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# DEVELOPMENTAL EFFECTS OF INCUBATION TEMPERATURE ON HATCHLING PINE SNAKES *PITUOPHIS MELANOLEUCUS*

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**Abstract**—1. In this study pine snake eggs were incubated at different temperatures (21–32°C) to examine effects on physiological and morphological development.

2. Snakes from eggs incubated at low temperatures (21–23°C) had significantly more morphological abnormalities, were shorter in body length, and had proportionally larger heads than those from eggs incubated at higher temperatures.

3. At low temperatures most embryonic mortality occurred before two weeks and after 50 days of development. In the low temperature group most eggs that developed beyond 50 days but failed to hatch, had fully-formed snakes.

4. The amount of yolk remaining unabsorbed was negatively correlated with total length.

5. Incubation temperature affected the age at which shedding occurred in hatchlings maintained at the same temperatures.

6. Snakes maintained at a temperature below their incubation temperature took longer to shed, while maintenance at a temperature greater than incubation temperature accelerated shedding.

7. The results are consistent with a physiologic set point determined by incubation temperature, which then influences rates for at least some physiologic phenomena.

## INTRODUCTION

Recently sex determination has been found to be influenced by incubation temperatures in several species of turtles (Yntema, 1976, 1979; Yntema and Mrosovsky, 1980; Bull, 1980, 1985), a lizard (Charnier, 1966) and an alligator (Ferguson and Joanen, 1982). In other reptiles, particularly snakes, sex is determined by heteromorphic sex chromosomes (Vorontsov, 1973; Gorman, 1973). Although differential embryonic mortality at different incubation temperatures has sometimes been noted in passing (Vinegar, 1973; Osgood, 1978; Gutzke *et al.*, 1985), actual hatching success rates at different temperatures have not been reported.

Although many papers cite abnormalities of development in reptile embryos as a function of temperature (e.g., Progscha and Lehmann, 1970; Fox, 1948) few present quantitative data. Vinegar (1973, 1974) demonstrated that eggs of *Python molurus* developed normally at 30.5°C, developed abnormally at 27.5°C and did not develop at 23°C. He described three types of abnormalities: (1) skin pattern abnormalities, (2) skeletal abnormalities and (3) scalation abnormalities. His report is based on two clutches, and he quantified only scalation differences. Osgood (1978) studied the effect of temperature on meristic characters in a large sample of *Natrix fasciata* and found an increase in vertebral number at both high and low temperatures as well as changes in other features of scalation. In this paper we present data on physiological and morphological abnormalities in relation to incubation temperatures in pine snakes *Pituophis melanoleucus*.

The genus *Pituophis* is widely distributed throughout the United States (Parker and Brown, 1980). The pine snake (*P. m. melanoleucus*) is limited mainly to the southeastern United States (Stull, 1940). The New Jersey pine barrens has a disjunct population which is at the northern limit of its range. Susceptibility to temperature differences during incubation, resulting in morphological or physiological abnormalities and differential survival and fitness, may vary over a species' range. Our work is based on this northernmost population of pine snakes.

## MATERIALS AND METHODS

Pine snake females (*Pituophis melanoleucus*) deposit eggs in underground chambers at the end of tunnels they dig in the sand (Burger and Zappalorti, 1986). When females leave the tunnel, a characteristic opening with a sand pile remains, making it possible to locate nests (Zappalorti *et al.*, 1983). With regular daily censuses nests can be located within hours after egg-deposition. Pine snake eggs from 12 clutches in 1984 and 26 clutches in 1985 were collected immediately following oviposition. The usual clutch size was about nine eggs (mean = 8.8).

In the laboratory each clutch was divided into three approximately equal groups, each placed in an individual container, and assigned a clutch number (relating to a particular female) and a box number (corresponding to a temperature treatment). Eggs were placed on moist sand and covered with damp sphagnum moss to ensure sufficient humidity.

In 1984, eggs were incubated at 21, 26 and 30°C; and in 1985 eggs were incubated at 23, 28 and 32°C. Temperatures were maintained ± 1°C. Eggs were normally checked every other day during incubation and the chamber was moistened to keep the sphagnum damp. Toward the end of

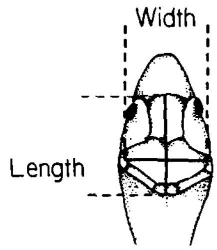


Fig. 1. Schematic of snake head showing measurements of occipital scales.

