1 How do duration, frequency, and intensity of exogenous CORT elevation affect immune
2 outcomes of stress?

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ABSTRACT

Stress is typically characterized as “acute” (lasting from minutes to hours) or “chronic” (lasting from days to months). These terms are of limited use as they are inconsistently used and only encompass one aspect of the stressor (duration). Short and long duration stress are generally thought to produce specific outcomes (e.g. acute stress enhances while chronic stress suppresses immune function). We propose that aspects of stress other than duration, such as frequency and intensity, are important in determining its outcome. We experimentally manipulated duration, frequency, and intensity of application of exogenous corticosterone, CORT, in *Sceloporus undulatus* (Eastern fence lizards) and measured the immune outcomes. Our findings reveal that immune outcomes of stress are not easily predicted from the average amount or duration of CORT elevation, but that intensity plays an important role. Although three of our treatments received the same average amount of CORT, they produced different effects on immune outcomes (hemagglutination). As predicted by the literature, short-duration exposure to *low*-dose CORT enhanced hemagglutination; however, short-duration exposure to *high*-dose CORT suppressed hemagglutination, suggesting that stressor intensity affects immune outcomes of stress. While both are traditionally termed “acute” based on duration, these treatments produced different immune outcomes. Long-duration (“chronic”) exposure to CORT did not produce the expected suppression of hemagglutination. Frequency of CORT application did not alter immune outcomes at low intensities. These results highlight the need to quantify more than just the duration of a stressor if we are to understand and manage the ecological consequences of stress. Specifically, we should consider stressor frequency and intensity, as well as duration, for a more complete characterization and understanding of stress.

Keywords: acute stress; chronic stress: corticosterone; *Escherichia coli*; hemagglutination;
lizard

1. INTRODUCTION

The influence of stressor duration on physiological outcomes is well recognized; however, less is known about the role of other stress aspects. Stress is typically characterized by its duration, as “acute” or “chronic”. Acute stress is characterized as “short” in duration (Burchfield, 1979; Romero, 2004), lasting from minutes to hours (Boonstra, 2012; Harbuz and Lightman, 1992; Martin, 2009), and the stressor is usually not repeated. The stress response is generally considered to be adaptive in the short term as it can facilitate the response to and recovery from a threat (Munck et al., 1984; Sapolsky et al., 2000). For example, the production of glucocorticoids (such as cortisol or corticosterone, CORT) in response to a stressor can mobilize energy to allow a fight or flight response (Sapolsky et al., 2000), enhance immune function to deal with increased risk of injury and infection (Dhabhar, 2009; Martin, 2009), and alter metabolism to help an individual maintain homeostasis (Stratakis and Chrousos, 1995). Chronic stress is characterized as “long” in duration (Burchfield, 1979; Romero, 2004; Sapolsky et al., 2000), lasting from days to months (Boonstra, 2012; Dhabhar, 2009; Martin, 2009), and includes persistent or frequent repeated stress (Harbuz and Lightman, 1992; Romero, 2004), such as frequent tourist visits or sub-lethal predator attacks (“repeated acute;” Burchfield 1979; Busch et al. 2008a; b). Persistent or long term activation of the physiological stress response can push animals into allostatic overload, whereby more energy is required for allostasis than can be obtained from the environment (McEwen and Wingfield, 2003). This can lead to energy being diverted away from other energy-sensitive functions, which in turn may lead to suppressed immune function

(Dhabhar, 2009; French et al., 2007; Guillette Jr. et al., 1994; Martin, 2009), growth rates (Chrousos and Gold, 1992; Laugero and Moberg, 2000), and reproductive output (Greenberg and Wingfield, 1987; McGrady, 1984). However, long-duration exposure to stress does not always have these suppressive effects (Chester et al., 2010; McCormick and Langkilde, 2014; Miles et al., 2007). It is also unclear at what point in time acute stress become chronic, and where repeated acute stressors actually fall on the acute - chronic spectrum (Busch et al., 2008a, 2008b). The terms “acute” and “chronic” may not be entirely useful, as they are inconsistently used and only encompass one aspect of the stressor (duration).

While it is clear that stressor duration influences the physiological outcome of stress (citations above), other characteristics of a stressor may also play a role. Intensity and frequency may have important influences on the outcomes of stress. These characteristics are largely ignored in the literature (but see intensity: Ottenweller et al. 1989; McEwen et al. 1997; frequency: McCormick et al. 1998; Busch et al. 2008), and are not considered simultaneously or in combination with duration. We suspect that considering only duration of stress is hampering progress towards understanding how stress can have different outcomes (Romero et al., 2009).

