

VIRGINIA JOURNAL OF SCIENCE
OFFICIAL PUBLICATION OF THE VIRGINIA ACADEMY OF SCIENCE

Vol. 65

No. 3 & 4

Fall/Winter

TABLE OF CONTENTS

ARTICLES	PAGE
A Comparison of Techniques Measuring Stress in Birds.....	133
Deborah A. O'Dell, Michael A. Carlo, Abigail Kimmitt, Ellen Bikowski, Katherine R. Morris and Andrew Dolby	
Survey of the Ectoparasites of the Invasive Small Indian Mongoose (<i>Herpestes auropunctatus</i> [Carnivora: Herpestidae]) on St. John, U.S. Virgin Islands.	151
Kelsey L. Townsend and Karen E. Powers	
NECROLOGY	
Roy L. Williams.....	157
Leonard O. Morrow.....	159
ANNOUNCEMENTS	
VAS Symposium of State of Virginia's Environment.	161

A Comparison of Techniques Measuring Stress in Birds

**Deborah A. O'Dell¹, Michael A. Carlo, Abigail Kimmitt,
Ellen Bikowski, Katherine R. Morris and Andrew Dolby**

Department of Biological Sciences
University of Mary Washington
Fredericksburg, VA 22401

ABSTRACT

Free-living birds are subjected to both external and internal stresses which can affect their health, activity, and reproductive success. To study stress in free living birds, they must be captured in nets and handled by the researcher to take blood samples for commonly used measures of stress, an activity which itself can induce stress and confound results. This study compares the effects of handling time on three different measures of stress: levels of the stress hormone corticosterone (CORT), levels of Heat Shock Protein 60 (HSP 60) and the ratio of heterophils to lymphocytes (H/L ratio) in tufted titmouse (*Baeolophus bicolor*) captured at feeders between December and January (2011-2013) in Fredericksburg, VA. Blood samples collected between two and 15 minutes from 12 birds were assayed for levels of CORT and HSP and from 24 birds for H/L ratios. Relationships were examined between these stress indicators and handling time, body mass and body condition. CORT was significantly correlated with handling time ($p < 0.01$), which reinforces existing evidence of CORT's sensitivity to the way subjects are handled immediately prior to blood collection. HSP or H/L ratios were not affected by handling time, suggesting that they may be preferable indicators of stress in free living birds under some circumstances.

INTRODUCTION

Free-living birds face a variety of internal and external sources of stress, which may affect physiological function and reduce fitness. Acute stress results from a specific stressful event, such as an attack by a predator or sudden storms, whereas chronic stress results from prolonged exposure to biologically challenging conditions, such as exposure to extreme temperatures (Vleck et al. 2000), periods of limited food availability (Herring et al. 2011), and anthropogenic pressures such as pollution, habitat disturbance (Arriero et al. 2008; Busch and Hayward 2009), and from prolonged psychosocial stressors (Cyr et al. 2007; Cyr and Romano 2007; Landys et al. 2011). To

¹ Corresponding author: dodell@umw.edu

cope with such stressors, birds and other animals have a protective physiological stress response that allows them to withstand immediate threats to their homeostatic balance. When this response is elevated chronically, however, it can become biologically costly and have negative impacts on birds' fitness by weakening the immune system (Dabbert et al. 1997), which could increase susceptibility to disease, and compromising growth and reproduction (Sapolsky et al. 2000). Thus, stress levels can indicate the general physiological condition of birds and point to possible environmental perturbations.

Biomarkers such as the glucocorticoid corticosterone (CORT), heterophil/lymphocyte ratios (H/L), and heat shock proteins (HSPs) have all been used as tools to assess chronic or long-term stress in wild populations. These markers may be predictably regulated according to environmental conditions and various biological challenges (Gross and Siegel 1983; Sapolsky et al. 2000; Vleck et al. 2000; Moreno et al. 2002; Martinez-Padilla et al. 2004; Tomas et al. 2004; Davis 2005; Herring and Gawlik 2007; Busch and Hayward 2009; Cockrem et al. 2009; Krams et al. 2010; Herring et al. 2011), and thus can provide researchers with consistent methods of detecting stress experienced by birds in their natural habitats. However, they may also be affected to varying degrees by acute stress caused by capture and handling leading up to blood sample collection. Therefore, drawing inferences about birds' long-term stress status prior to their capture by researchers may be problematic since the relationships between the different measures used have not been adequately studied.

Quantifying glucocorticoids, such as CORT, is presently the most frequently applied method of assessing individual stress in birds (Sapolsky et al. 2000; Tomas et al. 2004; Herring and Gawlik 2007). However, the release of CORT is highly influenced by acute stress associated with capture and handling during field research which may confound results. Capture and handling can rapidly mobilize CORT, which can make the interpretation of CORT measurements difficult under some circumstances (Sapolsky et al. 2000; Romero and Reed 2005; Fridinger et al. 2007; Herring and Gawlik 2007; Busch and Hayward 2009; Cockrem et al. 2009). When handling time before sampling lasts for more than 2 or 3 minutes, CORT levels may no longer accurately reflect birds' physiological status before their capture (Romero and Reed 2005; Cockrem et al. 2009). In studies involving free-living birds, field conditions may prevent sufficiently prompt blood collection, leading to less reliable measures of chronic stress. Additionally, fecal CORT, can degrade over time in frozen samples (Herring et al. 2007) which reduces reliability of measurements. Herring and Gawlick (2007) compared the use of CORT with that of HSPs as ways of measuring stress associated with allostatic overload (when the energy requirements needed to maintain homeostasis in the body exceed the capacity of the animal) and concluded that HSPs have some advantages over CORT and may represent a viable supplementary or even alternative indicator of chronic stress.

Circulating H/L ratios have also been used to measure chronic stress in birds. These ratios are used because the avian immune response to stress takes significantly longer to initiate, by hours to days, than the rapid CORT response, and changes in leukocyte numbers last longer than changes in CORT levels (Davis et al. 2008). Their slower response to stress and longer endurance indicate that H/L ratios may be informative, especially in obtaining baseline stress measurements. However H/L levels are impacted by disease and infection and may not reflect true levels of stress to external stressors. While studies on Adélie penguins (Vleck et al. 2000) and house finches (Davis 2005)

indicate that H/L ratios are resistant to handling up to 1 hour, a study investigating wintering male great tits (Cirule et al. 2012) found that acute stress due to capture and handling caused an increase in heterophil counts between 30 and 60 minutes and a decline in lymphocyte counts between 60 and 120 minutes after capture. Therefore, H/L changes may be species specific and may change more rapidly than originally thought, which may limit the reliability of results in a way similar to CORT.

A different cellular response to stress is mediated by HSPs. HSPs are a family of proteins whose expression is increased when cells are exposed to both cellular stressors such as parasites (Merino et al. 1998; Martinez-Padilla et al. 2004; Arriero et al. 2008; del Cerro et al. 2010), limited food availability (Zulkifli et al. 2002; Herring et al. 2011), and sibling competition (Martinez-Padilla et al. 2004; Merino et al. 2006) as well as psychosocial stressors such as crating in birds (Zulkifli et al. 2009), fear (Al-Aqil et al. 2013) and social interactions in fish (Currie et al. 2009). They have been found to exist in almost all organisms, including bacteria, plants, and animals (Feder and Hofmann 1999). HSPs are a special class of proteins referred to as molecular chaperones which protect proteins from degradation and correct damage caused by stress-induced instability (Merino et al. 1998; Feder and Hofmann 1999; Tomas et al. 2004; del Cerro et al. 2010). Essentially, they serve to restore and maintain cellular homeostasis during times of increased stress (Tomas et al. 2004; del Cerro et al. 2010).

These molecules may be reliable indicators of chronic stress because they are maintained at high levels for longer periods after stressors are applied, and some research has shown that handling stress does not cause their rapid up-regulation (Martinez-Padilla et al. 2004; Herring and Gawlik 2007; Herring et al. 2011). However, the resistance of HSPs to acute stress triggered by capture and handling has not been systematically compared to those of CORT and H/L ratios in the same study.

The tufted titmouse (*Baeolophus bicolor*) is a good model species to expand research on the relationships between these three stress indicators and their relative sensitivities to handling time. Close relatives of the tufted titmouse, the blue tit and the great tit, have been used in past studies examining both the intracellular and hormonal stress responses (Arriero et al. 2008; Cockrem et al. 2009; del Cerro et al. 2010). The tufted titmouse is a canopy-dwelling, omnivorous permanent resident species of eastern North American deciduous forests (Grubb and Pravosudov 1994). During the winter, they spend time in flocks of about 2 to 5 individuals. Caching food during cold weather, they commonly frequent feeders and carry 1 seed at a time to store within 40 meters of the feeders, allowing them to be easily captured with mist nets (Grubb and Pravosudov 1994).

