REMARKS ON SOME ADAPTIVE ECOLOGICAL TRENDS OF *PELOBATES CULTRIPES* FROM PORTUGAL: THERMAL REQUIREMENT, RATE OF DEVELOPMENT AND WATER REGULATION

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REMARKS ON SOME ADAPTIVE ECOLOGICAL TRENDS OF *PELOBATES CULTRIPES* FROM PORTUGAL: THERMAL REQUIREMENT, RATE OF DEVELOPMENT AND WATER REGULATION (*)

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ABSTRACT: Spawning, tadpole biology, rate of development, tolerance to water loss and water absorption, of *Pelobates cultripes* from Portugal, were studied, having in mind similar researchs carried out on western American spadefoots.

INTRODUCTION

The living Pelobatid frogs are widespread in North America and Eurasia. Their fossil lines extended there from Eocene through the Middle Miocene, but in accordance with the recent discussion by ESTES (1970), species of *Eopelobates* may have been present in the North American Cretaceous as well. Such a primitive genus is intermediate between megophryine and pelobatine lines, moreover the actual spadefoot toads or *Scaphiopus* were probably derived from it.

A very ancient divergence — Eocene, early Oligocene — may be indicated both of the American spadefoots and *Pelobates* from the megophryine--like eopelobatids (ZWEIFEL, 1956; KLUGE, 1966; ESTES, 1970). It was assumed that the primitive aquatic megophryine *Eopelobates* «gave rise to the terrestrial spadefoots in response to early Cenozoic climatic deterioration in both Europe and North America» (ESTES, 1970). Similarities between the two modern pelobatines, *Scaphiopus* and *Pelobates*, could support their probable common ancestry.

The morphological evolution of the Nearctic *Scaphiopus-Spea* complex, has been discussed by ZWEIFEL (1956) and KLUGE (1966). Ecological features of the spadefoot toads have received large attention from several American Authors and their extended treatments give a remarkable information on many aspects of the extreme behavioural and physiological adaptations of these fossorial anurans to the desert environments, such as the arid regions of the south-western United States (BRAGG, 1965; MAYHEW, 1965; McCLANAHAN, 1967; BROWN, 1967; ZWEIFEL, 1968; RUIBAL, TAVIS and ROIG, 1969; SHOEMAKER, McCLANAHAN and RUIBAL, 1969). Some species, such as *Scaphiopus couchii* and *S. hammondi*, are found there «in drier parts of the south-western deserts than any other anurans» (THINKHAM, 1962; MAYHEW, 1962).

Also the Eurasian genus *Pelobates* is terrestrial, fossorial and secretive. It is found in Central and Eastern Europe *(P. fuscus)*, in the Middle East *(P. syriacus)*, in the southern France and Iberian Peninsula *(P. cultripes)*, and in the opposite Moroccan lowlands *(P. varaldii)*.

Pelobates cultripes is strictly related to *P. varaldii* and *P. syriacus*. It is considered as one of the most primitive offshoots of the group, with closest relationships to the oligocenic *Macropelobates* and *Eopelobates*. It is a well known European toad, but because of its life underground it is very difficult to find, emerging only during a very short period of the year to breed and forage.

Therefore a very limited information is still available, concerning its environmental correlations such as water regulation, response to dehydration, water uptake, or temperature tolerance. In accordance with its evolutive affinities with the well known *Scaphiopus*, we studied preliminarly some ecological features of *Pelobates cultripes* from Portugal. A number of general adaptive trends was checked in comparison with the high specialization for a semiarid habitat which is stressed by the spadefoot toads from western North America. The present notes cover an annual period of field and experimental work (1970-71). Our investigations concern with adult and larval biology, rate of development and tolerance to environmental water loss.

Pelobates cultripes, or «Sapo da unha preta», is a rather scattered anuran of the Portuguese fauna, in spite of its burrowing habits and its very difficult location. It prefers sandy soil, but inhabits a number of different ecotypes as well. The map of fig. 2 indicates the localities where our field researches were undertaken, mainly since February to June 1971. Tadpoles of *Pelobates* have been observed in any of these localities, notwithstanding no evidence of adult toads was found, even examining carefully the neighbouring sites. The toads used to test water loss and absorption were captured in breeding in the Porto de Mós area, near Batalha, 16 km south of Leiria, Estremadura. That was the only capture of adult individuals in the checked region.

SEASONAL ACTIVITY AND LARVAL LIFE

Because of our failure to locate burrowed *Pelobates* in the neighbourhood of the lagoons, prior to the spawning, our personal experience of their fossorial behaviour is still based on captive specimens. Like *Scaphiopus* they dig vertical cavities, or cylindrical burrows, about 5 cm in diameter, being located at the bottom of their hollows, completely embedded in the looser soil of the digging. Sometimes at night some individuals are found outside of the cavities; probably foraging under natural conditions. Extension of the burrows was 10-30 cm under artificial conditions, but it is certainly deeper in the natural sites of the toads.

As above mentioned in the Introduction, chorus and spawning of *Pelobates* were registered only on March 19, 1971, in the Lagoa da Vala seasonal pool, near Porto de Mós, Estremadura. They were observed in breeding at 17.00 until 20.00 hr.: it was a rainy day, with irregular hailstorms. Air temperatures of 9.5° C, water temperatures of 11° C and RH of 76 % were registered. Floating girdles of egg masses were observed on the grassy surface of the shallow pond. Later, since March 19 to May 5, the Lagoa da Vala pool were visited periodically but no additional toads were found, in spite of our careful control and digging.

A number of temporary or almost permanent pools were studied furthermore in Algarve (southern Portugal), since March 24 until March 31. Both in the almost permanent shallow ponds near Odeceixe, and in the small muddy temporary pools on the sandy coast (Sagres, Espiche) noticeable quantities of large *Pelobates* tadpoles were collected; no adult toads were seen. Differences of the seasonal thermal curves are significant between the mild Algarve province and the Porto de Mós cooler region. Notwithstanding the size of these tadpoles (75-80 mm) could suggest a very pro-

longed larval life rather than a former winter spawning in the observed southern localities. A so ready larval growth should be too much rapid to be in accordance with the real climatic trends of the regional winter season, or with the normal rate of development of the species.

The size of tadpoles from the Porto de Mós egg laying, hatched on March 25, measured still 17-18 mm on June 25, at temperatures of 14° C to 20° C. Moreover tadpoles of 75 mm length, captured on March 27 in Sagres, metamorphosed since April 18. The froglets (26 mm in length) left the aquatic environment on May 8. They behave immediately such as the adult individuals and dig holes quickly underground. Conversely other tadpoles from the Odeceixe samples, on March, were still limbless on June (size 75 mm). Very abundant colonies of tadpoles at all stages were found in the permanent granitic pools of Alto Alentejo (Alpalhão, Nisa: 200-300 m) on May 19, 21. Metamorphosed froglets and limbless tadpoles were mixed there in the same pool as well.

The water temperatures of the shallow pools of Algarve on March 24-30 sound very variable, from 18° C or 22° C in the noon or afternoon (Espiche, Sagres) to 12° C in the late morning (Odeceixe): probably lower at night, when air temperatures of 9° C were registered. That is not in accordance with a too high rate of growth and development. Our field observations are anyhow in agreement with a very prolonged larval life of the Portuguese *Pelobates*, such as pointed out by former Authors for other European populations.

The ecological versatility of the Iberian spadefoots is really remarkable. They inhabit in Portugal so different biotopes such as the Algarve sandy coast scrub scattered by the «alfarrobeiras» trees *(Ceratonia siliqua)*, the open oak woodlands of Baixo Alentejo *(Quercus suber)*, the degraded sienitic or granitic landscape of Alto Alentejo, or the calcareous soils surrounding the rocky hills of the Porto de Mós Middle Jurassic massif. The wide adaptiveness of the tadpoles to the environmental conditions together with a very high demographic rate are strongly favourable inherent factors of the species. Tadpoles of *Pelobates* were found living in very polluted or muddy shallow waters. They feed on a variety of animal and vegetal substances. Their cannibalistic habits may alternate even with an exclusive cellulose feeding. In our experimental containers they fed frequently on paper sheets or small cartoons, with a curious preference for the red tracks of chemical inks of the markers! (Fig. 1).