In this study, we systematically investigated the effects of duration, frequency, and intensity of CORT on immune outcomes using *Sceloporus undulatus* (Eastern fence lizards). We experimentally elevated CORT, the primary glucocorticoid stress-relevant hormone in reptiles, using various application regimes that differed in duration (length of time exposed), intensity (concentration of CORT dosage), and frequency (how often exposed), and determined how these stress aspects affected immune outcomes (Fig. 1). We then determined whether the traditional classification of these treatments as “acute” or “chronic” matched the expected immune outcomes (immune enhancement vs suppression, respectively). We chose to manipulate CORT.

Many other components of the HPA axis, including hypothalamic and pituitary hormones, as well as the sympathetic nervous system are also important in the physiological stress response, and may respond differently to various stress aspects. CORT plays an important role in the stress response, its elevation is frequently linked to negative consequences (Dhabhar, 2009; French et al., 2007; Guillette Jr. et al., 1994; Martin, 2009), and our manipulation of CORT will provide insight into how organisms respond to stressors with different characteristics.

21 METHODS

2.1 Study System

Between May and July 2013, we collected 32 adult male *Sceloporus undulatus* (Eastern fence lizards) using a hand-held noose. Lizards were collected from five sites across the Southern United States: (1) Standing Stone State Park, Overton County, Tennessee; (2) Fall Creek Falls State Park, Van Buren County, Tennessee; (3) Holly Springs National Forest, Marshall County, Mississippi; (4) Conecuh National Forest, Covington County, Alabama; and (5) Blakeley State Park, Baldwin County, Alabama. We measured lizards for snout-vent length (SVL) at capture and placed lizards in individual cloth bags for transport.

Lizards were housed individually in plastic enclosures (42 x 28 x 27 cm L x W x H). Outer walls of these enclosures were wrapped with dark paper to prevent lizards from visually interacting, which could affect CORT concentrations. Each enclosure was lined with a paper towel and furnished with a water dish and plastic shelter for refuge and basking. Overhead lights were set to a 12:12 light:dark schedule, and a 60-W incandescent light bulb was placed above the shelter provided additional heat for 4 hours each day to allow lizards to thermoregulate. Lizards were fed crickets (*Acheta domestica*) every second day.

2.2 Treatments

Lizards were housed for at least 10 days (10-73 days) to allow lizards to acclimate and CORT concentrations to return to baseline (i.e. CORT levels declined and then remained relatively constant; Trompeter and Langkilde, 2011). Lizards from each site were assigned randomly to one of 5 treatment groups, accounting for site of capture: 4 CORT-application treatments and one control, all with appropriate handling controls (Fig. 1). These were selected to allow us to test the contribution of differences in duration, frequency and intensity of elevations in CORT concentrations in determining immune outcomes, ensuring all lizards received the same handling and exposure to CORT vehicle. Two treatments represent those typically termed acute (by duration), but with differing intensities (different dosages applied): a high-dose acute (HA; high but ecologically relevant) and a low-dose acute (LA). Lizards in these treatments received a high or low dose, respectively, every third day and oil vehicle only on the remaining days (described below). One treatment represented repeated acute (RA) and involved daily applications of the low dosage of CORT. The final treatment represented a chronic treatment (Ch), and lizards in this treatment received CORT via a slow-release patch. Lizards in the HA, RA, and Ch treatment groups all received the same total (and average) amount of CORT in each 3-day period (and thus over the entire treatment period).

Each day for 9 days, all lizards were given topical oil containing the appropriate amount of CORT (high-dose, low-dose, or none), and every third day all lizards were fitted with slow-release patches containing CORT or oil-vehicle only (Fig. 1). All lizards received topical doses daily and a slow-release patch every third day, resulting in similar handling for all lizards (only the presence of CORT in the oil solutions differed). The oil (and hormone, where relevant) were

quickly absorbed due to the lipophilic nature of lizard skin (Belliure and Clobert, 2004) resulting in physiologically and ecologically-relevant increases in plasma CORT concentrations (Knapp and Moore, 1997; Trompeter and Langkilde, 2011). The lower dosage was selected to mimic the CORT response that occurs in this species in response to an attack by a fire ant predator. We treated animals in the evening (between 1500 and 1700) because animals were less active at these hours, negating the need to handle lizards during topical application.

