## DEVELOPMENTAL FLEXIBILITY IN SPOTTED HYENAS (*CROCUTA CROCUTA*): THE ROLE OF MATERNAL AND ANTHROPOGENIC EFFECTS

By

Julia Rachel Greenberg

### A DISSERTATION

Submitted to Michigan State University in partial fulfillment of the requirements for the degree of

Integrative Biology -- Doctor of Philosophy Ecology, Evolutionary Biology, and Behavior -- Dual Major

### ABSTRACT

### DEVELOPMENTAL FLEXIBILITY IN SPOTTED HYENAS (CROCUTA CROCUTA): THE ROLE OF MATERNAL AND ANTHROPOGENIC EFFECTS

By

#### Julia Rachel Greenberg

Whereas human activities are leading to declines in many wildlife populations, some species appear to be faring relatively well in the face of environmental change by demonstrating a great deal of phenotypic plasticity. I investigated the mechanisms underlying such flexibility in spotted hyenas (*Crocuta crocuta*), a species that persists in areas rapidly changing due to human activity. Specifically, I tested an hypothesis that the developmental trajectory of spotted hyenas is shaped by anthropogenic disturbance, and that hyena mothers transmit information about anthropogenic disturbance in their environment to their offspring via behavioral and/or physiological maternal effects. To test this hypothesis, I used longitudinal analyses of hyena behavior and physiology as well as cross-sectional analyses comparing hyenas in "high-disturbance" and "low-disturbance" areas of the Masai Mara National Reserve in Kenya.

First, I conducted field experiments to assess differences in personality among juvenile spotted hyenas living in high- and low-disturbance areas. I found that juveniles in the highdisturbance area were significantly less neophobic and more exploratory, but also less bold, than those in the low-disturbance area. Because most of the subjects tested were residing at the communal den, where cubs are not directly exposed to anthropogenic activity, these findings suggested that maternal effects may be shaping these differences. Next, I determined socioecological correlates of juvenile fecal glucocorticoid (fGC) concentrations and tested the hypothesis that disturbance-related differences in fGC concentrations mediate differences in juvenile personality. I did not find strong support for this hypothesis because, although juvenile females showed higher fGC concentrations in high-disturbance than low-disturbance areas, there were no significant disturbance-related differences in fGC concentrations among juvenile males, lactating females, or pregnant females. Finally, I explored variation in maternal behavior among spotted hyenas. I found that mothers showed significant individual variation in the amount of time they spent grooming cubs, but little variation in the amount of time they spent nursing and in close proximity to cubs, or in how they maintained proximity with cubs. I also found support for the hypothesis that disturbance-related differences in maternal behavior mediated the differences I documented in juvenile personality. Using spatial data from mothers fitted with GPS collars, I found that mothers in the high-disturbance area showed significantly lower rates of den attendance than did mothers in low-disturbance areas. While at the den, mothers in high-disturbance spent more time nursing their cubs, but also less time close to their cubs and higher rates of leaving their cubs, than did mothers in low-disturbance.

Overall, our results provide insights into how plasticity may be affording spotted hyenas the ability to persist in disturbed areas. None of the maternal behaviors measured were significantly associated with offspring survival, although maternal effects on offspring boldness may positively affect cub survivorship in high-disturbance. Therefore, juvenile resilience to variation in maternal behavior and an early period buffered from disturbance at the communal den may allow mothers to alter their behavior in response to disturbance without negatively affecting offspring survivorship. Copyright by JULIA RACHEL GREENBERG 2017 For my father, Gary Greenberg, who told me from a young age, that I would one day earn a PhD just like he did, and fulfill my dream of studying animals in beautiful, far away places.