Tolerance of the tadpoles to increasing temperatures was tested. Larvae (65 mm length) from the Odeceixe samples were utilized. The constant water volumes of their containers were heated slowly, from an initial tempe-

rature of 20° C, by means of a Singleton Bros, Dial-O-Matic Thermostatic Heater.

Temperatures of 25° C were regularly tolerated. Temperatures of 32° C were tolerated during 48 hours, but a 35 per cent mortality was registered after 72 hours. The respiratory rate increased from 60 at 20° C to 108-110 at 32° C. Temperatures of 35° C are lethal: all the four tested samples (8 tadpoles each) suffered 50 per cent mortality after one hour, 100 per cent mortality after two hours. Respiratory rate increased to 120 pulses per minute. Data are insufficient for the establishment of the upper limiting temperatures in the natural environment of the tadpoles.
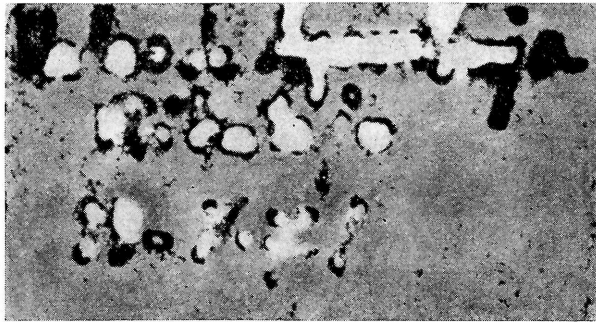

FIG. 1 — Marked card eaten by *Pelobates* tadpoles.

The demographic potentiality of *Pelobates cultripes* has been stressed by spanish Authors (VALVERDE, 1961), or by interesting remarks such as in the notes by PETIT and DELABIE (1951) and PETIT and LOMONT (1958), concerning the striking density of metamorphosed froglets and their migratory activity in the eastern Pyrenean region. Estimations by BALCELLS (1966: pers. commun.) give for only a pond about 10.000 m² a population of some 3.000.000 of tadpoles (locality of Bujaraloz, Zaragoza Prov., Spain). Likewise, densities of 100-120 larvae for m², or more, were a very common one in our checked Algarve and Alto Alentejo lagoons.

Natural control of population growth is specially exerted by a number of small vertebrates intervening in the *Pelobates* biocenotic communities, such as *Milvus migrans, Nycticorax, Rattus norvegicus, Natrix maura* (Guadalquivir swamps: VALVERDE, 1961). This latter snake is present in great number and constitutes the most active natural enemy during the spadefoot larval life in all the above mentioned Portuguese localities.

Sites from which *Pelobates cultripes* is reported lie generally in Portugal below 250-300 m. Amphibian associations assemble there some urodelans *(Pleurodeles waltl, Triturus marmoratus, Triturus boscai)* and anurans *(Alytes cisternasii, Pelodytes punctatus, Rana ridibunda perezi, Hyla meridionalis, Hyla arborea molleri).* The association between adult and larval individuals of *Pleurodeles* and *Pelobates* tadpoles is a very current one, but a relative scarcity of the invasive frog *Rana ridibunda* was impressive in any permanent or semipermanent water reservoirs if crowded by the *Pelobates* larvae.

RATE OF DEVELOPMENT

Amplexus and egg laying may take place at 11° C as in the case of the Porto de Mós mating pairs, on March 19. Some couples were in amplexus when captured: they ovulated naturally at night and fertilized eggs were utilized in our preliminary comparative studies on rate of development and limiting temperature of *Pelobates cultripes.*

Three experiments were conducted at different, relatively uniform temperatures (6° C; 14° C; 25° C), using several sets of fertilized eggs. They were placed in refrigerated or heated containers, avoiding delay in exposing them, in accordance to the experimental procedure suggested by Zweifel (1968). The range of variation was always slight and normally the thermal fluctuation stayed within a range of 1° C. The ambient room temperature was 14° C. At intervals the eggs were removed from their constant water baths and the stages of development registered under microscopical control. The staging system followed was the classical system (Pollister and Moore, 1937), further adapted by Shumway to *Rana pipiens* (1940).

Our present results are indicated by the graphic of fig. 3 and give a first basic information on the embryonic thermal requirement of this European pelobatid, in comparison with the extreme temperature adaptation of the western American spadefoots.

At temperature of 6° C there was no normal development. Cleavage took place but it was irregular and abnormal: no embryo developed beyond stage 8.

At temperature of 14° C the majority of the embryos survived to stage 20 with a slight per cent of abnormalities. Development was normal at the higher temperature, 25° C.

A comparison between the solid lines of the graphic shows better the different rate of development beyond stage 8, at temperature of 14° C and 25° C. The average elapsed time to stage 20 in embryos exposed at

temperature of 14° C was 240 hours, against 96 hours to the same stage in embryos exposed at temperature of 25° C. Hatching takes place generally in late stage 16 or stage 17.

Available data are still insufficient to estimate the upper limiting temperature. Also the lower limiting temperature is uncertain but the high degree of normality at 14° C and the water temperature of the natural environment in breeding site (11° C) lead us to suppose it tentatively about 9° C or 10° C. It would be in accordance with the ZWEIFEL'S statement that «...it is probably true that lowest temperature at which frogs will initiate oviposition is slightly above the lower limiting temperature for early embryonic development».

Plotting of fig. 4 summarizes the rates of development shown by several species of *Scaphiopus* from the arid western American biota, studied by ZWEIFEL (1968), in comparison with the rates of development of *Rana pipiens* and *Pelobates cultripes*. Rate of development of *Pelobates* appears nearly superposed to the rate of development of *Rana pipiens* but it diverges enough from those of the *Scaphiopus* species from Arizona. That is in agreement with the lower limiting temperatures, of 15.5° C for *Scaphiopus couchii*, or 13° C for *Scaphiopus bombifrons*. The former species does not breed, i.e., unless the environmental temperature is about 18° C or higher.

Our present results point out the physiological differences between the embryonic thermal tolerance of the primitive, less differentiated *Pelobates* and the extreme adaptive conditions of the *Scaphiopus* spadefoots. Some considerations on the evolutive significance of such a ecological divergence shall be given further in our general discussion.

TOLERANCE TO WATER LOSS AND WATER ABSORPTION

Pelobates cultripes is a true terrestro-fossorial anuran, capable of surviving underground for several months at a time, perhaps for years. It is of interest to determine the real limits of its physiological adaptation concerning water economy under eventual stressing environmental conditions.

Tolerance to body water loss, dehydration and rate of rehydration were comparatively investigated. Moreover rate of rehydration in wet soil and water exchanges between toad and soil were considered, in accordance with the digging habits and its almost permanent life underground.

Because of the interest repeatedly focussed on the phyletic relationship between the ancient European and American lines of spadefoot toads, our experimental observations were carried out having in mind the recent but

important researches by McClanahan (1967) on *Scaphiopus couchii* and by Ruibal et all (1969) on *Scaphiopus hammondi*. Our available information is so comparable with the data of these Authors. Some relative assumption can be got in shape as well as conclusion of the present preliminary reports.

TOLERANCE TO WATER LOSS

The four *Pelobates cultripes* used in these experiments were weighed without bladder urine, and slowly dehydraded in single glass jars during 6 days (134 hours) at uniform room temperature (20° C) and relative humidity of 70%. Eight aquatic frogs *(Rana ridibunda)* and four terrestrial toads *(Bufo bufo)* were tested simultaneously under identical experiment conditions. Weight losses were checked daily for any sample. All *Pelobates* and *Bufo* survived, but *Rana ridibunda* suffered 50 per cent mortality in the same period.

Averages and ranges of Standard Weights (g) were:
51.08 (50.35-53.50) for *Pelobates cultripes,*
70.80 (62.80-78.30) for *Bufo bufo,*
32.22 (20.30-48.20) for surviving *Rana ridibunda,*
30.22 (18.30-41.80) for dead *Rana ridibunda.*

There is not a significant correlation between mortality and Standard Weight of the latter species. The point of death by dehydration was not determined because of the scarcity of the available *Pelobates* individuals.

