ORIGINAL PAPER

Maintaining rear-fanged snakes for venom production: an evaluation of mortality and survival rates for *Philodryas olfersii* and *P. patagoniensis* in captivity

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Abstract: This study evaluates the mortality and average survival rates of captive female *Philodryas olfersii* and *Philodryas patagoniensis* snakes maintained for venom production. Also, two factors likely to reduce captive survival were studied – body condition at admission and seasonality. Mortality peaks occurred during the second month in captivity. More than half the individuals were dead at the end of the third month. This suggests that the first three months in captivity are the most critical in terms of survival and adaptation. Females collected and admitted during spring and summer lived less time than those collected in autumn and winter. As gravidity and egg-laying occur during spring and summer, we suggest that the lower survival rates in these seasons may be due to high costs and stress involved in these reproductive events. Unexpectedly, body mass and body condition were poor predictors of survival in captivity. Our results have important implications in maintaining snakes for venom production. We propose some prophylactic measures to minimize the deleterious impacts of captivity during the adaptation period.

Key words: animal husbandry, snake venom, seasonal variation, body mass index.

INTRODUCTION

Rear-fanged snakes are responsible for a significant number of snakebites in Brazil and are therefore considered a public health problem (1). Although considered nonvenomous, there are several important clinical manifestations such as abrasions, pain, local hemorrhage, swelling and erythema (1, 2). Furthermore, there is at least one report of death caused by a rear-fanged snake, *Philodryas olfersii* in southern Brazil (3). As a result, the biological activity and biochemical characterization of venom from these animals has received more attention in this decade (4-7). However, unlike venomous snakes, secretion from the venom gland of rear-fanged snakes is difficult to obtain in suitable quantities, so some species have started being kept in captivity at Butantan Institute Herpetology Laboratory

(LHIB) with the aim of producing venom from periodical extractions.

Establishing a large scale snake breeding program for venom production substantially differs from one destined for exhibition or biological study. This is because the captive environment for such a purpose must combine the practicability in maintaining a large number of individuals with their needs and welfare. Leloup (8) suggests three systems for maintaining snakes on a large scale for venom production: intensive, semi-extensive, and extensive captivity. Although all have advantages and disadvantages, intensive captivity is widely adopted in breeding snakes for venom production (8-11). This system simplifies maintenance by allowing constant easy inspections, individual control of feeding and health, temperature control according to specific requirements, and smaller installations (8).

A number of reviews on snake maintenance in captivity suggest that the combination of variables such as temperature, humidity, light, feeding, physical space, and sanitation is required for suitable husbandry (12-14). However, even with all this care, adaptation to captivity is not assured and many individuals die within two years of acquisition (15). Thus, any attempt to understand factors that may affect captive survival is central to the successful implementation of a snake colony for venom production.

The major cause of death in captive reptiles is judged to be their inability to adapt to the captive environment; this is called maladaptation syndrome (15, 16). This syndrome accounted for about 80% of captive snake deaths at Philadelphia Zoological Garden and can be defined as the pathological effects of stress on an animal associated with a captive environment (15). Maladaptation syndrome may cause anorexia, emaciation (even though animals may be feeding), tissue fragility, increased susceptibility to infection (even with normally innocuous organisms), parasite overgrowth, gut ulceration, reduced growth, and reproductive failure (15, 16). The end result is that animals are wasted and all fat reserves are depleted.

However, a number of other factors are likely to affect captive survival (e.g. seasonality and age). Herpetological literature on snake husbandry mostly deals with the general aspects of maintenance or with the deleterious effects of diseases and parasites (8, 10, 14, 15, 17-19). In this paper we examine the mortality rates and average survival in captive females of rear-fanged *P. olfersii* and *P. patagoniensis* snakes maintained under an intensive captivity system for venom production at LHIB. In addition, we explore factors other than disease which are likely to reduce survival in captivity. Specifically, we attempt to answer two questions:

- Does average survival in captivity differ depending on the season in which snakes were collected? Seasonality affects several biological processes in snakes (e.g. activity patterns and reproduction). Thus, the effects of maladaptation to captivity are also likely to vary between seasons. Indeed, differential survival between seasons has previously been observed in at least one snake species maintained in captivity for venom production. *Bothrops jararacussu* snakes captured during spring and summer (wet season) showed higher survival than those captured in autumn and winter (dry season) (9).

