

Stress in wild and captive snakes: quantification, effects and the importance of management

Stress bij in het wild en in gevangenschap levende slangen: kwantificatie, gevolgen en het belang van management

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ABSTRACT

As in other animals, distress and impaired welfare have a deleterious effect on the mental, physical and behavioral health of snakes in the wild and in captivity. Besides anthropogenic disturbance, the availability of food and shelter, the presence of predators, and environmental factors, such as seasonality and climatological changes, are important factors that affect the stress level and subsequent welfare in wild snake populations. In captive snakes, inappropriate management is the most prominent cause of chronic stress and impaired welfare. Chronic stress can be assumed by looking at the snake's behavior, but there is need for a standardized quantification method to pin-point more accurately (chronic) stress levels. The biomarker suitable in this framework is the level of corticosterone in plasma, feces and shed skin.

SAMENVATTING

Net zoals bij andere diersoorten hebben stress en verminderd welzijn een negatief effect op de mentale status, gezondheid en op het gedrag van zowel in het wild als in gevangenschap levende slangen. Naast antropogene verstoring zijn de beschikbaarheid van voedsel en schuilplaatsen, de aanwezigheid van predatoren en omgevingscondities, zoals seizoensale en klimatologische veranderingen, de belangrijkste factoren die stress en welzijn van wilde slangenpopulaties beïnvloeden. Bij in gevangenschap levende slangen is een ongeschikt management de meest prominente reden van chronische stress en verminderd welzijn. Chronische stress kan bepaald worden door gedragswijzigingen van de slang, maar een gestandaardiseerde kwantificatiemethode om (chronische) stresslevels accurater te bepalen, ontbreekt. Een biomarker die gebruikt kan worden, is het gehalte aan corticosterone in bloedplasma, feces en vervellingshuid.

INTRODUCTION

All vertebrates respond to unpredictable and stressful conditions with the so-called 'stress response' (Wingfield, 2005). Stressors can be defined as stimuli that put the individual in a state of uncertainty, lack of information and/or lack of control (Brown et al., 1991). Snakes faced with such stressful stimuli launch a stress response, not only through secretion of catecholamines, such as adrenaline and noradrenaline into the blood, but also through an endocrine stress response with activation of the hypothalamic-pitui-

tary-adrenal (HPA-) axis, to release glucocorticoids, in particular corticosterone into the blood (Sapolsky et al., 2000) (Figure 1). Corticosterone is analogous to cortisol, a glucocorticoid present in mammals, and can be seen as an adaptive hormone as it facilitates energy availability to body systems through gluconeogenesis in order to face the stressor(s) (Palacios et al., 2012; Silvestre, 2014; Tarlow and Blumstein, 2007). A stressor may be acute, eliciting an acute short-term response causing physiological and behavioral changes that counteract the stressor. This is only temporarily and allows the individual to quickly return

to its normal activities (Dantzer et al., 2014; Dickens and Romero, 2013). In contrast, a chronic, sustained response with non-adaptive levels of glucocorticoid secretion, will disturb chronically normal activities, leading to an impaired welfare (Claunch et al., 2017; Dantzer et al., 2014; Davis et al., 2008; Dickens and Romero, 2013; Tarlow and Blumstein, 2007).

Determination and quantification of stress are pivotal because of its relation to the general performance and health of the individual. Behavioral changes indicate disturbance, injury or disease, but may also lead to physical lesions and infections if prolonged. Higher corticosterone levels caused by chronic stress lead to immune suppression, making the individual more susceptible to viral, bacterial and parasitic infections (Dantzer et al., 2014; Neuman-Lee et al., 2015; Silvestre, 2014). However, the perception of stressors is highly individual; whereas one individual does not experience stress from a specific stimulus, the welfare of another individual could be strongly impaired. This individual perception is caused by intrinsic factors, such as sex, age, reproductive condition and former experiences (Dantzer et al., 2014) (Figure 2).

