Published with Open Access at Journal BiNET
Vol. 02, Issue 02: 88-100
Journal of Fisheries, Livestock and Veterinary Science
Journal Home: https://www.journalbinet.com/jflvs-journal.html

Stress biomarker: A review on glucocorticoids concentration pattern & its impact on captive animals

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Article received: 13.05.22; Revised: 28.07.22; First published online: 10 August, 2022.

ABSTRACT

As a stress biomarker, glucocorticoids (GCs) concentration in wild animals shows how well a species copes with confinement. In captivity, the animal may suffer from many health problems such as weight loss, irreversible glucocorticoid changes, immune system abnormalities, and reproductive suppression. The review mainly focuses on the effect of transferring wild-caught animals to captivity on stress-related physiological systems such as weight changes, glucocorticoid modulation, adrenomedullary control, and effects on immune and reproductive systems. The GCs concentration pattern on species-wise variation is recorded, and the detection technique and alleviation process is also discussed.

Key Words: *Captivity, Stress, Glucocorticoids, Impacts, Detection technique and Alleviation.*

Cite Article: Islam, P., Saha, P., Khatun, P., Hossain, M. I., Islam, A. and Sachi, S. (2022). Stress biomarker: A review on glucocorticoids concentration pattern & its impact on captive animals. Journal of Fisheries, Livestock and Veterinary Science, 02(02), 88-100.
Crossref: https://doi.org/10.18801/jflvs.020222.10

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I. Introduction

Stress is a condition that endangers or appears to endanger the homeostasis of an individual, and in response to stress, an individual adapts a series of mechanisms aimed at protecting and restoring homeostasis (Möstl and Palme, 2002; Sapolsky, 2000). Stress responses play a crucial role in allowing animals to cope with change and challenges to face both environmental certainty and uncertainty (Sheriff et al., 2011). The goal of keeping wild animals in captivity is for conservation, research, agriculture, the exotic pet trade, and entertainment (Morgan and Tromborg, 2007). Animals kept in captivity have a comfortable home and plenty of food. Although the basic needs of animals are met in captivity, the conditions of confinement can result in physiological stress, especially in newly captured animals (Fischer and Romero, 2019). Human presence, an unfamiliar environment, artificial light conditions, confinement, dietary changes, and handling of animals are all unpredictable and uncontrollable inputs that may activate the stress response (Morgan and Tromborg, 2007). The behaviour and adrenal activity of animals are also influenced by visitors (Pifarre et al., 2012).

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The stress response is mediated by several hormones, including ACTH, glucocorticoids, catecholamines, prolactin, and others (Moberg, 2000). In stress conditions, glucocorticoid hormones, mainly cortisol, are secreted by the adrenal glands and regulate energy and maintain homeostasis (Sapolsky et al., 2000). Increased plasma levels of glucocorticoid hormones like corticosterone are critical physiological reactions to stressful events in vertebrates (Moore and Jessop, 2003; Romero, 2004). Most taxa, including birds (Cockrem and Silverin, 2002), reptiles (Cash et al., 1997), mammals (Lopez-Olvera et al., 2007), and amphibians (Mosconi et al., 2006), exhibit this response. Cortisol levels that are too high can impact the behaviour of animals (O'Connor et al., 2000). In wild animals, short-term captivity is known to cause a hormonal stress response (Mosconi et al., 2006). In long term captivity, the increased level of cortisol may influence behaviour (more aggression, moving, and grooming activities) and physiological functions such as depression, hypertension, loss of body weight, reproductive failure, immunosuppression, and a shorter life span (Birke, 2002; Breazile, 1987; Palme, 1997).