### ACKNOWLEDGEMENTS

I have been fortunate in my academic career to learn from a number of "big thinkers." First and foremost, I want to thank Dr. Kay Holekamp, who, as an expansive scientist and thinker, has encouraged me to "think big" in my approach to my dissertation and to science. I am particularly grateful that Kay gave me the freedom to pursue my curiosities, take risks, and combine my varied research interests in creative ways. I have learned so much from studying with a scholar whose expertise cannot be easily classified into one sub-field or methodological approach. I am grateful to have studied with a scientist who values keen observational skills, encouraged me to ground my ideas in direct observations, and to spend time simply watching my study subjects before making too many specific plans. I will always cherish the time that I spent observing hyenas with Kay in the field and learning things that cannot be taught in any textbook. Finally, I am beyond grateful to have spent seven years working with a woman who sees me as a person and not simply a scientist. In an academic climate in which there is increasing pressure to specialize, to de-value the art of deep observation in favor of quick results, and to adopt a cut-throat mentality towards peers, I am flooded with the recognition of how fortunate I have been. I have also been humbled by the opportunity to utilize a long-term dataset that you and your students built over the course of three decades. Thank you.

Similarly, the opportunity to work among lab-mates whose interests and expertise are so incredibly diverse has helped to expand my thinking into scientific realms I could not have foreseen. I cannot express how much I have learned from my many lab-mates and fellow researchers in Kenya throughout the years. I know how lucky I am to have worked in an environment where people want to help each other, share ideas and data, and collaborate with

vi

one another even if it does not have some immediate reward. I specifically want to thank several researchers and friends who have helped me in specific aspects of my dissertation work. Tracy Montgomery and Sarah Jones, I honestly do not know how I would have survived lab work without you. Jenna Parker, Lily Johnson-Ulrich, Wes Binder, Benson Pion, and Wilson Kilong, you not only made my field experiments and observations possible, but I became a better field researcher and observer from working alongside you. Andrew Denhardt (aka "Stats Man"), I started meeting with you at a time when I was at my wits end with stats and you gave me the confidence and skills to overcome analytical hurdles. Zach Laubach, if it weren't for your interest and skill in working with messy behavioral data, I might currently be summing up the data for two chapters of this dissertation on pencil and paper. Thank you for being such a great collaborator and I'm looking forward to continuing to work with you in the future. Joseph Kamaamia, Jackson Kamaamia, Philomen Naigurian, Moses Naigurian, and George Kilinyet, thank you for opening up your hearts and worlds to me in Kenya. The things I learned from living and learning with you cannot be expressed in the text of a dissertation. I also am grateful to have worked with numerous talented undergraduates, several of which not only made my research possible, but also helped to inform my thinking, specifically Shannon Carvey, Kaycee Morra, Samantha Patterson, and Karee Lesko.

There is not enough room for me to describe how each of the following people have helped me along the way, but I want to thank them for not only being great colleagues, but also for being supportive friends who have made my years in graduate school and Kenya an insane amount of fun: Leslie Curren, Katy Califf, Andy Flies, Andy Booms, David Green, Marc Wiseman, Siri Okamoto, Emily Dittmar, Eli Swanson, Eli Strauss, Kenna Lehmann, Julie Turner, Nora Lewin, Hadley Couraud, Erin Pearson, Aaron Sandel, Rachna Reddy, Bethany

vii

Hansen, Elizabeth Johnson, Rachael Eaton, Bene Bachelot, Rohan Maddamsetti, Kari Dammerman, Austin Dreyer, Pat Bills, and Dee White.

I want to thank my committee members for their guidance. Tom Getty, the person who always asks that tough question at seminar, I have benefited from your discerning eye. Joe Lonstein, I'm grateful you made sure I read foundational papers for my comprehensive exams, and I've greatly benefited from having my research critiqued by someone who studies similar topics in a laboratory setting. Laura Smale, your class and our discussions over the years have made me think deeply about developmental processes and I am grateful to have had the opportunity to utilize some of the data you collected in the early days of the project.

I also want to thank several other faculty members who helped in my training and development during graduate school. Dr. Jeff French and Dr. Jacinta Beehner, thank you for opening up your endocrinology labs to me and helping me to understand and resolve numerous thorny issues involving hormone assays and long-term hormonal data sets. Thanks also to your lab managers, Drew Birnie and Teera Parr for assisting in my training. Dr. John Mitani, your Friday afternoon lab meetings served as a kind of surrogate academic home for me while I was conducting research in Ann Arbor. I want to thank you for being so welcoming, for your enthusiasm and interest in talking about my research, and for the numerous times that you shared your own "big thoughts" about science and the world with me.

