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Short communication

Assessing survival of wild-caught snakes in open venom production systems

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ABSTRACT

We evaluate adaptation of eleven species of wild-caught snakes maintained in captivity for venom production using two procedures for estimating survival rates. Kaplan-Meier estimations of survival time provide a better account of subsistence in captivity than estimations based solely on mean time to death. Highland and mid-elevation species are better adapted to our captive settings, but factors such as body condition at admission, locality of origin, seasonality, and maintenance protocol, affect the studied species differently. Periodic estimations of the collection's mortality rates, coupled with necropsy analyses, are recommended to assess adaptation and to develop acceptable species-specific management practices in captivity.

Maintaining a large collection of snakes is a critical step to secure venom for toxin research, the production of antivenoms (Gutiérrez et al., 2009), and the acquisition of molecules for various pharmaceutical uses (Fox and Serrano, 2007; Escoubas and King, 2009; Koh and Kini, 2012). Many production centers that keep venomous snakes in captivity are "*open systems*" in which snakes are constantly brought from the wild (Chanhome et al., 2001; Powell et al., 2006; Arias-Ortega et al., 2016). Keeping wild-caught snakes is not an easy endeavor, as the accurate capture of the animals and proper health condition at arrival cannot always be ensured (Pasmans et al., 2008; Fry et al., 2015). Additionally, maladaptation to captivity, physiological stress, species-specific requirements, and the risk of infectious diseases in artificially high densities further challenge adaptation in these systems (Leloup, 1984; Fry et al., 2015). Consequently, constant health surveillance and periodic estimation of the collection's mortality rates are recommended to assess adaptation and to develop acceptable species-specific management practices. Unfortunately, information on mortality rates in venom production centers is seldom available, making it hard to attain standards for acceptable mortality levels in those facilities.

Here, we provide firsthand information of survival time for wild-caught venomous snakes kept at the Instituto Clodomiro Picado (ICP), a research facility and antivenom production center in Costa Rica. We empirically tested the advantage of using survival analysis procedures (Kleinbaum and Klein, 2012) over the more conventional approach of mean time-to-death comparisons under parametric tests.

Snakes were brought to ICP by the general public from all over the country. On admission, a unique identification number was assigned to each individual; and species, sex, and locality of origin were recorded, as well as its general health condition. Snakes were kept individually in cages (60 × 85 × 45 cm) in a quarantine room for at least four weeks and then placed in acclimated maintenance rooms. Water was provided *ad libitum* in all cases. Pit vipers were fed mice (*Mus musculus*) in a quantity corresponding to approximately 5–10% of the snake weight, whereas coralsnakes were fed with gray earth snakes (*Geophis* sp), banded coffee snakes (*Ninia maculata*), or fish strips (see Chacón et al., 2012). Only species with at least 20 individuals with complete entrance records and confirmed survival status during the period 2000–2014 were included in the analyses. Time in captivity was defined as the number of 30-day months between the admission date and the date of death (for non-censored data) or the study termination date (12/31/2014, for censored data). Two procedures were performed to analyze survival. First, mean survival time was estimated from non-censored data and compared using ANOVA. Shapiro-Wilks test

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of normality and Levine's test of homogeneity of variances were employed to assess parametric assumptions (Altman, 1991; Levesque, 2007). Second, the survival function was assessed from censored and non-censored data using the nonparametric Kaplan-Meier estimator (Kleinbaum and Klein, 2012).

We analyze the effect of species, sex, body length, body condition, and management protocol on survival. Body condition is an attempt to quantify fat reserves at admission and is estimated for each sex from the residuals of the linear regression of body mass on body length (Braz et al., 2012). Two management protocols were implemented at two distinct periods (2000–2008 and 2009–2014); these protocols differ in the frequencies of feeding and venom extraction, as well as in the implementation of heating tapes in cages during the second period. We also evaluate the effect of travel distance (vicinity of ICP in the Central Plateau, a 100 km ratio, or more than100 km ratio from ICP), and season in which snakes were collected (dry season: December–April, transition: May–July, and rainy season: August–November) on survival. To assess these factors, we compared survival curves among different groups using *log-rank test* (Mantel-Cox test, Kleinbaum and Klein, 2012), implemented in the packages SPSS (version 19.0, IBM©) and plotted in Prism® (version 5.0; GraphPad software, Inc. 2007).

