RESPONSE OF WHITE'S TREEFROG (*LITORIA CAERULE)* TO COMMON HOUSEHOLD CAPTIVITY STRESSORS

A Report of a Senior Study

by

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ABSTRACT

While numerous studies have been conducted on the effects of captivity on other classes of vertebrates, very few studies have investigated the effects of captivity and its related stresses on amphibians. Chronic stress results in elevated levels of corticosterone (CORT) released from the adrenocorticol cells, which in turn result in hyperglycemia, anorexia, and changes in behavior. This study investigated the effects of household stressors (irregular light patterns, noise, and handling) on the White's tree frog, *Litoria caerulea,* a common exotic pet that is nocturnal in the wild. It was hypothesized that *Litoria caerulea* exposed to the aforementioned household stressors would exhibit elevated blood glucose levels, decreased appetite and thus decreased body mass, and other stress-induced behavior changes in comparison to frogs housed in a more natural, less disturbed environment (control). After 12 days of observation (and handling of the treatment group), the frogs were massed, blood samples were collected, and blood glucose and hematocrit levels were evaluated. Control and treatment frogs exhibited no significant difference in mass (p=0.785), hematocrit (p=0.375), or blood glucose levels (p=0.680). The frogs also did not show any significant difference in the frequency of behaviors exhibited between the control and treatment groups. However, over time the control group showed an increase in traveling throughout their enclosure (p=0.020) and a decrease in the frequency of bathing (p=0.049), which was not seen in the stressed frogs. Therefore, in this study, household captivity did not appear to have any significant

physiologic effects on *Litoria caerulea*; however, activity level was decreased over time in stressed White's tree frogs.

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ACKNOWLEDGEMENTS

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CHAPTER I

INTRODUCTION

Physiology of Stress

The physiological response to stress is conserved among vertebrates. The body exhibits a stress response in two ways: immediately via the nervous system and also more slowly via the endocrine system. During the latter response, stress causes the release of hormones from endocrine organs, resulting in metabolic, mental, and physical responses by the animal. The hormones related to stress for several classes of vertebrates can be seen in Table 1. Specifically in amphibians, stressful stimuli, such as anxiety or pain, excites the hypothalamus and causes the production of corticotropin-releasing factor (CRF) which initiates the release of corticosterone (CORT) from the adrenal cortex.

Table 1: The stress-associated hormones of a selection of vertebrate classes (based on Norris 2007).

Class	Stress Associated Hormones
Amphibia	Corticosterone Cortisol Aldosterone 18-hydroxycorticosterone Epinephrine
Reptilia	Corticosterone Aldosterone Epinephrine
Aves	Corticosterone Aldosterone Epinephrine
Mammalia	Cortisol Corticosterone 11-deoxycortisol Aldosterone Deoxycorticosterone Epinephrine

The principle source of CRF in amphibians is the preoptic nucleus, which is homologous to the principle source of CRF in mammals, the paraventricular nucleus of the hypothalamus (Yao, Westphal, and Denver 2004). Once produced, CRF travels to the corticope cells of the anterior pituitary gland (APG) by way of the hypothalamic-hypophysial portal vessels (Guyton and Hall 2006). The anterior portion of the pituitary, also known as the adenohypophysis, is glandular tissue derived from Rathke's pouch during embryonic development and is therefore composed of epithelial tissue. In amphibians, CRF travels through the hypothalamic-hypophysial portal vessels to a well developed APG that is divided into the pars distalis, the pars tuberalis, and the pars

intermedia (Norris, 2007). CRF has also been located in the cerebellum, rhombencephalon, and the rostral spinal cord in frogs (Yao, Westphal, and Denver 2004).

After receiving the CRH from the hypothalamus, the corticotrope cells of the non-mammalian APG hydrolyze proopiomelanocortin to form adrenocorticotropin hormone (ACTH). This hormone enters circulation and travels to the adrenal glands. This pathway is known as the hypothalamic-pituitary-adrenal axis and is outlined in Figure 1.

Amphibians and chelonians have adrenocorticol tissue found in irregular nodules (Norris 2007). This tissue is loosely arranged into glands located interrenally on the kidneys' ventral side. Two types of cells, chromaffin and adrenocortical cells, are typically found in the adrenal glands. The chromaffin cells are responsible for the release of epinephrine and the adrenocortical cells release the chronic stress hormones (Norris 2007).