Water losses range from 20.3 per cent to 23.2 per cent of the initial Standard Weight in *Pelobates cultripes,* from 13.9 per cent to 20.0 per cent in *Bufo,* but they increase from 23.1 per cent to 37.8 per cent in *Rana ridibunda.* Such a water loss is largely lethal for our aquatic frogs but it is interesting to report that *Scaphiopus couchii* of similar Standard Weight can tolerate losses of over 40 per cent and recover (McClanahan, 1967).

Comparative date for *Pelobates cultripes, Scaphiopus couchii* and *Rana ridibunda* have been plotted on graph of Fig. 5. Concerning its tolerance to water loss *Pelobates cultripes* is intermediate between the aquatic frog and the American spadefoot. However stronger adaptation to arid environments of *Scaphiopus couchii* towards the European *Pelobates* is stressed by their noticeable difference in the average and range of initial weights.

Likewise it would be full agreement in the graph for the per cent of water loss of *Scaphiopus* and the unreported per cent of water loss of *Bufo*

bufo. Striking difference in initial Standard Weights can still emphasize the adaptive advantages of the water regulation by the small American form under similar environmental stress.

RATES OF REHYDRATION

Dehydrated *Pelobates cultripes* (to an average of 78 per cent of initial weight) were rehydrated in tap water within a 2 hr period. They were not completely immersed in water but it covered only their ventral surface. *Bufo bufo* and *Rana ridibunda* can also fully rehydrate within a 2 hr and 3 hr period. Like *Scaphiopus*, dehydrated *Pelobates* fill their lungs when placed in the jars, «floating» at the surface of the water. *Rana ridibunda* and *Bufo bufo* do not inflate lungs while rehydrating.

Table I summarizes rates of rehydration giving comparative per cent wt/min. in different species. Rates of rehydration are moreover indicated in graphs 6 and 7.

Specific trends of water absorption have been pointed out there. The broken and solid lines of the graph report rates of rehydration of *Pelobates*, *Bufo* and *Rana* within a 24 hr period of immersion. It may be assumed that only the aquatic frog *Rana ridibunda* regulates its water uptake when in tap water, since the stage (third hour) from which it recovered 100 per cent of the original weight is mantained.

RATE OF REHYDRATION IN WET SOIL:
WATER EXCHANGES BETWEEN TOAD AND SOIL

A very important information on this interesting subject was assembled by the careful field observations and experiments performed by RUIBAL, TAVIS and ROIG (1969) on *Scaphiopus hammondi*. These Authors studied behavioural features, water uptake at different soil moisture tensions and osmotic regulation of this spadefoot, both during its fossorial underground life and brief seasonal emergence. Comparative measures of osmolarity in body fluids of *Pelobates cultripes* and their relationships with soil moisture tensions were not possible in our present preliminary step of Laboratory researches on this species. Notwithstanding a number of experimental observations have been carried out usefully to test water absorption from the soil in *Pelobates cultripes* and *Bufo bufo* and their relative ability to absorb moisture from different types of soil having the same water content.

Such in the experiments by RUIBAL et all. our toads were dehydrated for a four day period prior to testing, by allowing them to burrow into dry sand (particles 1 mm-0.05 mm) at room temperature of 20° C. Three experimental tests were then performed.

A group of four *Pelobates cultripes* was allowed to burrow singly in glass containers having 500 g of sand with 3 per cent water. Other group of four individuals was tested singly in glass containers having 500 g of sand with 6 per cent water. A third group of four individuals, at last, was allowed to burrow under the same experimental conditions but in a soil mix having 68 per cent of sand and 32 per cent of clay (particles 0.05 mm), also adjusted to contain 6 per cent of water. Analogous tests, under identical laboratory conditions, were contemporaneously performed with the common toads *(Bufo bufo)*. Results are reported in Table II.

Both the experimented species can absorb water easily from a sandy soil. Spadefoot toads and common toads were weighed daily, and a constant weight increase was evident, such as in the American spadefoots studied by RUIBAL et all. Moreover significant differences of weight gain were stressed by *Pelobates cultripes* burrowing in sandy soil at different percentage water content (3% — 6%). On the other hand these anurans were unable to absorb moisture from a soil mix having the same water content (6%) but a different percentage of clay and sand particles. The water loss of *Pelobates cultripes* was comparable to that of *Scaphiopus hammondi* as well, because of the remarkable weight difference between the American and European spadefoots.

Also the terrestrial *Bufo bufo* was able to absorb water from the soil. Moreover the common toad burrows incompletely in the experimental soils of the jars, which covered only the ventral and lateral skin of the animals. Thus a more irregular availability of soil water is pointed out for *Bufo bufo* by the data in Table II. The availability of soil water also decreases clearly in a soil mix having a 32 per cent of clay particles. However the weight losses of *Bufo bufo* are somewhat lesser than the weight losses registered for the burrowed *Pelobates cultripes*, in the same soil.

A last control was performed for *Pelobates cultripes*, to test its water absorption from a soil sample taken in Porto de Mós, near to its spawning site (June, 1971). It was adjusted so that it contained also 6% water and the utilized four spadefoot toads were allowed to burrow into the soil sample, under the above mentioned experimental conditions. Results are indicated in Table III. It is evident that the animals were unable to absorb from the Porto de Mós soil, such as from the laboratory soil mix, just reported. Their water losses ranged from 20% to 30% after 4 days. These toads readily rehy-

drated when placed in identical jars but having 500 g of sand, adjusted to 6 per cent water, and a weight gain of 24.8 % to 28.3 % of the body weight after dehydration in the Porto de Mós soil, may be emphasized only after 24 hours there, at the same moisture level.

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In Table IV and fig. 8 some additional data are reported about the soil moisture tension exerted by our experimented soils at different percentage soil moisture levels. The moisture tension curves up to 1 atm. have been determined by the ceramic plate extractor (RICHARDS's Method); above 1 atm. the RICHARDS's method of pressure membrane was utilized. It is evident that at the 6 % moisture level our Laboratory mix (sand and silt--clay) and the Porto de Mós soil held water respectively, at 3.4 atm and 15 atm. of pressure. On the contrary, at the same moisture level, sand helds water at such a low rate as 0.038 atm. Also lowering at the 3 % moisture level, sand helds water at only 0.050 atm. The curves easily explain our experimental results, and a striking ecological analogy could be claimed, having in mind the results by RUIBAL et all. on *Scaphiopus hammondi* and its tested environmental soils from Arizona. Both in our Laboratory mix and Porto de Mós soil water was not available to *Pelobates* at the 6 % moisture level and an experimental water loss resulted. Our spadefoots on the other hand were able to absorb soil moisture in the sand holding the water very loosely, at 0.038 or 0.050 atm of pressure.

DISCUSSION

Through different kinds of evidence, our observations indicate that, like the American spadefoots, the *Pelobates cultripes* has adapted itself to a very long terrestrial existence. However the environmental conditions of its Iberian biotopes differ from the extreme semiarid habitats reported for the south-western *Scaphiopus*, such as *S. couchii* or *S. hammondi*. Annual rainfalls and soil moistures of many of the sites in which tadpoles and adults of *Pelobates cultripes* were found are in contrast with very short summer rainy season and the autumn, winter and spring dryness of the Sonoran deserts.

Portugal benefits of a mild climate with oceanic influences. Winter and spring rainy seasons are followed by a dry summer period. Water deficit (25 mm) may extend 5 months in some of its southern districts (Coast of Algarve or Baixo Alentejo), but in the central and northern provinces

3 to 1 arid months are the rule.   Average annual precipitation is still 358 mm in Sagres, or 489 in Lagos, Algarve, these localities being situated in the dried up regions of the country (semiarid regions in accordance with the TOHRNTHWAITE approach: MORAIS, 1966).   Also the observed reproductive activity and egg laying of *Pelobates* fit into the cold and humid seasonal period (March).   Average annual precipitation in Leiria, near Porto de Mós, is 976.9 mm, with some 368.4 mm falling during the months of February, March, April and May (1932-1960: AMORIM FERREIRA, 1965).   Severe droughts (monthly averages 6.9 mm, 9.3 mm) and high daytime temperatures were reported there only during July and August.   But at that time the toads presumably fall into a true physiological lethargy, when buried in the ground at the greatest depth.