- Are survival rates in captivity dependent on snake fat reserves at admission? Because the most noticeable result of maladaptation syndrome is the depletion of all fat reserves in an individual, we hypothesized that animals weighing more in relation to their body size (and presumably with larger fat reserves) might survive longer in captivity (15).

We therefore investigated the periods of higher susceptibility to the captive environment with the aim of increasing knowledge on factors that may impact survival. Consequently, we expected to gather information to help develop effective husbandry practices which assure animal welfare.

MATERIALS AND METHODS

Study Species

P. olfersii and *P. patagoniensis* (Figure 1) are medium-sized (up to 1.2 m snout-vent length, 280 g) diurnal snakes widely distributed in South America and are largely sympatric (20-23). *P. olfersii* is mainly found in forested areas and forest edges and displays semi-arboreal habits, whereas *P. patagoniensis* is mostly found in open habitats and is predominantly terrestrial (21, 22). Both species are diet generalists feeding on frogs, lizards, birds, and mammals (21, 22). Female reproduction is seasonal in both species with vitellogenesis occurring from May to January for *P. olfersii* and from August to December for *P. patagoniensis* and gravid females are found from September (late winter) to January (early summer) (24). These species are responsible for a significant number of snakebites in Brazil (1, 25, 26). The mortality rates and average survival times of 36 adult female *P. olfersii* and 56 adult female *P. patagoniensis* maintained in captivity for venom harvest were analyzed. Most females were found in São Paulo state, Brazil, between May 2003 and July 2008.

Maintenance Protocol

Newly-arrived snakes in the laboratory were submitted to visual surveys. Only active adult individuals of healthy appearance were selected. All individuals were washed with 0.2% trichlorfon solution (Neguvon®, Bayer, Brazil) at admission
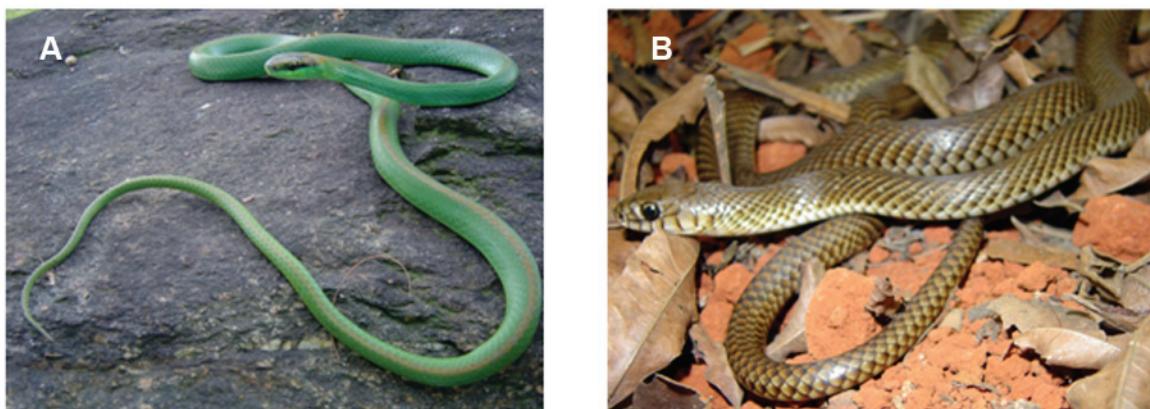
Figure 1. Adult individuals of (**A**) *Philodryas olfersii* and (**B**) *Philodryas patagoniensis*. Photos by Silvia R. Travaglia-Cardoso.

and seven days later to eliminate ectoparasites (27). Snakes were sexed (by hemipenis eversion), measured (to nearest millimeter), and weighed (to nearest 0.1 g). The snakes were maintained under an intensive captivity system (8).

Specimens were kept individually in plastic cages (SanRemo, Brazil, 975; 564 × 385 × 371 mm for larger individuals and SanRemo, Brazil, 965; 400 × 270 × 362 mm for smaller ones), with corrugated cardboard as substrate, a water bowl (replaced every other day), and previously disinfected furniture including stones, plastic tubes (for shelter), and tree branches. Cages, bowls, and furniture were changed weekly and disinfected with 4% sodium hypochlorite solution. Room temperature averaged 25°C (range 20-30°C). Photoperiod corresponded to natural light entering room windows.