This study compared the sensitivity of HSP60, CORT, and H/L to acute stress induced by the capture and handling of tufted titmice to evaluate their reliability as tools in avian stress research. The relationships between CORT, H/L, and HSP60 and the time elapsed between capture of subjects and blood collection, as well as the relationships among these bioindicators, were analyzed. We predicted that CORT levels, but not H/L ratios or HSP60 levels, would be positively correlated with handling times longer than 2 minutes after capture of subjects. Finally, we examined whether CORT, H/L, and HSP values were correlated within subjects and related to body condition.

MATERIALS AND METHODS

Capture, Handling, Blood Sampling and Slide Formation

Tufted titmice were captured using mist nets set around feeders at sites in Spotsylvania and Stafford counties in Virginia. A total of 41 subjects were captured between December 2011 and February 2013. Of these, 24 were used to assay H/L ratios while 12 were used for CORT and HSP60 assays.

Blood samples were drawn after varying handling times after capture. Handling time was defined as the elapsed time in seconds from when a subject flew into the mist net to the start of blood sampling. Handling time was intentionally varied between 120 and 915 seconds (2-15 minutes) to simulate the range of handling times typical of this type of field research.

A 26-gauge needle was used to puncture the left brachial vein of each subject. A capillary tube was used to collect the blood and transfer it to an individually labeled 5.0 mL centrifuge tube. The blood samples were kept cool with a freezer pack in a small cooler for transport from the sampling site to the laboratory. Immediately upon return to the laboratory (1-4 hours after capture and sampling), the blood samples were centrifuged at 500 X G for 10 minutes to separate the plasma supernatant from the hematocrit. The plasma and hematocrit samples were kept frozen, separately, in individually labeled 0.5 mL centrifuge tubes in a subzero (-80 °C) freezer.

Preparation of Slides

At the time of blood sampling, a small drop of blood collected from each bird was used to create a blood smear on an individually marked microscope slide using the two-slide wedge method (Houwen 2000). The blood smears were air dried before being transported from the sampling site to the laboratory. Immediately upon return to the laboratory (1-4 hours after capture and sampling), blood smears were fixed in methanol for 30 seconds then stained using the Wright-Giemsa stain procedure. Slides were analyzed using a Leica compound light microscope.

Body Condition Measures

Right wing chord length was measured to the nearest 0.5 mm and mass to the nearest 0.5 g for each subject before its release. A body condition index (BCI) was calculated for each subject as a value obtained from the equation $\frac{mass}{winglength} \times 10^6$, a

modification of the condition index used by Owen and Cook (1977).

Heterophil to Lymphocyte Ratios

Stained blood smears were examined under a compound microscope with 1,000x magnification using oil immersion. Areas of each slide with a monolayer of erythrocytes were searched for leukocytes. Identification of leukocytes was based on information and images found in the *Atlas of Clinical Avian Hematology* (Clark et al. 2009).

For each individual slide, only fields of view with similar erythrocyte densities and those containing countable leukocytes were examined. If a field of view did not contain any leukocytes, it was skipped as the slide was systematically searched from field to field. For each leukocyte-containing field of view, counts were recorded for numbers

of heterophils, lymphocytes, total leukocytes, and total erythrocytes. Counting was arrested when at least 100 leukocytes were found on a slide.

To account for variability among cell counts owing to uneven distribution of cells in the blood smears, each slide was evaluated in duplicate by separate researchers and the resulting counts were averaged. H/L was calculated as the number of heterophils divided by the number of lymphocytes. To standardize the counts for comparison, the total number of leukocytes to the total number of erythrocytes counted on each slide was converted to the number of leukocytes per 10,000 erythrocytes. An H/L index was calculated for each slide by multiplying the H/L by the number of leukocytes per 10,000 erythrocytes.

Corticosterone Levels

Frozen plasma samples were brought back to room temperature and analyzed using a Corticosterone Enzyme Immunoassay (EIA) kit from Enzo Life Sciences International, Inc. The assay buffers and reagents were prepared according to the instructions in the kit. Corticosterone standards were prepared in 5 labeled tubes with concentrations of 20,000, 4,000, 800, 160, and 32 pg/mL. Plasma samples were diluted 1:30 with assay buffer to produce a 100 μ L sample as previously determined to provide the best results with this assay (Dolby et al. 2011). Samples were assayed in duplicate in a 96-well microwell plate according to the layout sheet provided in the EIA kit.

Following the EIA protocol, a 100 μ L of standard diluent, standards, and diluted plasma samples and 50 μ L of assay buffer, conjugate, and corticosterone antibody were dispensed into wells of the plate. The plate was incubated at room temperature on a plate shaker for 2 hours at 500 rpm, washed 3 times using the prepared wash buffer, and then aspirated to remove remaining wash buffer. After washing, 5 μ L of conjugate and 200 μ L of pNpp substrate solution were added to the wells. The plate was incubated at room temperature for 1 hour without shaking. Then 50 μ L of stop solution was added to every well to stop the reaction. The plate was immediately read at 405 nm optical density using an Eon microplate reader and Gen5 microplate reader software from BioTek Instruments, Inc.

Using the optical densities bound as reported by the software, the average net optical density bound for each standard and sample was calculated and the binding of each pair of standard wells was calculated as a percentage of the maximum binding wells. These calculated values were used to create a standard curve with which the concentration of corticosterone in each sample could be determined in pg/mL diluted serum. The concentrations calculated according to this procedure were recalculated to adjust for the initial dilution of the plasma samples, and the resulting concentrations were recorded for each sample in ng/mL serum.

HSP60 Concentrations

Frozen hematocrit samples were thawed and washed by adding 1.0 M PBS (BioRad) using gentle pipetting to disperse the cells. The samples were centrifuged at 500 X G for 10 minutes, and the supernatant removed and discarded. The cells were lysed by adding 100 μ L of extraction buffer (Enzo Life Sciences) containing 1 μ L of protease inhibitor cocktail (Thermo Scientific) to each sample. The cells were vortexed for 5 minutes, sonicated for 1 minute, and then centrifuged at 2500 X G for 15 minutes. Using the BioRAD DC Protein Kit, protein concentrations of the supernatant for each

sample was measured in duplicate along with standards of BSA (bovine serum albumin, BioRad). Sample absorbance was measured using an Eon microplate reader and Gen5 microplate reader software (BioTek Instruments, Inc.) at 750 nm. Protein levels for each sample trial were calculated from the generated standard curve, and the protein levels for the 2 trials for each sample were averaged to give total protein in mg/ml for each sample.

An Enzyme Linked Immunoassay (ELISA) kit for Human HSP60 (Enzo Life Sciences) was used to determine the concentration of HSP60 in each sample. This kit was previously shown to detect avian HSP60 in our lab (Humayon et al. 2010). The protocol from the kit was followed. HSP60 protein standards ranging from 50 ng/mL to 1.56 ng/mL were produced by dilution of the supplied protein standard with sample buffer. For the assay, samples were applied at 200 μ g of total protein/well, and each sample was assayed in duplicate. Sample buffer was added to each sample to give a final volume of 100 μ l. The samples, standards, and negative control (buffer only) were applied to the assay plate using 100 μ L total volume per well, and the plate was incubated for 1 hour at room temperature. The plate was washed with a 1X Wash Buffer to remove unbound antigens, followed by the addition of 100 μ L of biotinylated anti-HSP60 (Enzo) to each well. The plate was incubated for a further 30 minutes at room temperature, and washed again with wash buffer. Addition of 100 μ L of HRP conjugate (Enzo) to each well, was followed by incubation for 10 minutes at room temperature. After washing, 100 μ L of TMB substrate (Enzo) was added, and the reaction was allowed to proceed for 5-10 minutes producing a detectable color change. Stop solution was added, and the absorbance was measured using an Eon microplate reader and Gen5 microplate reader software (BioTek Instruments, Inc.) at 450 nm. The total mass (ng) of HSP60 in each sample was calculated based on absorbance relative to the standard curve. This value was then divided by 200 μ g to express the results as ng of HSP60/ μ g of total hematocrit protein for each sample.

Statistical Analysis

Blood samples were collected from a total of 41 birds. After obtaining the CORT and HSP60 concentrations and H/L for each sample, only samples with less than a 10 percent difference between duplicates were included in further analysis. Additionally, birds with missing data were excluded from analysis, which reduced the sample size to 12 subjects for CORT and HSP60 analysis. Samples from 24 birds were used for analysis of H/L ratios. IBM SPSS Statistics software was used to evaluate the relationships between the following variables: HSP60, CORT, H/L, H/L Index, body mass (Mass), BCI, and handling time (Time). Linear regression and analysis of variance (ANOVA), were used to describe the following relationships between variables in determining correlations: CORT vs Time, H/L vs Time, H/L index vs Time, H/L vs Mass, H/L Index vs Mass, H/L vs BCI, H/L Index vs BCI, HSP vs Time, HSP vs BCI, and HSP vs Mass.