A plethora of stimuli may influence the welfare of snakes. The most common stressors for snakes in the wild are food deprivation, habitat loss due to anthropogenic activity and changes in climate (Ajtić et al., 2013; Gregory, 2016; Robert and Bronikowski, 2009). Other potential stressors are the presence of predators and non-indigenous species in the same habitat (Burger, 1998; Gregory, 2016; Pasmans et al., 2017; Šukalo et al., 2014). Snakes in captivity are mainly stressed by management related aspects, such as temperature, humidity, enclosure size and crowding (Silvestre, 2014).

The quantification of chronic stress in a wild snake population is pivotal for their management and habitat, while for snakes in captivity, it is important regarding welfare. However, this is challenging due to the individual nature of the chronic stress response, but also because of the lack of a uniformly used and standardized quantification method and matrix in the pertinent literature. In the literature, various matrices such as blood, feces and shed skin have been described to measure corticosterone as a biomarker for chronic stress, but comparing results of these studies using different methods and matrices is challenging (Dantzer et al., 2014; Sheriff et al., 2011). In the last section, the commonly used matrices to quantify corticosterone, i.e. blood, feces and shed skin are discussed.

STRESS IN WILD SNAKES

Over the last decade, the welfare of captive as well as wild animals has become increasingly important. In this framework, Bonnet et al. (2016) studied the rapid pace of urbanization and its negative impact on biodiversity, the functioning of ecosystems and the

subsequent impairment of animal welfare in snakes. Assessment of stress in wild snake populations is challenging and has been underexamined. However, having a good understanding of stressful stimuli and their effects on welfare impairment is needed in managing snake populations.

The direct effect of food resources on the stress level has been little studied; however, it is known that they influence the size of a population. In a study by Sewell et al. (2015), the population size of grass snakes (*Natrix natrix*) positively correlated with common frog (*Rana temporaria*) spawn clumps and peak counts of pool frogs (*Pelophylax lessonae*) on a site in eastern England restored for the reintroduction of these amphibians. This was explained by the migration of snakes when food resources in this site increased. Management of forests and heathland which damages prey species populations, has been shown to have a negative impact on the preservation of wild snake populations (Reading and Jofré, 2013).

Secondly, loss of habitat caused by urbanization also affects wild snake populations. Dantzer et al. (2014) have shown that anthropogenic disturbances are frequently correlated with increased levels of plasma baseline glucocorticoids. Bonnet et al. (2016) has shown that shrub habitats, being important shelters for several snake species, have been dramatically altered for practical and esthetical reasons. This shift results in a decrease of sufficient habitat for snake species, which in turn has a negative effect on their population size and biodiversity. In addition, the presence of snakes sometimes triggers rather negative perceptions by the public, making local authorities remove snakes

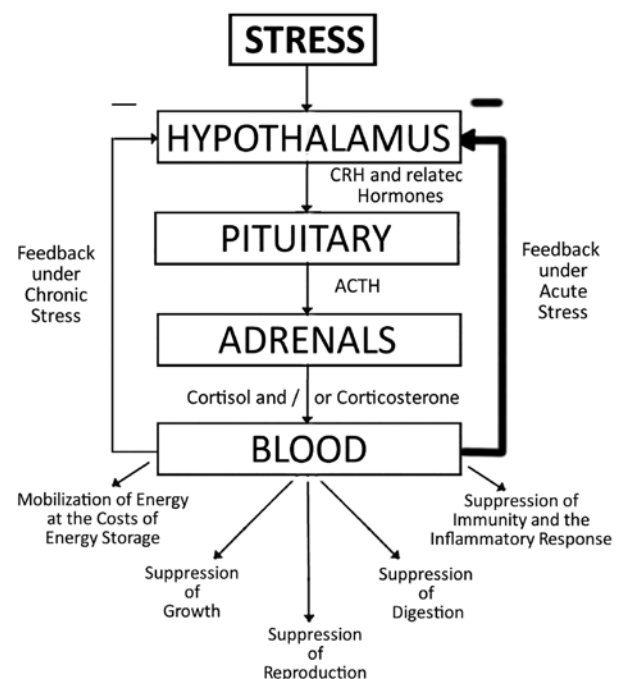


Figure 1. HPA-axis and its effect on systemic processes and the negative feedback response under acute stress compared with response under chronic stress (Sheriff et al., 2011).