We dissolved CORT ($\geq 92\%$, Sigma C2505, Saint Louis MO) in commercial sesame oil at two different physiologically relevant concentrations: 1 μg CORT / 1 μL sesame oil and 3 μg CORT / 1 μL sesame oil. We applied 6 μL of the appropriate dose to provide 6 μg (“low” dose) or 18 μg (“high,” but physiologically relevant, dose) of CORT ($= \sim 0.66 \mu\text{g}$ or 2 μg CORT / g lizard respectively; Fig. 1). Topical oil and oil-CORT solutions were applied using a repeat pipette, resulting in elevated CORT levels 30 minutes after application (Trompeter and Langkilde, 2011). Patches were constructed from the padded section of an adhesive bandage, which held the oil or oil-CORT solution, and electrical tape and transparent surgical adhesive, which secure the patch to the back of a lizard (Knapp and Moore, 1997). Patches were loaded with 6 μL of either the high CORT solution (18 μg CORT) or oil-vehicle alone (Fig. 1) and provided a slow release of hormone to the lizard over the 3-day period (Knapp and Moore, 1997). Patches were replaced on Days 1, 4, and 7 of the treatment period at the usual time of treatment, and handling animals was necessary to attach patches.

2.3 Blood Collection

After the 9 days of treatment, we collected blood samples from the lizards’ post-orbital sinus using 70 μL heparinized microhematocrit tubes (VWR, San Francisco CA). Blood was

collected within 3 minutes of capture (51 – 198 seconds). Blood samples were centrifuged, and plasma was drawn off and immediately frozen (-20°C) until assays were performed (24hr for hemagglutination assay; 4 days for bacterial killing assay; Graham *et al.* 2012).

2.4 Hemagglutination assay

As a measure of innate immunity, we measured the ability of lizard plasma to hold sheep red blood cells (SRBC) in suspension *in vitro*. We washed SRBC (Innovative Research, Novi, MI) with phosphate buffered saline (PBS) and brought it to a 2% solution with PBS. We thawed 25µL of plasma, diluted it 1:1 with PBS (25 µL), and serially diluted this solution to 1:64 in a 96-well plate using a multichannel pipette. Control wells contained PBS only (25 µL). We then added 25µL of 2% SRBC solution to each well and gently vortexed plates. After incubation at room temperature for 1 hour, we scored plates for agglutination (ability of antibodies in plasma to hold SRBC in suspension). Scores were calculated as the negative log₂ of the last dilution at which agglutination was attained – higher scores indicate higher concentration of SRBC-specific antibodies in the plasma (Matson et al., 2005). Half scores were recorded when SRBC precipitated partially but not to the extent of control wells. Lysis of erythrocytes was not scored since this process has been shown to be subjective (Matson et al., 2005).

2.5 Bacterial killing ability

As an additional measure of innate immunity, we measured the ability of plasma to lyse *Escherichia coli* bacteria. We mixed thawed plasma (14 µL) with *E. coli* (10 µl of 200 CFU bacteria dilution). We allowed this solution to react and combined it with a growth medium dilution (126 µl CO₂ L-glutamine, containing 400 µL L-glutamine and 19.6 mL CO₂ medium).

We spread 50 μ l of each sample in duplicate on agar plates, which were incubated at 37°C for 15 hr. Colonies on each plate were then counted, averaged (across duplicates) and compared to mean colony counts of two replicated control plates that contained no plasma (140 μ l CO₂ L-glutamine medium + 10 μ l *E. coli*). Percent bacterial killing was calculated as 100 - (mean plasma treatment colony count/mean control colony count) x 100. For detailed methods, see (Graham et al., 2012). Plates that ranged from 0 to negative 10% killing (n=11) were corrected to 0%. Plates with less than negative 10% killing (n=9) were discarded (see Fig. 2b for final sample sizes).

2.6 Data Analysis

SVL was log transformed and percent killing data were angular transformed prior to analysis to meet assumptions of parametric tests. We analyzed hemagglutination of plasma and percent killing separately using ANCOVA with treatment and source population as factors and snout-vent length (SVL) and time in captivity as a covariate. Time in captivity did not significantly explain variation in the immune data ($p > 0.78$), suggesting that being held in captivity for longer than 10 days does not affect these physiological measures. It was thus removed from the final model. We used a post-hoc least square means student's t-test to test for differences in hemagglutination scores between treatments. All statistical analyses were performed using JMP (version 7.0, SAS Institute Inc., Cary NC) with $\alpha = 0.05$.

