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Growth and metabolism in snake embryos

By RAZI DMI'EL[1]
From the Department of Zoology, Tel-Aviv University

Since the early papers of Bohr & Hasselbalch (1900, 1903) and Murray (1925), considerable information concerning the growth and metabolic processes in the avian embryo has been accumulated (Romanoff, 1967). Respiratory and growth processes during embryonic life in many species of fishes, amphibians and mammals are also fairly well known. Only a few studies have been reported, however, on the metabolism and growth of the reptilian embryo. Lynn & Brand (1945) presented a detailed description of these processes in four species of turtles. Some data on respiration in the egg of the snake *Coluber natrix* are given by Bohr (1904). The respiration of four eggs a few days prior to hatching in the snake *Liopeltis vernalis* was studied by Zarrow & Pomerat (1937). Scattered information on the embryonic growth of snakes may also be obtained from papers dealing with the development of certain anatomical systems in the embryo, such as venom glands and the musculature related to them (cf. Fukada, 1958; Kochva, 1963, 1965). Growth or metabolic curves are not available for snake embryos, however.

It is the aim of the present paper to describe the growth and metabolic patterns in embryos of several species of snakes common in Israel, in order to obtain a general pattern for growth and metabolism in snake embryos, and to facilitate comparison among the different species.

MATERIALS AND METHODS

Eggs were obtained from *Natrix tessellata* and *Spalerosophis cliffordi* (Colubridae), and *Vipera xanthina palaestinae*, *Echis colorata* and *Aspis cerastes* (Viperidae), kept in the Research Zoo of the Department of Zoology, Tel-Aviv University. They were incubated at a temperature of 30 ± 1 °C, as described previously (Dmi'el, 1967). Incubation of the eggs started within 8 h of laying. Eggs were taken at fixed intervals, generally between 3 and 4 days. They were dissected, and the embryos were taken out, weighed and their total length measured. Some of the eggs of each clutch were taken for metabolic measurements at $30 \pm 0{\cdot}01$ °C. These eggs were incubated separately and allowed to develop normally without interruption. Oxygen consumption was determined in a closed manometric system, by using a modified Haldane respirometer (Fig. 1).

[1] *Author's address:* Department of Zoology, 155 Herzl St., Tel-Aviv, Israel.

CO_2 was absorbed by a 10 % KOH solution. The respirometers were made of polyethylene with two connexions (*g* in Fig. 1), one with the KOH dish and the other with the bottom of the respirometer surrounding this dish. These connexions made it possible to renew the KOH solution and to add water without opening the respirometer, thus preventing loss of water by the eggs (Mendelssohn, 1963; Dmi'el, 1967). Determinations of oxygen consumption were carried out for periods of 24–30 h every 3 days. Values were obtained at hourly readings. More than 95 % of the eggs used in this procedure hatched successfully. The

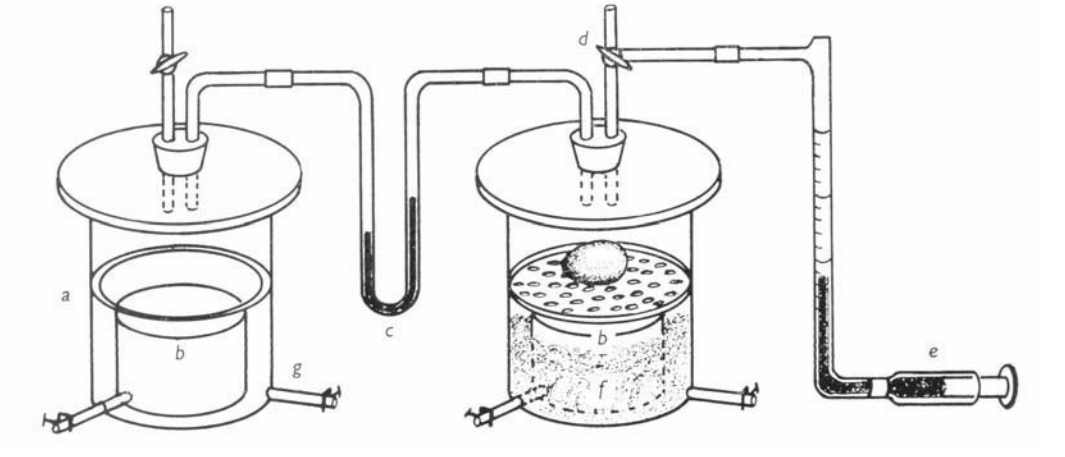

Fig. 1. Respirometer used for measuring oxygen consumption. *a* = thermobarometric compensator; *b* = dish, for KOH solution; *c* = manometer; *d* = three-way stopcock; *e* = syringe connected to a calibrated pipette; *f* = cotton wool saturated with water; *g* = pipes for renewing water and KOH solution.