Table 2. Morphological abnormalities in pine snakes incubated at 21 and 23°C. Given are number with abnormalities and per cent in parentheses

| Abnormalities | Females | Males | Total |
|---|---|---|---|
| Live snakes | 45 | 21 | 66 |
| Vential hernia | 6 (13) | 5 (24) | 11 (17) |
| Enlarged eye | 1 (2) | 1 (5) | 2 (3) |
| Kinked tail | 3 (7) | 2 (10) | 5 (8) |
| Kinked body | 1 (2) | 2 (10) | 3 (5) |
| Redundant skin growth on head | 2 (4) | 0 | 2 (3) |
| Total[a] | 13 (28) | 10 (49) | 23 (35) |

[a]$X^2 = 2.21$, difference between sexes not significant.

incubation the eggs were checked daily for signs of hatching so that exact hatching times could be determined. Whenever early embryonic death occurred (noticeable because eggs lost water and became hard), they were dissected and examined to determine the embryonic stage, any abnormalities and sex (where possible).

Residual yolk remaining in eggs after hatching was weighed immediately. Following hatching, at 15 days of age, young were weighed, measured, and placed in individual, numbered plastic boxes. If eggs from the same female and temperature regime failed to hatch several days after their siblings had hatched, they were opened, examined, measured, and sexed. All young in 1984 and 77% in 1985 were maintained at 24 ± 1°C. In 1985 23% of the young were divided randomly into three groups to be maintained at 28, 30, or 32°C until shedding, to ascertain temperature effects on shedding.

Measurements taken on all hatchlings and on all fully developed (fully pigmented), but dead embryos included: body length, tail length from cloacal opening to tip of the tail, weight, number of head scales, and width and length of head scales (Fig. 1). Full pigmentation resulting in a well-developed color pattern occurs at stage 37 (of 37 stages) in *Thamnophis sirtalis* (Zehr, 1962). Abnormalities were described whenever they were encountered. Total length measurements are notoriously difficult to obtain on live, adult snakes, but in young snakes it was possible, with patience to obtain a good and repeatable estimation to ± 1 cm.

## RESULTS

### *Hatching and abnormality rates*

Hatching and abnormality rates varied significantly as a function of incubation temperature (Table 1). There were several types of gross morphological abnormalities in hatchling pine snakes: ventral hernias, enlarged or desiccated eyes, kinked tails or bodies, and skin growths on the head or neck (Table 2, Fig. 2). Ventral hernias were the most common abnormalities and ranged in severity from a superficial lack of fusion of the scales to a complete opening with a prolapse of the intestine (Fig. 2). Minor hernias fused in time (2 weeks to 2 months), but those with prolapses never fused. Of those hatched, 49% of males and 28% of females showed one or more abnormalities (Table 2). This difference was not significant ($X^2 = 2.21$) at this sample size.

For live snakes there were no differences in hatching weights (Table 3), but snakes from eggs incubated at low temperatures were shorter and had wider and longer occipital scales (larger heads) than those from eggs incubated at higher temperatures (Fig. 3). Larger occipital scales reflected actual difference in scale size, and not simply a change in scale number.

### *Developmental and physiological effects*

Embryonic mortality in low and high incubation temperature groups was not evenly distributed

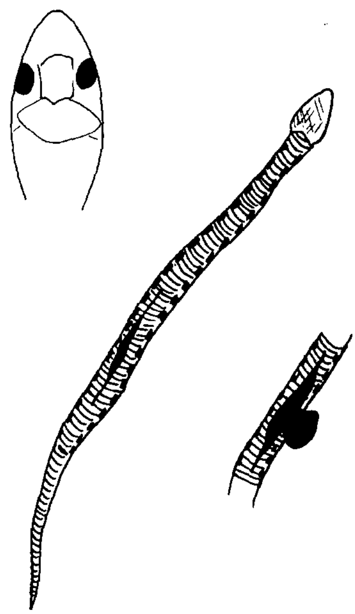

Fig. 2. Schematic of morphological abnormalities in pine snakes (drawn from photographs). Top = enlarged eyes, bottom = ventral hernias.