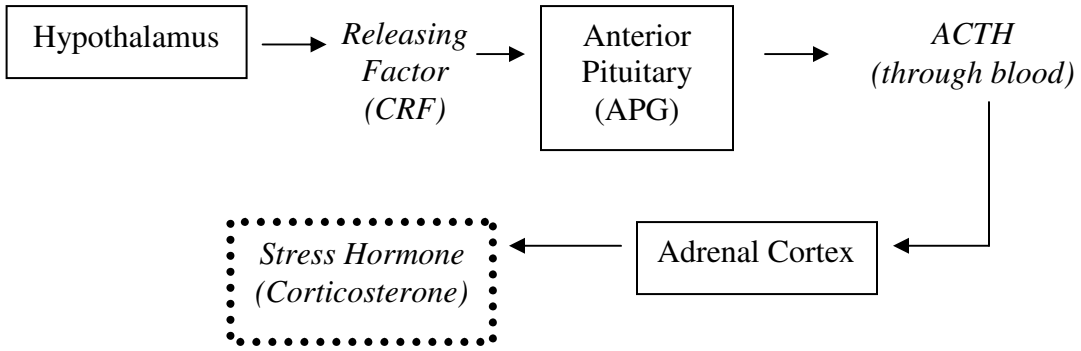

Figure 1: The hypothalamic-pituitary-adrenal axis (HPA axis)

In at least one group of frogs, ranids, a third cell type identified as Stilling cells are found. These cells appear during the summer months and recede during the winter. The function of Stilling cells is still unknown. Similar to other classes of vertebrates, amphibians respond to elevated ACTH by synthesizing a variety of corticosteroids including aldosterone, CORT, and 18-hydroxycorticosterone, which is a precursor for aldosterone. In metamorphosing or permanently aquatic amphibians the major corticosteroid is cortisol rather than CORT, which is the dominant corticosterone in terrestrial amphibians. It is hypothesized that the role of this steroid in aquatic amphibians is the maintenance of proper sodium balance, which is the steroid's primary role in fishes (Norris 2007).

Adaptive and Maladaptive Stress Responses

The main function of chronic stress response in amphibians is the preparation for an alarm reaction (commonly referred to as a fight or flight response) to escape what is perceived by the animal to be a life threatening situation (Guyton and Hall 2006). The excitation of the hypothalamus by acute stress causes the utilization of these additional energy stores created by chronic stress. An alarm reaction enables the body to perform additional, strenuous activity that is not normally required. Some effects of the sympathetic nervous system as a result of the fight or flight response are an increase in arteriole pressure, increased blood flow to muscles, decreased blood flow to organs not directly associated with motor activity, and increased metabolism (Guyton and Hall 2006). Hyperglycemia caused by cortisol, CORT, and aldosterone, is compounded by the fast acting, neurologic stress hormone, epinephrine. This neurologic hormone has hyperglycemic effects as a result of glycogenolysis, the breakdown of glycogen, in the liver and muscle tissue. This increase in glucose allows for increased mental activity and

increased muscle strength (Norris 2007). Additionally in amphibians, rises in CRF have been shown to activate movement in tadpoles and frogs (Boorse and Denver 2004).

The effects of stress hormones have been indepthly studied in mammals, which release the hormone cortisol in response to chronic stress; however, the stress hormones CORT and aldosterone elicit many of the same physiologic responses in amphibians. One of the primary effects of cortisol, CORT, and aldosterone metabolically is stimulating the formation of new glucose (gluconeogenesis) at the liver and muscle (Norris 2007). Gluconeogenesis occurs when the liver responds to these hormones by transcribing the enzymes that convert stored amino acids into glucose. Additional amino acids are also released from muscles as a result of cortisol, CORT, and aldosterone (Guyton and Hall 2006). These amino acids circulate to the liver and are used in gluconeogenesis. Aldosterone exhibits less significant effects on gluconeogenesis than corticosterone due to the direct role of CORT in gluconeogenic enzyme activation (Norris 2007). Nevertheless, in organisms with elevated levels of cortisol, CORT, and aldosterone the blood glucose level rises resulting in hyperglycemia.

In addition to the hyperglycemia resulting from gluconeogenesis, these stress hormones make tissues more resistant to insulin, which further elevates blood glucose (Guyton and Hall 2006). Insulin resistance describes the phenomenon of glucose not being readily absorbed and utilized by skeletal muscle and other tissues. As a response to the decrease in glucose utilized for metabolism, cells begin to mobilize fatty acids from adipose tissue and additional noncontractile proteins from muscle to use as energy sources.

Chronically elevated CORT has the potential to elicit detrimental effects as well as the aforementioned benefits. During reproduction, these elevations in CORT are likely due to the stress of competing for mates as well as the increased metabolic demands for calling (Burmeiser, Somes, and Wilczynski 2001). Unlike in birds and mammals, physiological levels of CORT are not shown to have any ill effects on reproduction; however, induced elevations of CORT to more than two times levels seen in stressed frogs results in suppression of calling in male green treefrogs (Burmeiser, Somes, and Wilczynski 2001). Stress has not shown any definitive effects on the plasma concentrations of sex steroids in amphibians, which is commonly seen in other vertebrate classes (Coddington and Cree 1995). Naturally high levels of corticosterone can make amphibians more susceptible to environmental toxins, however. In the tree frog species, *Hyla versicolor*, exposure to the pesticide carbaryl, which is marketed under the name Sevin, caused up to 4 times greater mortality rates when the frogs were stressed by a natural predator's presence (Relya and Mills 2000). The cause of the increased mortality rates in stressed tadpoles exposed to pesticide is not fully understood, but it is hypothesized that the fear caused by the release of chemical cues from the predator results in too much stress for the tadpole's physiology to cope with when also coping with toxic pesticides. Elevated CRF levels, which lead to elevation in CORT levels, also have a powerful anorectic effect on tadpoles and juvenile frogs (Boorse, Denver, 2004).