Table 1. *Data on eggs used for length and weight measurements and respiration experiments*

Family and species	No. of clutches	Mean weight of eggs upon laying (g)	No. of eggs used for respiration experiments	No. of eggs used for length and weight measurements	Days of development at 30 °C (from laying to day of hatching)
Colubridae					
Natrix tessellata	6	7	32	79	37
Spalerosophis cliffordi	12	22	60	88	60
Viperidae					
Vipera xanthina palaestinae	7	14	40	89	41
Echis colorata	6	10	20	45	43
Aspis cerastes	3	11	14	29	62

For further details on reproduction and egg development in *Vipera*, *Echis* and *Spalerosophis* see Mendelssohn (1963, 1965) and Dmi'el (1967).

hatching process in this group occurred on the same day as that of the rest of the eggs used for length and weight measurements. The experimental data obtained for length and weight increase and those for metabolism were statistically analysed in the Computation Centre of the Tel-Aviv University. Data on the eggs used in the various experiments are given in Table 1, which also includes data on weight and length of embryos from studies carried out in the Department of Zoology on the development of oral glands in snakes (Fain, 1969; Ovadia, 1969).

RESULTS

Absolute values for total length, wet weight and oxygen consumption in the five species of snakes at the beginning and end of the incubation period are given in Table 2. Empiric equations and numerical values related to each growth process are given in the following graphs and tables, using the common statistical

Table 2. *Mean of measured values for oxygen consumption (ml O_2/egg/h), wet weight (g) and length (mm) in five species of snakes on the first day of incubation and upon hatching (standard deviations in parentheses)*

	Oxygen consumption	Weight	Length
	First day		
Natrix	0·160 (0·07)	0·173 (0·06)	6·42 (1·33)
Spalerosophis	0·153 (0·05)	0·199 (0·06)	3·38 (0·62)
Vipera	0·162 (0·07)	0·394 (0·09)	51·93 (8·45)
Echis	0·197 (0·07)	0·466 (0·11)	88·55 (12.34)
Aspis	0·201 (0·07)	0·317 (0·08)	24·43 (4·76)
	Day of hatching		
Natrix	1·432 (0·21)	5·11 (1·31)	228·0 (11·2)
Spalerosophis	2·791 (0·43)	16·33 (2·09)	359·6 (8·8)
Vipera	1·456 (0·22)	10·68 (1·63)	234·5 (9·3)
Echis	1·027 (0·14)	6·22 (1·52)	254·0 (7·6)
Aspis	1·277 (0·19)	6·47 (1·61)	180·8 (10·2)

terms: a is the intercept of the regression line on the Y axis; b, the slope of the curve, gives the increase in growth or metabolism for an increase of unity in the value of X; r is the coefficient of correlation between X and Y; F, the variance ratio, tests the significance of the correlation coefficient.

Increase in length

The results for the length increase in the five species are summarized in Fig. 2 and Table 3. These results show clearly that length increase in all the species examined is linear. However, there are marked differences in rate of increase between the two families and among different species of the same family. The embryos of the two colubrids, *Natrix* and *Spalerosophis*, are the smallest ones when laid (a values in Table 3) but have the highest rates of length increase

(*b* values), that of *Natrix* being the higher of the two. The vipers, on the other hand, are larger when laid but have lower rates of length increase. Among the viperids, *Vipera* embryos have a higher rate of length increase than *Echis* and *Aspis*.

Table 3. *Length of snake embryos during incubation (predicted values)*

Growth equation: Length (mm) = $a+bX$ (X = day of incubation).

	a	*b*	*r*	*F b*
Natrix	6·3790	5·9897	0·95333	**
Spalerosophis	3·3663	5·8318	0·96885	**
Vipera	52·9596	4·4694	0·94531	**
Echis	89·4702	2·8648	0·93094	**
Aspis	23·3502	2·4400	0·98260	**