Snakes were fed monthly with mice (*Mus musculus*) in quantities corresponding to approximately 10% the snake weight. If snakes refused to feed for two consecutive months they were force-fed with freshly killed mice. Venom was also extracted monthly, but three weeks after feeding. Snakes were intraperitoneally (IP) inoculated with 10 mg/kg pilocarpine solution diluted in 0.85% saline solution to induce venom secretion from Duvernoy glands (28). The venom was collected by placing a 100-μL capillary tube over each fang (29). During venom extraction, snakes were manipulated for 5 to 10 minutes. Capillary tubes were changed after each venom extraction and fangs disinfected with 0.2% alcoholic iodine solution to prevent bacterial

infection. Necropsy or histopathological exams were not performed to determine causes of death in studied snakes.

Data Analysis

All data were tested for normality using the Kolmogorov-Smirnov test and for homogeneity of variances using the Bartlett's test. Data were log-transformed when necessary to satisfy the assumptions for parametric tests. Values are presented throughout as mean ± 1 standard error and the significance level of all tests was α = 0.05.

Survival in captivity was defined as the number of days between the date admitted to captivity and the date of death. Differences in average survival between species were examined with the *t*-test. For analysis of mortality rates during the captivity period we considered a class interval of 30 days because that was also the period between feeding sessions and venom extractions. Differences in distribution of deaths across class intervals were examined with chi-square analysis. Mortality rates were defined as the ratio of number of deaths over total number of individuals analyzed.

In an attempt to estimate female fat reserves we calculated their body condition, which indicates whether individuals are relatively light or heavy for their body size. Body condition was defined as the residuals from the linear regression of log-transformed body mass on log-transformed body length (both at admission) (30). Thus, high body condition values indicate relatively large mass per unit of body length and presumably larger stored energy.

Linear regression analyses were performed to test for the influence of morphological traits at admission (body condition, body mass, and body size) on survival. Seasons were assigned as summer (January to March), autumn (April to June), winter (July to September) and spring (October to December) and female reproductive status (gravid or non-gravid) was determined by palpation or the occurrence of egg-laying in captivity. Seasonal differences in survival rates were compared by one-way ANOVA followed by Tukey's *post hoc* multiple comparisons test to determine how survival differed each season.

RESULTS

Mortality Rates

The proportion of deaths for both species was not equally distributed over the months (Figure 2). The highest mortality level was observed during the second month in captivity when 33.3% of female *P. olfersii* (χ^2 = 84.34, df = 18, *p* = 0.0000; Figure 2) and 28.8% of *P. patagoniensis* (χ^2 = 217.52, df = 30, *p* = 0.0000; Figure 2) died.

Mortality curves were also similar for both species (Figure 2). At the end of the second month in captivity the number of accumulated deaths was about 40% of the total and at the end of the third month more than half (about 61%) the individuals had died (Figure 3). Mortality curves continued to increase similarly in both species up to of the end the fourth month, when the death rate for *P. olfersii* slightly slowed against *P. patagoniensis* (Figure 3). At the end of the tenth month about 94% of females from both species had died. The longest surviving captive *P. olfersii* individual lived 544 days and *P. patagoniensis* lived 902 days after entering the laboratory.

Does average survival in captivity differ depending on the season in which snakes were collected?