Table 1. Hatching rate and abnormality rate in pine snakes as a function of incubation temperature

| Incubation temperature °C | Number of eggs | Hatching rate (per cent) | Morphological abnormality[a] | Per cent of hatchlings with unused yolk |
|---|---|---|---|---|
| 21 | 34 | 27 | 22 | 100 |
| 23 | 73 | 78 | 32 | 79 |
| 26 | 43 | 87 | 0 | 8 |
| 28 | 73 | 93 | 0 | 0 |
| 30 | 39 | 97 | 0 | 0 |
| 32 | 62 | 77 | 0 | 5 |

[a]Some snakes had two abnormalities.

Table 3. Morphological characteristics of hatchling pine snakes incubated at different temperatures. Given are means ± SE

| Characteristics | Incubation temperatures Low | Medium | High | $X^2 (P)$ |
|---|---|---|---|---|
| 1984 | | | | |
| Number of live young | 9 | 39 | 38 | — |
| Temperature (°C) | 21 | 26 | 30 | — |
| Weight (g)[a] | 30.6 ± 1 | 32.1 ± 0.6 | 32.6 ± 0.6 | NS |
| Body length (cm) | 37 ± 1 | 47 ± 0.2 | 49 ± 0.4 | 33.51 (0.0001) |
| Tail length | 4.7 ± 0.2 | 5.9 ± 0.7 | 6.9 ± 0.4 | 24.54 (0.0001) |
| Occipital scale length | 9.8 ± 1.4 | 9.4 ± 1.1 | 9.4 ± 1.7 | 3.91 (0.05) |
| Occipital scale width | 8.9 ± 1.6 | 8.1 ± 2.3 | 8.0 ± 2.1 | 13.34 (0.0001) |
| Number of occipital scales | 1.5 ± 0.1 | 1.5 ± 0.1 | 1.3 ± 0.1 | NS |
| 1985 | | | | |
| Number of live young | 57 | 68 | 48 | — |
| Temperature (°C) | 23 | 28 | 32 | — |
| Weight (g)[a] | 32.3 ± 8 | 33.5 ± 6 | 32.3 ± 8 | NS |
| Body length (cm) | 44 ± 5 | 47 ± 3 | 45 ± 6 | 29.7 (0.0001) |
| Tail length (cm) | 5.6 ± 1 | 6.4 ± 0.5 | 6.3 ± 0.9 | 37.8 (0.0001) |
| Occipital scale length | 9.9 ± 0.9 | 9.9 ± 1.5 | 9.8 ± 0.8 | NS |
| Occipital scale width | 9.0 ± 1.3 | 8.2 ± 0.7 | 8.1 ± 0.7 | 34.00 (0.0001) |
| Number of occipital scales | 1.4 ± 0.1 | 1.5 ± 0.1 | 1.4 ± 0.1 | NS |

[a]At 15 days.

throughout development (Fig. 4). Instead, mortality occurred mainly before 2 weeks and after 50 days. Under medium (26–28°C) and high (30–32°C) incubation temperatures pine snakes hatched in 60–75 days, while low incubation snakes hatched in 95–120 days.

For hatchling pine snakes body length and weight were positively correlated ($r = 0.88$, $P < 0.0001$, Fig. 5). This relationship held for snakes from eggs incubated at low temperatures, regardless of whether or not they hatched.

For all embryos that died late in development dissection indicated a completely formed snake with a well-developed color pattern. Such embryos had unabsorbed yolk remaining in the eggs. Similarly, snakes hatching from eggs incubated at low temperature also left unabsorbed yolk in the egg. For both 1984 ($r = -0.87$, $P < 0.001$) and 1985 ($r = -0.71$, $P < 0.0001$) the amount of remaining yolk for low incubation temperatures was negatively correlated with snake weight for unhatched embryos and hatchlings combined (Fig. 6). The regression curve for yolk against snake weight had a steeper slope at 21 than at 23°C.