Since the relationship between CORT, HSP60, and H/L may depend on the time elapsed between initial capture and sampling, the analysis was also performed using the residuals of CORT vs Time (CORT residuals) to account for the variation in CORT values owing to differences in handling time. Linear regression and ANOVA were performed on H/L vs CORT residuals, H/L Index vs CORT residuals, and HSP60 vs CORT residuals. Regressions were also run to determine if body condition or body

Table 1. Summary of descriptive statistics of data obtained from field measurements, corticosterone assay, HSP 60 assay, and stained-slide cell counts.

Variable	N	Mean	StDev	Range
CORT ^a	12	16.42	14.56	1.13-44.41
CORT residuals ^b	12	0.385	7.97	-14.69-11.89
H/L ^c	24	0.157	0.082	0.047-0.362
H/L Index ^d	24	95.869	79.878	9.37-320.4
HSP60 ^e	12	0.076	0.028	0.032-0.125
Mass (g)	12	21	1.11	19-22
BCI ^f	12	39.86	2.3	35.8-45.1
Time (s) ^g	12	400.25	265	120-915

^aCorticosterone concentration in ng/mL serum

^bStandard residuals of CORT vs. Time linear regression

^cHeterophil to lymphocyte ratio

^dH/L multiplied by number of leukocytes per 10,000 erythrocytes

^eHeat Shock Protein 60 concentration in ng/μg total hematocrit protein

^fBody condition index calculated from mass (g) divided by wing length cubed (mm³)

^gHandling time, defined as the time elapsed between capture and blood sampling

mass was correlated with CORT (CORT residuals vs BCI and CORT residuals vs Mass) and HSP60 (HSP60 vs BCI and HSP60 vs Mass).

The SPSS software was also used to calculate Spearman's rho, a nonparametric correlation coefficient, to compare within-subject values of H/L and CORT residuals, and CORT residuals and HSP60. Results were determined to be significant when $p \leq 0.05$.

RESULTS

The average handling time before blood sampling was 400 seconds (N = 12, Mean = 400.25 ± 265 , Range 120 to 915) (Table 1) or 6.6 minutes. CORT was positively and significantly correlated with handling time ($R^2 = .707$, N = 12, $P < .001$) (Fig. 1, Table 2). No other relationships were significant (Table 2), including H/L and CORT residuals (Table 2), H/L and handling time (Fig. 2), HSP60 and handling time (Fig. 3), or HSP60 and CORT (Fig. 4).

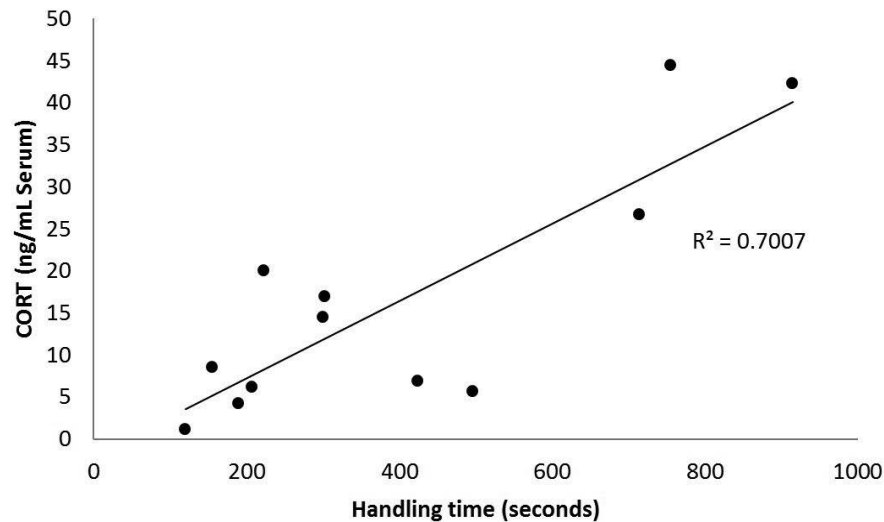


FIGURE 1. Corticosterone (CORT) concentration (ng/mL serum) is significantly correlated with handling time (seconds). CORT levels increase as handling time after capture increases ($R^2 = 0.7007$, $N = 12$, $p < 0.001$).

DISCUSSION

Corticosterone and H/L comparison

CORT was positively and significantly correlated with handling time, as expected based on the results of previous research (Romero and Reed 2005; Fridinger et al. 2007; Herring and Gawlik 2007; Herring et al. 2011; Busch and Hayward 2009; Cockrem et al. 2009). Also as expected, no correlation was found between H/L and handling time, or between H/L Index and handling time. These results support the hypothesis that H/L is less sensitive than CORT to handling time in samples taken 2-8 minutes after capture and supports previous studies which show that H/L ratios are not as responsive to acute stress such as in handling (Vleck et al. 2000). This supports the conclusion that the use of H/L may be preferable for measuring chronic stress levels in free living birds when unavoidably prolonged handling after capture for blood sampling may affect CORT levels.

The results for heterophil counts, lymphocyte counts, and H/L in this study are similar to those for Siberian tits in managed habitats (Krams et al. 2010) and for wintering male great tits (Cirule et al. 2012) and were within ranges reported by Davis (2005). The ranges of leukocyte counts and H/L in this study are consistently lower than the those reported in several studies on chickens (Gross and Siegel 1983; Davis et al. 2000; Zulkifli et al. 2000; Al-Murrani et al. 2006), Adélie penguins (Vleck et al. 2000), pied flycatcher nestlings (Moreno et al. 2002), and Eurasian kestrel nestlings (Martinez-Padilla et al. 2004). The disparities among these studies may reflect species-specific differences in leukocyte profiles.

Table 2. Summary of results from statistical analysis. For each relationship listed, SPSS software was used to perform linear regression and ANOVA^a. Relationships were determined to be significant when $P < 0.05$.

Variable	df	F	R ²	p
CORT ^b vs. Time ^c (s)	11	23.409	0.707	< .001
H/L ^d vs. Time (s)	23	1.21	0.053	0.28
H/L Index ^e vs. Time (s)	23	1.782	0.075	1.19
H/L vs. CORT residuals ^f	11	1.2	0.114	0.28
H/L Index vs. CORT residuals	11	0.975	0.088	0.34
H/L vs. Mass (g)*	21	0.022	0.001	0.88
H/L Index vs. Mass (g)*	21	0.142	0.007	0.71
H/L vs. BCI ^g *	21	0.612	0.030	0.45
H/L Index vs. BCI*	21	0.708	0.034	0.41
HSP60 ^h vs Time (s)	11	0.517	0.49	0.48
HSP60 vs Mass(g)	11	0.210	0.020	0.65
HSP60 vs BCI	11	0.188	0.185	0.67
HSP vs CORT	11	0.207	0.020	0.66
HSP vs CORT residuals	11	0.048	0.004	0.83

^aAnalysis of variance

^bCorticosterone concentration in ng/mL serum

^cHandling time, defined as the time elapsed between capture and blood sampling

^dHeterophil to lymphocyte ratio

^eH/L multiplied by number of leukocytes per 10,000 erythrocytes

^fStandard residuals of CORT vs. Time linear regression

^gBody condition index calculated from mass divided by wing length cubed (mm³)

^h Heat Shock Protein 60 concentration in ng/μg total hemocrit protein concentration

*Mass was only obtained from 22 birds for these analyses

No correlation between CORT and any leukocyte variable after removing the variation in CORT owing to the effect of handling time was detected. These findings agree with Gross and Siegel (1983), who found no correlation between H/L and CORT

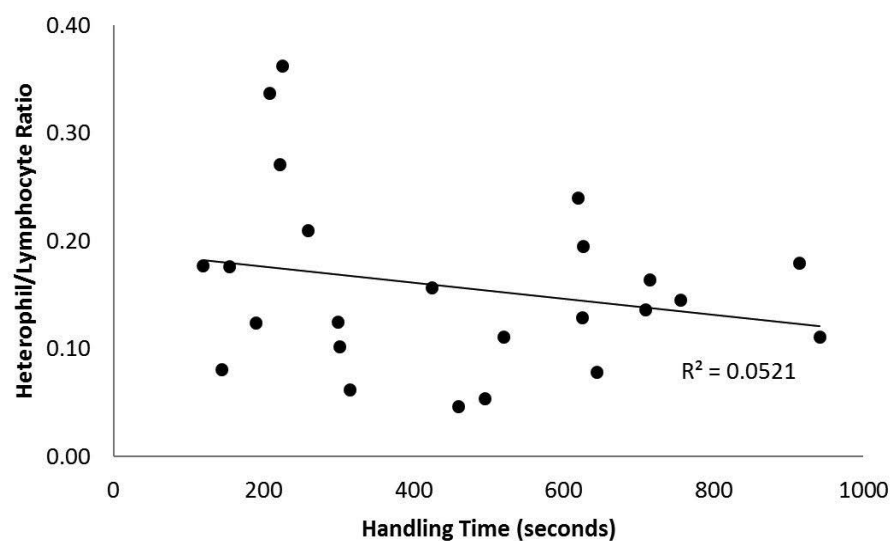


FIGURE 2. Heterophil/lymphocyte ratios (H/L) are not significantly correlated with increases in handling time (seconds) ($N = 24$, $R^2 = 0.0521$, $p = 0.39$).