3. RESULTS

3.1 Hemagglutination

Hemagglutination scores were significantly affected by treatment (Fig. 2a; $F_{4,21}=4.503$, $p=0.009$; SVL $F_{1,21}=1.747$, $p=0.200$; source population $F_{5,21}=1.816$, $p=0.153$). Post-hoc tests revealed that lizards in the HA treatment had significantly lower hemagglutination scores than those from LA, RA, and Control treatments; and lizards from the Chronic treatment had intermediate hemagglutination scores.

3.2 Bacterial Killing Ability

Bacterial killing ability did not significantly differ among our treatment groups (Fig. 2b; $F_{4,17}=0.865$, $p=0.510$; SVL $F_{1,17}=0.099$, $p=0.757$; source population $F_{5,17}=4.685$, $p=0.007$).

4. DISCUSSION

The four treatments in which we manipulated CORT (mimicking the physiological stress response) and the control treatment produced very different immune outcomes in this study: lizards in the High Dose Acute (HA) treatment expressed lower concentrations of sheep red blood cell (SRBC)-specific antibodies in their plasma (lower hemagglutination scores) than those from the Low Dose Acute (LA), Repeated Acute (RA), and Control (Ctl) treatments, and lizards from the Chronic (Ch) treatment expressed intermediate hemagglutination (Fig. 1 and 2). This raises a number of concerns with considering duration alone when investigating the physiological consequences of stress, as discussed below. Here we describe our results for the effects of total, average, duration, frequency, and intensity of CORT application on immune function, and the potential implications for furthering our understanding of the consequences of stress more broadly. Future research examining how other aspects of the stress response, such as

the sympathetic nervous system or hypothalamic and pituitary hormones, might affect these general conclusions is needed.

4.1 Total or Average Stress (CORT)

Three of our treatments (HA, RA, Ch) all received the same amount of corticosterone (18µg CORT in each 3-day period, for 9 days total, Fig. 1). The immune outcomes of these treatments, however, differed. Plasma hemagglutination of novel protein (sheep red blood cells) was significantly lower for lizards receiving short infrequent elevations of high doses of CORT (High Dose Acute, HA) than for lizards receiving short frequent elevations of low doses of CORT (Repeated Acute, RA), and plasma of lizards receiving a slow release of high doses of CORT (Chronic, Ch) had an intermediate level of hemagglutination. While hemmagglutination of lizards in the HA and Ch groups did not differ significantly from controls, this nonetheless supports the notion that not all stress is the same: the average or total amount of CORT to which an individual is exposed (or the associated stress it experiences) is not a good predictor of the immune outcome, and specific details of the stress regime are needed to understand outcomes. The treatments in this study were selected to reflect differences in duration, frequency, and intensity of exposure to CORT (Fig. 1). Under these circumstances, intensity, but not frequency or duration of CORT elevation, affected the immune outcome.

4.2 Duration

Standard terminology implies that duration of exposure to a stressor affects immune outcome: repeated or continuous (days to months, “chronic”) exposure to a stressor should suppress immune function (Dhabhar, 2009; Martin, 2009; Sapolsky et al., 2000), whereas short-

duration (minutes to hours, “acute”) exposure to a stressor should enhance or have no effect on immune function (Deak et al., 1999; Dhabhar, 2009; Martin, 2009). We found no significant difference in plasma hemagglutination of lizards exposed to continuous elevation of CORT (Chronic, Ch) for 9 days versus that of lizards given short exposure to CORT (High Acute, HA) at the same dosage, every 3 days, suggesting that duration of exposure to a single stress event may not always have important consequences for immune function. However, we tested duration of the discrete events (e.g. CORT was elevated for hours vs. days; Knapp and Moore, 1997) while keeping exposure to the entire stress series consistent (all CORT treatments were applied over 9 days). It is therefore possible that we could find that duration affects immune outcomes if we alter the total duration of exposure to CORT (e.g. 1 day vs. 30 days of exposure to continuously elevated CORT).