General survival in captivity averaged 120.8 ± 19.9 days (range = 14-544; n = 36) for *P. olfersii* and 108.2 ± 19.2 days (range = 20-902; n = 52) for *P. patagoniensis*, but did not differ between species (*t* = 0.71, df = 86, *p* = 0.48). Survival rates varied significantly depending on the seasons in which snakes were captured both for *P. olfersii* (one-way ANOVA: $F_{3.32}$ = 8.73; *p* = 0.0002) and *P. patagoniensis* (one-way ANOVA: $F_{3.48}$ = 4.40; *p* = 0.008). The lowest survival rates for *P. olfersii*
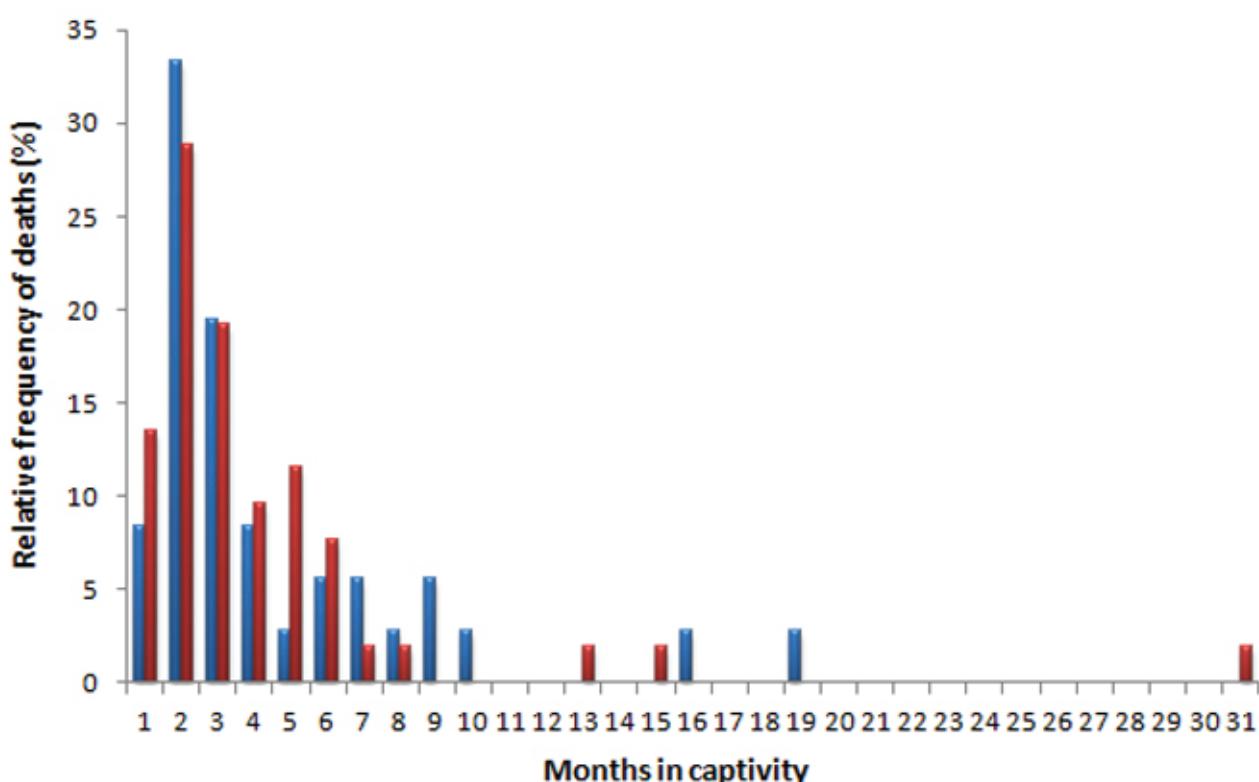
Figure 2. Relative monthly mortality frequencies for *Philodryas olfersii* (blue bars) and *Philodryas patagoniensis* (red bars) held in captivity for venom extraction.
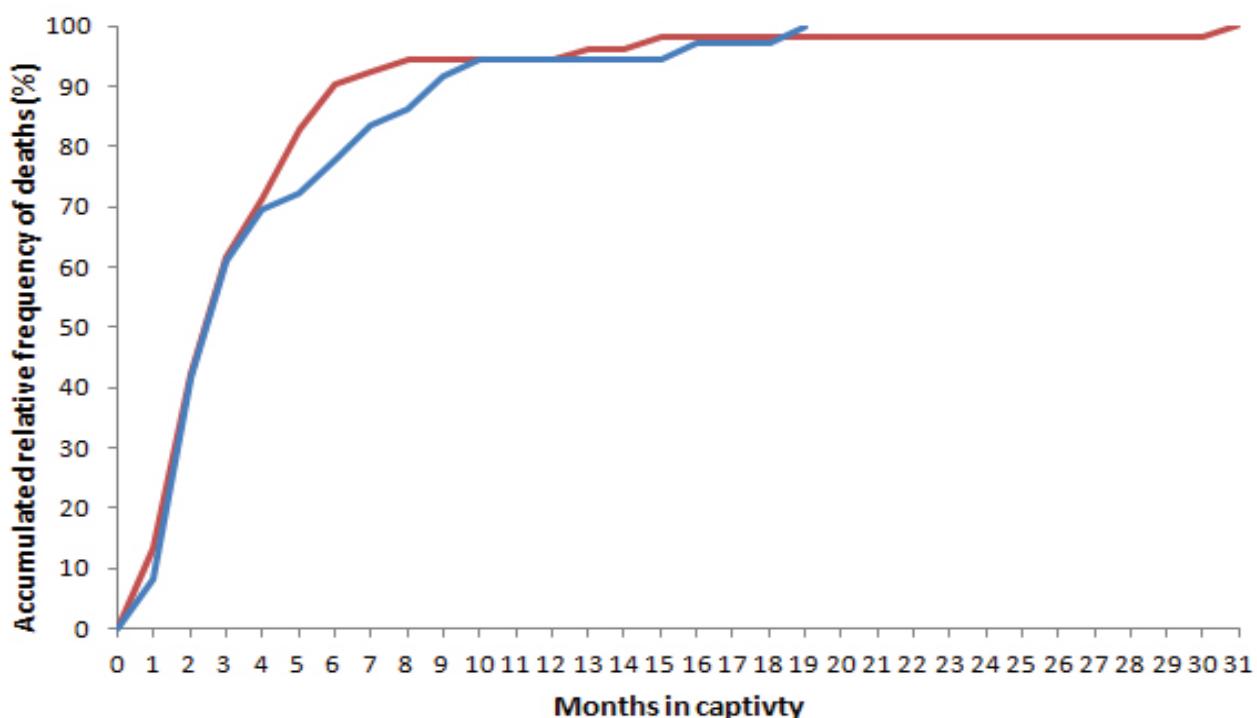
Figure 3. Mortality curves for captive *Philodryas olfersii* (blue line) and *Philodryas patagoniensis* (red line) after admission in laboratory.
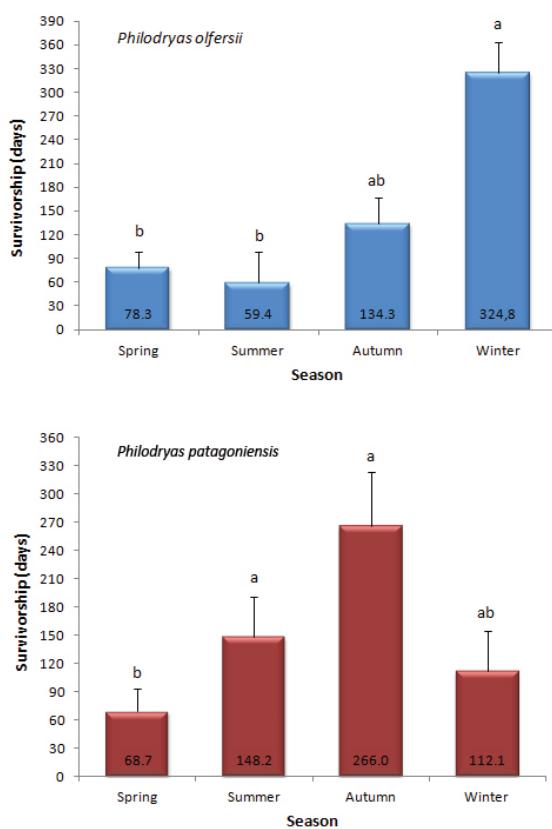