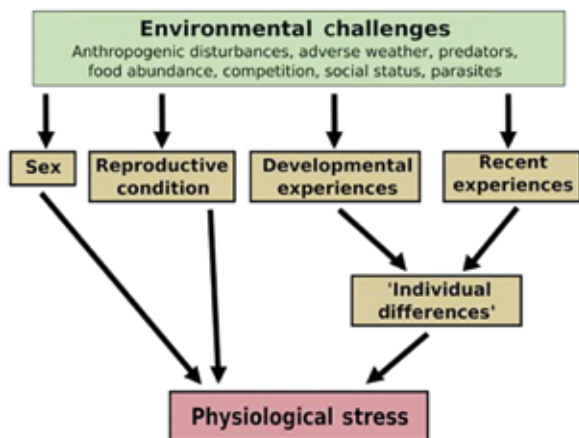


Figure 2. Influence of the intrinsic factors of the individual on its stress response (Dantzer et al., 2014).

from parks and green areas (Ballouard et al., 2013). For example, changes in French legislation in 2007 allowed the clearing of the aspic viper (*Vipera aspis*), a species previously marked as ‘protected’ (Bonnet et al., 2016).

Lastly, climatological changes are believed to have an impact on the overall performance of wild snake populations. Snakes, like all reptiles, are ectothermic, relying on the environment to maintain their body temperature. Snakes living in the temperate-zone are well-adapted to harsh wintering conditions as they hibernate. During this dormant period, their body temperature is generally lower than during active periods in contrast to plasma corticosterone, which is generally higher during hibernation (Dupoué et al., 2013; Nordberg and Cobb, 2016). Subsequently, it has been assumed that mild winter conditions trigger a state of chronic stress and a decrease of body condition when emerging. However, in a study by Brischoux et al. (2016), lower levels of plasma corticosterone were demonstrated when hibernation temperatures were higher, even though there was more body mass loss in snakes maintained in mild wintering conditions. Increased body mass loss may be caused by higher metabolism resulting from significant energy use during mild wintering conditions (Brischoux et al., 2016). Furthermore, it is possible that the experimental design of the study and the matrix, in which corticosterone was measured, affected the quantification of corticosterone. Therefore, further studies must be conducted to assess the effect of global warming on chronic stress levels.

STRESS IN CAPTIVE SNAKES

Inappropriate management conditions are the main reason of stress and impaired welfare in snakes in captivity (Silvestre, 2014). Factors like temperature, humidity, enclosure size and crowding all influence snake welfare, hereby restricting the physiologic

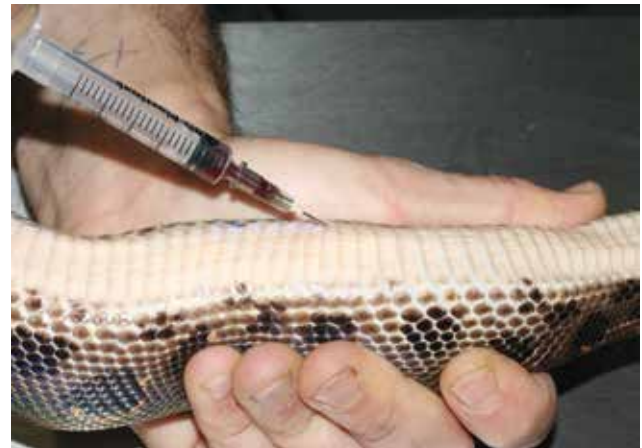


Figure 3. Blood collection from the heart of a snake.

needs of the animals. Persistent inadequate conditions lead to chronic stress and, with time, to dwindling (DeNardo, 2006). Raising awareness of the effect of bad management on mental, physical and behavioral health is the key to improve snake welfare in captivity (Silvestre, 2014). In some countries, guidelines for husbandry standards for reptiles are available providing a good framework of management factors for snakes kept in captivity (Table 1).