CRF has additional effects on amphibian metamorphosis (as cited in Boorse, Denver 2004). CRF in larval amphibians can act as a thyrotropin releasing factor (TRF), which assists in accelerating metamorphosis. Thus, CRF acts as a dual purpose hormone,

increasing the survival rate of tadpoles that are experiencing the stress of a dessicating environment by directly speeding up their development into mature frogs (Denver 1997).

Household Environmental Stressors

Environmental stressors are not limited to the natural world. In fact, many of the stressors most often associated with nature are just as evident in a household environment when considering a frog's perception of its surroundings. Irregular light patterns, loud noises, high population densities, and predators such as dogs, cats, and humans are just some of the stresses experienced by amphibians kept as pets.

Although few studies have been conducted on the effects of these stressors on amphibians, household stressors result in elevated CORT levels, suppressed immune responses, and behavior changes in other classes of vertebrates. Mice exposed to white noise for a period of four weeks exhibited CORT levels double basal levels and significantly decreased immune response (Zheng and Arhzumi 2007). Elevated levels of CORT increase the production of free radicals in the body, which suppresses the immune system (as cited in Zheng and Arhzumi 2007). Overcrowding, which is often common in a home terrarium, also produces elevations in CORT for fish and tadpoles (Glennemeier and Denver 2002). Lastly, when rats are put into the same enclosure as ferrets, without the ferrets having direct access to the rats, CORT levels rise to 700% above basal levels (Roseboom et al. 2007). The stressed rats also exhibit behavioral inhibition, or the absence of movement except for the requirements of breathing, for significant amounts of time whereas control rats exhibited no inhibition.

The stress associated with a household environment is compounded for nocturnal animals due to the fact that most households are active during the daylight hours whereas

the animals are active at night. Basal CORT levels have a natural circadian rhythym with the highest values seen at night in nocturnal animals (Roseboom et al. 2003). However, the HPA axis of nocturnal animals is more susceptible to stress during the day when their basal CORT levels are lowest. Stressful stimulation during daylight hours causes the greatest increase in CORT levels.

Purpose

While the effects of stress hormones in amphibians have been widely studied, most of these studies have been conducted using tadpoles of captive bred strains of *Xenopus lavis* or wild caught populations of a variety of frogs that have been kept captive for a relatively short amount of time. Very few, if any, studies have been conducted on the stress response of adult frogs that remain in captivity for extended amounts of time or the stress on species of frogs that are kept by individuals as pets. One species in particular, *Litoria caerulea* or White's tree frog, is commonly kept as a pet yet there are no available studies on the effects of household captivity. Therefore, nothing is known about the effects of the abundance of household stressors these frogs endure on a daily basis. Because previous studies indicate that most species of frogs show increases in CORT levels during short term captivity, it is hypothesized that *Litoria caerulea* that are kept as pets (and therefore endure the stressors of pet-life such as irregular light and moderate handling) versus those housed in a more natural setting will exhibit elevated blood glucose levels, decreased appetite and thus decreased body mass, and other stress induced behavior changes.

CHAPTER II

MATERIALS AND METHODS

To begin the study, six glass aquaria (10 gallon) were washed and assembled as follows. An approximately ¾ inch layer of aquarium gravel was spread out on the bottom on each aquarium to provide drainage. A 1 ½ inch layer of dampened sphagnum peat moss was laid over the gravel for substrate. A silk plant was hung by a suction cup on one corner of the tank and a wide brimmed ceramic bowl was placed at the opposite end of the tank. The bowl was pressed into the peat moss so that the edge of the bowl rested only slightly above the level of moss. Dechlorinated tap water was added to each of the bowls. After the tanks were assembled, 18 unsexed White's tree frogs (*Litoria caerulea*) from HaHa Reptiles (www.hahareptiles.com) were distributed by size into each tank. Several of these frogs were noted to have open wounds on their snouts as a result of struggle during shipment. Three frogs of approximately the same size were placed into each tank in order to prevent cannibalism of smaller frogs. The frogs were allowed to acclimate for three weeks to recover from any stress caused by shipping. During this time the frogs were observed for typical behavior patterns and an ethogram of behaviors was created (see Appendix A).

Following the acclimation period, each frog was massed using Mettler College1300 balance. Each individual was identified using the pattern of non-pigmented spots on its back and assigned a name. The frogs were then divided into two groups, each with nine frogs and three tanks. The frogs were assigned randomly to each group and a t-test assuming equal variances was conducted to ensure that the two groups did not contain frogs with masses that significantly differed (p=0.90).