Thermal requirement and tolerance to temperature of *Pelobates cultripes* are lower than thermal requirement or tolerance to temperature of *Scaphiopus*. It is stressed by our field notes on its larval life, beside of the tested tolerance of *Pelobates* tadpoles to experimental increasing temperatures (lethal point: 32° C, 35° C).   Our results (graph 4 and 5) show remarkable differences regarding the lower limiting temperatures and rate of embryonic development of both *Pelobates cultripes* and *Scaphiopus* species.   The rate of development of *Pelobates cultripes* diverges from that of the south-western spadefoots, but superposes to the rate of development of the aquatic frog *Rana pipiens* from Chiricahua Mnts. (crf. ZWEIFEL, 1968), belonging to the «slow» races of such a highly differentiated complex as the *pipiens* specific group (RUIBAL, 1962).   Moreover it has been assumed that *Scaphiopus couchii* or *S. bombifrons* does not breed unless the environmental temperature is about 18° C or higher; meanwhile spawning of *Pelobates cultripes* may occur in Porto de Mós at water temperature of 11° C.

Adaptive advantages of extremely rapid rates of both embryonic and larval development has been successfully discussed by ZWEIFEL (1968) for the specialized south-western spadefoots.   These toads breed in temporary summer pools from which rain water is often gone by evaporation before the larvae are able to metamorphose.   Thus a clear correlation may be emphazised between the ecological trends and the breeding sites of *Scaphiopus couchii* or *S. hammondi* and their rapid rates of development, one of the highest rates attained by anurans.   *Rana pipiens,* on the other hand, with a quite low rate of development, breeds normally only where the water supply appears permanent.   One might assume that analogous correlation is in accordance with the low rate of development of *Pelobates cultripes,* breeding in more permanent pools and lagoons during the wet season.   Our general remarks on larval life and distribution of the Portuguese spadefoot sounds

to be in agreement with the general ZWEIFEL's statement that «species with rapid rates of embryonic development have rapid rates of larval development». That is not the case of the big *Pelobates* tadpoles, living i.e. in the deep permanent granitic lagoons of Alto Alentejo (Nisa, Alpalhão) or in the permanent pools near Odeceixe, Algarve, in the neighbourhood of irrigated fields.

Because of its late winter or spring mating period *Pelobates cultripes* does not seem so much restricted in breeding sites than the desert spadefoots, whose rapid development and tolerance to high temperatures were probably attained under the stress of a particularly rigorous climatic selection. Likewise spawning and egg laying of the closest species *Pelobates varaldii* from the wooded Moroccan lowlands occur during the mild period of the humid autumn or winter months (PASTEUR and BONS, 1959). Divergences in the rate of development of the southern European and western American spadefoots may readily be explained in terms of their different seasonal cycles, since dry summer months are not available for any reproductive activity of *Pelobates*, in its Iberian or North African areas. Perhaps the past influence of habitat selection and breeding environment has been there a rather weak extrinsic factor for wide adaptations to extreme natural conditions, such as in the case of the fastest developing species. We could have in mind the evolutive and paleoecological relationships of Pelobatid toads, just reviewed by ESTES (1970). Primitive trends of the Pelobatine line and of *Pelobates cultripes* itself were pointed out through the Eurasiatic cenozoic *Macropelobates* branch. This anuran seems very close to the primitive eastern spadefoot *Scaphiopus hoolbrocki* and to the actual genus *Pelobates*, whose modern species may probably have been delimited by Miocene time, being later restricted by the advancing Pleistocene ice sheets. Unspecialized features and aquatic biology of the ancestral Pelobatid lines have been suggested by different kinds of paleontological evidence. Some conservative ecological trends of Iberian *Pelobates cultripes* may be assumed for its rate of development within the general evolutive tendency of its phyletic group. Nevertheless they emphazise the advanced diversification for extreme deteriorating climatic conditions suffered by several actual species of the *Scaphiopus-Spea* complex, such as *Scaphiopus couchii*, *S. bombifrons* or *S. hammondi*.

Our data on tolerance to water loss of *Pelobates cultripes* make evident a remarkable adaptation for a prolonged underground survival. Its water loss is significantly greater than the water loss of *Scaphiopus couchii* i.e., under similar experimental conditions, but its ability to tolerate weight loss by dehydration is still noticeably high when confronted with comparable but lethal weight losses of the aquatic frog *Rana ridibunda*. On the contrary

the tolerance to dehydration of the terrestrial toad *Bufo bufo* is not significantly different from that of *Pelobates cultripes*.

The rates of rehydration for these Portuguese anurans were reported in our Table I and graphs. It was evident that they are all comparably rapid within the first hour, gradually decreasing for the following hours. To the 90 per cent of the original body weight a very favourable rate of rehydration may be assumed for *Rana ridibunda* (0.30 per cent body weight/min.), then it drop sensibly (0.16) to the 100 per cent of the original body weight. However only *Rana ridibunda* appeared to be capable of regulating water exchanges when placed in tap water, within the following 24 hours. *Pelobates cultripes* and mostly *Bufo bufo* increased continually their initial corporal weight during the same period and the water absorption was at last equilibrated by *Pelobates cultripes* only at the end of 24 hours. It indicates that a very effective regulating mechanism of water balance plays an important ecological role above all for *Rana ridibunda*, a fundamentally aquatic frog of every Portuguese biocenotic community. It seems therefore of smaller adaptive value for such a terrestrial type of anurans as the common toad or the fossorial *Pelobates cultripes*. Also the very rapid initial rate of rehydration above reported for *Rana ridibunda* suggests a significant adaptive advantage. These frogs live all the year in permanent waters but jump outside very frequently to forage, by day or in the night, often far enough from the shores. Thus a fugacious immersion as well can rapidly equilibrate their eventual water losses in the surrounding dry summer environment.

Hydric exchanges between fossorial toads and soil are the most important ones for the underground existence of the animals. Having in mind the exhaustive studies carried out by RUIBAL et all. and McCLANAHAN on the American spadefoots, our comparative experiments provide a first background of information on such an ecological feature of *Pelobates cultripes*. Periodic observations on this species in its natural sites were still practically not available because of the extreme difficulty to locate the burrowed toads at any moment.

In spite of the relative versatility of its distribution in Portugal *Pelobates cultripes* is said to prefer sandy soils and the coastline habitats. It would be in agreement with the evident suitability of a more friable, not compacted ground for digging, but even with a ready, easier utilization of the soil moisture to store water and to reduce corporal fluid losses. RUIBAL, TAVIS and ROIG (1969) investigated very carefully the physiological mechanisms of *Scaphiopus hammondi* from Arizona facing the seasonal variations of the water content in different soils. This model is not directly comparable to the field situation in Portuguese biotopes, on account of the strong differences existing between

regional climates. However our present data on *Pelobates cultripes* may suggest a general adaptive parallelism between the American and European spadefoots regarding their ability to mantain an equilibrium between the osmotic concentration of the body fluids and the moisture tension of the ambient soil.

RUIBAL et all. demonstrate that the availability of soil water by *Scaphiopus hammondi* is not crudely measured by the moisture content, but it is in accordance with the moisture tension of each soil at different moisture contents. In other terms the water availability by the permeable skin of the fossorial toads is determined by the forces by which the water is held by the soil particles in different types of grounds. Such as in *Scaphiopus*, it was shown by our experimental tests that *Pelobates cultripes* burrowed in a sandy soil, was able to absorb water easily, at a regular weight increase rate. Significant difference of weight gain was obviously observed at different soil moisture (3 per cent, 6 per cent water content). However, like *Scaphiopus hammondi*, the Portuguese spadefoots were unable to absorb water from a laboratory mix having the same moisture percentage that the sandy soil but a content of 32 per cent of clay particles. In this case the weight losses of *Pelobates cultripes* ranged from 0.6 to 3.8 percent of initial body weight. If they were somewhat slighter, i.e., than the weight losses of *Scaphiopus hammondi* experimentally burrowed in the Boss Ranch soil (crf. RUIBAL et all., 1969), they would yet be comparable having in mind the striking different size of both forms. Further more, severe water losses were presented by *Pelobates cultripes* when experimentally allowed to burrow in a clay soil sample taken from the same breeding site: Porto de Mós. Weight loss ranged there from 20 to 30 per cent of the initial body weight, obviously being in agreement with the increase of the clay particles of the sample (1). Physical structure of the ground must also play a very important part in the not random choosing of burrow sites by *Pelobates cultripes*. It is a fundamental ecological trend for an anuran spending a very long time underground and it may explain the irregularly scattered distribution of the submerged adult individuals and the extreme difficulty to locate them in the field.