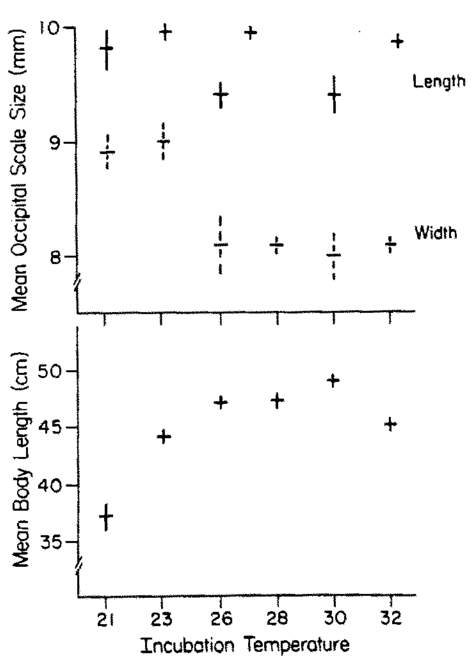


Fig. 3. Mean body length and occipital scale size in pine snake hatchlings as a function of incubation temperature.

There was a broad range of snake weights (15–33 g) and unabsorbed yolk weights (3–22 g) where embryos either died before hatching or during hatching, or hatched successfully (Fig. 6). Snakes that died before hatching were fully formed, with complete pigment pattern and an egg tooth. Unhatched embryos were dissected 1 week after their siblings raised in the same temperature had hatched.

Pine snakes shed within the first 10–14 days following hatching. In the 1984 sample we found age at shedding significantly less for snakes incubated at 21°C ($\bar{X} = 10.6 \pm 0.9$) than at 26°C ($\bar{X} = 11.4 \pm 0.2$, Median Test $P < 0.05$). For the 1985 sample, age at shedding again varied significantly with incubation temperatures (Kruskal-Wallis $X^2 = 38.6$, $P < 0.001$) with low temperature snakes shedding faster than medium or high temperature snakes. When hatchlings were maintained at different temperatures, shedding times decreased at higher temperatures for all snakes regardless of incubation temperatures (Fig. 7). Using a general linear model procedure it was found in 1985 that 45% of the variability in the time between hatching and the first shedding was explained ($F = 3.03$, $P < 0.0001$) by incubation temperature ($F = 4.21$, $P < 0.003$), maintenance temperature of hatchlings ($F = 9.61$, $P < 0.0001$) and parentage (identity of females; $F = 2.09$, $P < 0.004$).

## DISCUSSION

As has been reported in previous studies, pine snakes exhibited morphological abnormalities as a function of incubation temperatures. Secondly, hatchlings that emerged from eggs incubated at low temperatures left unabsorbed yolk in the egg, and unused yolk and snake weight were negatively cor-

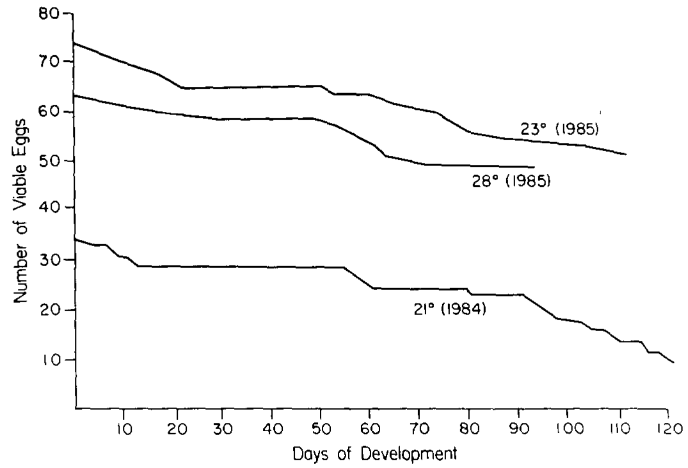


Fig. 4. Embryonic death in pine snakes as a function of day in incubation.

related. However, the slopes of the regression lines for hatchlings incubated at 21°C and those incubated at 23°C differed. For an equivalent hatching weight, snakes incubated at low temperatures left more of the yolk unused. Thus, development produced a similarly-sized hatchling without apparently using as much of the energy reserves stored in the egg.

Snakes that in the process of development reached a weight threshold of 35 g (23°C) or 30 g (21°C) always hatched and remained alive and those that absorbed all but 3 g or less of yolk survived. Below these limits embryos either hatched alive, died hatching, or never hatched. Thus, there was a broad range of weights which could result in live hatchlings or dead embryos. The factors that affect whether an embryo hatches or dies without hatching are unclear, and bear further examination.