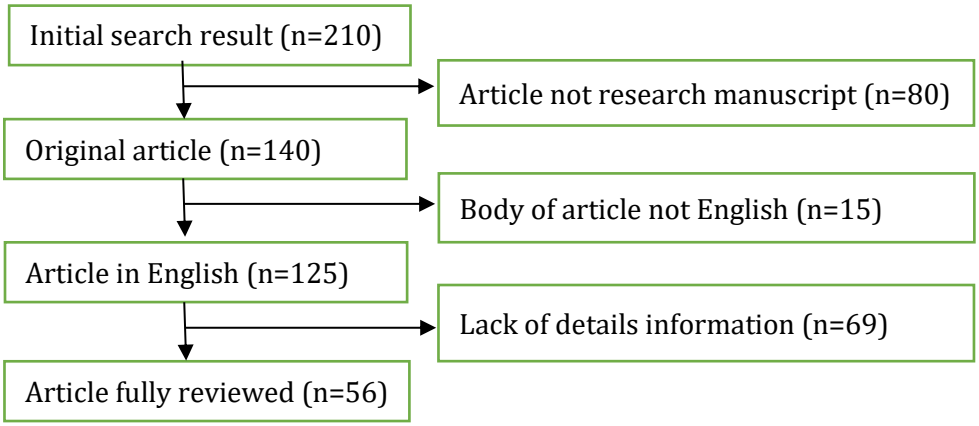
To keep the animals healthy, stress symptoms should be decreased or eliminated. In mammals, the relationship between stress hormones and leukocytes has been extensively established (Dhabhar et al., 1994). Ornithologists are increasingly using the neutrophil/heterophil ratio to demonstrate the consequences of various stressful conditions, such as transit stress (Groombridge et al., 2004) and reproductive output (Moreno et al., 2002). The success of modern zoos and safaris is dependent on achieving and maintaining high animal care standards. Therefore, stress levels should be eliminated, and better management should be provided to ensure animal welfare. The review documented the effects of stress on captive animals, especially the patterns of change in baseline and integrated GCs. Finally, some mitigation process is discussed to improve the condition of captive animals.

II. Materials and Methodology

Published literature on glucocorticoids as a stress indicator was collected from PubMed, Scientific Gate, Google Scholar, and E-Journals of ISI (Institute for Scientific Information). The following keywords were used to search the literature: "glucocorticoids," "captivity," "stress," "physiology," or "endocrinology," and related words. The searched items or publications were thoroughly checked, downloaded in detail, and reviewed. Only the original research data containing publications written in English were included for our review. The abstracts of the research articles that had data regarding our interests were selected for review of the full content. The research work was thoroughly revised and sorted out to meet the field of interest. The full articles were managed in PDF format using Mendeley, a reference management software. We, therefore, devised the following criteria to determine whether papers should be included: (a) GCs elevated in captivity (b) GCs increase at capture then decrease (c) GCs lower in captivity (d) High initial GCs decrease after the captivity (e) No effect of GCs (f) Impact of stress on captive animal

Publications

Primarily, we found 210 articles that could meet the area of interest. A total of 140 articles contained original research data, and the English language was used to write up to 125. Out of 125, we considered 56 reports for our study due to a lack of detailed information in the remaining papers (both quantitative and qualitative) on glucocorticoid concentration in captive wild animals.



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Species-wise analysis

Among the surveyed species, the highest number of works, 15 (40.54%), were accomplished on mammals, followed by birds, 9 (24.32%); reptiles, 7 (18.91%) and amphibians, 6 (16.21%) (Table 01). It is observed that more research studies on glucocorticoid concentration were performed on mammalian species than on other animals (Figure 01).