A total of 1892 venomous snakes entered ICP during the study period; 85% of them have complete records that meet the inclusion criteria. This amount represents ten pitviper and one elapid species (Table 1). The Central American coralsnake *Micrurus nigrocinctus*, the Tropical rattlesnake *Crotalus simus*, and the terciopelo *Bothrops asper*, were the most frequently brought species, with 28%, 27%, and 16% of the entrances, respectively.

Overall, snake arrival varies through the year ($\chi^2 = 51.4$, $df = 11$, $P < 0.001$) with May, June, and July exhibiting more admissions. These months coincide with the rain transition period in Costa Rica, a phase of increasing activity in snake populations in the country (Solórzano, 2004). In contrast, frequencies of deaths in captivity are more evenly distributed over the months ($\chi^2 = 17.42$, $df = 11$, $P = 0.06$).

Records of survival time ranged from a few days to over 138 months: the longest time was observed in jumping pitvipers *Atropoides mexicanus* and *A. picadoi.* Other long-term survivors include a bushmaster *Lachesis stenophrys* (129 months) and a coralsnake *M. nigrocinctus* (120 months). Despite these extreme values, mean time to death was substantially lower in all species and differed among them (Table 1, $F_{10,1135} = 29.40$; $P < 0.001$). If only non-censored data is considered, both species of jumping pitvipers show the highest survival time, whereas for *B. asper*, *C. simus,* the eyelash pitviper *Bothriechis schlegelii,* and the montane pitviper *Cerrophidion sasai*, 50% of the individuals survive less than a year (Table 1).

In contrast, under the Kaplan-Meier function (KM), survival time estimates that include both censored and non-censored data are statistically higher (Table 1). For instances, if censored data is also contemplated, at least 50% of the *C. sasai* survived more than 82 months in captivity (Table 1), which reveals the actual adaptation capacity for this species. Likewise, median estimates of survival for *B. asper*, *C. simus,* and *B. schlegelii* improved under the KM analysis (Table 1).

We detect four statistically distinct survival patterns (Fig. 1): (1) High-survival: over 70% survival during the first 24 months and less than 40% of deaths occurring after the 60th month in captivity, exhibited by both jumping pitviper species and *C. sasai* (Fig. 1A). (2) Steady decay: less than 50% of deaths occurring during the first 24 months in captivity, and a gradual reduction of survival therein, displayed by *M. nigrocinctus* and *Bothriechis nigroviridis* (Fig. 1B). (3) Rapid decline: close to 40% survival during the first 24 months in captivity but mortality rate decreasing after 48 months, exhibited by *B. asper*, *B. schlegelii* and *C. simus* (Fig. 1C). (4) Severe decline:

Table 1
Survival time (in months) for wild-caught venomous snakes brought to Instituto Clodomiro Picado during 2000–2014. Mean and median estimations including and excluding censored data are shown.

Species	# Records included	# Censored individuals	Mean (±SE) survival time Median (±SE) survival time Censored data excluded	 Censored data included
Viperidae				
Atropoidesmexiacanus	39	13	60.4 (8.1) 61.4 (11.3)	66.3 (7.2) 68.0 (9.4)
Atropoides picadoi	97	47	47.8 (4.6) 53.4 (5.2)	74.8 (6.3)** 64.9 (4.3)
Bothrops asper	273	87	12.9 (1.1) 6.6 (0.8)	38.7 (3.8)** 12.9 (1.9)**
Bothriechis lateralis	79	21	18.9 (2.6) 12.1 (1.2)	29.4 (4.3) 14.1 (1.9)
Bothriechis nigroviridis	31	6	24.6 (5.0) 9.2 (5.8)	27.8 (5.1) 16.9 (10.2)
Bothriechis schlegelii	88	21	13.3 (1.8) 7.8 (1.3)	34.8 (6.0)** 12.7 (2.2)
Cerrophidium sasai	60	41	22.1 (7.1) 6.8 (0.7)	72.0 (9.1)** 82.7 (25.4)**
Crotalus simus	467	149	9.1 (0.7) 5.5 (0.3)	40.1 (2.7)** 8.1 (0.5)**
Lachesis stenophrys	22	3	25.1 (8.3) 7.3 (5.9)	33.7 (9.4) 13.4 (6.8)
Porthidium nasutum	38	10	20.9 (4.5) 12.5 (3.7)	27.7 (5.3) 17.0 (3.6)
Elapidae				
Micrurus nigrocinctus	423	94	25.7 (1.4) 17.3 (2.0)	32.5 (1.6)** 26.4 (2.4)**
Overall	1638	492	19.4 (0.7) 8.5 (0.5)	38.4 (1.3)** 16.9 (1.1)**

**P < 0.01 compared with estimations excluding censored data.