** Highly significant: $P < 0{\cdot}001$. F values are between 174·240 and 982·650.

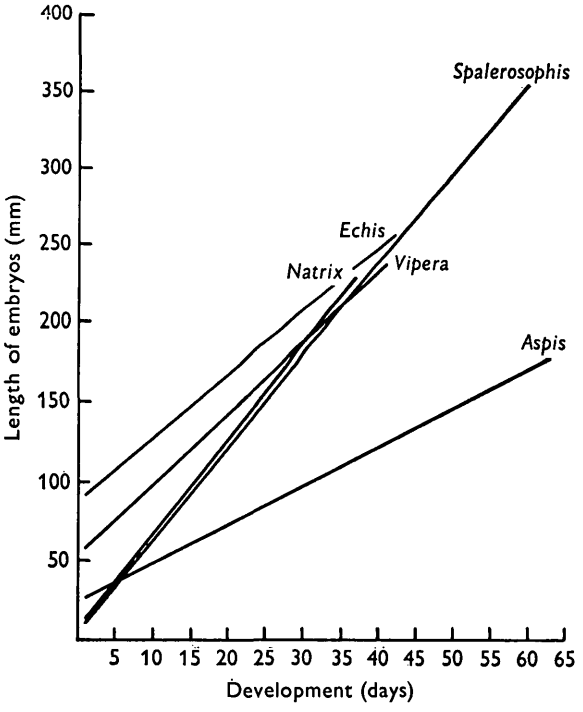

Fig. 2. Length of snake embryos during incubation. Curves calculated by the method of least squares (Table 3).

Increase in weight

Weight increase in the snake embryos is exponential. Plotting the logarithmic values of the wet weight against age yields essentially a straight line (Fig. 3, Table 4). Here again, the colubrids are smaller at the beginning of the incubation period, but grow more rapidly, whereas the vipers, being larger when

laid, show lower increase rates. *Vipera* embryos are different in this respect. Although upon laying they weighed more than *Aspis* and each of the two colubrids, the slope of their rate of increase is intermediate between the slopes obtained for *Spalerosophis* and *Natrix*.

Table 4. *Weight of snake embryos during incubation (predicted values)*

Growth equation: log wet weight (g) = log $a+bX$ (X = day of incubation).

	Log a	b	r	F b
Natrix	−0·7613	0·03956	0·91085	**
Spalerosophis	−0·7035	0·03187	0·94565	**
Vipera	−0·4041	0·03505	0·95719	**
Echis	−0·3319	0·02587	0·93626	**
Aspis	−0·4988	0·02076	0·97519	**

** Highly significant: $P < 0{\cdot}001$.

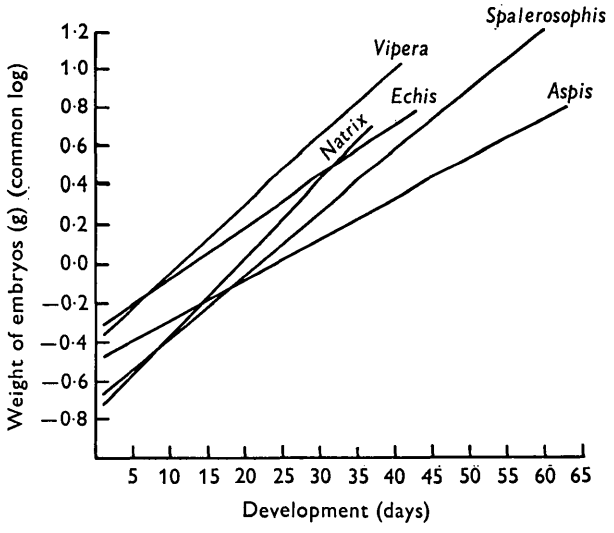

Fig. 3. Weight of snake embryos during incubation. Curves calculated by the method of least squares (Table 4).

Oxygen consumption of the snake eggs

The metabolic pattern of the eggs is very similar to the pattern of the embryo weight increase, i.e. it is an exponential process. Plotting the metabolic values on an arithlog grid gives a straight line (Fig. 4, Table 5). As in the weight increase, the slope of increasing rate of oxygen consumption in *Vipera* is steep and intermediate between those of the two colubrids, whereas *Echis* and *Aspis* have the most moderate slopes. The values of the correlation coefficients obtained for the metabolic curves (Table 5) are somewhat lower than those for the growth curves. This is mainly because of the daily fluctuations found in the metabolic

rate of the eggs, which may perhaps show a circadian rhythm (unpublished data). Nevertheless, all probability values calculated for (r) in Table 5 were, without exception, highly significant ($P < 0{\cdot}001$).

Table 5. *Oxygen consumption of snake eggs during incubation (predicted values)*

Metabolic equation: log ml O_2/egg/h = log $a+bX$ (X = day of incubation).