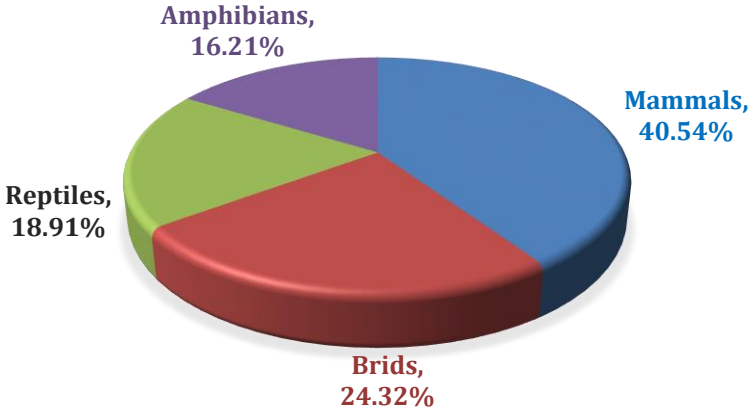

Figure 01. Research on GCs (Species-wise analysis)

III. Glucocorticoids level in wild animals

Under stress conditions, GCs are secreted from the adrenal glands and cause many health problems to the animal. So GCs can be a valuable source of knowing the stress level in animals. The review documented the data about the species, the duration of the study, the sample used for GC determination, ranges of GC concentration, as well as the GC concentration in different conditions to know the worldwide scenario of the stress level in animals.

Table 01. Patterns of change in baseline and integrated GCs when wild animals are brought into captivity

Mammals						
Species	**Study duration**	**Sample used for GC determination**	**Detection technique**	**Detection ranges**	**GC concentration pattern in captivity**	**Normal level of cortisol**
Canada lynx (*Lynx canadensis*) (Fanson et al., 2012)	Long term (unknown)	Faecal Glucocorticoids Materials (FGMs)	EIA	350 ng/ml		25 ng/ml
Spider monkey (*Ateles geoffroyi yucatanesis*) (Rangel-Negrín et al., 2009)	Long term (unknown)	FGMs	RIA	1925.4 ng/g		1224.2 ng/g
Grevy's zebra (*Equus grevyi*) (Franceschini et al., 2008)	6 weeks	FGMs	RIA	25.1 ng/gm	Elevated	14.6 ng/gm
Black rhinos White rhinos (*Ceratotherium simum*) (Linklater et al., 2010; Yang et al., 2019)	75 days	FGMs	RIA	Black Rhino (Male 23.9 ng/gm; Female 24.5 ng/gm) White Rhino (Male 12.3 ng/gm; Female 16.3 ng/gm)		9.34 ng/gm
Degu (*Octodon degus*) (Quispe et al., 2014)	>1 year	Plasma	RIA	150 ng/ml		100ng/ml
Harbor seal (*Phoca vitulina*) (Trumble et al., 2013)	>4 weeks	Plasma	RIA	125 ng/ml	No effect	148 ng/ml

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Mammals						
Species	**Study duration**	**Sample used for GC determination**	**Detection technique**	**Detection ranges**	**GC concentration pattern in captivity**	**Normal level of cortisol**
Tuco-tuco (*Ctenomys talarum*) (Vera et al., 2011)	30 days	Plasma	RIA	18.97ng/ml		14.40 ng/ml
Chacma baboon (*Papio ursinus*) (Steyn, 1975)	Peak: 4 weeks approach long term captives by 7 weeks	Plasma	RIA	141 mg/100 ml		99.90 mg/100 ml
African green monkey (*Cercopithecus aethiops*) (Suleman et al., 2004)	Peak: 1 day approach free-living by 2 days	Plasma	RIA	530 nmol/L	Increase at capture, then decrease to approach wild baseline	391 nmol/L
Richardson's ground squirrel *(Urocitellus richardsoni*) (Hare et al., 2014)	Peak: 3-5 days approach free-living by 6 days	FGMs	RIA	161.04 ng/ml		104.99 ng/ml
Harbor porpoise (*Phocoena phocoena*) (Siebert et al., 2011)	Long term	Plasma	RIA	122 µg/L		338.6 µg/L
Gilbert's potoroo (*Potorous gilbertii*) (Stead-Richardson et al., 2010)	Long term	FGMs	RIA	6 ng/ml	Lower	10.5 ng/ml
Harbor seal (*Phoca vitulina*) (Gardiner and Hall, 1997)	Long term	Plasma	ELISA	444.2 nmol/l		829.5 nmol/l
Bushtail possums (*Trichosurus volpecula*) (female) (Baker et al., 1998)	Decreased from week 1 to 20	Plasma	RIA	34 ng/ml	Elevated at wild baseline decrease over capture period	13 ng/ml
Meadow vole (*Microtus pennsylvanicus*) (Olsen and Seabloom, 1973)	Decreased from day 1 to day 70	plasma	Fluorometric Determination	Male 1397 ng/ml Female 1562 ng/ml		Male 300 ng/ml Female 590 ng/ml