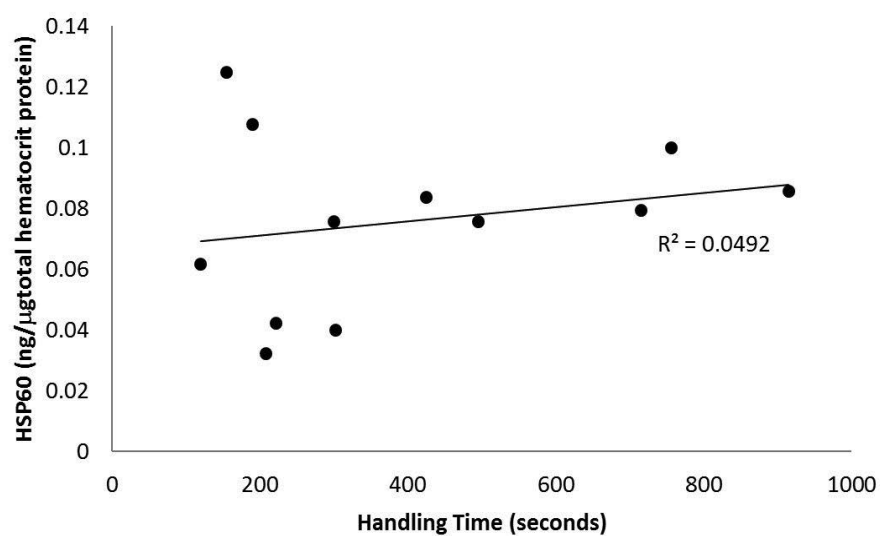


FIGURE 3. Heat shock protein 60 (HSP60) concentrations (ng/ μ g total hematocrit protein) are not significantly correlated with handling time (seconds) ($N = 12$, $R^2 = 0.0492$, $p = 0.48$).

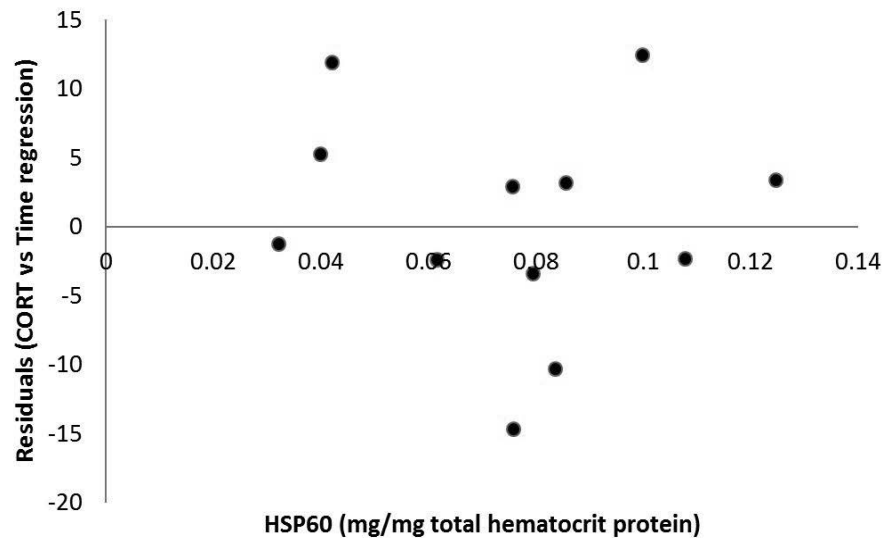


FIGURE 4. Heat shock protein 60 (HSP60) levels (expressed as a fraction of total hematocrit protein concentration) measured in tufted titmouse blood samples were not correlated with the residuals found from the CORT (ng/mL serum) vs. time in seconds regression (N = 12).

in response to various stressors. The only situation in which Gross and Siegel (1983) found a correlation between H/L and CORT was in well-socialized groups of chickens in low-stress environments with stable social hierarchies before any experimental stressors were applied. The absence of a correlation between H/L and CORT in our study does contradict the results of more recent research. Prolonged increases in levels of glucocorticoids have been shown to have both an immunosuppressive effect resulting in reductions of circulating leukocytes (Sapolsky et al. 2000), although studies using exogenous administration of CORT can also increase levels of H/L (Müller et al. 2011) in free living birds. According to Sapolsky et al. (2000), immune activation in response to stressors contributes to the subsequent release of glucocorticoids such as CORT by synthesizing molecules similar to adrenocorticotrophic hormone and cytokines that stimulate the adrenocortical axis. Thus decreases in leukocyte counts in response to prolonged increases in glucocorticoids may not be due to the depletion of leukocytes, but to the diversion of leukocytes to local areas of need in response to specific stressors. Therefore, the overall leukocyte proportions in a subject's system should not be significantly affected by variations in CORT levels, a scenario which would be supported by the findings of this study. Leukocyte counts, however, may vary in such a case, depending where in the system leukocytes are being diverted as well as where and how samples are taken. Based on our methods, we would not have detected this. The results of a study by Muller et al. (2011) using free-living Eurasian kestrels subjected to increased CORT using exogenous implants demonstrated that H/L levels can be increased by prolonged CORT elevation. However, their study also

demonstrated that CORT and H/L will normally respond to different stressors, indicating that the body will respond to different types of stress using different mechanisms. Additionally, El-Lethey et al. (2003) have noted that there are both stress resistant and stress susceptible antigen responses leading to immune suppression, which can further confound results. Therefore, when attempting to measure chronic stress in birds, multiple measures of stress should be used.

Because H/L did not change with differences in handling time, H/L may be a useful indicator of chronic stress in field research. It is relatively resistant to the effects of acute stress and would, therefore, allow researchers more time to obtain blood samples in the field than CORT. In addition to decreasing the pressure on researchers to minimize handling times, the use of H/L as a stress measurement tool offers other benefits that the use of CORT may not. First, the volume of blood necessary to prepare a blood smear is much smaller than that required for CORT assays. A blood smear requires 1-2 μ l of blood whereas typical assays to measure CORT concentrations require at least 10 μ l of blood to get an appropriate sufficient amount of plasma. The average tufted titmouse body mass in this study was just over 21 g, and the smallest bird sampled was 19 g. A smaller blood sample might produce fewer negative effects on small songbirds than larger blood samples (Brown and Brown 2009). Second, the relative costs of obtaining H/L and CORT measurements for research are substantially different. CORT assay kits are expensive, and analysis of samples requires the use of sensitive and expensive equipment and specialized software. The method for measuring H/L requires only stain, microscope slides, a microscope, and immersion oil.

There are challenges to the use of H/L, though, as previous researchers such as Davis et al. (2008) have pointed out. First, H/L measured from a single blood smear is only indicative of the relative proportions of leukocytes that were circulating in the blood at the moment and the specific bodily location of sampling. It is, therefore, possible that H/L may not be representative of heterophils or lymphocytes present in other areas of the body. Without taking multiple samples and creating multiple blood smears, there is no way to know if such a difference is present or the magnitude of that difference. Second, H/L is not a direct measure of chronic stress levels, but a relative measure that requires comparison either to other samples or to a reference of some kind. References do not exist for many species which describe typical measures under low-stress and high-stress conditions, which can create issues in interpreting the data. Finally, H/L is influenced by other factors that induce an inflammatory or an antibody response, such as injury or disease (Davison et al. 1983) or prolonged elevations of glucocorticoids (Müller et al. 2011), which could be a response to either physical or psychosocial stress leading to greater confusion in interpretation of data. Therefore, researchers must be cautious in choosing the appropriate stress assessment tool for use in specific research applications, and ideally use several different measures to gain an accurate picture.

Corticosterone and HSP60 level comparison

HSP60 concentrations were not significantly correlated with handling time, while CORT levels in the same subjects were. No correlation between CORT levels and HSP60 levels within individuals was seen. Those subjects with higher CORT levels did not necessarily have higher HSP60 levels. These results support the hypothesis that HSP60 is less sensitive to acute stress caused by handling time than CORT. Therefore,

like H/L ratio, HSP60 may also be a preferable or informative complementary indicator of stress along with CORT when blood samples must be collected under challenging field conditions.