My work would not have been possible without funding to myself and to my advisor from several sources. My research was supported by an NSF Graduate Research Fellowship, the Hensley Fellowship from the MSU College of Natural Sciences, and grants from MSU's Program in Ecology, Evolutionary Biology, and Behavior. This work was also supported by NSF Grants OISE 1556407 and DEB 1353110. I also want to thank the Kenyan National

viii

Council for Science and Technology, the Narok County Council, the Kenya Wildlife Service, and the Mara Conservancy for permission to conduct field research with hyenas.

Finally, I want to thank several people in my non-academic life. Michelle (mom), you have always believed in me. In graduate school, the fact that you have seen such value in me pursuing a PhD, and this line of research in particular, has helped me to believe in myself and my abilities. Your "pep talks" and writing advice have been invaluable. Jared, my biggest fan, thank you for your enthusiasm, and thanks to you and Jordie for making such a memorable visit to Kenya. Aura, you came into my life at a difficult time in my PhD career, and you helped me move through it. Thank you for forcing me to leave my computer and go for beautiful walks and swims with you, and for always humbling me when I begin to think I know anything about animal behavior. John, meeting you mid-way through grad school and your presence in my life ever since has kept me grounded in why I did this to begin with. Moving through the world with you helps me to remember that no scientific analysis can replace what is learned by being in nature, and that I should never let an academic matter distract me from my fundamental curiosity and connection to the natural world. Your help formatting this dissertation wasn't bad either! Finally, thank you to my father. Although you couldn't be here during this time in my life, I have imagined you with me in all phases of this process-- visiting me in Kenya and playing "research assistant" like you always said you would, meeting my colleagues and friends, or having long chats on the phone about the details of research that most people don't want to hear. My PhD has been the fulfillment of a childhood dream that you helped to foster.