The study began after the two groups were placed into separate rooms with different environmental stimuli. The room containing the treatment group was set to an irregular light pattern and sporadic noise stimulus in the form of a radio. The schedule for these stimuli, or stressors, can be seen in Table 2. The control group was placed into a room with no disturbance on a 12h light: 12h dark schedule. Both rooms were maintained at an average temperature of 22.7C during the day and 23.1C at night. The frogs were observed at the beginning of the experiment before any stressful stimuli had been applied to the treatment group in order to ensure similar behavior between the groups initially. Following the initial observation the frogs were observed, fed, and handled, if in the treatment group, on a four day rotation. The groups were observed for ten minutes without stressful stimuli once during daylight hours and again at night under extremely low-light conditions.

Table 2: Patterns of light and noise stress in treatment room

Light Hours	Dark Hours	Noise Stress Hours	Noiseless Hours
7am – 10am	10am - 1pm	8am – 11am	11am – 2pm
1pm – 6pm	6pm – 8pm	2pm – 6pm	6pm – 7 pm
8pm – 11pm	11pm – 2am	7pm – 8pm	8pm - 9pm
2am – 2:30am	2:30am – 7am	9pm – 11pm	11pm – 2am
		2am – 2:30am	2:30am – 8am

The next day, the treatment frogs were handled for two minutes each with minimal restraint except when required due to fleeing. The frogs were held at eye level and prior to handling, the holder's hands were washed. The control group was left undisturbed. Later that evening, 2-3 crickets per frog were placed into each tank. The same number of crickets was used per frog at each feeding; however, the number of crickets per frog varied between feedings due to supply. The frogs were observed for ten minutes after the crickets were released into the tanks and the behaviors were recorded on an ethogram. After the ten minute observation period the water bowls were rinsed clean and fresh, de-chlorinated water was added in each tank. The third day, the treatment group was observed for ten minutes with stressful stimuli during the day and also at night. The control group was observed during the same time frame. On the fourth day, the treatment group was handled as described above. This schedule was repeated for a total of 17 days. In addition to the previously listed schedule, on day five, the plants and bowls were removed from the treatment groups' tanks, washed, and placed on the opposite side of the tank from which they had been removed. Any obvious feces were also removed from the tanks and the peat moss was churned to redistribute moisture.

Throughout the course of the study a total of five frogs died of uncertain causes. Three of the deceased frogs had wounds from shipping that had not healed, one had no signs of illness, and one frog began convulsing and exuding a white, sticky mucous during the final handling session which resulted in the frog being euthanized. The deceased frogs were removed from the tanks and in total two frogs died in the control group and three frogs died in the treatment group.

At the conclusion of the study, the frogs were removed from there respective rooms and re-massed using the same Mettler College1300 balance. The frogs were then anesthetized in order to collect blood samples by cardiac puncture (National Wildlife Health Center 2001). The frogs were partially immersed in a solution of 0.50 grams/L of MS222 (Cecala et al. 2007). Each frog took at least an hour to fully anesthetize after which time each frog was checked for pain response by toe pinching. Once unresponsive, the frogs were laid on their backs on a paper towel dampened with the MS222 solution. The heart was located under the center of the sternum at the same level that the frogs' elbows rested against its body. The area was sprayed with Bactine before a 100 U/I insulin syringe was inserted into the heart. Once a small amount of blood entered the hub of the syringe the needle was carefully rotated to maintain a flow of blood. Once the flow of blood stopped, the needle was removed and soft compression was place on the area with a Kimwipe. Bactine was reapplied to the insertion site and the frogs were placed into a tub of de-chlorinated water to recover from anesthesia.

The blood samples were injected into heparinized capillary tubes which were sealed at both ends and centrifuged. The hematocrit value for each blood sample was then calculated. Plasma was removed from the capillary tubes and stored in bullet tubes at -72°C. A significant amount of plasma (>12 µl) was collected from only six frogs (3 control: 3 treatment). One week after collection a blood glucose assay was conducted using the plasma samples and Cayman's Glucose Assay Kit (kit#10009582, www.caymanchem.com). The samples were prepared as directed and read at an absorbance of 515 nm. A standard curve was created and used to determine the blood

glucose concentration of each frog's blood plasma. A cortisol assay could not be conducted due to an insufficient amount of plasma collected.

After all data were collected, statistical analyses were conducted to determine if a difference existed between the treatment and control group. A t-test assuming equal variances was used to determine if the groups showed significant difference in mass, hematocrit level, or blood glucose concentration. Lastly, behavioral observations were analyzed using StatView. An ANOVA was used to determine if behaviors differed significantly between the day and night observations. The frequency that each behavior occurred in each of the control and treatment groups was analyzed by a t-test assuming equal variances to determine if any significant discrepancy in behaviors existed between the stressed and unstressed frogs. Linear regression analysis was conducted to determine if the frequency of behaviors changed over time for each group.