Figure 4. Mean survival against capture season for female *Philodryas olfersii* and *Philodryas patagoniensis* maintained in captivity for venom extraction. Seasons with the same letter are not significantly different (Tukey's *post-hoc* test, α = 0.05, a > b). Error bars represent standard error.

were observed in females captured during spring and summer (i.e. the whole wet season) and for *P. patagoniensis* captured in spring (first half of wet season; Figure 4). However, the highest survival rates for *P. olfersii* were observed in females captured during winter and for *P. patagoniensis* captured in summer and autumn (Figure 4).

Are survival rates in captivity dependent on snake fat reserves (body condition) at admission?

At admission, female *P. olfersii* (n = 36) had an average snout-vent length (SVL) of 883.1 ± 24.5mm (range: 510-1080 mm), body mass 161.5 ± 13.3 g (range: 22-320 g), and body condition indexes ranging from –0.275 to 0.253. For *P. patagoniensis* females (n = 52), SVL averaged 902.9 ± 28.2 mm (range: 455-1550 mm), body mass 220.4 ± 17.0 g (range: 30-668 g), and body condition indexes ranged from –0.278 to 0.824.

For both species, linear regression analysis showed that longevity in captivity was not related to any of these morphological traits at admission. Snakes did not live longer or less because of their initial body size (*P. olfersii*: $r^2 = 0.004$; n = 36; $p = 0.71$ and *P. patagoniensis*: $r^2 = 0.019$; n = 52; $p = 0.33$), body mass (*P. olfersii*: $r^2 = 0.025$; n = 36; $p = 0.35$ and *P. patagoniensis*: $r^2 = 0.055$; n = 52; $p = 0.09$), or body condition (*P. olfersii*: $r^2 = 0.091$; n

= 36; *p* = 0.07 and *P. patagoniensis*: r^2 = 0.049; n = 52; *p* = 0.11) at admission.

Because gravidity usually affects body mass measurements and may cause bias we reran analysis using only nongravid snakes. This also showed that longevity in captivity is not related to body size (*P. olfersii*: *r* = 0.002; n = 22; *p* = 0.83 and *P. patagoniensis*: r^2 = 0.006; n = 38; *p* = 0.64), body mass (*P. olfersii*: r^2 = 0.021; n = 22; *p* = 0.52 and *P. patagoniensis*: r^2 = 0.006; n = 38; *p* = 0.64), or body condition (*P. olfersii*: r^2 = 0.127; n = 22; *p* = 0.10 and *P. patagoniensis*: r^2 = 0.0003; n = 38; *p* = 0.92).

Body measurements at admission were compared between long-lived (survival over three months) and short-lived (under three months) individuals. Long-lived and short-lived *P. olfersii* individuals showed similar body size, body mass, and body condition (Table 1). The same was observed in *P. patagoniensis* only for body size (Table 1), as short-lived individuals were heavier (relative to body size) at admission than their long-lived counterparts (Table 1).

DISCUSSION

From our data we were able to identify periods with higher mortality rates of *Philodryas olfersii* and *P. patagoniensis* kept in captivity for venom extraction. The data also supported the idea of differential survival across the seasons, but did not support the hypothesis that heavier animals (presumably with larger fat reserves) might survive longer in captivity.

The literature does have information dealing with the maintenance of captive snakes for venom production (8-11, 17, 27). However, data on mortality and survival rates are only available for a few species, such as the venomous snakes *Bothrops jararacussu* and *Micrurus corallinus* (9, 11). Both mortality and average survival rates differ between such species. General mean survival in these species was slightly higher (~6 months) than for *P. olfersii* (~4.0 months) and *P. patagoniensis* (~3.6 months). Also, mortality peaks seem to occur later for *M. corallinus* and *B. jararacussu* than for *Philodryas*. For instance, about 60% of deaths in captivity were after three months for *Philodryas* but were after six months for *B. jararacussu*. It could be argued that this was an artifact of admitting unhealthy individuals.

In this study, newly acquired individuals were not dewormed or put quarantine before admission to captivity (12, 14). The only prophylactic measures performed were visual surveys and preventive treatment against ectoparasites. Injured or apathetic individuals were not admitted to the animal house.

We judged that other factors may have been more important in reducing *Philodryas* mean survival time in captivity. Firstly, venom extraction from viperids and elapids at our laboratory is accomplished with animals almost unconscious after submission to anoxia by CO_2 (31, 32). However, venom extraction from rear-fanged snakes is performed with the animals completely conscious. This is because of

Table 1. Mean body measurements at admission for long-lived (over three months) and short-lived individuals (under three months). Values in bold indicate significant differences between long and short-lived individual

Species/variable	Short-lived		Long-lived		Statistical test
	n	Mean ± SD	n	Mean ± SD	
P. olfersii					
Body size (mm)	22	896.9 ± 150.4	14	861.4 ± 143.7	*t* = 0.70; df = 34; *p* = 0.49
Body mass (g)	22	164.8 ± 80.4	14	156.3 ± 81.5	*t* = 0.30; df = 34; *p* = 0.76
Body condition	22	−0.013 ± 0.083	14	0.021 ± 0.107	*t* = 1.08; df = 34; *p* = 0.29
P. patagoniensis					
Body size (mm)	32	934.8 ± 201.3	20	852.0 ± 200.8	*t* = 1.44; df = 50; *p* = 0.15
Body mass (g)	32	252.6 ± 129.2	20	168.9 ± 93.6	**t = 2.51; df = 50; p = 0.01**
Body condition	32	0.036 ± 0.178	20	−0.058 ± 0.089	**t = 2.55; df = 50; p = 0.01**