Our HSP60 results cannot be directly compared to past studies because of the methods used. Most of these previous studies used cell fractionation and Western blots to evaluate HSP concentrations (Merino et al. 2002; Barbosa et al. 2007; Arriero et al. 2008; del Cerro et al. 2010; Martinez-de la Puente et al. 2010) whereas we used ELISA. Western Blots report results using optical density measures while our results are expressed in micrograms per milligrams of hematocrit protein although trends in relative amounts may be compared. Herring and Gawlik (2013) evaluated HSP60 concentrations using ELISA. However, they reported their results in nanograms per milliliter, which also makes a direct comparison difficult. Our method of reporting HSP60 levels relative to total protein levels standardizes the HSP measurements and removes variation in results owing to experimental technique and blood composition in subjects.

In a study of the relationship between food availability and both CORT and HSP levels in the White Ibis (*Eudocimus albus*), HSP60 levels increased in a predictable manner when food was limited (Herring et al. 2011). Plasma corticosterone levels (PCORT) were found to be ambiguously related to nutritional condition, indicating that the PCORT results might have reflected handling stress. Our results are consistent with the results of this study.

Additionally, there was no significant relationship between HSP60 concentrations and either CORT or CORT residuals (Table 2, Fig 4). This shows that HSP60 concentration was not related to the remaining variation in CORT levels not accounted for by handling time, and that there is no relationship between increases in CORT levels and HSP levels due to acute stress caused by handling. CORT is present at low levels under normal conditions. However, when the brain recognizes a stressor, it signals the HPA axis to release more CORT (Herring and Gawlik 2007), and levels may remain high when an individual faces chronic stress. HSPs are also present under normal conditions and are up-regulated during periods of both physical (Feder and Hoffman 1999) and psychosocial stress (Isosaki and Nakashima 1998; Currie et al. 2009; Al-Aquil et al. 2013) though increases in HSP levels would be slower in onset and have a longer duration than CORT. Therefore, their levels may more accurately represent the presence of chronic stress. Since CORT and HSPs function in different responses to stress, they should not necessarily be correlated, which is supported by our results.

Body mass, body condition (Table 2) and standardized mass (data not shown) were not correlated with CORT, H/L or HSP60, indicating that our specimens were either not chronically stressed or that the measures we used are not good predictors of chronic stress in this species. Other measures of condition, such as a rating of the pectoral muscle size, may be a more accurate way to index differences in physical condition attributable to factors other than energetic status given that our subjects were food supplemented.

This study was conducted to determine the effect of acute stress due to capture and handling on levels of plasma CORT, H/L, and HSP60 to determine possible correlations between these physiological stress indicators. As described above, the results of this study demonstrate that both H/L and HSP60 are less sensitive to the

effects of handling time than the more commonly used CORT, and may be preferable stress measures in some instances. This study was intended to help researchers identify which indicators may be more effective for particular research applications. In light of these results, this research encourages more frequent use of H/L and HSP60 as complementary measures of chronic moderate stress in avian field research.

ACKNOWLEDGEMENTS

We would like to thank Louis Kartoudi for assistance in capturing birds, and Diana Gutierrez for assistance in collecting heterophil/lymphocyte data. We thank the following homeowners for allowing access to their property for bird capture: Mike and Joella Killian, Jim Hazzard and Sally Knight, Mike Lott and Jackie Gallagher, Larry Valade, and Gerry Weinberger. We thank the University of Mary Washington for use of grounds, facilities and equipment, and we would like to thank Virginia Ornithological Society and the Larry Valade Foundation for providing funding of this research.

LITERATURE CITED

- Al-Aqil, A, I. Zulkifli, M. Hair Bejo, A. Q. Sazili, M. A. Rajion, and M. N. Somchit. 2013. Changes in heat shock protein 70, blood parameters, and fear-related behavior in broiler chickens as affected by pleasant and unpleasant human contact. *Poultry Science* 92(1):33-40.
- Al-Murrani, W. K., A. J. Al-Rawi, M. F. Al-Hadithi and B. Al-Tikriti. 2006. Association between heterophil/lymphocyte ratio, a marker of 'resistance' to stress, and some production and fitness traits in chickens. *British Poultry Science* 47:443-448.
- Arriero, E., J. Moreno, S. Merino and J. Martinez. 2008. Habitat effects on physiological stress response in nestling blue tits are mediated through parasitism. *Physiological and Biochemical Zoology* 81:195-203.
- Barbosa, A., S. Merino, J. Benzal, J. Martínez and S. García-Fraile. 2007. Population variability in heat shock proteins among three Antarctic penguin species. *Polar Biology*. 30:1239-1244.
- Brown, M. B. and C. R. Brown. 2009. Blood sampling reduces annual survival in cliff swallows (*Petrochelidon pyrrhonota*). *The Auk*. 126:853-861.
- Busch, D. S. and L. S. Hayward. 2009. Stress in a conservation context: A discussion of glucocorticoid actions and how levels change with conservation-relevant variables. *Biological Conservation* 142:2844-2853.
- Cirule, D., T. Krama, J. Vrublevska, M. J. Rantala and I. Krams. 2012. A rapid effect of handling on counts of white blood cells in a wintering passerine bird: a more practical measure of stress? *Journal of Ornithology* 153:161-166.
- Clark, P., W. S. J. Boardman and S. Raidal. 2009. *Atlas of Clinical Avian Hematology*. Wiley-Blackwell, United Kingdom. 200pp.
- Cockrem, J. F., D. P. Barrett, E. J. Candy and M. A. Potter. 2009. Corticosterone responses in birds: Individual variation and repeatability in Adélie penguins (*Pygoscelis adeliae*) and other species, and the use of power analysis to determine sample sizes. *General and Comparative Endocrinology* 163:158-168.

- Currie S, S. LeBlanc, M. A Watters and K. M. Gilmour. 2009 Agonistic encounters and cellular angst: social interactions induce heat shock proteins in juvenile salmonid fish Proceedings of the Royal Society of London B [internet] doi:10.1098/rspb.2009.1562. Available from <http://rspb.royalsocietypublishing.org/content/early/2009/11/17/rspb.2009.1562.full.pdf+html> accessed: November 11, 2014.
- Cyr, N. E., K. Earle, C. Tam, L. M. Romero. 2007. The effect of chronic psychological stress on corticosterone, plasma metabolites, and immune responsiveness in european starlings. General Comparative Endocrinology 154(1-3):59-66.
- Cyr, N. E., L. M. Romero. 2007. Chronic stress in free-living European starlings reduces corticosterone concentrations and reproductive success. General Comparative Endocrinology 151(1):82-89.
- Dabbert, C. B., R. L. Lochmiller and R. G. Teeter. 1997. Effects of acute thermal stress on the immune system of the Northern Bobwhite (*Colinus virginianus*) The Auk 114(1):103-109.
- Davis, G. S., K. E. Anderson and A. S. Carroll. 2000. The effects of long-term caging and molt of single comb white leghorn hens on heterophil to lymphocyte ratios, corticosterone and thyroid hormones. Poultry Science 79:514-518.
- Davis, A. K. 2005. Effect of handling time and repeated sampling on avian white blood cell counts. Journal of Field Ornithology 76:334-338.
- Davis, A. K., D. L. Maney and J. C. Maerz. 2008. The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. Functional Ecology 22:760-772.
- Davison, T. F., J. G. Rowell and J. Rea. 1983. Effects of dietary corticosterone on peripheral blood lymphocytes and granulocyte populations in immature domestic fowl. Research in Veterinary Science 34:236-239.
- del Cerro, S., S. Merino, J. Martinez-de la Puente, E. Lobato, R. Ruiz-de-Castañeda, J. Rivero-de Aguilar, J. Martinez, J. Morales, G. Tomas and J. Moreno. 2010. Carotenoid-based plumage colouration is associated with blood parasite richness and stress protein levels in blue tits (*Cyanistes caeruleus*). Oecologia 162:825-835.
- El-Lethey, H., B. Huber-Eicher and T. W. Jung. 2003 Exploration of stress-induced immunosuppression in chickens reveals both stress-resistant and stress-susceptible antigen responses. Veterinary Immunology and Immunopathology 95: 91-101.
- Feder, M. E. and G. E. Hofmann. 1999. Heat-shock proteins, molecular chaperones, and the stress response: evolutionary and ecological physiology. Annual Review of Physiology 61:243-282.
- Fridinger, R. W., K. M. O'Reilly, S. D. Kildaw and C. L. Buck. 2007. Removal of a nest-mate elicits an age-dependent increase in plasma corticosterone of nestling black-legged kittiwakes. Journal of Field Ornithology 78:93-99.
- Gross, W. B. and H. S. Siegel. 1983. Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. Avian Diseases 27:972-979.
- Grubb, T. C., J. R. and V. V. Pravosudov. Tufted titmouse. 1994. The Birds of North America. 86: 1-16.