Birds						
Species	**Study Duration**	**Sample used for GC determination**	**Detection technique**	**Detection ranges**	**GC concentration pattern in captivity**	**Normal level of cortisol**
White crowned sparrow (*Zonotrichia leucophrys*) (Marra et al., 1995)	35 days	Plasma	RIA	16.45 ng/ml		13.25 ng/ml
Blackbirds (*Turdus merula*) (Adams et al., 2011)	22 days	Plasma	RIA	6.8 ng/ml	Elevated	2.2 ng/ml
House sparrow (*Passer domesticus*) (Fischer and Romero, 2016)	7 days	Plasma	RIA	4.8 ng/ml		1.2 ng/ml
Southern pied babblers (*Turboides bicolor*) (Jepsen et al., 2012)	5 days	FGMs	EIA	73.49 ng/ml		21.46 ng/ml
House sparrow (*Passer domesticus*) (Martin et al., 2011)	Up to 4 weeks	Plasma	ELISA	20 ng/ml	No effect	1.2 ng/ml

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Birds						
Species	**Study Duration**	**Sample used for GC determination**	**Detection technique**	**Detection ranges**	**GC concentration pattern in captivity**	**Normal level of cortisol**
House sparrow (*Passer domesticus*) (Kuhlman and Martin, 2010)	Peak: Days 1-2 approach free-living by 1 month	Plasma	EIA	20 ng/ml	Increase at capture, then decrease to approach wild baseline	1.2 ng/ml
White crowned sparrow (*Zonotrichia leucophrys*) (Wingfield et al., 1982)	Peak: Days 1-2 approach free-living by day 14	Plasma	RLA	25.39 ng/ml		15.77 ng/ml
European starling (*Stumus vulgaris*) (Cyr and Romero, 2008)	Unknown	FGMs	RIA	7 ng/ml	Lower	55 ng/ml
Red knot (*Calidris canutus*) (Piersma and Ramenofsky, 1998; Piersma et al., 2000)	Decreased from first sample to 2 years	Plasma	RIA	30 ng/ml	Elevated at wild baseline decrease over capture period	40ng/ml

Reptiles						
Species	**Study Duration**	**Sample used for GC determination**	**Detection technique**	**Detection ranges**	**GC concentration pattern in captivity**	**Normal level of cortisol**
Tuatara (*Sphenodon punctatus*) (female) (Tyrrell and Cree, 1994)	Unknown	Plasma	RIA	Captive juvenile female 4.21 ng/ml Wild juvenile female 2.44 ng/ml		1.28-4.65 ng/ml
Tree lizard (*Urosaurus ornatus*) (Moore et al., 1991)	Up to 3 weeks	Plasma	RIA	20 ng/ml	Elevated	10ng/ml
Water snake (Nerodia sipedon) (Sykes and Klukowski, 2009)	5-8 days	Plasma	ELISA	310 ng/ml		275 ng/ml
Brown tree snake (*Boiga irregularis*) (Mathies et al., 2001)	3 days	Plasma	RIA	Male 49.30 ng/ml Female 56.87 ng/ml		Male 4.41 ng/ml Female 8.71 ng/ml
Tuatara (*Sphenodon punctatus*) (male) (Tyrrell and Cree, 1994)	Unknown	Plasma	RIA	3.61 ng/ml	No effect	2.48 ng/ml
Kutum (*Rutilus frisii kutum*) (Nikoo et al., 2010)	3 days	Plasma	RIA	308 ng/ml		378 ng/ml
Skink (*Egernia whitii*) (Jones and Bell, 2004)	Peak: 1 day-1 week approach free-living by 4 weeks	Plasma	RIA	24.8 ng/ml	Increase at capture, then decrease to approach wild baseline	5.3 ng/ml