The above general considerations were confirmed by the common toad *Bufo bufo*, tested under identical laboratory conditions (Table II). Rates of weight gain were similar to those of *Pelobates cultripes* when placed in

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(1) Porto de Mós sample: sand fraction, 45.3 per cent; silt and clay fraction, 54.7 per cent (in accordance with the American Scale).

sandy soil; likewise weight losses were shown, but quite smaller or insignificant, when placed in the laboratory mix with 32 per cent of clay particles. Behavioural features of *Bufo bufo* may be stressed here, because common toads do not burrow completely in the experimental soil in the jars, such as *Pelobates*. Water uptake can account probably in this species at a somewhat similar rate through the pelvic integument. That interesting adaptive pattern of Bufonids was demonstrated by McCLANAHAN and BALDWIN (1969) for the Desert toad *Bufo punctatus*, sympatric with *Scaphiopus couchii* in California.

Studies on the immediate relationships between the osmotic concentration of the body fluids in our spadefoots and the soil moisture tension were not carried out at the present preliminary step of our observations. Perhaps some physiological mechanism of internal osmotic control by raising urea concentration could have been developed by *Pelobates cultripes* too, to avoid water loss when soil moisture tension reaches a remarkably high level. The physiological problems the toad has to solve to conserve hydric equilibrium are possibly not too much important since late autumn to late spring months. Rainfalls are generally sufficient to prevent a dangerous increase of soil moisture tension in most part of the Portuguese habitats of *Pelobates cultripes*, during that time. The critical phase may come about in June, until late October, covering the whole dry and hot summer months. Hydric deficit is very high everywhere in the country, and a rapid rising of the steepest portion of the soil tension curve may be obviously assumed (Crf. Fig. 8). The same gradient between the osmotic concentration of the buried spadefoots and soil moisture tension will then steeper than in the winter or spring natural conditions. Thus a more rapid and critical dehydration may be postulated unless adaptive osmotic compensation or other protective mechanism will intervene to prevent large water transfer to the soil. Layers of keratinized skin, or coocon-like coatings, reducing water exchanges, have been described for a number of Australian desert toads (LEE and MERCER, 1967) and for *Scaphiopus couchii* itself (MAYHEW, 1965; McCLANAHAN, 1967). They are also known structures in the hibernating Ceratophryd toads from Argentina (*Ceratophrys ornata, Lepidobatrachus llanensis:* personal unpublished data). They are specifically supported by cyclical neurohypophysical regulations. But nothing has been reported hitherto for *Pelobates cultripes* whose underground biology remains practically unknown.

<div align="center">*</div>

SUMMARY

Spawning and tadpole biology of *Pelobates cultripes* from Portugal were studied, with special interest for the climatic patterns of the habitat. Rate of development of this south European spadefoot was controlled. It is slower than the rate of development of several American forms of *Scaphiopus* from the arid south-western environment, but superposes the rate of development of *Rana pipiens*. Tolerance to water loss and water absorption were experimentally studied, having in mind similar researches carried out on western American spadefoots. Adaptive trends of dehydrated *Pelobates cultripes* were demonstrated, however, at a lesser extent than those of *Scaphiopus couchii*.

Rate of rehydration of *Pelobates cultripes* is not significantly different from that of Portuguese anurans *Bufo bufo* and *Rana ridibunda*. On the contrary, the dehydrated aquatic frog *Rana ridibunda* shows a very rapid rate of rehydration up to 90% of the initial body weight. That may be of adaptive value in its natural environment.

Both *Pelobates cultripes* and *Bufo bufo* were able to absorb moisture from a sandy soil at different percentages of water content, notwithstanding they were unable to absorb water from a mixed clay soil at the same water percentages due to the different soil moisture tension. Probable ecological parallelisms with the adaptive osmotic water regulation of the American spadefoots were discussed.

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TABLE I

Comparative rate of hydration in dehydrated toads and frogs.
The rates of hydration were expressed in per cent of body weight per minute

		RATE OF HYDRATION
To 90% original body weight:	*Pelobates cultripes*	0.20
	Scaphiopus couchii	0.26
	Bufo bufo	0.21
	Rana ridibunda	0.30
	Rana pipiens	0.16
To 100% original body weight:	*Pelobates cultripes*	0.20
	Bufo bufo	0.21
	Rana ridibunda	0.16

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TABLE II

WEIGHT GAINED OR LOST (AS WATER, IN GRAMS) BY *Pelobates cultripes* AND *Bufo bufo*, WHEN
BURROWING IN DIFFERENT SOILS HAVING DIFFERENT PER CENT OF WATER CONTENT (+ SIGN
GAIN IN WEIGHT; — SIGN LOSS IN WEIGHT) — (ALL TESTS AT 20º C)

INITIAL BODY WEIGHT (g)		WT AFTER 5 DAYS (g)	% WT CHANGE AFTER 5 DAYS (g)	
Sandy soil: 3% water content				
Pelobates cultripes	60.6	63.4	+4.6	
	32.0	37.7	+17.8	MEAN: +14.8
	29.2	35.4	+21.2	
Bufo bufo	32.9	36.9	+12.1	
	27.0	30.0	+11.1	MEAN: +17.4
	30.3	39.2	+29.8	
Sandy soil: 6% water content				
Pelobates cultripes	45.0	53.7	+19.3	
	34.6	45.4	+31.2	MEAN: +22.9
	25.3	30.5	+20.6	
	49.5	59.7	+20.6	
Bufo bufo	57.7	61.2	+6.0	
	42.5	49.4	+16.2	MEAN: +13.3
	63.9	76.6	+19.8	
	59.7	66.6	+11.5	
Soil Mix: 32% clay particles, 68% sand.				
Pelobates cultripes	21.5	21.1	—1.9	
	47.5	46.8	—1.5	MEAN: —1.9
	36.4	36.0	—0.6	
	39.5	38.0	—3.8	
Bufo bufo	87.0	84.9	—2.5	
	46.9	46.7	—0.5	MEAN: —0.5
	56.7	57.0	+0.5	
	42.0	42.1	+0.2	

TABLE III

WEIGHT LOSS BY *Pelobates cultripes* IN A SOIL SAMPLE FROM PORTO DE MÓS, NEAR LAGOA
DA VALA, ESTREMADURA — (6% WATER CONTENT; TEMP. 20° C)

INITIAL BODY WEIGHT (g)	WT AFTER 4 DAYS (g)	% WT CHANGE AFTER 4 DAYS (g)
40.0	31.9	—21
24.0	18.0	—25
37.7	26.5	—30
45.5	36.4	—20

TABLE IV

SOIL MOISTURE TENSION CURVES

MOISTURE TENSION (atm.)	MOISTURE TENSION log (atm. × 1000)	MOISTURE (%)		
		SAND	SAND + CLAY	PORTO DE MÓS SOIL
0,01449	1,16	21,8	26,1	29,4
0,02415	1,38	17,9	24,7	28,7
0,02692	1,43	16,3	—	—
0,02884	1,46	14,0	—	—
0,03020	1,48	13,2	—	—
0,03802	1,58	5,9	—	—
0,04831	1,68	3,2	19,5	27,3
0,09662	1,99	1,5	14,7	25,5
0,19360	2,29	0,9	11,5	22,3
0,58070	2,76	0,8	9,1	16,3
2,90350	3,46	0,7	6,3	10,3
4,83920	3,68	0,6	5,4	8,6
9,67840	3,99	0,5	4,7	7,0
15,00000	4,18	0,4	4,2	6,0