	Log a	b	r	F b
Natrix	−0·7997	0·02583	0·92823	**
Spalerosophis	−0·8157	0·02120	0·88233	**
Vipera	−0·7914	0·02321	0·85460	**
Echis	−0·7049	0·01666	0·85079	**
Aspis	−0·7003	0·01280	0·88045	**

** Highly significant; $P < 0{\cdot}001$.

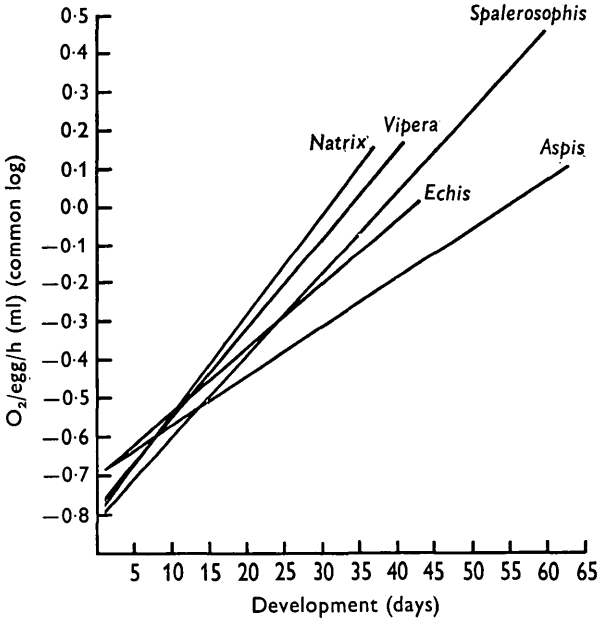

Fig. 4. Oxygen consumption in snake eggs during incubation. Curves calculated by the method of least squares (Table 5).

DISCUSSION

General

As shown in Fig. 2 and Table 3, the length increase in the embryonic snakes is best described by an arithmetic straight-line equation. This means that the embryo length grows at a constant time rate. This pattern is in contrast to that found in turtle embryos, in which the growth in carapace length is sigmoid (Lynn & Brand, 1945). The linearity of the elongation process in embryonic

snakes is, however, in full agreement with the general pattern of embryonic and prepubertal elongation found in mammals as well as in many other animals (cf. Brody, 1945, pp. 602–5, 633).

The straight-line curves which resulted from semilogarithmic plotting of the data for weight and metabolism (Figs. 3, 4 and Tables 4, 5), demonstrated processes each of which progresses at a constant percentage increase of rate (Brody, 1945, chapter 16, and p. 633). The more theoretical growth curve, namely that of the sigmoid type, could not be shown statistically in these embryos. Inflexion points, if any, in the growth of metabolic curves of the embryonic snakes may occur only a day or two prior to hatching. It may also be pointed out that inflexion points in the weight increase curves of avian embryos occur after

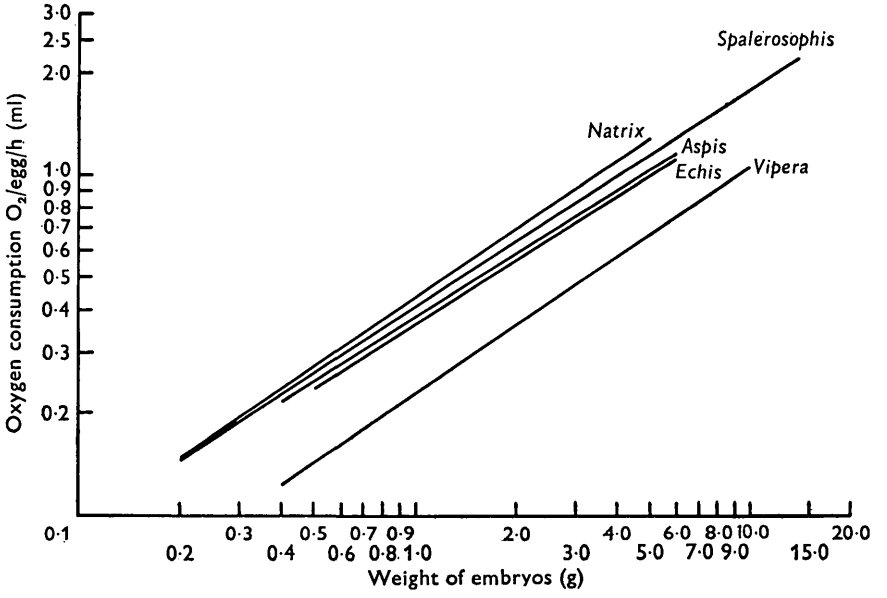

Fig. 5. Oxygen consumption in snake eggs as a function of the embryonic weight. Curves calculated by the method of least squares (Table 6).