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Amphibians						
Species	**Study Duration**	**Sample used for GC determination**	**Detection technique**	**Detection ranges**	**GC concentration pattern in captivity**	**Normal level of cortisol**
Cururu toad (*Rhinella icterica*) (De Assis et al., 2015)	3 months	Plasma	EIA	65 ng/ml	Elevated	9 ng/ml
Toad (*Rhinella schneideri*) (Titon et al., 2017; Narayan et al., 2012)	60 days	Plasma	EIA	4.24 ng/ml		31.3 pg/µg
Cane toad (*Rhinella marina*) (Narayan and Hero. 2011)	Peak: Day 5 approach free-living by Day 12	Urine	RIA	600 pg/µg	Increase at capture, then decrease to approach wild baseline	200 pg/µg
Cane toad (*Rhinella marina*) (Narayan et al., 2012)	Peak: Day 4 approach free-living by Day 14	Urine	EIA	66.3 pg/µg		31.3 pg/µg
Fijian ground frog (*Platymantis vitiana*) (Narayan and Hero, 2011)	Peak: Day 5 approach free-living by Day 25	Urine	EIA	113.93 pg/µg		34.74 pg/µg
Tod (Rhinella icterica) (Titon et al., 2018)	Decreased from days 30 to 60	Plasma	RIA	3ng/ml	Lower	18 ng/ml

IV. Glucocorticoids concentration pattern-wise analysis

Most of the research findings have shown that elevated glucocorticoids concentrations at 14 (37.83%) in captivity, followed by increased glucocorticoids at capture, then decreased to approach wild baseline, 8 (21.62%), the same percentage of lower and no effect on glucocorticoid concentration in captivity, 6 (16.21%), and then elevated glucocorticoids concentrations at wild baseline decreased over the capture period, 3 (8.1%) (Figure 02).