- Herring, G., M. I. Cook, D. E. Gawlik and E. M. Call. 2011. Food availability is expressed through physiological stress indicators in nestling white ibis: a food supplementation experiment. *Functional Ecology* 25:682-690.
- Herring, G. and D. E. Gawlik. 2007. The role of stress proteins in the study of allostatic overload in birds: use and applicability to current studies in avian ecology. *The Scientific World Journal* 7:1596-1602.
- Herring, G. and D. E. Gawlik. 2013. Differential physiological responses to prey availability by the Great Egret and White Ibis. *Journal of Wildlife Management* 77:58-67.
- Houwen, B. 2000. Blood film preparation and staining procedures. *Laboratory Hematology* 6:1-7.
- Humayon, W., A. Dolby and D. A. O'Dell. 2010. Heat Shock Protein 60 Detection by Enzyme-Linked Immunosorbent Assay in House Sparrows (*Passer domesticus*). *Virginia Journal of Science* 61:25-26.
- Isosaki, M. and T. Nakashima. 1998. Psychological stress induces heat shock protein 70 expression in rat aorta. *Japanese Journal of Pharmacology* 76(3):305-308.
- Krams, I., D. Cirule, T. Krama, M. Hukkanen, S. Rytönen, M. Orell, T. Iezhova, M. J. Rantala and L. Tummeleht. 2010. Effects of forest management on haematological parameters, blood parasites, and reproductive success of the Siberian Tit (*Poecile cinctus*) in northern Finland. *Annales Zoologici Fennici* 47:335-346.
- Landys, M. M., W. Goymann and T. Slagsvold. 2011. Rearing conditions have long-term consequences for stress responsiveness in free-living great tits *General Comparative Endocrinology* 174(2):219-24.
- Martinez-de la Puente, J., S. Merino, G. Tomas, J. Moreno, J. Morales, E. Lobato, J. Martinez. 2010. Nest ectoparasites increase physiological stress in breeding birds: an experiment. *Naturwissenschaften* 98:99-106.
- Martinez-Padilla, J., J. Martinez, J. A. Dávila, S. Merino, J. Moreno and J. Millán. 2004. Within-brood size differences, sex and parasites determine blood stress protein levels in Eurasian Kestrel nestlings. *Functional Ecology* 18:426-434.
- Merino, S., J. Martinez, A. Barbosa, A. P. Moller, F. de Lope, J. Pérez and F. Rodríguez-Caabeiro. 1998. Increase in a heat-shock protein from blood cells in response of nestling house martins (*Delichon urbica*) to parasitism: an experimental approach. *Oecologia* 116:343-347.
- Merino, S., J. Moreno, G. Tomas, J. Martinez, J. Morales, J. Martinez-de la Puente and J. L. Osorno. 2006. Effects of parental effort on blood stress protein HSP60 and immunoglobulins in female blue tits: a brood size manipulation experiment. *Journal of Animal Ecology* 75:1147-1153.
- Moreno, J., S. Merino, J. Martinez, J. J. Sanz and E. Arriero. 2002. Heterophil/lymphocyte ratios and heat shock protein levels are related to growth in nestling birds. *Ecoscience* 9:434-439.
- Müller, C., S. Jenni-Eiermann and L. Jenni. 2011. Heterophils/Lymphocytes-ratio and circulating corticosterone do not indicate the same stress imposed on Eurasian kestrel nestlings. *Functional Ecology*. 25:566–576.
- Owen, M. and W. A. Cook. 1977. Variations in body weight, wing length and condition of Mallard *Anas platyrhynchos platyrhynchos* and their relationship

- to environmental changes. *Journal of Zoology: Proceedings of the Zoological Society of London* 183:377-395.
- Romero, L. M. and J. M. Reed. 2005. Collecting baseline corticosterone samples in the field: is under 3 min good enough? *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology* 140:73-79.
- Sapolsky, R. M., L. M. Romero and A. U. Munck. 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews* 21:55-89.
- Tomas, G., J. Martinez and S. Merino. 2004. Collection and analysis of blood samples to detect stress protein in wild birds. *Journal of Field Ornithology* 75:281-287.
- Vleck, C. M., N. Vertalino, D. Vleck and T. L. Bucher. 2000. Stress, corticosterone, and heterophil to lymphocyte ratios in free-living Adélie penguins. *The Condor* 102:392-400.
- Zulkifli, M. T., C. Norma, C. H. Chong and T. C. Loh. 2000. Heterophil to lymphocyte ratio and tonic immobility reactions to preslaughter handling in broiler chickens treated with ascorbic acid. *Poultry Science* 79:402-406.
- Zulkifli, M. T., C. Norma, D. A. Israf and A. R. Omar. 2002. The effect of early-age food restriction on heat shock protein 70 response in heat-stressed female broiler chickens. *British Poultry Science* 43:124-145.
- Zulkifli, I, A. Al-Aqil, A. R. Omar, A. Q. Sazili and M. A. Rajion. 2009. Crating and heat stress influence blood parameters and heat shock protein 70 expression in broiler chickens showing short or long tonic immobility reactions. *Poultry Science* 88 (3): 471-476.

Survey of the Ectoparasites of the Invasive Small Indian Mongoose (*Herpestes auropunctatus* [Carnivora: Herpestidae]) on St. John, U.S. Virgin Islands

Kelsey L. Townsend² and Karen E. Powers³

Biology Department, Radford University,
Radford, VA 24142

ABSTRACT

In March 2012, live trapping surveys were conducted for invasive small Indian mongoose (*Herpestes auropunctatus*) on St. John, U.S. Virgin Islands. Forty mongoose were sampled (31♂, 9♀) for ectoparasites, and cat fleas (*Ctenocephalides felis*) were discovered on 17 individuals. There was no difference in the number of ectoparasites per mongoose across age classifications ($r = 0.109$, $P = 0.579$). However, males had more cat fleas than females, even when mass was taken into account (males are generally heavier). Future behavioral studies may explain these sex differences. Although management suggestions from this research are limited, these data contribute to an understanding of ectoparasite distributions on these invasive mongoose in the Caribbean.

INTRODUCTION

The small Indian mongoose (*Herpestes auropunctatus*) is a 120-1000-gram carnivore, feeding opportunistically on all major vertebrate groups, invertebrates, and occasionally, plants (Lewis et al. 2011). Although uncertainty exists about the extent of its geographic range, this mongoose is believed to be native to the Middle East, India, and Myanmar (Veron et al. 2007). The uncertainty lies in its confusion with a sympatric mongoose, the Javan or small Asian mongoose (*H. javanicus*), for which *H. auropunctatus* had been treated as a conspecific. Indeed, nearly all literature published prior to 2007 assumed that the mongoose released onto Hawaiian and Caribbean islands was *H. javanicus*. However, Veron et al. (2007) confirmed with mtDNA analyses that *H. auropunctatus* and *H. javanicus* were two distinct species, and Bennett et al. (2011), definitively determined through mtDNA barcoding that mongoose currently inhabiting Hawaiian and Caribbean islands were *H. auropunctatus*.

¹ Current address: Dept. of Environmental Studies, Univ. of Illinois at Springfield, Springfield, IL 62703

² Corresponding author: kpowers4@radford.edu

The small Indian mongoose was originally introduced in the late 1800s (likely the 1870s) to control the invasive black rat (*Rattus rattus*) population on St. John, St. Croix, and other nearby Caribbean islands (Nellis and Everard 1983; Horst et al. 2001). The primary diet of mongoose in this region is not the black rat but instead includes native species on the U.S. Virgin Islands (USVI) such as eggs of the brown pelican (*Pelicanus occidentalis*) and the green sea turtle (*Chelonia mydas*; Seaman and Randall 1962), and, more commonly, lizards, amphibians, ground-nesting birds, and invertebrates (Nellis 1989; Lewis et al. 2011).

Besides hawks (on some islands), the small Indian mongoose has no other natural predators in the USVI, and wildlife managers lack the time, effort, and funds to eradicate the species from the islands (Nellis and Everard 1983). However, as a method of managing for rare breeding birds or reptiles, localized, seasonal removal efforts can be fairly successful. For example, Coblentz and Coblentz (1985) estimated 86% of the mongoose populations were removed in five nights of trapping a number of bays and trails on St. John. However, immigrants and young dispersing mongoose quickly take the place of those lethally removed (Coblentz and Coblentz 1985). If removal efforts only temporarily decrease the mongoose population, what else might negatively impact this invasive species? We suggested that if mongoose on St. John, USVI, carried a heavy parasite load, this could negatively impact the health of the mongoose. Rust and Dryden (1997) report that high flea loadings can cause skin irritation, skin allergies, and anemia in affected individuals.