Table 6. *Rates of metabolism in snake eggs as a function of embryonic weight (predicted values)*

Metabolic equation: log ml O_2/egg/h = log a + b log W (W = embryonic weight in g).

	Log a	b	r	F b
Natrix	−0·3559	0·67970	0·93431	47·484**
Spalerosophis	−0·3949	0·64584	0·94810	55·621**
Vipera	−0·6455	0·66963	0·87958	54·438**
Echis	−0·4419	0·62839	0·91010	45·831**
Aspis	−0·4236	0·62340	0·96870	47·687**

** Highly significant: $P < 0{\cdot}001$.

ca. 95 % of the incubation period, or even closer to the day of hatching (Romanoff, 1967, p. 263, fig. 72).

The differences between the values of oxygen consumption presented here and those observed in the snake *Liopeltis vernalis* (Zarrow & Pomerat, 1937) and in turtle embryos (Lynn & Brand, 1945), reflect generic differences as well as different incubation conditions (30 °C in the present experiments as against 12–30 °C in the others). Nevertheless, metabolic values for all species mentioned above are of the same order of magnitude. These metabolic rates are 10–20 times less than that of the avian embryo.

The similarity between the metabolic pattern of the snake embryos and their weight increase curves is remarkable. The metabolic rates of the eggs as a function of embryo weight are given in Fig. 5 and Table 6. It should be noted in this respect that oxygen consumption was determined for the whole intact egg. There was no possibility of separating the respiration of the embryo from the respiration of the extra-embryonic membranes (especially that of the amnion and allantois) in these, and as far as is known, in any other reptilian species. The contribution of the extra-embryonic membranes to the total rate of metabolism, though not great, should be taken into account (cf. Romanoff, 1967, p. 279 and table 28); therefore, the values given above should be considered only as close approximations to the metabolism of the embryo alone. As shown in Table 6, the correlation between metabolic rate and embryonic weight is high for all species. It is worthwhile to mention the steepness of the slopes, since in all species, regardless of the differences in size and duration of the incubation periods, the numerical value of the slopes is close to 0·66 (range: 0·623–0·679). This numerical value is the well-known exponent which describes the relationship between weight (volume) and surface area. It seems, therefore, that the metabolism of embryonic snakes is related to the surface area of the embryo rather than directly to its weight. It is interesting that the same value ($b = \frac{2}{3}$) was found both in adult snakes (Vinegar, 1968; R. Dmi'el, unpublished) and in adult lizards (Bartholomew & Tucker, 1964).

Comparison between the species

In spite of the similarity between the growth and metabolic patterns found in all the species studied, some differences are obvious. Generally speaking, the viperids are laid at a more advanced developmental stage and have lower rates of growth. It should be noted in this connexion that the Viperidae in general are viviparous and do not reproduce by egg-laying (for a detailed discussion on this subject, see Mendelssohn, 1963, pp. 147, 153). This is in contrast to the colubrids. One may expect, therefore, a simple correlation between a small embryo size at oviposition and a high rate of growth during development, and vice versa. However, this is not the case, as may be noted especially among the viperids, in which *Aspis* has the smallest embryo but also the lowest rate of increase, whereas *Vipera* with its medium-sized embryos has the highest growth rates.

The same is true for the metabolic process. There are indications that such differences may be attributable to ecological factors, especially humidity, as is illustrated in Table 7.

Table 7. *Instantaneous relative increase of weight and metabolism in the five species, expressed as %/day (100* b) *and as the number of days required for each process to double itself (log 2/*b)

Values of *b* were taken from Tables 4 and 5.

	Weight increase		Metabolic increase	
	%/day	Days required to double body weight	%/day	Days required to double metabolism
Natrix (*m*)	3·95	7·59	2·58	11·65
Vipera (*m*)	3·50	8·58	2·32	12·96
Spalerosophis (*d*)	3·18	9·44	2·12	14·19
Echis (*d*)	2·58	11·63	1·66	18·06
Aspis (*d*)	2·07	14·50	1·28	23·51

(*m*) = Mediterranean species; (*d*) = desert species.