differences in venom apparatus between taxa (33, 34). While venom in viperid is easily obtained by gentle compression of the venom gland, venom volume from Duvernoy's gland is extremely low; this can be improved through moving the head of the snake (29). However, this procedure may be more detrimental and stressful to snakes and could have contributed to the low survival rates in our snakes. Secondly, this may have also resulted from differences in physiology, habits and consequently in captive requirements between taxa. Captivity conditions that provide higher survival rates for viperids and elapids may not be the most suitable for *Philodryas* and, therefore, adaptation to captive conditions may be more difficult for the latter.

Seasonality was an important factor affecting captive survival in both species. Survival was significantly lower for individuals captured and admitted in the wet season (spring and summer for *P. olfersii* and spring for *P. patagoniensis*). This contrasts with the results found by Leinz *et al.* (9) on *Bothrops jararacussu*, in which captive survival is seemingly lower when animals are captured during autumn and higher when captured during spring and summer (wet season). Those authors suggested that higher survival rates in the wet season could be related to higher resource availability and consequently higher energy stores when faced with the captive environment (9). We suggest that the lower *Philodryas* survival rates observed in the wet season are more likely due to the high costs and stress involved in reproductive events.

In *P. olfersii* and *P. patagoniensis* gravidity and egg-laying mostly take place during the wet season (September to February) for both species (24). In snakes, this period is characterized by higher physiological stress and costs (35, 36). During the reproduction season females cease feeding, lose body mass, and experience increased mortality rates (35, 36). Thus, female *P. olfersii* and *P. patagoniensis* may be faced with high physiological frailty during this season. In captivity, stressor agents are common, and simple maintenance routines such as cage cleaning, are potential stressors (37). Additionally, handling, restraint, and fluid collection, the procedures involved in venom extraction, are generally associated with higher plasma corticosterone concentrations and thus regarded as stressful incidents in captive animals (38-40). Therefore,

what is already a difficult time in the natural life cycle of these animals may be aggravated by laboratory routines.

We hypothesized that animals with larger fat reserves could survive longer in captivity. This hypothesis was tested by verifying the relationship between admission body mass and body condition with survival. However, our analyses did not corroborate this hypothesis. Thus, body mass and body condition were shown to be a poor predictor of captive survival, at least for *P. olfersii* and *P. patagoniensis*. This remains true even after removal of gravid snakes from the analyses. In addition, heavier *P. patagoniensis* individuals lived significantly less than lighter ones. This does not invalidate findings by Cowan (15) of the depletion of all individual fat reserves, nor does it mean that body mass is unimportant, but suggests that larger fat reserves are not crucial to longevity in captivity. We suggest that the consumption of all fat stores observed by Cowan (15) may vary individually and independently of smaller or larger initial fat reserves.

Our results present important implications for the captive management and welfare of snakes maintained for venom production. Being aware of higher vulnerability periods allows us to design effective protocols for further husbandry and management practices with the aim of increasing captive individual survival and welfare. Faced with this, some preventive measures could be adopted looking towards reducing the deleterious impacts of captivity. For example, minimization of excessive handling and cage cleanings and increase of the interval between venom extractions are suggested (13, 41).

Taken together, the analysis of the mortality periods and mortality curves indicated that the first three months of captivity are by far the most critical in terms of individual survival and adaptation to captive conditions. This time should therefore be regarded as an adjustment period to the captive environment and animals should be disturbed as little as possible. In addition, venom extraction should only start after this period. Special care should be given to individuals collected and admitted during the wet season. Our work also showed that some individuals had high longevity. Further important research should focus on the longest surviving

individuals, especially asking: what features do the longest surviving individuals possess over those surviving less?

CONCLUSIONS

Firstly, for *Philodryas olfersii* and *Philodryas patagoniensis* the first three months in captivity are by far the most critical in terms of individual survival and adjustment to captive conditions. Secondly, capture season strongly affected captive survival. The most critical season for survival in both species was the wet season (spring to summer). And finally, body mass and body condition were poor predictors of captive survival, at least for *P. olfersii* and *P. patagoniensis*.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ETHICS COMMITTEE APPROVAL

This study was approved by the Butantan Institute Ethics Committee for Animal Use (CEUAIB 482/08).

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