FIG. 2 — Map of prospected areas — Numbers and filled circles indicate sites of observed tadpoles of *Pelobates cultripes;* open circle indicates the breeding site (Porto de Mós, Estremadura). (1 — Praia S. Cruz; 2 — Azambuja; 3 — Nisa; 4 — Alpalhão; 5 — Pavia; 6 — Moura; 7 — Odeceixe; 8 — Sagres; 9 — Espiche).
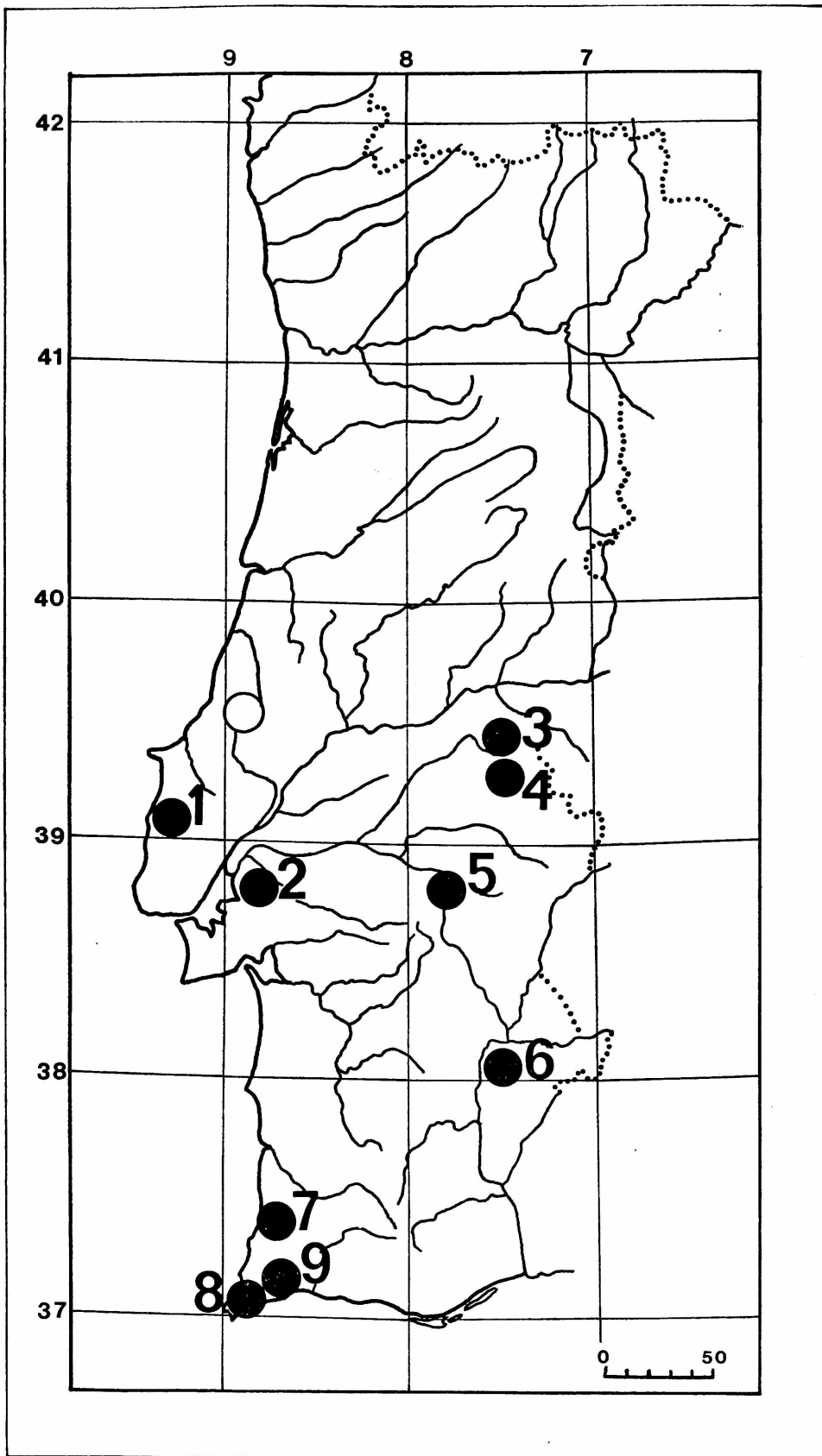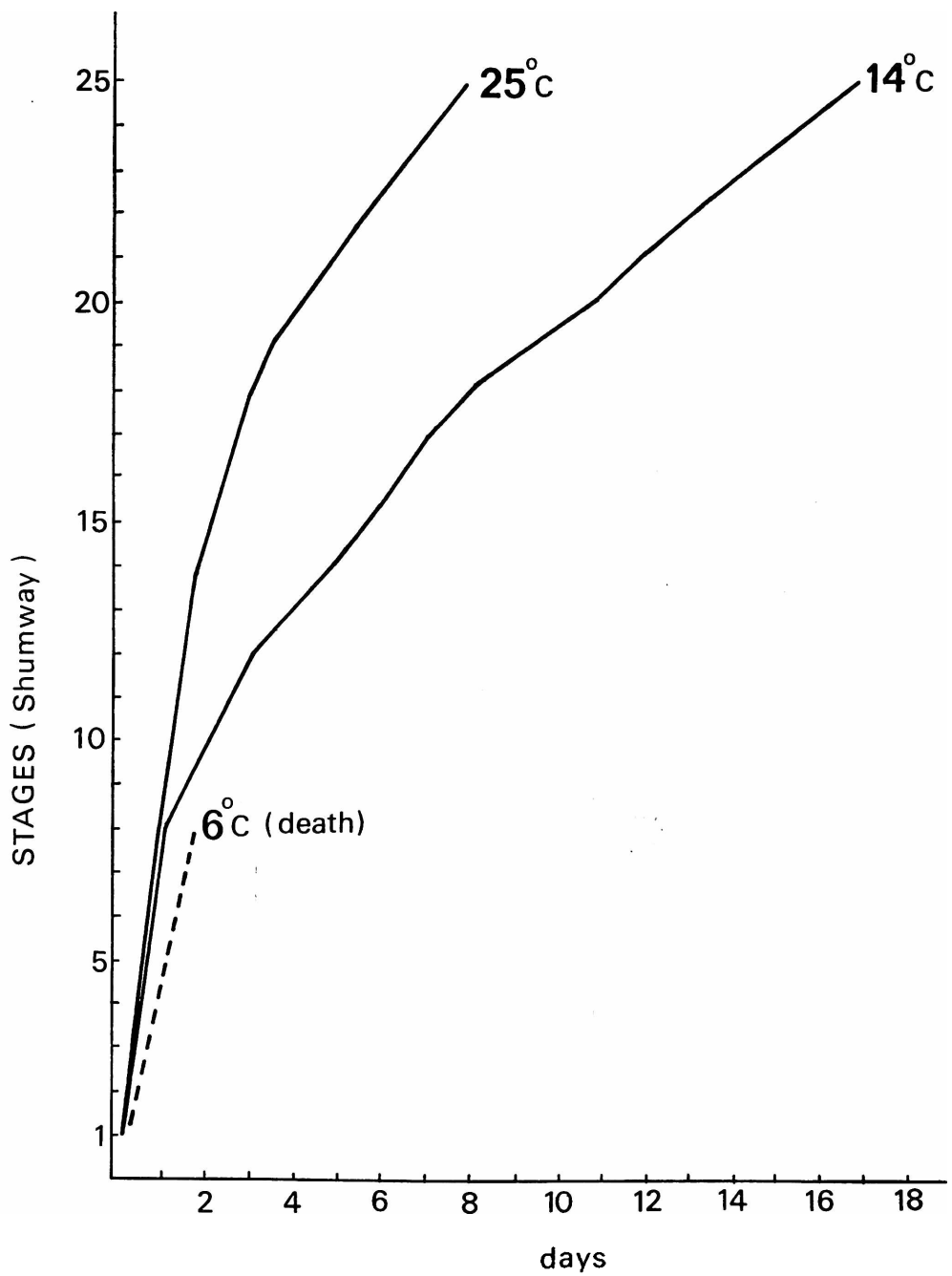
FIG. 3 — Rate of development of *Pelobates cultripes* at temperatures of 25° C, 14° C and 6° C.
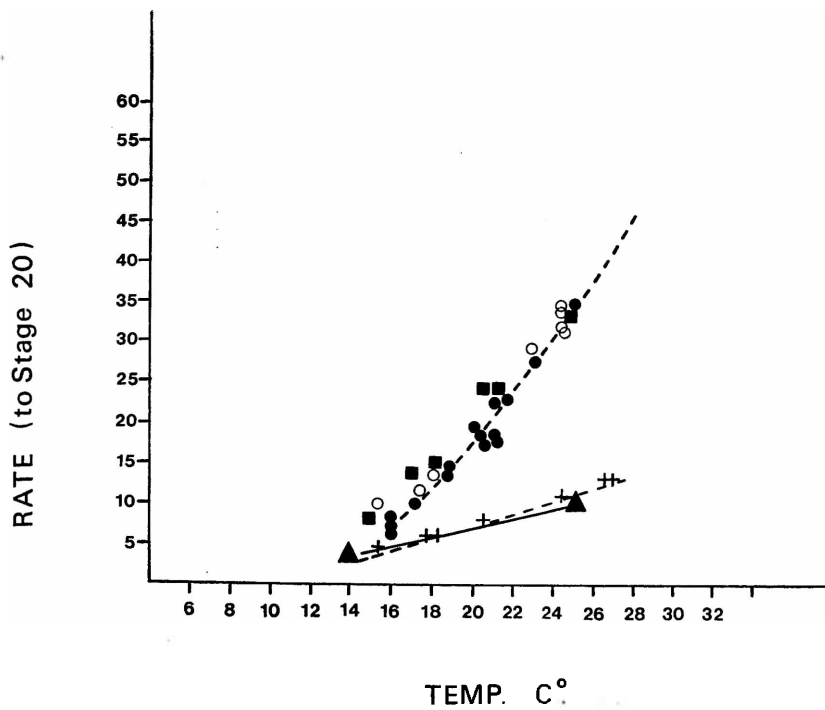
FIG. 4 — Comparative rates of development (1/T × 1000) of *Pelobates cultripes* (▲), Rana pipiens (+) and *Scaphiopus* species from Arizona (● — *S. couchii*; ■ — *S. bombifrons*; ○ — *S. hammondi*). Data on *Rana pipiens* and *Scaphiopus* reported from Zweifel's work (1963). (1/T × 1000: rate of development expressed as 1000 divided by time in hours to stage 20).