CHAPTER III

RESULTS

At the conclusion of the study, the masses of the thirteen living frogs (7 control:6 treatment) were averaged and plotted, including standard error (Figure 3). The masses of treated and control frogs were not statistically different (p=0.785). The masses of the frogs did change on an individual basis throughout the course of the experiment; however, this change was evident in both groups and resulted in no significant difference in mean mass of the frogs in each group. In both groups, the larger, more mature frogs (>16 grams initially; p=0.05) significantly lost weight during the course of the study while the smaller, more juvenile frogs (<16 grams initially; p=0.59) showed no such weight loss (see Figure 2). The mean hematocrit levels for the control and treatment groups at the conclusion of the study were also found to show no significant difference (p=0.375, see Figure 4). Only enough blood was collected from 6 frogs (3 control:3 treatment) to analyze blood glucose (BG) levels. The BG levels were determined based on the standard curve and line of best fit in Figure 5. No significant difference was found between the BG levels of the control or treatment groups (p=0.68, see Figure 6).

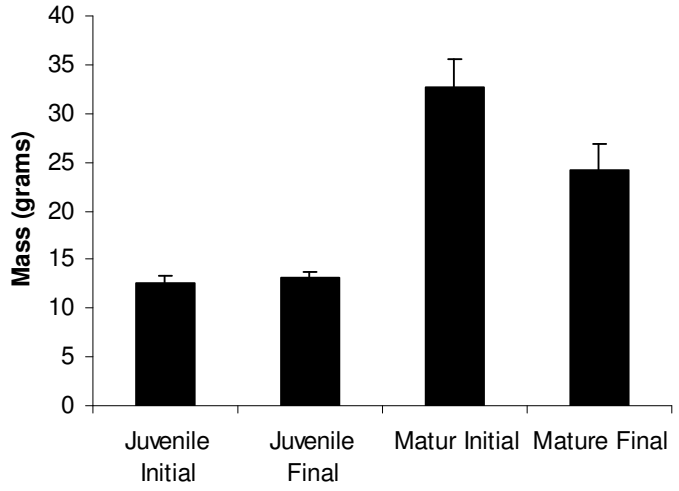

Figure 2: Average mass (+1SE) of juvenile and mature *Litoria caerulea* initially and at the conclusion of the study.

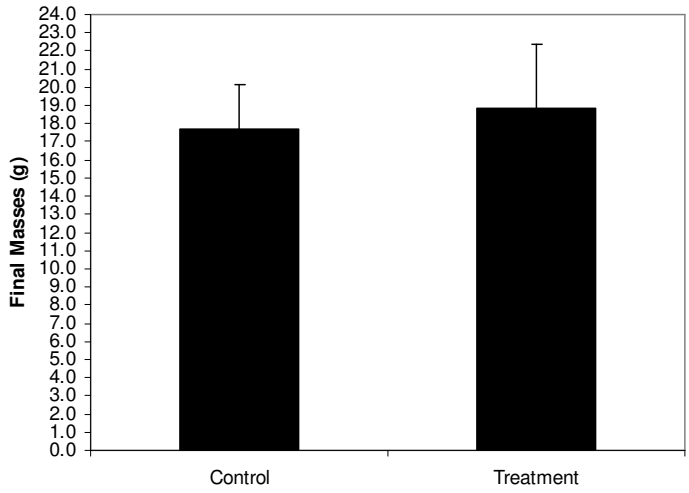

Figure 3: Final masses (+1SE) of *Litoria caerulea* in each group ($p = 0.785$)

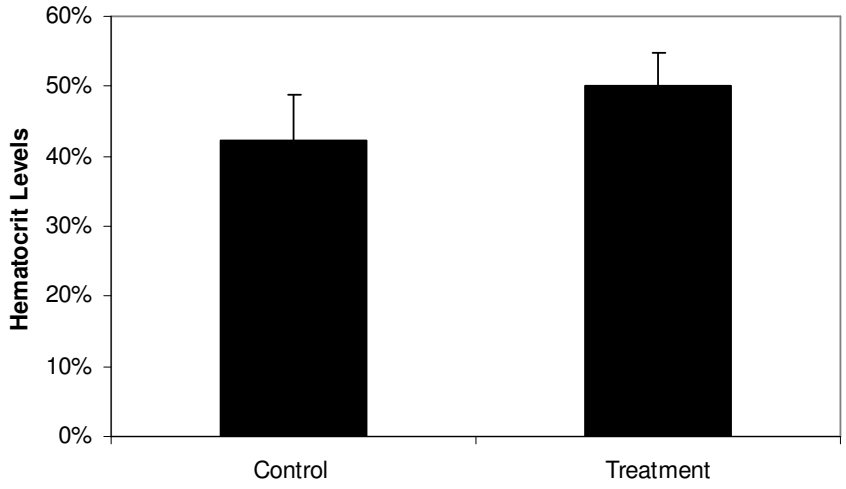

Figure 4: Hematocrit levels +1SE of *Litoria caerulea* (p=0.375)

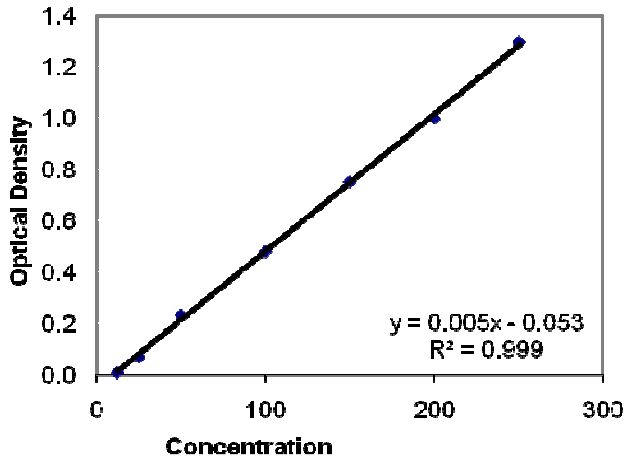

Figure 5: Standard curve of glucose concentrations created using Cayman's Glucose Assay Kit.