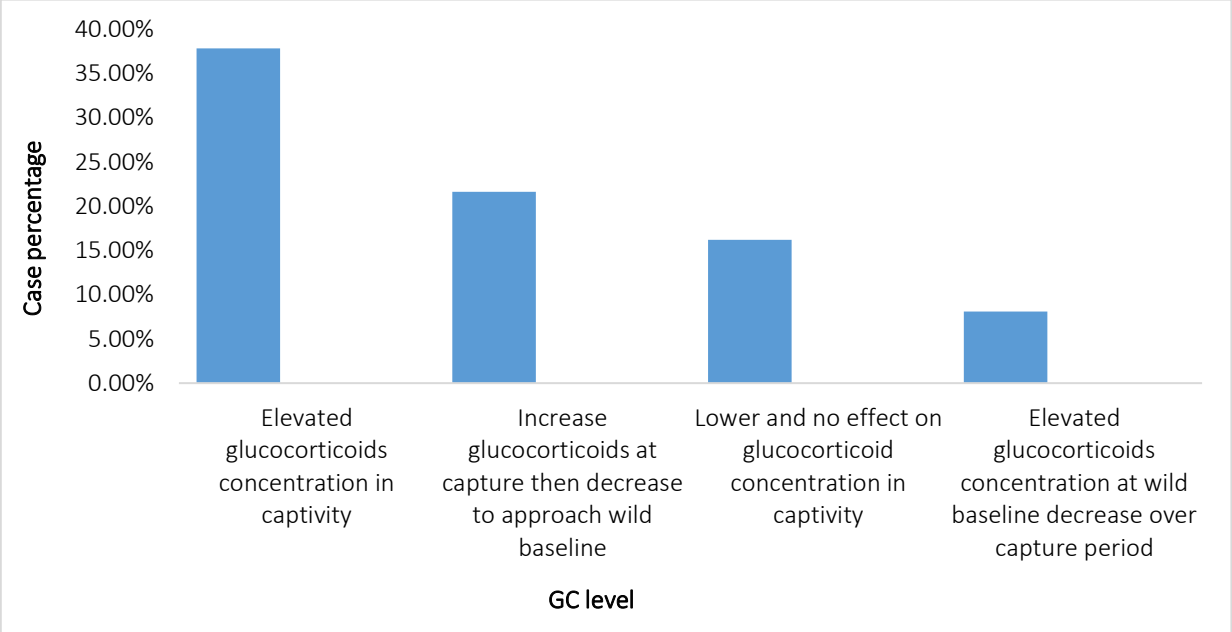

Figure 02. Patterns of change in baseline and integrated GCs when wild animals are brought into captivity

V. Detection technique

GCs concentrations are one of the most commonly utilized measures to assess captivity stress. Acute stress causes a brief increase in GCs. Long-term stressor exposure, on the other hand, typically causes alterations in GC regulation that vary depending on the species and conditions (Dickens and Romero, 2013). Thus monitoring glucocorticoid levels can be a valuable tool to provide information on the stress level experienced by the animals. Glucocorticoid levels can be found in blood, faeces, hair, etc. (Cockrem, 2005; Goymann et al., 1999). The most frequent method for measuring GCs is quantifying them in circulating plasma (Sheriff et al., 2011). Nowadays, the assessment of hormone matrix using

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faeces is a widely accepted approach. The benefit of faeces collection is, easy to collect, animals are usually not disturbed during sample collection, and sampling is feedback-free due to the absence of capture and handling (Ganswindt et al., 2012).

Enzyme-Linked ImmunoSorbent Assay (ELISA), Chemiluminescent Immuno Assays (CLIA), Radio Immuno Assays, mass spectrometry (MS), and other immunoassays are the most commonly used for detection and quantification of cortisol.

In immunoassays, antibodies are used to produce a signal and subsequently determine the concentration of the target compound (i.e., the analyte); in an ELISA, one of the employed antibodies is modified (enzyme-conjugated) to be able to produce an optical signal after administration of the substrate; in a competitive CLIA, the target compound (e.g., cortisol or cortisone) is labeled by chemiluminescent tracer substance (Hogenelst et al., 2019). When a specific antibody is added to the labelled antigen, they will bind to each other. Another immunoassay method (LFIA) is the Lateral Flow Immuno Assay, typically employed for qualitative evaluations (Posthuma-Trumpie et al., 2009). In particular, a liquid is placed on a strip and moved over a test strip through several compartments as it passes over a liquid (such as urine or saliva). As a result, a coloured strip will appear if the analyte is present.

Mass-spectrometry (MS) is a technique that separates compounds based on their polarity and detects them based on their mass and charge. Because of its great specificity and sensitivity, it is regarded as the gold standard for cortisol measurement (Miller et al., 2013; Perogamvros et al., 2010; Baid et al., 2007).

The Radio Immune Assay (RIA) and High-Performance Liquid Chromatography are the common detection techniques to measure faeces' GCs levels (Ganswindt et al., 2012).