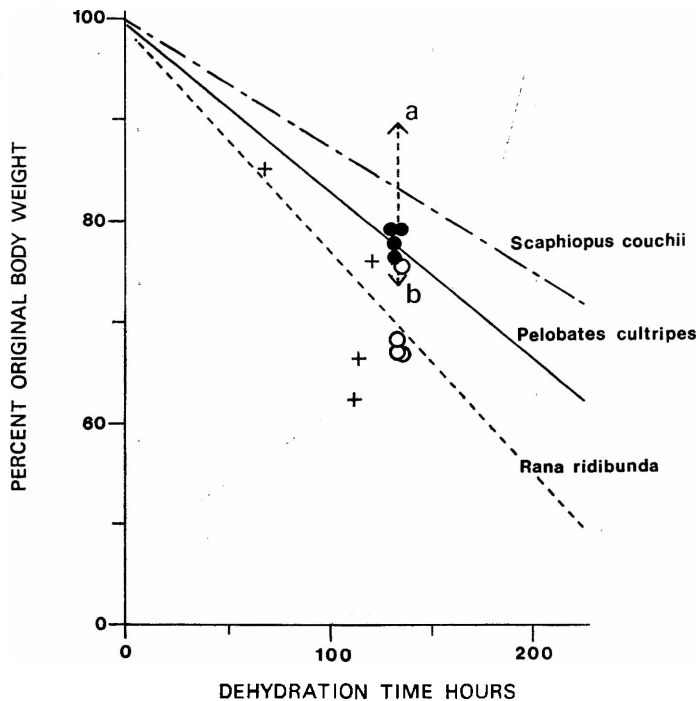
FIG. 5 — Tolerance to water loss in *Pelobates cultripes* dehydrated at 20° C, with a relative humidity of 70%. For comparison tolerance to water loss in *Rana ridibunda* at the same temperature. Vertical broken line (a <——————> b) indicates the range of tolerance in *Scaphiopus couchii* at 20° C (in accordance with the McClanahan's reports and graph, 1967). ● *Pelobates cultripes*, ○ *Rana ridibunda*. (Point of death for *Rana ridibunda* indicated by crosses).
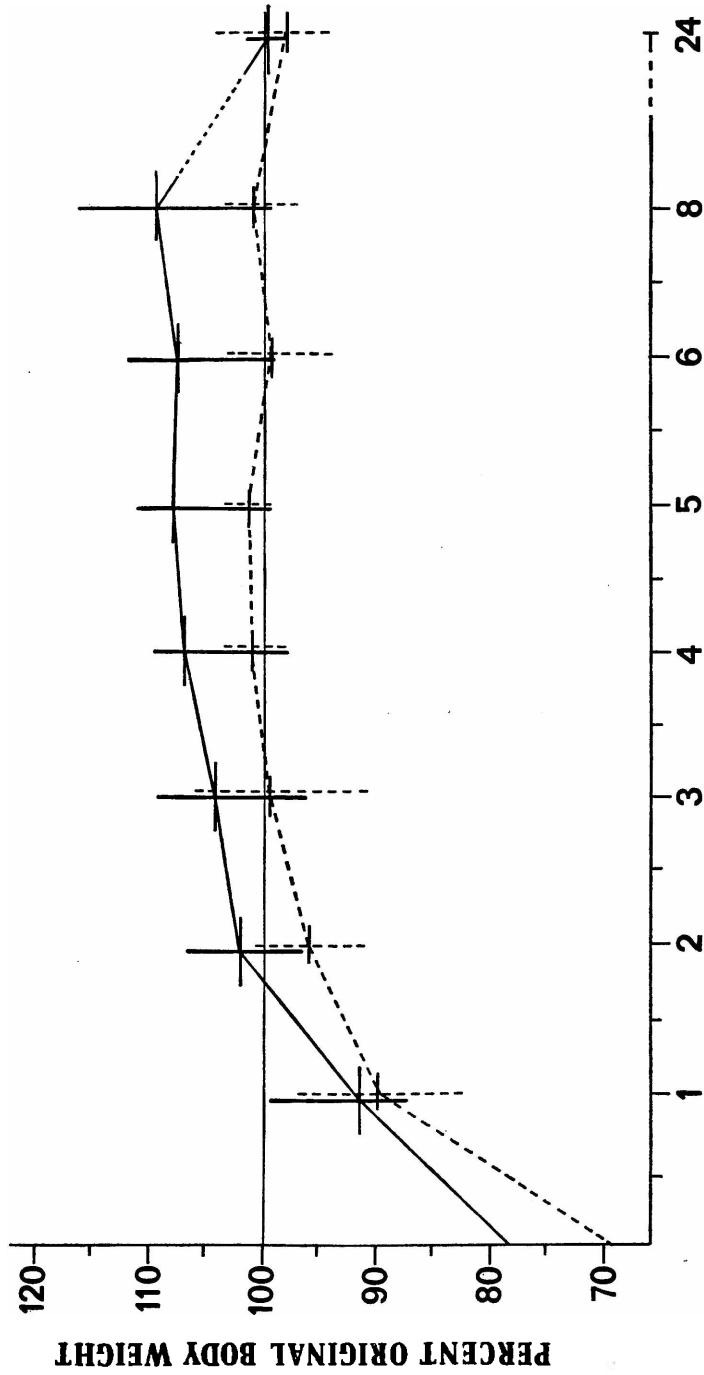
FIG. 6 — Rates of rehydration in dehydrated *Pelobates cultripes* and *Rana ridibunda* when placed in tap water. Solid and broken vertical lines represent ranges; horizontal lines are