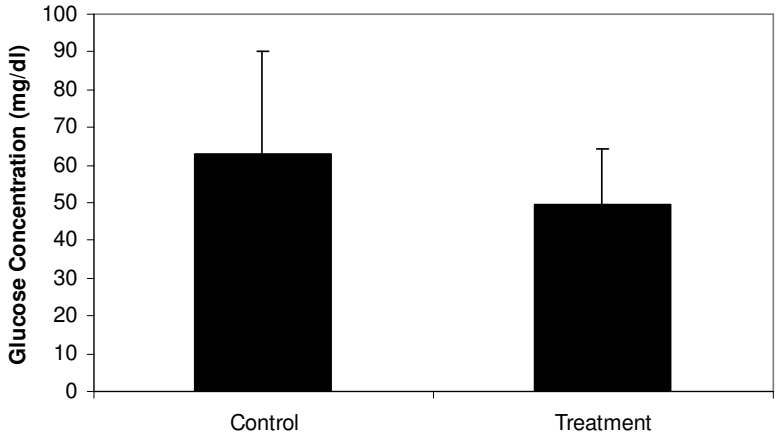

Figure 6: Blood glucose concentrations (mg/dl) +1SE of *Litoria caerulea* (p=0.680).

The behaviors of *Litoria caerulea* were analyzed for statistical significance. Due to observations being conducted at three different times of day, it was necessary to determine if any variation in the frequency of behavior existed between these times. According to an ANOVA, no difference in the frequency of behavior existed among the morning, evening, and feeding observation times (p=0.835) allowing all of the observation times to be pooled and analyzed together. In comparing the frequency of each behavior in the control and treatment groups throughout the entire course of the experiment, none of the behaviors differed significantly in frequency between the control and treatment groups (see Table 3).

The observed behaviors were additionally analyzed for change in frequency over the course of the experiment within each group. The treatment group remained consistent in behavior frequency from day one of the study until day 12, which is evident from the p-values listed in the third column of Table 3. The control group did show significant change over time for two behaviors. For the control frogs, the frequency of bathing

decreased over time (p=0.049, see Figure 7), whereas traveling increased through the course of the experiment (p=0.020, see Figure 8).

Table 3: ANOVA results for behavior in treatment and control groups of *Litoria caerulea.* Significant values are shown in bold.

Behaviors	Control vs. Treatment behavior frequency (p-values)	Control – Change in behavior frequency over time (p-values)	Treatment – Change in behavior frequency over time (p-values)
Sleeping	0.438	0.703	0.096
Bathing	0.628	**0.049**	0.158
Stalking	0.979	0.265	0.835
Eating	0.964	0.925	0.576
Escape	0.441	0.521	0.731
Sedentary	0.686	0.143	0.222
Traveling	0.593	**0.020**	0.946
Repositioning	0.168	0.830	0.253

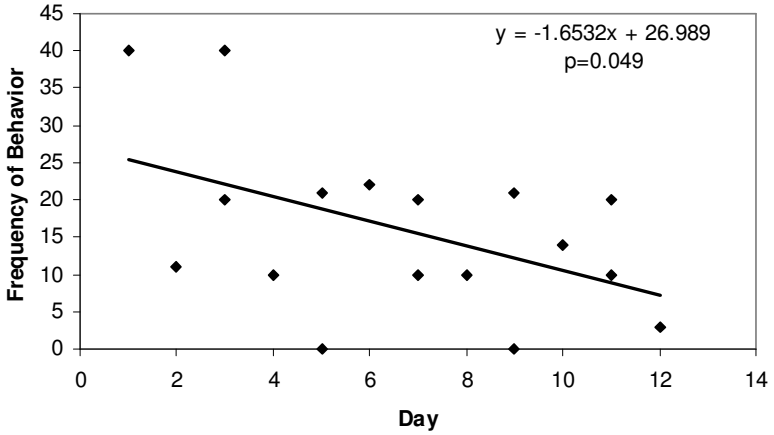

Figure 7: Frequency of bathing for *Litoria caerulea* in control group throughout course of study.

