the development of heavily sudanophilic tubules.

Thumbpads and interstitial tissue cycles

The tumbpads of temperate zone anurans undergo pronounced annual developmental cycles. In early breeders, the thumbpads usually reach maximum development before hibernation, e.g. *B. bufo* (Heusser, 1969) and *R. temporaria* (Lofts *et al.*, 1972). In late breeders the thumbpads start developing prior to hibernation to resume growth and differentiation of papillae and cornification after emergence, prior to breeding, e.g. *R. esculenta* (Lofts,1964; de Kort, 1971).

It is now well established that this annual cycle in development of the tumbpads is closely correlated with the cycle in development and functional state of the interstitial tissue (Lofts and Boswell, 1960; Lofts, 1964; de Kort, 1971; Obert, 1973). This correlation could also be demonstrated in the toad, which showed a seasonal waxing and waning of cholesterol and lipids in the interstitial tissue similar to that noted in other seasonally breeding vertebrates (Lofts, 1968). The cholesterol and lipid cycle is indicative of the steroid secretory activity of this tissue (see reviews by Lofts and Bern, 1972; Lofts, 1974). The rapid accumulation of dense sudanophilic masses of cholesterol rich substances immediately after the breeding period is probably due to build-up of precursor material as a consequence of the cessation of androgen production, and conversely, its rapid depletion later in the year marks a resumption of secretory activity with rapid utilization of these cytoplasmic lipids and sterols. In *R. temporaria* and *R. esculenta* the interstitial cells show loss of 3β-hydroxysteroid dehydrogenase activity as the cells enter this postnuptial sudanophilic stage (Lofts, 1974), and in some reptiles at least this phenomenon is also associated with a decline in testicular *in vitro* testosterone production (Lofts, 1969; Tam *et al.*, 1969).

A recovery of interstitial cell activity in *B. bufo* in late August is similar to the cyclic events in *R. temporaria* (Lofts and Boswell, 1960; Lofts *et al.*, 1972) and *R. esculenta* (Lofts, 1964).

It is close at hand to suggest that it is the seasonal cycle in secretion of androgen that controls the thumbpad cycle in *B. bufo* and in other anurans. This suggestion is supported by the finding that castration causes thumbpad regression that can be reverted by treatment with testosterone (*Bufo arenarum,* Burgos, 1950; *B. bufo,* Müller *et al.*, 1977; *R. esculenta,* Botte *et al.*, 1972; *Bombina variegata,* Obert, 1975).

The development of the thumbpad in anurans may be controlled by other factors than testosterone, e.g. other androgens secreted by the testis. *Rana pipiens* testes secrete androstendione besides testosterone (Kirby, 1970). Moreover, the mechanism of control may vary between species. Thus, Guyénot *et al.*, (1932) observed that implanted pituitaries restored the thumbpads in castrated males of *Bombinator pachypus,* but not of *B. bufo.*

Non-hormonal factors may be of importance for thumbpad development, especially the nutritional state (Heusser, 1969; own unpublished observations).

Relationships between fat bodies and gonads

A functional relationship has been suspected to exist between the fat bodies and the gonads in amphibians ever since it was observed in the last century that the fat bodies tended to be small when the gonads mature and large in immature individuals (Duvernoy, 1844; Wiedersheim, 1886; Giglio-Tos, 1896). The nature of the relationship between fat bodies and gonads, however, still remains unsettled (Bargmann and von Hehn, 1969).

The inverse relationship between size of the fat bodies and size and development of ovaries and testes is well documented in a large number of amphibians from the temperate regions. Some recent examples are the anurans *Bufo bufo* (present work), *B. arenarum* (Allende and Orias, 1955), *Rana esculenta* (Roca *et al.*, 1970), *R. clamitans* and *Acris crepitans* (Brenner, 1969), *Hyla crucifer* (Oplinger, 1966), and the urodeles *Ambystoma tigrinum* (Rose and Lewis, 1968) and *Desmognathus ochrophaeus* (Fitzpatrick, 1973).

It has often been suggested that the fat bodies play a direct nutritional role for the vitellogenetic growth of oocytes in the ovaries and for spermatogenesis in the testes (Mazzocco, 1940a; Allende and Orias, 1955; Oplinger, 1966; Rose and Lewis, 1968; Lewis and Rose, 1969; Brenner, 1969; Fitzpatrick, 1973). It has also been suggested that the fat bodies produce specific substances, hormones, that maintain normal gonadal function (Rose, 1967; Roca *et al.*, 1970; Chieffi *et al.*, 1975).

Several attempts have been made to assess the role of the fat bodies by ablation of the organs, but the results have been varying. Some authors observed no effects of extirpation of the fat bodies. Thus, in *Triton* sp., Aron (1924) found no effects on the secondary sex characters of the males. In *Rana* sp., Kennel (1912) observed that frogs of both sexes operated in autumn went into amplexus the following spring. The ovaries contained normal eggs and the testes normal spermatozoa. Also *Bufo bufo* males with completely extirpated fat bodies exhibited normal annual sexual cycles with respect to thumbpad development and amplexus (Ponse, 1924).

Later authors observed effects of fat body extirpation. Thus, in the urodeles *Triturus viridescens* (Adams and Rae, 1929) and *Amphiuma means* (Rose, 1967) ablation of the fat bodies resulted in degeneration of the gametes. Adams and Rae (1929) made the interesting observation that unilateral elimination of a fat body resulted in degeneration of gametogenesis in the ipsilateral gonad without affecting the function of the contralateral gonad. Recently, Chieffi *et al.* (1975) obtained similar results in male frogs, *Rana esculenta*. They, moreover, found that treatment of the operated frogs with extracts of fat bodies maintained spermatogenesis and prevented degeneration of spermatogenetic cysts.

We have made a comparable experiment in female toads, *Bufo bufo*, in which the fat body was unilaterally extirpated early in the final growth phase of the oocytes. Complete elimination of the fat body did not affect the ipsilateral ovary. Oocyte growth proceeded normally and could not be distinguished form oocyte growth in the contralateral ovary (unpublished data). It is thus indicated that in the toad ovarian function does not depend upon the ipsilateral fat body.

The inverse relationship observed between fat body size and ovarian maturation in toads may not reflect a direct functional relationship between the two organs, but may result from competition for energy obtained with the food. Ovary and fat body both deposit energy during the period of the year when the toad is active and feeding. Deposition is in the form of yolk in the ovary and of fat in the fat body. It may therefore be postulated that vitellogenetic growth has highest priority and that less energy becomes available for deposition in the fat bodies of female toads that are developing large oocytes than in toads that are not.

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