means of N = 4 *Pelobates cultripes* and N = 4 *Rana ridibunda* (Solid lines — *Pelobates cultripes*; broken lines — *Rana ridibunda*).
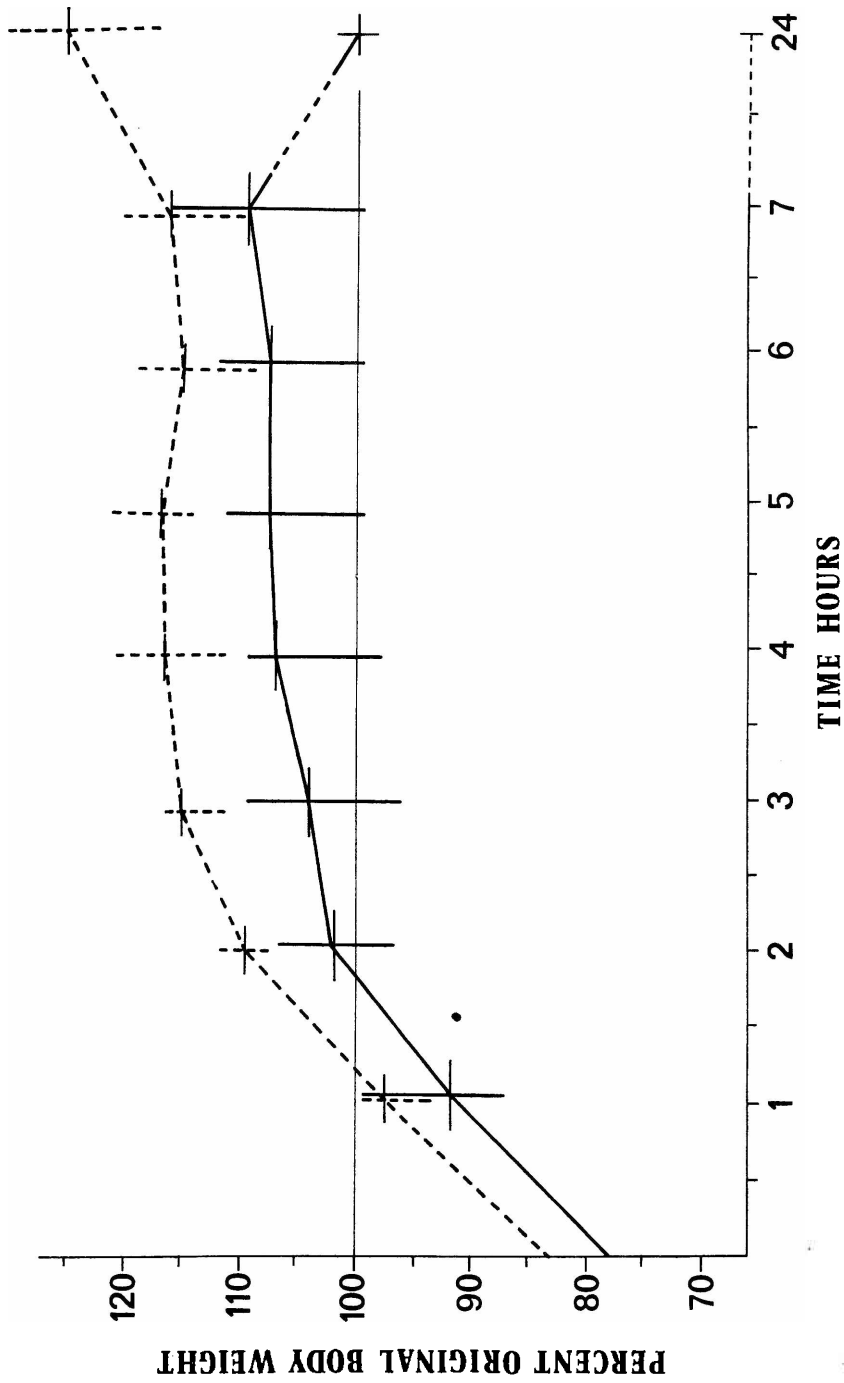
FIG. 7 — Rates of rehydration in dehydrated *Pelobates cultripes* and *Bufo bufo* when placed in tap water. Solid and broken vertical lines represent ranges; horizontal lines are means of N = 4 *Pelobates cultripes* and N = 4 *Bufo bufo*. (Solid lines — *Pelobates cultripes*; broken lines — *Bufo bufo*).
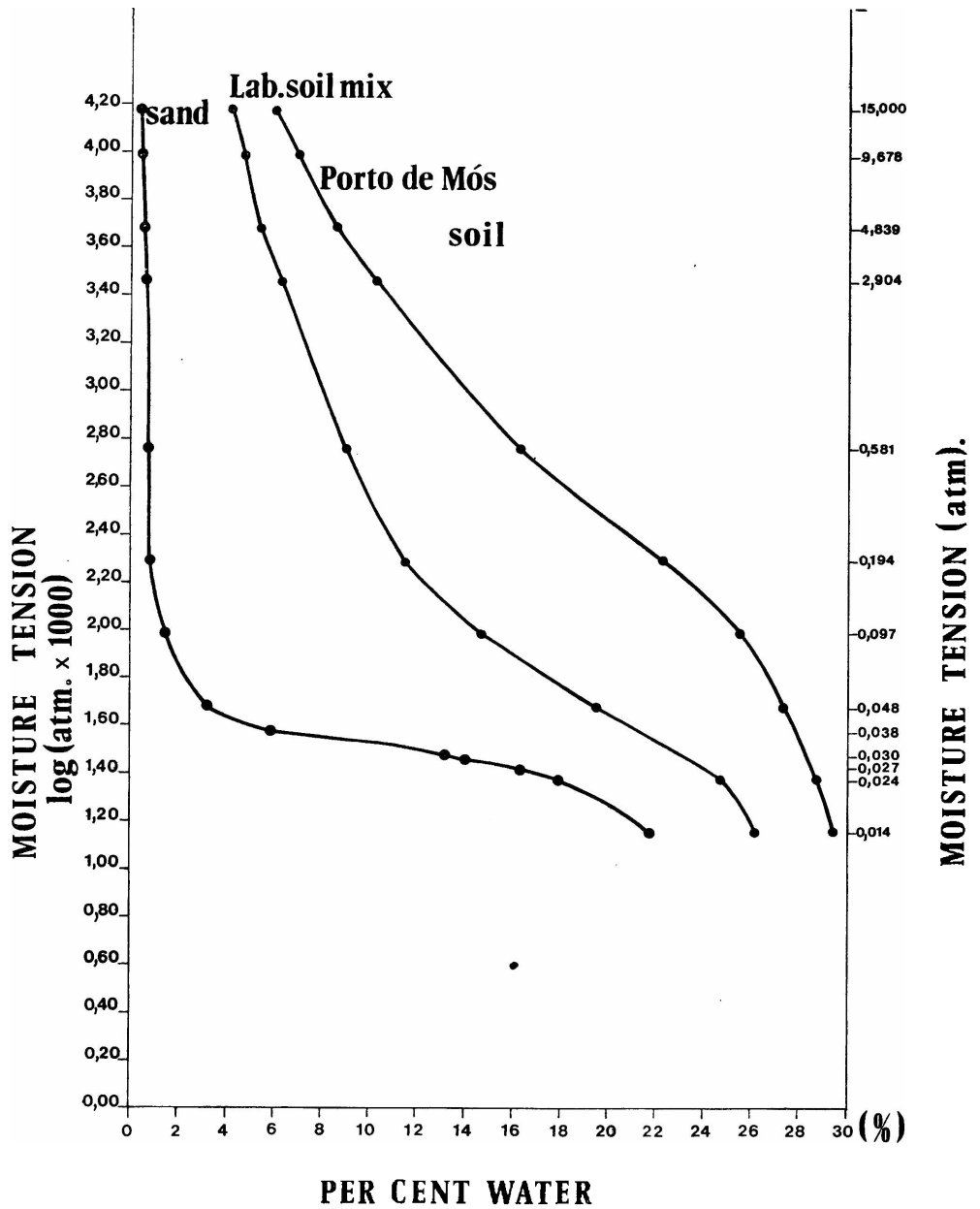
FIG. 8 — Soil moisture tension curves for Porto de Mós soil, Lab soil mix and sand. Vertical axis on left indicates logarithmic expression of the moisture tension, reported as atmosferes × 1000. Vertical axis on right indicates measures of the moisture tension (atm) for any point of the curves.