In a study by Sparkman et al. (2014) captivity of wild gravid female snakes (*Tamnophis Elegans*) resulted in increased plasma corticosterone levels as well as heterophile to lymphocyte (H:L) ratios over time. Maladaptation syndrome is a phenomenon occurring in reptiles that are acquired from the wild but do not adapt to captivity, subsequently leading to their death (DeNardo, 2006).

Assessment of chronic stress

In the pertinent literature, chronic stress is measured in various ways (Tables 2 and 3). In some studies, the snake’s behavior is observed, especially when studying snakes in captivity (Silvestre, 2014). If the snake is unable to flee from its stressor, which is mostly the case in captivity, the animal will be unable to respond in an appropriate manner and will therefore express abnormal, non-functional behavior (DeNardo, 2006; Silvestre, 2014). Behavioral change in captive reptiles may indicate disturbance, injury or disease (Warwick et al., 2013). These changes can be used to evaluate their condition and welfare, but must be considered carefully in their context. A normally active reptile may become lethargic due to stress, whereas an individual with a calm nature may become very active. Also physical lesions and topical infections, e.g. rostral lesions incurred by rubbing their heads against transparent boundaries, are regarded as indicators for impaired welfare. However, in a study by Claunch et al. (2017), corticosterone administered to the Southern Pacific rattlesnake (*Crotalus helleri*) by

Table 1. Management factors for five popular snake species bred in captivity (From: Belgian law of animal welfare, 2004; guidelines from German federal ministry of food and agriculture, 1997; LICG, 2017).

Management of popular snake species			
	Boa constrictor (<i>Boa constrictor</i>) (¹ <4 m)	Reticulated python (<i>P. reticulatus</i>) (¹ <10 m), Tigerpython (<i>P. molurus</i>) and African rock python (<i>P. sebae</i>) (¹ <7 -8 m)	Corn snake (<i>Elaphe guttata</i>)
Temperature	Gradient; 20 – 35 °C, at night 20 – 22 °C	Gradient; 26 °C – 38 °C, at night 5 – 10 °C	Gradient; 22 – 32 °C, at night 18 – 20 °C
Humidity	Moderate; 60-85%	High; 70 – 90%	Low; 40 – 60%
Light	12 hours/day	12 hours/day	12 hours/day
Stocking density	1 - ² snakes per tank	1 - ² snakes per tank	1 – 2 snakes per tank
Terrarium lay-out	Heating place, hiding places, swimming pool and climbing areas	Heating place, hiding places, swimming pool and climbing areas	Heating place, hiding places, small swimming pool and climbing areas. Hibernation recommended
Terrarium size per snake (length x width x height)	¹ Under 1,5m: 0.75 m x 0.5 m x 0.75 m ¹ Above 1.5m: 1.0 m x 0.5 m x 0.75 m ² 2 m ² surface with 1.5 m height	¹ Under 2.5 m: 0.75 m x 0.5 m x 0.5 m ¹ Above 2.5m: 1.0 m x 0.5 m x 0.75 m ² 8 m ² surface with 1.5 m height	¹ 1.0 m x 0.5 m x 1.0 m ² 0.5 m ² surface with 0.60 m height
Substrate	Paper, pebbles or woodchips	Paper, pebbles or woodchips	Paper, pebbles or woodchips

¹ Minimum standards according to German federal ministry of food and agriculture

² Standards for reptiles kept in zoos, according to the Belgian law of animal welfare, June 23, 2004.

an implant with 3.6 mg (snakes less than 800 g) or 6.1 mg (snakes of more than 800 g) did not mediate their behavioral trait expression. Therefore, observing snake's behavior might not be the best way to ascertain chronic stress.