The colubrid *Natrix tessellata* is a semi-aquatic species, abundant in and near rivulets, ponds and ditches, throughout the Mediterranean region of Israel (Bodenheimer, 1935; Barash & Hoofien, 1956, pp. 47, 134). Of the five species studied, this snake has the highest relative rates of increase in metabolism and growth, as well as the least time required to double these rates (Table 7). Second to it is the viperid *Vipera xanthina palaestinae*, which inhabits almost all the biotopes of the Mediterranean parts of the country, i.e. areas with high precipitation and moderate temperatures (Mendelssohn, 1963). The colubrid *Spalerosophis cliffordi* is the third in this series; it lives in sandy deserts of North Africa and Arabia (Marx, 1959) and throughout the desert region of Israel (Barash & Hoofien, 1956, p. 146; R. Dmi'el, unpublished). Its relative rates of increase are between those calculated for *Vipera* and for the desert vipers *Echis* and *Aspis*, which are both vipers and desert snakes. *Echis colorata*, however, occurs from the extreme southern desert of Israel to Mount Gilboa in the north, a place which has almost Mediterranean climatic conditions (Mendelssohn, 1965), whereas *Aspis cerastes* is limited to sandy desert biotopes (Mendelssohn, 1962, 1965), and apparently never meets in nature the combination of high humidities and low temperatures that *Echis* sometimes does. This ecological situation is in good agreement with the relative increase rates found in the embryonic processes of these species. Both species have the lowest rates of relative increase in weight and metabolism, and also require the longest time for doubling these rates. *Echis*, however, has somewhat higher rates than *Aspis*, the latter being the species apparently best adapted to desert biotopes.

SUMMARY

The increase in length and weight, and the oxygen consumption, in embryos of five species of snakes common in Israel were studied. These species represent two families (Colubridae and Viperidae) and are of different ecological types (semi-aquatic, terrestrial Mediterranean, and desert-dwelling).

Length increase of embryos as a function of age is a linear process progressing at a constant rate.

Weight increase and oxygen consumption of the embryos are exponential processes. The rates of weight increase in the different species are between 3·95 %/day in the semi-aquatic *Natrix* and 2·07 %/day in the desert viper *Aspis;* those of oxygen consumption being 2·58 and 1·28 %/day, respectively. The equation best fitting these processes is: $\log Y = \log a + bX$, Y being ml O_2/egg/h or embryonic weight in g, while X is the day of incubation. Oxygen consumption as a function of the embryonic weight is best described by the equation: log ml O_2/egg/h = $\log a + b \log W$, W being the embryonic weight in g. The mean value of b (the slope of the curve), calculated for all species, is 0·66.

Eggs of the Viperidae are laid when the embryos are at advanced developmental stages, and generally have lower relative rates of increase, as compared to the Colubridae. A correlation was found between the ecological status of the species, and the relative rates of increase in growth and metabolic processes. The best adapted species to desert biotopes appears to have the lowest rates of increase in metabolism and growth.

RÉSUMÉ

Croissance et métabolisme des embryons de serpent

La présente étude porte sur la croissance en longueur et en poids, et la consommation en oxygène, chez les embryons de 5 espèces de Serpents communs en Israël. Ces 5 espèces appartiennent aux deux familles (Colubridés et Vipéridés) et sont de types écologiques différents (semi-aquatique, terrestre côtier et désertique).

L'accroissement en longueur des embryons en fonction de l'âge est un processus linéaire (croissance constante).

L'accroissement pondéral et la consommation en oxygène sont des processus exponentiels. Le taux d'accroissement pondéral pour les différentes espèces se situe entre 3,95 et 2,07 %/jour et la consommation en oxygène est de 2,58–1,28 %/jour. L'équation décrivant les variations de l'accroissement pondéral, ou de la consommation en oxygène, en fonction du temps, est de la forme: $\log Y = \log a + bX$, Y étant la consommation d'oxygène (en ml par œuf par h) ou le poids de l'embryon (en g) et X étant le temps d'incubation. La consommation d'oxygène en fonction du poids de l'embryon est décrite par l'équation: log ml O_2/œuf/h = $\log a + b \log W$, W étant le poids de l'embryon en g. La valeur moyenne de b (pente de la droite), calculée pour toutes les espèces, est 0,66.

Les œufs des Vipéridés sont pondus à un stade de développement avancé et ils ont généralement des taux d'accroissement relatifs plus faibles que ceux des Colubridés. Une corrélation a été trouvée entre l'état écologique de l'espèce et les taux d'accroissement relatifs de la croissance et des processus métaboliques. Les espèces les mieux adaptées aux biotopes désertiques paraissent avoir les taux d'accroissement les plus bas.

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