4.3 Frequency

Frequent exposure to a short-duration *high* intensity stressor may result in continuous elevation of CORT, if CORT concentrations do not have the opportunity to return to baseline between events. Continuously elevated CORT could lead to outcomes typical of chronic stress (Dhabhar, 2009). For example, exposure to CORT in our High Acute (HA) treatment (every 3 days) may have constituted a physiological response to persistent (chronic) exposure to a stressor, which may explain why lizards in this treatment had similar immune outcomes to those continuously exposed to CORT in the Chronic (Ch) treatment, despite the fact that lizards were given three days between exposures to CORT in the HA treatment. Measuring circulating CORT levels would inform whether frequent, high intensity stressors prevent CORT concentrations from returning to baseline. These measurements would need to be non-invasive to avoid

affecting CORT levels and compromising the treatments. Our results, however, also suggest that repeated exposure to acute stressors do not always produce “chronic” (immune) outcomes. Lizards exposed to *daily* short-duration *low* dosage elevations of CORT (Repeated Acute, RA) produced outcomes typical of acute stress: this treatment elevated plasma hemagglutination similarly to lizards exposed to short duration low-dosage elevations of CORT *every 3rd day* (Low Dose Acute, LA). Repeated exposure to *low*-intensity elevations of CORT (Repeated Acute, RA) may have allowed CORT concentrations to return to baseline (though our previous study reveals that CORT concentrations are somewhat elevated above controls after 21 days of exposure; McCormick & Langkilde 2014). Therefore, frequency of CORT application did not appear to have important consequences for immune outcomes at low intensities, but frequency may be important at high intensities. Interactions between frequency and intensity of perturbations have also been shown to result in complex effects on species diversity in community ecology (Miller et al., 2011). Understanding how frequency and intensity of exposure to stress interact to affect the recovery of CORT concentrations to baseline, and subsequent immune outcomes, would be valuable for understanding the consequences of stress.

Our results suggest that repeated exposure to acute stressors may not always produce “chronic” (immune) outcomes, despite the fact that repeated acute stressors are frequently considered chronic (Harbuz and Lightman, 1992; Romero, 2004) and indeed are sometimes referred to as “chronic intermittent stressors” (Burchfield, 1979; Martí et al., 1994). These results support previous findings that repeated exposure to short-duration elevations in CORT enhanced immune function in this species and had no effect on immune function in other species (Table 1). However, many studies have found that repeated exposure to short-duration stress suppressed immune function (Table 1) and/or had negative effects on other fitness relevant traits including

growth rate (Barton et al., 1986; McCormick et al., 1998) and condition (Busch et al., 2008b). Together with these studies, our results demonstrate the range of physiological outcomes associated with repeated acute stress, and that repeated exposure to short-duration stressors does not always produce “chronic” effects (at least at low intensities). Delimiting the boundary at which the outcomes of stress are reversed would be hugely informative.

4.4 Intensity

Our results suggest that intensity of CORT exposure can affect immune outcomes. Short-duration exposure to stress is typically predicted to enhance or not affect immune function (Deak et al., 1999; Dhabhar, 2009; Martin, 2009). However, our two short-durations treatments produced very different immune outcomes: plasma hemagglutination of lizards exposed to short-duration *low-intensity* CORT (Low Acute, LA) were significantly greater than those exposed to short-duration *high-intensity* CORT (High Acute, HA); with hemagglutination of lizards in the control treatment being intermediate to these two (Fig. 2a). This suggests that “chronic” outcomes (e.g. immune suppression) could result even in response to short-duration stress if the stressor is sufficiently intense. Immune suppression has been observed in response to short-duration *pharmacological* levels of stress (Dhabhar, 2009, 2000), but our results suggest that immune suppression in response to short-duration stress is possible even at physiologically relevant levels. Such lasting consequences of high intensity stressors on immune function are also observed in response to post-traumatic stress disorder (PTSD) in humans (reviewed in: Altemus et al., 2006; Gill et al., 2009), suggesting the potentially broad importance of understanding how exposure to stress can modulate immune outcomes.

It is possible that our results showing different immune outcomes to acute elevations of
CORT arise in part from our binary treatment design (we only applied two levels of each stress
aspect). For example, when three levels of stressor intensity were examined, both mild and
intense short-duration stressors suppressed while moderate stressor enhanced cellular immune
responses (in rats: McEwen et al. 1997; Dhabhar & McEwen 1997). It may be that there is a non-
linear, unimodal response of immune function to stress, where mild and intense short-duration
stressors enhance immune function, while moderate stressors suppress immune function. Our
study may have constituted moderate (LA, immune enhancement) and intense (HA, immune
suppression) levels of stress. Only a study that systematically examines outcomes for all possible
combinations of frequency, intensity and duration of stress can comprehensively delineate the
complete stress response surface (Miller, Roxburgh, & Shea, 2011). And these stress aspects will,
of course, need to be scaled to the relevant traits of the organism of interest (e.g. life cycle, HPA
function) in order to make them generally applicable (Roxburgh et al., 2004).