Levels of circulating cortisol and corticosterone are widely accepted as biomarkers for stress in vertebrates. Both are pleiotropic having besides their role as a stress hormone, various functions in non-stress related physiological processes (Dantzer et al., 2014; Silvestre, 2014; Sparkman et al., 2014). In addition, individual differences in HPA-axis reactivity related to intrinsic factors, such as sex and age, make the interpretation of corticosterone levels challenging (Sparkman et al., 2014). Corticosterone can be determined by means of radioimmunoassay (Palacios et al., 2012; Sparkman et al., 2014), enzyme immunoassay in blood or skin (Berkvens et al. 2013), or by measuring fecal glucocorticoid metabolite (FGM) using the referred methods (Berkvens et al., 2013).

Plasma corticosterone is used to quantify acute stress in snakes. The samples can be obtained quickly, and only a small volume (ca. 25–50 µL) is required to quantify glucocorticoids (Tarlow and Blumstein, 2007) (Figure 3). The response time of plasma corticosterone to acute stress caused by capture and handling is fast; hence, biasing may occur when samples are not taken in time (Sparkman et al., 2014). Palacios et al. (2012) showed that a difference of approxima-

tely 300 ng/mL corticosterone can occur between blood sampling of garter snakes (*Thamnophis elegans*) at capture time and 50 minutes later. Nonetheless, an elevation in baseline blood corticosterone can be used to determine chronic stress (Dantzer et al., 2014; Palacios et al., 2012). An elevated baseline of blood corticosterone may be caused by acute stressful stimuli and/or the reduced ability to terminate the stress response. This principle is based on the assumption that chronically stressed individuals will initially respond greater to acute stimuli such as the blood sampling itself (Dantzer et al., 2014), which is an invasive method (Tarlow and Blumstein, 2007). In addition, plasma levels of corticosterone are influenced by circadian as well as other rhythmicities making plasma unsuitable for chronic stress quantification (Silvestre, 2014).

Measuring FGM levels is a way to assess HPA-axis activity over a specific time lapse, and has gained interest for the quantification of stress in multiple species. In snakes, specifically, fecal corticosterone metabolite (FCM) is measured (Berkvens et al., 2013). It is assumed to be a cumulative exposure of corticosterone reflecting the average of the blood corticosterone over a specific duration (Dantzer et al., 2014; Sheriff et al., 2011). Measuring FGM levels is non-invasive, and therefore not altering results due to sampling (Dantzer et al., 2014; Palme et al., 2005). However, this matrix has its limitations. In mammals and birds,

Table 2. Comparison of different criteria to score stress in reptiles (part 1). N/A = not assessed (Adapted from: Brischoux et al., 2016; DeNardo, 2006; Dickens and Romero, 2013; Fitze et al., 2009; Silvestre, 2014; Warwick et al., 2013).

Scoring stress in reptiles (in theory): part 1				
Criterion	Behavioral change	Higher occurrence of lesions and infections	Change in color	Weight loss
Specificity	Low	Low	Low	Low
Sensitivity	Low	High	Low	High
Quantification	Not possible	Not possible	Not possible	Not possible
Invasiveness	Non-invasive	Non-invasive	Non-invasive	Non-invasive
Applicability	Non-species specific	Non-species specific	Species specific	Non-species specific
Expertise needed	High	Moderate	High	Low
Validation	N/A	N/A	N/A	N/A
Biasing factors	Observer, intrinsic factors	Observer, other agents causing lesions and infections	Observer, dietary differences	Infections, dietary differences

dietary differences and metabolic rate have been shown to have an influence on FGM levels (Dantzer et al., 2011; Goymann et al., 2006). In a study of Dantzer et al. (2011), higher dietary fiber consumption seemed to increase fecal cortisol metabolite (FCM) concentration in North American red squirrels (*Tamiasciurus hudsonicus*). Moreover, microbial interconversion, degradation after defecation or wash-out by rainfall may bias results (Tarlow and Blumstein, 2007; Washburn and Millspaugh, 2002).