Past studies of mongoose on St. Croix and Puerto Rico found them to be carriers of cat fleas (*Ctenocephalides felis*), ticks (*Ornithodoros puertoricensis*), and mange mites (*Notoedres cati*; Pimentel 1955; Garrett and Haramota 1967; Webb 1980; Corn et al. 1994; Corn et al. 2009). Nellis (1989) states that cat fleas are the most common ectoparasites in the mongoose's introduced range, and suggests that these fleas were not present in their native habitat in the late 1800s. However, Baldwin et al. (1952) cite a 1934 Hawaiian public health survey, suggesting cat fleas were so common on mongoose on Hawaiian Islands, this animal must be a natural host. With so little natural history research completed on this mongoose in its native range (Horst et al. 2001), absolute confirmation of the cat flea in the native range is not possible. Further, it is clear that flea density varies among locations. Counter to the Hawaiian surveys, Pimentel (1955) found just a single cat flea on 1/210 individuals examined in Puerto Rico. To date, although reported studies of mongoose ectoparasites exist for St. Croix (Webb 1980; Nellis and Everard 1983), Puerto Rico (Pimentel 1955), and Hawaii (e.g., Haas 1966; Garrett and Haramota 1967), we find no published findings of ectoparasites on St. John, USVI, nor their potential negative impact on the mongoose on this island. Further, some studies collected ectoparasites from museum specimens (e.g., Garrett and Haramota 1967), and the authors' goal was to conclusively determine presence on live individuals.

MATERIALS AND METHODS

To determine the parasite loads of mongoose on St. John, USVI, the authors live-trapped individuals from 8-10 March 2012 using 45 Tomahawk #202 traps (Tomahawk

Live Trap, Hazelhurst, Wisconsin, USA). Hiking trails in early and late successional habitats were selected, and were restricted to the south-central portion of the island. Sites were at or within 2 km of the Virgin Island Environmental Resource Station (VIERS; UTM Zone 20, 2026684 N, 317964 E).

Forty mongoose were captured and combed: 31 males, aged 5.5–46 months (AVG. age = 21.4 ± 2.8 [SE]), and 9 females, aged 5.5–26 months (AVG. age = 14.3 ± 2.5) via tooth wear (Pearson and Baldwin 1953). Using a flea comb, mongoose fur was brushed for approximately one minute, and ectoparasites were collected. Results, therefore, do not assume that all ectoparasites were collected from each individual; instead, equal brushing effort allowed for a relative comparison among individuals in this study.

Collected ectoparasites were stored in 70% ethanol, and transferred back to Radford University for identification. Authors identified the contents of each vial using keys and data from past studies (Webb 1980) and flea guides (Ewing and Fox 1943; De Campos Pereira 2012).

This study was approved by the Radford University Institutional Animal Care and Use Committee, protocol #F12-03, and permitted by the National Park Service, VIIS-2012-SCI-0001. Arthropod voucher specimens are housed in the Radford University, Biology Department's Natural History Collection.

RESULTS

Ectoparasites were discovered on 62.5% of 40 captured individuals (22♂, 3♀), but authors were only able to capture and preserve the ectoparasites from 18 of these 25 individuals. Such ectoparasite escapes are not uncommon, given the difficulty in sampling live mongoose under sometimes intense field conditions (Haas 1966). Seventeen of the 18 individuals from which ectoparasites were successfully collected harbored cat fleas (*Ctenocephalides felis*). A single tick of the genus *Amblyomma* also was documented on the 18th individual; an incomplete specimen prevented identification to the species level. However, this tick genus was detected on mongoose by Nellis (1989) from the West Indies (specific island not reported) and by Corn et al. (1994; *Amblyomma variegatum*) in Antigua, West Indies.

When examining all 40 individuals for which ectoparasite relative counts (number of ectoparasites per 1 minute of brushing) were available, there was no difference in the number of fleas per mongoose across age classifications (Pearson's product-moment correlation; $r = 0.109$, $P = 0.579$; SAS Institute 2009). However, Student's t-tests revealed that males had significantly more parasites (AVG number of parasites for ♂: 1.7 ± 0.4 [SE]) than females (AVG number of parasites for ♀: 0.4 ± 0.2 ; $t = 2.65$, $df = 19$, $P = 0.016$). Even when mass was taken into account (AVG mass for ♂: 596 ± 18 g [SE], AVG mass for ♀: 514 ± 18 g), this gender difference remained in place (Student's t-test; $t = 2.07$, $df = 16$, $P = 0.027$).

DISCUSSION

The discovery of the cat flea was not surprising, given its documentation in multiple locations by multiple sources (e.g., Baldwin et al. 1952; Seaman 1963; Haas 1966;

Webb 1980). However, few reported actual ectoparasite loads, only taxon lists of ectoparasites discovered. Seaman (1963, as reported in Nellis and Everard 1983; AVG = 8.6), Haas (1966; March AVG for ♂: 4.7, AVG for ♀: 1.0), and Webb (1980; AVG = 2.7 fleas per mongoose) found similar parasite loads and trends in male vs. female parasite loads. However, with some authors failing to report the methods of parasite sampling (Webb 1980) and others only cursorily describing methods (Haas 1966), direct comparisons of parasite loads across studies may be of little scientific value. Therefore, this study was limited to relative comparisons.

This study's finding that males typically had more parasites than females is in agreement with the findings from these other locales, and this supports their theory that behavioral differences between sexes may impact ectoparasite loads (Haas 1966; Webb 1980). Although Webb (1980) and Haas (1966) suggested that size differences between sexes is a primary reason for unequal parasite load, the statistics from this study suggest that males had greater parasite loads regardless of mass differences. Webb (1980) further suggested that, on St. Croix, this sex difference might be influenced by males' greater home range size and higher activity level than females, especially during the breeding season. The authors suggest that future studies of these mongoose examine which differences in behavior (e.g., territory size, microhabitat use, or grooming mechanisms) most influence the presence and density of these parasites.

Webb (1980) did find a difference in ectoparasite density in comparison to age (juvenile females tended to have more ectoparasites than adults). Although the current study did not find such patterns, it's possible that the sample size (just 3 females carrying parasites, 2 of them juveniles) may not have been representative. Further, Webb was unaware of the development of an aging method for mongoose, which was employed in the current study (Pearson and Baldwin 1953); instead, he used weight as a predictor of age.

Similar to findings in Hawaii (Haas 1966) and St. Croix (Webb 1980), it appears that mongoose parasite loads on St. John are insufficient to detrimentally affect this invasive species. Unlike Rust and Dryden (1997), no individual captured showed obvious symptoms of excessive flea loads (skin irritation, allergies, missing fur patches) which could have been associated with health issues in the mongoose. However, direct observations of mongoose in traps and in hand were limited to approximately five minutes per individual. Currently, this relationship between the small Indian mongoose and its ectoparasites does not affect management for this species. However, these data contribute to an understanding of ectoparasite loads and distributions in the Caribbean.

ACKNOWLEDGMENTS

The authors are grateful to B. Hyzy and H. Moore for field assistance. Assistance with species identification was provided to Townsend by M. Kinsella and B. O'Connor. Thanks also to Radford University for providing a travel scholarship to Townsend. STATEMENT OF RESPONSIBILITY: Both authors contributed equally to field work, statistical analyses, and writing of this manuscript.

LITERATURE CITED

- Baldwin, P. H., C. W. Schwartz, and E. R. Schwartz. 1952. Life History and Economic Status of the Mongoose in Hawaii. *Journal of Mammalogy* 33(3):335-356.
- Bennett, C. E., B. S. Wilson, R. DeSalle, 2011. DNA Barcoding of an Invasive Mammal Species, the Small Indian Mongoose (*Herpestes javanicus*; E. Geoffroy Saint-Hillaire 1818) in the Caribbean and Hawaiian Islands. *Mitochondrial DNA* 22(1-2):12-18.
- Coblentz, B. E., and B. A. Coblentz. 1985. Control of the Indian Mongoose *Herpestes auropunctatus* on St. John, US Virgin Islands. *Biological Conservation* 33:281-288.
- Corn, J. L., D. M. Kavanaugh, T. E. Creekmore, and J. L. Robinson. 1994. Wildlife as Hosts for Ticks (Acari) in Antigua, West Indies. *Journal of Medical Entomology* (31)1:57-61.
- Corn, J. L., P. Berger, and J. W. Mertins. 2009. Surveys for Ectoparasites on Wildlife Associated with *Amblyomma variegatum* (Acari: Ixodidae)-Infested Livestock in St. Croix, U.S. Virgin Islands. *Journal of Medical Entomology* 46(6):1483-1489.
- De Campos Pereira, M. 2012. The Veterinary Parasitology Images Gallery, Arthropoda: Insecta and Acari. Retrieved from <http://www.icb.usp.br/~marcelcp/>. Date accessed: 18 November 2012.
- Ewing, H., and I. Fox. 1943. The Fleas of North America: Classification, Identification, and Geographic Distribution of These Injurious and Disease-Spreading Insects. U.S. Dept. of Agriculture, Miscellaneous Publication No. 500.
- Garrett, L. E. and F. H. Haramota. 1967. A Catalog of Hawaiian Acarina. *Proceedings of the Hawaiian Entomological Society* 19:381-414.
- Haas, G. E. 1966. Cat Flea-Mongoose Relationships in Hawaii. *Journal of Medical Entomology* 2(4):321-326.
- Horst, G. R., D. B. Hoagland, and C. W. Kilpatrick. 2001. The Mongoose in the West Indies: The Biogeography and Population Biology of an Introduced Species. Pp. 409-424, in *Biogeography of the West Indies: Patterns and Perspectives*. C. A. Woods and F. E. Sergile (eds.), Second Edition. CRC Press, Boca Raton, FL.
- Lewis, D. S., R. van Veen, and B. S. Wilson. 2011. Conservation Implications of Small Indian Mongoose (*Herpestes auropunctatus*) Predation in a Hotspot within a Hotspot: The Hellshire Hills, Jamaica. *Biological Invasions* 13(1):25-33.
- Nellis, D. W. 1989. *Herpestes auropunctatus*. *Mammalian Species* 342:1-6.
- Nellis, D. W. and C. O. R. Everard. 1983. The Biology of the Mongoose in the Caribbean. *Studies of the Fauna of Curacao and Other Caribbean Islands* 64:1-162.
- Pearson, O. P. and P. H. Baldwin. 1953. Reproduction and Age Structure of a Mongoose Population in Hawaii. *Journal of Mammalogy* 34(4):436-447.
- Pimentel, D. 1955. Biology of the Indian Mongoose in Puerto Rico. *Journal of Mammalogy* 36(1):62-68.