*Hatching*

The hatching process itself was a critical event. Many embryos never even made a cut in the egg shell while others died during hatching. Those that died hatching were not victims of other snakes, sand or any other object. More of the 21°C snakes died while hatching than those incubated at 23°C, and they did so at lighter weights (refer to Fig. 5). Taken altogether these points indicate that the physiological processes of development are slowed down in the embryos incubated at 21°C compared to the embryos incubated at 23°C. A vulnerability threshold where young die while hatching exists at both incubation temperatures, but it is displaced toward heavier snakes at the higher incubation temperatures.

*Shedding*

Reptiles shed their skins periodically as they grow, and ecdysis rates in snakes are influenced by ambient temperatures (Semlitsch, 1979). However, in this study, initial shedding rates were not only influenced by ambient temperature, but also by incubation temperature. Snakes incubated at low temperatures shed earlier than those incubated at higher temperatures; even though they were not different with respect to weight or clutch and none of them ate until after they shed the first time. Furthermore, at high ambient temperatures, the same differences existed but shedding time was uniformly decreased (refer to Fig. 7).

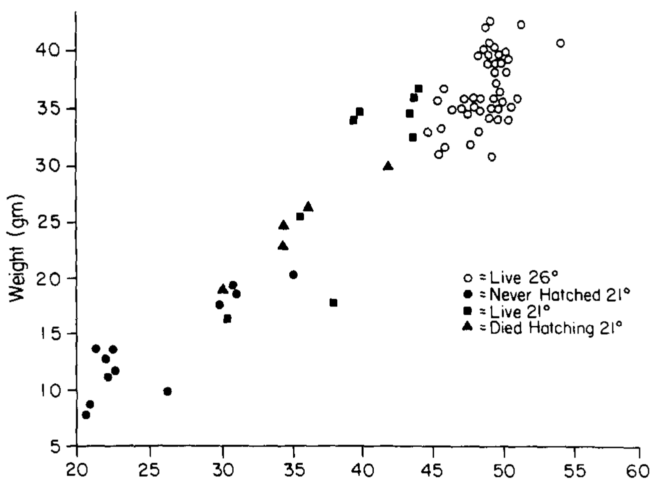


Fig. 5. Relationship of body length and weight at shedding for pine snakes.

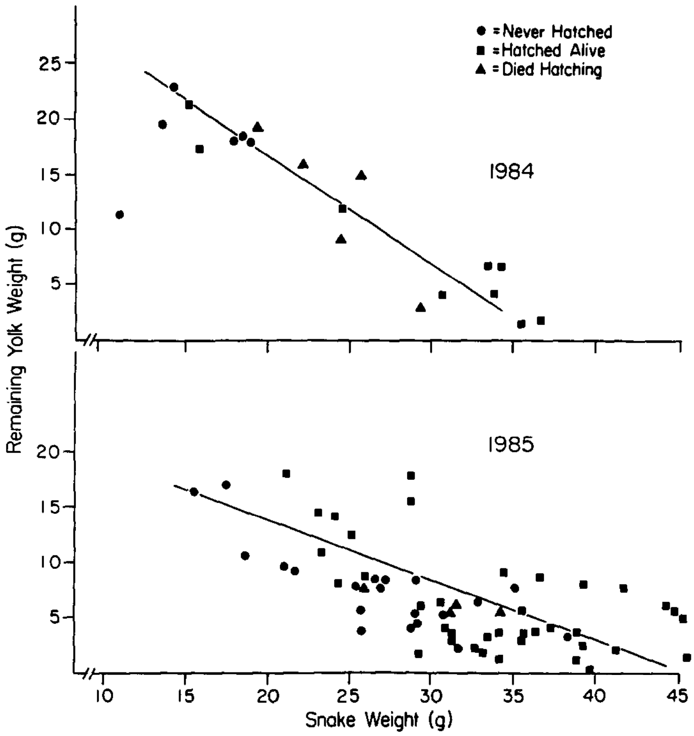


Fig. 6. Relationship of unused yolk to snake weight for low temperature incubation for live snakes, those that died during hatching and those that died just prior to hatching. 1984 temperature was 21°C; 1985 temperature was 23°C.