Although little studied, corticosterone in shed skin originated by ecdysis could be the future matrix for chronic stress quantification. Ecdysis is the process by which an old outer keratinized epithelial layer is shed, revealing new keratinized epidermal layers underneath (Maderson, 1965). Blood corticosterone

is presumed to incorporate in the skin, but this level is possibly biased by extra-adrenal production as is seen in human skin (Taves et al., 2011). Berkvens et al. (2013) showed a positive correlation between fecal and shed skin corticosterone in African house snakes (*Lamprophis fuliginosus*) and Eastern Massasauga rattlesnakes (*Sistrurus catenatus catenatus*), indicating that the status of the HPA-axis over a specific time frame influences fecal and skin corticosterone in a similar manner. Just as in feces, chronic stress results in a higher cumulative exposure of corticosterone in skin; and as corticosterone in shed skin represents the status of the HPA-axis over a time lapse, it could be used to determine chronic stress (Dantzer et al., 2014; Sheriff et al., 2011). However, up till now, positively correlated levels of corticosterone in shed skin with

Table 3. Comparison of different criteria to score stress in reptiles (part 2). N/A = not assessed, ? = unknown. (Adapted from : Berkvens et al., 2013; Brischoux et al., 2016; Dantzer et al., 2014; Davis et al., 2008 ; Dickens and Romero, 2013; Kalliokoski et al., 2012; Silvestre, 2014).

Scoring stress in reptiles (in theory): part 2				
Criteria	Heterophile/Lymfocyte ratio (H:L ratio)	Corticosterone in plasma	Fecal glucocorticoid metabolite (FGM)	Corticosterone in shed skin
Specificity	Low to ?	?	?	?
Sensitivity	? to high	?	?	?
Quantification	Possible	Possible	Possible	Possible
Invasiveness	Invasive	Invasive	Non-invasive	Non-invasive
Applicability	Species specific	Species specific	Possibly species specific	Species specific
Expertise needed	High	High	High	High
Validation	N/A	N/A	In iguana's	N/A
Biasing factors	Infections and inflammations	Intrinsic factors, acute stress, daily and seasonal cycles	Intrinsic factors, dietary differences, metabolic rate and degradation after defecation	Intrinsic factors, possibly extra-adrenal pathways

stressful environmental stimuli have not been reported yet (Berkvens et al., 2013; Sheriff et al., 2011).

CONCLUSION

Frequently, wild as well as captive snakes are confronted with chronic stress, affecting their overall performance and health. The quantification of stress in snakes, particularly of chronic stress, is challenging and quantification of the stress hormone corticosterone in different matrices, such as plasma, feces and skin, has its advantages and disadvantages. Regardless of the matrix used, the interpretation of results is difficult as the perception of stressors differs between individuals and is influenced by intrinsic factors, such as age and sex. Therefore, further research is needed to provide a scientifically validated quantification method for chronic stress in snakes.

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Uit het verleden

DE 'WORM' ALS ZIEKTEOORZAAK

Een eeuwenoud geloof schrijft allerhande ziekten toe aan geheimzinnig woekerende 'wormen' van demonische krachten voorzien. Hierbij moet men niet denken aan de ons bekende plat- en rondwormen, maar aan meestal onzichtbare ziekteverwekkers. Denk ook aan het Franse vermine (klein ongedierte, zoals bladluizen en rupsen), afgeleid van het Latijnse vermis, in feite hetzelfde woord als worm.

Vermoedelijk is dit geloof ontstaan vanuit de observatie van knagende, schade verwekkende wormen of insecten in de vrije natuur. Hoewel de aard van die zozegde wormen nooit duidelijk was, werden er wel allerhande namen aan gegeven. Soms werden ze genoemd naar de letsels die ze verwekten. Zo is ringworm (dermatofytose) in het Engels nog steeds een courante term. De meeste namen werden afgeleid van de locatie: haarworm, tandworm, navelworm, vingerworm. Een speciaal geval was de 'worm in de staart' waaraan allerhande ziekten werden toegeschreven. Denk aan 'het venijn zit in de staart' (in cauda venenum), waar men vermine verwacht of mengt met venijn (gif). Ook in het West-Vlaams gebeurt dat: 't fernin even goed als 't fenin. Om daaraan te verhelpen maakte men een sneetje in de staart of sneed men de tip af. De ziekteverwekker zou met het uitdruppende bloed verdwijnen.

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