- Rust, M. K., and M. W. Dryden. 1997. The Biology, Ecology, and Management of the Cat Flea. *Annual Review of Entomology* 42(1):451-473.
- SAS Institute. 2009. SAS 9.2 User's Guide, 2nd Edition. Retrieved from Accessed <https://support.sas.com/documentation/cdl/en/statug/63033/HTML/default/viewer.htm#titlepage.htm>. Date accessed: 29 April 2014.
- Seaman, G. A. 1963. Quarterly Progress Report, Wildlife Resources Survey of the Virgin Islands. March. P-R Project V-4-R-13. Unpublished document prepared for U.S. Fish and Wildlife Service.
- Seaman, G. A., and Randall, J. E. 1962. The Mongoose as a Predator in the Virgin Islands. *Journal of Mammalogy* 43(4):544-546.
- Veron, G., Patou, M. L., Pothet, G., Simberloff, D., and A. P., Jennings. 2007. Systematic Status and Biogeography of the Javan and Small Indian Mongooses (Herpestidae, Carnivora). *Zoologica Scripta* 36(1): 1-10.
- Webb, J. W. 1980. Parasites of Small Indian Mongoose on St. Croix, Virgin Islands. *The Journal of Parasitology* 66(1):176-178.



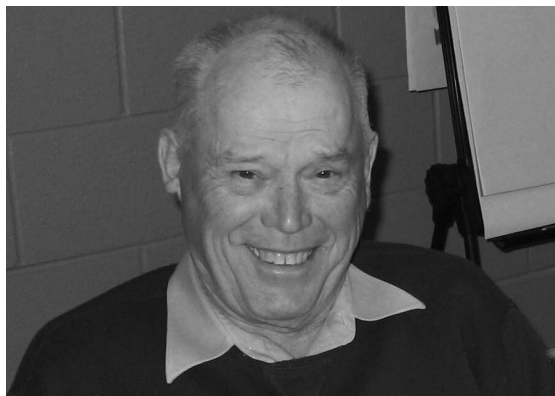
Roy L. Williams

Roy L. Williams, 77, longtime Virginia Academy of Science member, and Old Dominion University Emeritus Professor of Chemistry died Monday, July 28, from complications that developed after he underwent surgery to repair a fractured pelvis.

Few people in the institution's nearly 85-year history could match Williams' length of association with ODU. He received a bachelor's degree in sciences from the Norfolk Division of The College of William & Mary in 1960 and later received his Ph.D. in chemistry from the University of Delaware. In 1965 he returned to an alma mater that had become Old Dominion College as an assistant professor of chemistry. He retired from ODU in 2005, but often visited campus in later years, sometimes to share his wine knowledge.

Williams' first conducted research in polymers, and then drug synthesis. He began work on the chemistry of natural products, which included grapes and wine. This resulted in the founding of ODU's Enological Research Center in 1991 where he served as director until 2004. In 2001 Roy was named "Virginia Wine Industry Person of the Year" by the Commonwealth. He served as a wine columnist for the Hampton Roads Daily Press and was the creator and organizer of the Town Point Virginia Wine Competition for 22 years. Longtime Hampton Roads wine educator, Roy taught wine appreciation classes for over 25 years.

Survivors include his wife, Sherry Williams; daughters, Elise Cofer of Virginia Beach and Valerie Williams of Silver Spring, MD; son-in-law Richard Cofer and granddaughters Elena and Carmen; son-in-law Gregory Harry and granddaughter Morgan. In lieu of flowers, the family requests that donations be made to scholarships in his memory--the Roy L. Williams Carisbrooke Scholarship Fund or the Roy L. Williams Isle of Wight League Artist Scholarship at <https://www.regonline.com/roylwilliamsscholarships>.



Leonard O. Morrow

Leonard O. Morrow, 79, died August 24, 2014. Dr. Morrow first became a member of the Virginia Academy of Science in 1960. Len received his bachelors (1957) and masters (1960) degree from the University Richmond and went on to complete his PhD at Cornell University. His dissertation was "Anatomy and Morphology of Certain Coryphoideae (Palmae)." Upon his return to Richmond he was a regular participant of the Botany Section at the VAS annual meetings.

Dr. Morrow held positions at Virginia Commonwealth University, Randolph-Macon College, Maymont Park and J. Sargent Reynolds Community College. He retired from the latter position only a few years ago. Len had a long history of service work which included screening of science fair projects organized by the Richmond Math and Science Center (now the Richmond Math Science Innovation Center) from the late 1980s until just last year. In the fall of 2009 Len donated his personal herbarium (780 specimens) to the University to Richmond; the specimens include many collected during field trips organized in conjunction with VAS annual meetings. He is survived by three daughters, Pamela Anne Morrow, Sue Adele Morrow and Cynthia Morrow Harris; a sister, Anne Morrow Donley and family; grandchildren, Page and Chelsea Sparks and Robin and Constance Harris; and great grandchildren, Anson and Keira Sparks.

**Virginia Academy of Science
Symposium on State of Virginia's Environment**

Thursday, May 21, 2015
at James Madison University

What is the current state of our

Forests, Dr. Christine Small, Biology Department, Radford University

Rivers in Central Virginia, Dr. Paul Bukaveckas, Department of Biology, Virginia Commonwealth University

Air Quality, Dr. James Blando, School of Community & Environmental Health, Old Dominion University

Tidal Wetlands, Dr. William G. Reay Director, Chesapeake Bay National Estuarine Research Reserve, Virginia Institute of Marine Science

Water Quality, Dr. R. Christian Jones, Potomac Environmental Research and Education Center, George Mason University

Ocean & Marine Environments, Dr. Larry Atkinson and Dr. Tal Ezer, Department of Ocean, Earth & Atmospheric Sciences, Old Dominion University

Land Use, Dr. David S. Hardin, Department of Biology & Environmental Science, Longwood University

Mammals, Dr. Nancy D. Moncrief, Virginia Museum of Natural History & Dr. John F. Pagels, Department of Biology, Virginia Commonwealth University and Research Associate, Virginia Museum of Natural History

Birds, Brian Watts, Director, Center for Conservation Biology of The College of William and Mary & the Virginia Commonwealth University

Reptiles, Dr. Tom Akre, Director of Virginia Working Landscapes, Smithsonian Mason School of Conservation, Smithsonian Conservation Biology Institute

Amphibians, Dr. Jennifer Sevin, Center for Leadership in Global Sustainability, Virginia Tech & Research Associate at the Smithsonian Institution and John Kleopfer, Virginia Department of Game and Inland Fisheries.

Insects, Dr. Steve Roble, Natural Heritage Program, Virginia Department of Conservation & Recreation

Fish, Dr. Paul Angermeier, Cooperative Fish and Wildlife Research Unit & Department of Fish and Wildlife Conservation, Virginia Tech and Mike Pinder, Virginia Department of Game and Inland Fisheries

Mollusk, Dr. Jess W. Jones, U.S. Fish and Wildlife Service, Co-Director, Freshwater Mollusk Conservation Center, Department of Fish and Wildlife Conservation, Virginia Tech

Plants, Dr. Michael H. Renfroe, Department of Biology, James Madison University

Proceedings to be Published by VAS

For more information contact:

vas@smv.org OR call 804-864-1450