*Set point*

These observations are consistent with the influence of incubation temperature on a physiological set-point. Figure 8 illustrates our interpretation of this relationship. Snakes hatched from eggs incubated at low temperatures would exhibit low levels of activity while those incubated at higher temperatures would be more active when they are maintained at the same temperature as during incubation (top of Fig. 8). Snakes maintained at a temperature higher

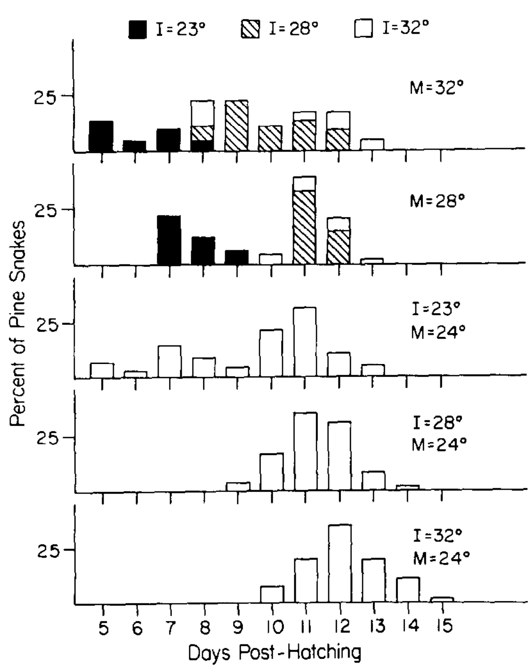


Fig. 7. Shedding ages as a function of incubation temperature for snakes maintained at 24 (bottom), 28 (middle) and 32°C (top) after hatching.

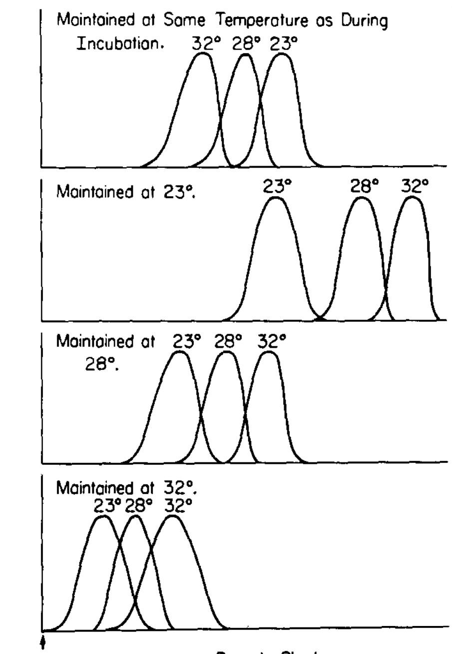


Fig. 8. Theoretical graph showing how a physiological set-point influenced by incubation temperature would affect age at shedding. Number over the line equals incubation temperature. See text for explanation.

than their incubation temperature would show accelerated activity and physiological rates (such as shedding), while those placed at maintenance temperatures lower than their incubation temperature would exhibit lower rates (Fig. 8, middle graphs). For any given incubation temperature, shedding time would decrease when placed at higher temperatures and increase at lower maintenance temperatures. Thus to predict shedding time it is necessary to know both the incubation temperature during development, and the maintenance temperature of the hatchlings.

In Fig. 8, the top panel shows the distribution of some physiologic rate, in this case age at shedding, for three groups of snakes in which post-hatching maintenance temperature ($T_m$) equalled incubation temperature. The second panel illustrates events where $T_m = 23°C$. The shedding age for the group with $T_i = 23°C$ would be unchanged, but the two groups with $T_i > 23°C$ would show retarded shedding age. The third panel illustrates a $T_m = 28°C$. The group with $T_i = 28°C$ shows the same distribution as in the top panel. However, the group with $T_i = 23°C$ shows a much advanced shedding age, while the group with $T_i = 32°C$ is still slower. Finally, the bottom panel illustrates a $T_m = 32°C$. The group with $T_i = 32°C$ now shows the same distribution as the top panel. The groups with $T_i < 32°C$ shows much accelerated rates at this higher temperature.

In snakes, temperature has previously been reported to affect ecdysis (Semlitsch, 1979), activity patterns and seasonality (Heckrotte, 1962) and digestion (Naulleau, 1983) as well as scale and skeletal abnormalities (Vinegar, 1973, 1974; Osgood, 1978) and rate of development (Zehr, 1962). It is suggested that low incubation temperatures in pine snakes result in lower hatching rate, higher morphological abnormality rates, changes in allometry, and the determination of a physiological set point that influences shedding time in hatchlings.

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