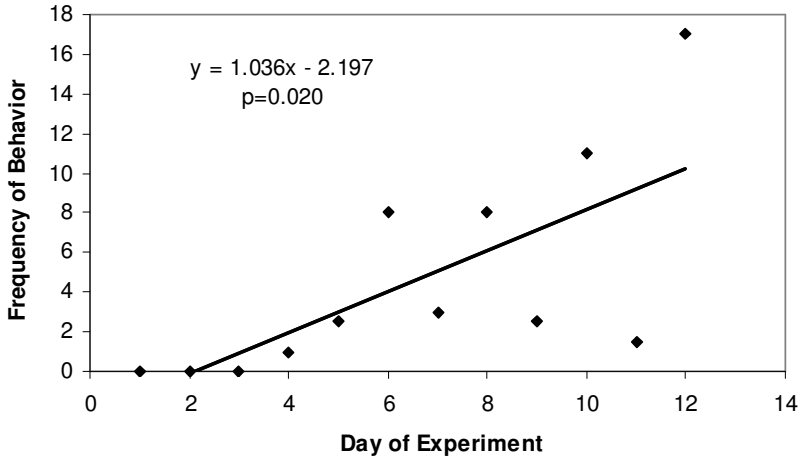

Figure 8: Frequency of traveling for *Litoria caerulea* in control group throughout course of study.

During the course of the study 2 frogs died in the control group and 3 frogs died in the treatment group; however, according to a chi square test with Yates correction the death rates did not differ significantly between the groups (p=0.599). All but one of these frogs had a wound from shipping remaining in its snout. At the conclusion of the study, the frogs were anesthetized using 0.5 g/L MS-222. All of the frogs anesthetized fully within approximately an hour and were unresponsive to pain while anesthetized. The frogs awoke fully approximately 30 minutes after blood collection and rinsing in de-chlorinated water. The frogs assumed normal behaviors after recovering from anesthesia; however, within 6 weeks, 12/13 of the frogs died. The majority of frogs died within two weeks after blood collection; however, only one or two of these frogs had snout wounds.

CHAPTER IV

DISCUSSION

The effects of household stress on *Litoria caerulea* through the course of this study do not support the original hypotheses that frogs enduring typical household stressors would exhibit elevated levels of blood glucose and decreased body mass. These findings also suggest that if enough blood was obtained from a majority of the frogs, the hypothesis that stressed frogs would exhibit elevated cortisol levels would also not be supported; however, this study was not able to determine whether household stressors cause elevated CORT levels in White's tree frogs.

Hematocrit levels did not differ significantly between control and treatment frogs, although treated frogs did have slightly elevated hematocrits. Previous studies on the hematocrit of hydrated and dehydrated toads indicate that the hematocrit levels measured in this study correspond to levels in toads dehydrated to 75% of their mass (41.8 +/- 8.5%; Warren and Vitalis 2005). These elevations in hematocrit levels are believed to aid in gas exchange in dehydrated frogs by increasing the capillary residence time of erythrocytes and increasing carbon dioxide excretion. Carbon dioxide excretion is increased as a result of the increased carbonic anhydrase per volume of blood (Warren and Vitalis 2005). This finding could indicate that even though they were provided with

ample water, the frogs used in this study were mildly dehydrated due to low relative humidity in the building in which they were housed. In comparison, one older study on *Xenopus laevis* indicates that hematocrit levels did not change in aquatic *Xenopus* during dehydration death (Hillman 1978).

The blood glucose levels measured in control and stressed *Litoria caerulea* frogs, while not significantly different, are elevated in comparison to two other species of amphibians, *Rana catesbeiana* and *Bufo parachemis* (Steiner 2000). The basal BG levels for these anurans are 40.35 +/- 7.25 and 27.25 +/- 1.14 mg/dl for *Rana catesbeiana* and *Bufo parachemis* respectively whereas the mean BG level in *Litoria caerulea* was determined in the present study to be 56.3 mg/dl . In the American bullfrog, *Rana catesbeiana,* short term handling stress resulted in no significant increase in plasma glucose levels (Mbangkollo and deRoos 1983). The observed elevations in BG may correlate with the elevated hematocrit due to dehydration and the resulting stress on both control and treatment *Litoria caerulea*. Alternatively, control frogs could be experiencing mild physiological stress. Future studies of endogenous blood glucose concentrations are required to determine if chronic stress in amphibians results in elevations seen in other vertebrate species (Edwards and Silver 1972).

The elevation in circulating glucose and hematocrit levels in control and treatment *Litoria caerulea* likely indicate acute stress in both groups resulting from struggle during anesthetizing and blood collection by cardiac puncture. Data compiled on the hyperglycemic effects of blood collection by cardiac puncture indicate that this technique results in minimal elevation of BG levels (Baranowski-Smith and Smith 1983); however, lactate levels, a metabolite indicated as a more precise measure of acute stress, are shown