VI. Impact of elevated glucocorticoid concentration on animals

GC concentrations were one of the most common approaches to quantifying captivity stress. GC hormones (mostly cortisol in fish and most mammals; primary corticosterone in reptiles, birds, amphibians, and rodents) are produced by the adrenal cortex and have a variety of actions throughout the body (Fischer and Romero, 2019). Acute stresses cause a spike in GCs that is quickly controlled by negative feedback. Changes in GC regulation are common after long-term stressor exposure. But depending on the species and the environment, different parts of the GCs response (baseline concentrations, stress-induced concentrations, or negative feedback) and the direction of change are impacted (Dickens and Romero, 2013). The GCs of different animals are not affected by captivity. In 16.21% (6 of 37) of the studies, there was no recorded difference in GCs during or after captivity compared to free-living levels. However, in most studies, captivity caused a change in baseline or integrated GCs. In 37.83% of studies (14 of 37), wild animals had increased GCs at the end of the capture period compared to concentrations in free-living animals (3 days to several years). Traditionally, elevated GCs have been considered a sign that animals are persistently stressed. This stress state can have serious consequences for animals, some of which are discussed in this article.

Weight loss

When wild animals are brought into captivity, they frequently lose weight for a time. According to a study of wild animals taken into captivity, 64 % of the animals examined exhibited a decrease in bulk as a result of being imprisoned at least during the first catch phase (Fischer and Romero, 2019). In captivity, chronic stress is most likely to blame for weight loss. Weight loss has been observed in mammals (Flügge, 1996), birds (Rich and Romero, 2005) and fish (Rich and Romero, 2005). Weight loss is the most prevalent symptom of persistent stress (Dickens and Romero, 2013). Weight loss was not the only trend observed in captivity. Fischer and Romero, 2019 found that 17% of the studied animals acquired weight beyond their initial condition during their investigation (Fischer and Romero, 2019). Because of the greater food available in confinement, certain animals may maintain their weight. On the other hand, other animals may grow obese due to unrestricted availability to food and exercise limits and suffer the negative consequences of having a big body mass or body fat content (West and York, 1998). In a study with domesticated budgerigars, birds were fed ad libitum and housed in cages with limited movement, and high body mass at the end of 28 days was linked to greater DNA damage (Larcombe et al., 2015).

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Immune consequences

Stress has some effects on the immune system. Increased GCs have a temporary or long-term impact on leukocyte populations, accounting for these changes. Lymphocytes moving from the bloodstream to the skin, spleen, and lymph nodes, where they will be ready in the event of a wound, can occur as a result of GCs (Dhabhar and McEwen, 1997). Neutrophils (most vertebrates) and heterophils (birds and some reptiles) can both multiply and mobilize in response to GCs (Dale et al., 1975; Gross and Siegel, 1983). As a result of these effects on leukocyte populations, the neutrophil or heterophil to lymphocyte ratio (N or H: L ratio) alters (Dhabhar and McEwen, 1997). A change in the N or H:L ratio does not always mean that the immune system of an animal is underactive or overactive. Instead, it serves as a supplementary indicator, similar to GC secretion. Davis *et al.* (2008) found that a long-term increase in the N or H:L ratio, equal to a long-term increase in circulating GCs, can suggest that an animal is stressed. Overall, it appears that confinement has no distinct immune regulatory pattern. Although it has been shown that captivity reduces immune activity in some animals (e.g., red knots and toads), it has also increased immune function in others. Gene expression for pro-inflammatory cytokines was higher in imprisoned house sparrows (2 and 4-week captives) than in recently acquired animals in house sparrows, indicating that captive birds are more prone to inflammation (Martin et al., 2011). CGs release is most likely linked to changes in the immune system's response to prolonged stress. The impact of GCs on the immune system, on the other hand, can be complicated. GCs often induce an immunological response in the short term, but they may be immunosuppressive in the long run (Dhabhar and McEwen, 1997; Martin, 2009). It is impossible to predict whether captivity conditions will result in appropriate or inappropriate immune activity because the interaction between GCs and immunity is complex and context-specific.