5. Conclusions

Our results reveal that average, total, or other single measures of stress (CORT) do not
satisfactorily encompass either how stress is experienced (aspects such as frequency, intensity,
and duration) or the physiological outcomes (immune function). Additionally, categorizing stress
as acute or chronic – by duration alone – and the lack of consistency in use of these terms may be
hindering progress in this field. Our results suggest that additional underlying aspects of stress,
such as stressor intensity and frequency, can affect the outcomes of stress and should also be
considered and reported if we are to adequately describe and assess the ecological and human
health consequences of stress.

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DATA ACCESSIBILITY

Data are available through The Pennsylvania State University's data repository, Scholarsphere, at <https://scholarsphere.psu.edu/files/pv63g128r>.

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FIGURE CAPTIONS

Fig. 1 A) The frequency, intensity, and duration of CORT application in each of the treatments used in this study, and the total amount of CORT received in each 3-day period. Text in parentheses indicates: for Frequency, how frequently a CORT-oil solution was applied (oil-vehicle only was applied on remaining days); for Intensity, the amount of CORT applied during each application; and for Duration, whether the period of CORT elevation was short or long. *Italicized pairs in each column represents treatments that differ in only the parameter shown in that column.* B) A graphical representation of the amount of CORT applied for each of the treatments used in this study (Control (Ctl) had no CORT applied). This is provided for illustrative purposes, to convey the expected duration of CORT release following application.

Fig. 2 A) Hemagglutination scores and B) Percent Bacterial Killing of plasma of lizards after 9 days of treatment (see Fig. 1). Lizards in the Low Acute (LA), Repeated Acute (RA), and Control (Ctl) treatments had significantly higher hemagglutination scores than did those in the High Acute (HA) treatment, and those in the Chronic (Ch) treatment had hemagglutination scores that were intermediate to these groups. Lines above the columns connect treatments that do not significant differ from one another. Bacterial Killing ability did not significantly differ across treatments. Error bars represent means $\pm$ one standard error. The sample size for each group is given within each bar.

474 **Table 1.** This table shows the different immune outcomes (enhancement, no change, or suppression) to repeated exposure to stress
475 (exogenous application of CORT, handling stress, or an ecologically-relevant stressor), indicating the study species and immune
476 component measured. Exogenous CORT was elevated using topical application or feeding. Handling includes handling or chasing,
477 placement in a bag, or air exposure (fish). Ecologically relevant stressors include food deprivation, social isolation, social defeat, or
478 exposure to predator scents. *Animals were simultaneously restrained. Abbreviations as follows: Hemag. = hemagglutination; BKA =
479 bacterial killing ability; DTH = delayed-type hyper sensitivity; Antibody Resp. = antibody response.

480

	Enhancement	No Change	Suppression
Exogenous CORT Application	Hemag. Lizard (McCormick & Langkilde) 2014	BKA Lizard (McCormick & Langkilde 2014) Squirrel (Brooks & Mateo 2013) Antibody response Chicken (El-Lethey et al 2003)	BKA (post immune-challenge) Squirrel (Brooks & Mateo 2013) Hemag., DTH, Antibody Resp. Chicken (El-Lethey et al 2003)
Handling		BKA Fish (Eslamoo et al 2014)	Wound Healing Lizard (French et al 2006) Hemag. Fish (Eslamoo et al 2014)
Ecologically Relevant Stressor	BKA Hamster (Chester et al 2010)	Antibody response Chicken (El-Lethey et al 2003) Hamster (Chester et al 2010) Hemag. Hamster (Chester et al 2010) Wound Healing Rat* (Vegas et al 2012)	Hemag., DTH, Chicken (El-Lethey et al 2003) Antibody response Hamster (Chester et al 2010) Wound healing Hamster (Detilliona et al 2004)

481

Figure 1

A)

Treatment	Frequency	Intensity	Duration	Total CORT (over 3 days)
High Acute (HA)	Infrequent (every 3 days)	High (18 μ g)	Short (hours)	18ug
Low Acute (LA)	Infrequent (every 3 days)	Low (6 μ g)	Short (hours)	6ug
Repeated Acute (RA)	Frequent (daily)	Low (6 μ g)	Short (hours)	18ug
Chronic	Infrequent (every 3 days)	High (18 μ g)	Long (days)	18ug

B)

Figure 2