to be 7 or more times greater in frogs where blood was collected by cardiac puncture rather than a surgically placed cannula (Mbangkollo and deRoos 1983, Farrar and Frye 1979a,b). Studies on the American bullfrog indicate that elevation in plasma lactate and hematocrit levels result from a neural response to acute stress while severe or prolonged stress activates the HPA axis (Mbangkollo and deRoos 1983, Rosenthal and deRoos 1985). In this study, the stress caused by struggle during anesthetizing and handling during blood collection resulted in direct nervous stimulation of muscles which caused increased lactate production and hematocrit. Increase in lactate production results from anaerobic glycolysis in the muscles that is used to power short bursts of activity, such as kicking in the anesthetic solution to escape (Bennett 1978). Future studies need to be conducted to quantify the effects of struggle during anesthetizing on lactate levels in the White's tree frog. Handling stress also stimulates the spleen to release stored erythrocytes resulting in increased hematocrit levels in control and treatment frogs (Mbangkollo and deRoos 1983). In amphibians, the response to stress is a sequential release of neurotransmitters, catecholamines, and corticosterone that work synergistically to regulate physiologic responses to stress (Mbangkollo and deRoos 1983). Elevation in corticosterone possibly functions as homeostatic mechanism to replenish liver and muscle glycogen stores by increasing the rate of gluconeogenesis and is therefore not a primary response to stress (Coulson 1979).

This study showed that household stressors have some significant effects on the behavior of *Litoria caerulea* with respect to the activity frequency of the control frogs. The control frogs that were minimally disturbed through the course of the study increased their level of activity through traveling within the tank while decreasing their sedentary

behavior of bathing. These findings indicate that while White's tree frogs do not show gross measures of physiological stress, their behavior is altered by household stressors and in order for households to enjoy these animals and their natural activities, stresses should be avoided. By placing these animals in a quiet room away from heavy traffic and minimizing handling, White's tree frogs kept as pets will likely be significantly more active.

Future research is greatly needed on the effects of household stress on White's tree frogs as well as most other exotic pets. Corticosterone levels are a valuable measure of the effects of chronic household stress; however, quantifying these levels in small anurans is limited by the difficulty of blood sample collection. The present study, while employing the well-studied and recommended method of blood collection in frogs (National Wildlife Health Center 2001), indicates that cardiac puncture should not be used for clinical testing of White's tree frogs, especially from those being kept as pets. Deaths are reportedly rare for this method (<1%) (National Wildlife Health Center 2001). While in the present study no animals died directly after bleeding, the death rate two weeks post bleeding was greater than 90%. For stress studies in the larger American bullfrog, the use of a nonocclusive cannula placed in the left truncus arteriosis is effective for the collection of multiple blood samples; however, this method is invasive, requires an extensive surgical procedure, and is not a viable option for use in veterinary procedures on the much smaller White's tree frog (Mbangkollo and deRoos 1983, Rosenthal and deRoos 1985). As the field of exotic animal veterinary medicine grows, research is greatly needed to develop less invasive clinical procedures for anurans.

In summary, exposure of White's tree frogs to common household stressors does not appear to induce physiological stress. However, several subtle behavioral changes are noted. In addition, the blood collection method often recommended for anurans (cardiac puncture) is not a viable collection technique for White's tree frogs. Future studies should (1) further investigate the effects of stressors on bathing and traveling behaviors, (2) develop a non-invasive blood collection technique for small anurans, and (3) investigate the incidence of delayed mortality caused by cardiac puncture in wild anurans.

APPENDICES

APPENDIX A

Group: Control Date: Time: Temperature:

Behavior (in the minute)	1	2	3	4	5	6	7	8	9	10
Sleeping										
Bathing										
Stalking										
Eating										
Escape										
Sedentary										
Traveling										
Repositioning										

Group: Treatment Date: Time: Temperature:

Behavior (in the minute)	1	2	3	4	5	6	7	8	9	10
Sleeping										
Bathing										
Stalking										
Eating										
Escape										
Sedentary										
Traveling										
Repositioning										

APPENDIX B

MARYVILLE COLLEGE
Institutional Animal Care & Use Committee (IACUC)
Animal Research Form

Provide information after each bold item

Student Name: Erin French

Student Email Address: erin.french@maryvillecollege.edu

Date: 3/1/2007

Senior Study Advisor: Dr. Drew Crain

Species to be used: White's tree frog (*Litoria caerulea*)

Age of animals: Adults (2-4 inch SVL)

Number of animals in study:
18

Duration of study:
2 months total, ~3 week experiment (animals will be held and observed until testing in the summer)

Location of animals during the study (building and room) :
Sutton – non-aquatic animal room

List personnel to call if problems with animals develop:

Name	Daytime Phone	Nighttime Phone	Emergency No.
Erin French	335-7629	N/A	977-6958 (Work)
Jeff Dols	406-2166	N/A	N/A
Lindsey Hill	256-0754	N/A	N/A
Vickie French	748-3674	984-1981	748-3674

Husbandry Requirements: Is anything other than routine care and equipment required?
YES___ No _X_ If "YES", please list below.

What will happen to the animals at the end of the study? If euthanasia is required, state the methods.
The animals will be adopted out to educated homes with the help of Animal Rescue and Fostering Networks (ARFNets). ARFNets is a local rescue group that places animals into foster homes until they are adopted.

(Do not write below line: For MC IACUC Use)

Maryville College IACUC Approval Number: ___200702_____
Date Approved: __April 3, 2007_____
Signed:

_____

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