Reproductive function

The adverse effects of captivity on reproductive biology have been well documented. High GC levels are likely linked to a reduced ability to reproduce. GCs may help reduce the negative effects of reproductive hormones (Sapolsky et al., 2000). Long-term GC exposure can influence gonad growth, egg maturation, sperm production, and behaviour by lowering testosterone and estradiol levels. A drop in sex hormones was followed by increased GCs in green tree frogs. (Zerani et al., 1991). Despite GC levels being below free-living values for most of the confined period, males had suppressed faecal testosterone, and females had suppressed faecal progestins in black rhinos (Linklater et al., 2010).

VII. Alleviation of stress in captivity

One of the simplest methods to minimize chronic stress symptoms is to change the physical conditions of captivity. In European starlings (long term captivity) (Dickens and Bentley, 2014), transferring from outdoor to indoor cages resulted in lower reproductive hormones and behaviours, as well as weight loss and immunological function (Dickens and Bentley, 2014). The size and density of the cage play a role in the development of chronic stress. During the initial captivity period high-density housing resulted in elevated GCs compared to low-density housing (Bolasina, 2011). Adding a female to a cage of male brown-headed cowbirds (who had previously been housed alone) resulted in lower plasma GCs and higher testicular regeneration. For visual species, lighting conditions could be critical. Under low flicker rate fluorescent lights, European starlings display more behavioural symptoms of chronic stress than under high flicker rate fluorescent lights (Evans et al., 2012), but low flicker rate fluorescent lights do not elicit a GC response (Greenwood et al., 2004). Particularly for poikilotherms, temperature conditions should be carefully considered. During the first transfer to captivity, warm conditions resulted in significant mortality in sardines (Marçalo et al., 2008) and increased GCs in cane toads (Narayan et al., 2012).

VIII. Conclusion

Captivity, a new site, travel, social tension and hostility, human disturbances, and predator exposure have all been used to identify adrenocortical activity using GCs. Captivity has been proven to raise baseline GCs and impair adrenal sensitivity in the literature. Even if the animal's basic needs are supplied, high GCs in many species can persist for months or years after capture, resulting in chronic stress. As a result, stress may impact the physical, psychological, and reproductive behaviour of zoo animals. Individual animal factors (such as species traits, genetics, temperament, and previous experience) play a role in how well an animal copes with environmental conditions (e.g., social

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grouping, enclosure design, and sensory environment). The most noteworthy finding of this study is that GCs are generally higher in captivity and have a deleterious influence on captive animals.

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HOW TO CITE THIS ARTICLE?

MLA
Islam, P. et al. "Stress Biomarker: A Review on Glucocorticoids Concentration Pattern & its Impact on Captive Animals". Journal of Fisheries, Livestock and Veterinary Science 02(02) (2022): 88-100.

APA
Islam, P., Saha, P., Khatun, P., Hossain, M. I., Islam, A. and Sachi, S. (2022). Stress Biomarker: A Review on Glucocorticoids Concentration Pattern & its Impact on Captive Animals. *Journal of Fisheries, Livestock and Veterinary Science*, 02(02), 88-100.

Chicago
Islam, P., Saha, P., Khatun, P., Hossain, M. I., Islam, A. and Sachi, S. "Stress Biomarker: A Review on Glucocorticoids Concentration Pattern & its Impact on Captive Animals". Journal of Fisheries, Livestock and Veterinary Science 02(02) (2022): 88-100.

Harvard
Islam, P., Saha, P., Khatun, P., Hossain, M. I., Islam, A. and Sachi, S. 2022. Stress Biomarker: A Review on Glucocorticoids Concentration Pattern & its Impact on Captive Animals. Journal of Fisheries, Livestock and Veterinary Science, 02(02), pp. 88-100.

Vancouver
Islam, P, Saha, P, Khatun, P, Hossain, MI, Islam, A and Sachi, S. Stress Biomarker: A Review on Glucocorticoids Concentration Pattern & its Impact on Captive Animals. Journal of Fisheries, Livestock and Veterinary Science. 2022 August 02(02):88-100.

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