ix

# TABLE OF CONTENTS

LIST OF FIGURES	LIST OF TABLES	xii
CHAPTER 1 GENERAL INTRODUCTION.  1    OVERVIEW OF CHAPTERS  4    CHAPTER 2 HUMAN DISTURBANCE AFFECTS PERSONALITY  8    DEVELOPMENT IN A WILD CARNIVORE  8    ABSTRACT.  8    NTRODUCTION  9    METHODS.  12    Subjects & Study Populations  12    Experimental Stimuli  14    Ethical Note  16    Data collection  16    Data extraction  17    Statistical Analysis  18    Independent Variables  18    Models of variation in neophobia, exploration and boldness.  20    Consistency across time and context  22    Relationship between personality traits and survival  23    Relationship between personality traits and survival  24    Exploration  25    Boldness  27    Consistency across time and context  29    Relationship between personality traits and survival  31    DISCUSSION  32    ACKNOWLEDGEMENTS  38    CHAPTER 3 SEX- AND AGE-SPECIFIC CORRELATES OF FECAL  39    CHAPTER 3 SEX- AND AGE-SPEC	LIST OF FIGURES	xiv
OVERVIEW OF CHAPTERS  4    CHAPTER 2 HUMAN DISTURBANCE AFFECTS PERSONALITY  8    DEVELOPMENT IN A WILD CARNIVORE  8    ABSTRACT  8    INTRODUCTION  9    METHODS  12    Subjects & Study Populations  12    Experimental Stimuli  14    Models of variation in neophobia, exploration and boldness.  20    Consistency across time and context  22    Relationship between personality traits and survival <td>CHAPTER 1 GENERAL INTRODUCTION</td> <td>1</td>	CHAPTER 1 GENERAL INTRODUCTION	1
CHAPTER 2 HUMAN DISTURBANCE AFFECTS PERSONALITY DEVELOPMENT IN A WILD CARNIVORE	OVERVIEW OF CHAPTERS	4
DEVELOPMENT IN A WILD CARNIVORE	CHAPTER 2 HUMAN DISTURBANCE AFFECTS PERSONALITY	
ABSTRACT	DEVELOPMENT IN A WILD CARNIVORE	8
INTRODUCTION	ABSTRACT	8
METHODS12Subjects & Study Populations12Experimental Stimuli14Ethical Note16Data Collection16Data Collection17Statistical Analysis18Independent Variables18Models of variation in neophobia, exploration and boldness20Consistency across time and context22Relationship between personality traits and survival23RESULTS24Ncophobia24Exploration25Boldness27Consistency across time and context29Relationship between personality traits and survival31DISCUSSION32ACKNOWLEDGEMENTS38CHAPTER 3 SEX- AND AGE-SPECIFIC CORRELATES OF FECAL39GUCOCORTICOID CONCENTRATIONS IN SPOTTED HYENAS.39INTRODUCTION39METHODS44Study Site and Subjects.44Fecal sample collection, extraction, and immunoassay of hormone concentrations.46Statistical Analysis47Longitudinal analysis of juvenile fGC concentrations 1993-201448	INTRODUCTION	9
Subjects & Study Populations12Experimental Stimuli14Ethical Note16Data Collection16Data Collection17Statistical Analysis18Independent Variables18Models of variation in neophobia, exploration and boldness20Consistency across time and context22Relationship between personality traits and survival23RESULTS24Neophobia24Exploration25Boldness27Consistency across time and context29Relationship between personality traits and survival31DISCUSSION32ACKNOWLEDGEMENTS38CHAPTER 3 SEX- AND AGE-SPECIFIC CORRELATES OF FECAL38CHAPTER 3 SEX- AND AGE-SPECIFIC CORRELATES OF FECAL39METHODS44Study Site and Subjects44Study Site and Subjects44Acknowlice Concentrations46Statistical Analysis47Longitudinal analysis of juvenile fGC concentrations 1993-201448	METHODS	12
Experimental Stimuli14Ethical Note16Data Collection16Data Collection17Statistical Analysis18Independent Variables18Models of variation in neophobia, exploration and boldness.20Consistency across time and context22Relationship between personality traits and survival23RESULTS24Neophobia24Neophobia25Boldness27Consistency across time and context29Relationship between personality traits and survival31DISCUSSION32ACKNOWLEDGEMENTS38CHAPTER 3 SEX- AND AGE-SPECIFIC CORRELATES OF FECAL39GLUCOCORTICOID CONCENTRATIONS IN SPOTTED HYENAS39INTRODUCTION39METHODS44Study Site and Subjects.44Study Site and Subjects.44Study Site and Subjects.44Actistical Analysis47Longitudinal analysis of juvenile fGC concentrations 1993-201448	Subjects & Study Populations	12
Etical Note16Data Collection16Data extraction17Statistical Analysis18Independent Variables18Models of variation in neophobia, exploration and boldness.20Consistency across time and context22Relationship between personality traits and survival23RESULTS24Neophobia24Exploration25Boldness27Consistency across time and context29Relationship between personality traits and survival31DISCUSSION32ACKNOWLEDGEMENTS38CHAPTER 3 SEX- AND AGE-SPECIFIC CORRELATES OF FECAL39GLUCOCORTICOID CONCENTRATIONS IN SPOTTED HYENAS39INTRODUCTION39METHODS44Study Site and Subjects.44Fecal sample collection, extraction, and immunoassay of hormone concentrations46Statistical Analysis47Longitudinal analysis of juvenile fGC concentrations 1993-201448	Experimental Stimuli	14
Data Collection16Data extraction17Statistical Analysis18Independent Variables18Models of variation in neophobia, exploration and boldness20Consistency across time and context22Relationship between personality traits and survival23RESULTS24Neophobia24Exploration25Boldness27Consistency across time and context29Relationship between personality traits and survival31DISCUSSION32ACKNOWLEDGEMENTS38CHAPTER 3 SEX- AND AGE-SPECIFIC CORRELATES OF FECAL39GLUCOCORTICOID CONCENTRATIONS IN SPOTTED HYENAS.39METHODS44Study Site and Subjects44Fecal sample collection, extraction, and immunoassay of hormone concentrations46Statistical Analysis47Longitudinal analysis of juvenile fGC concentrations 1993-201448	Ethical Note	16
Data extraction17Statistical Analysis18Independent Variables18Models of variation in neophobia, exploration and boldness20Consistency across time and context22Relationship between personality traits and survival23RESULTS24Neophobia24Exploration25Boldness27Consistency across time and context29Relationship between personality traits and survival21Data extraction25Boldness27Consistency across time and context29Relationship between personality traits and survival31DISCUSSION32ACKNOWLEDGEMENTS38CHAPTER 3 SEX- AND AGE-SPECIFIC CORRELATES OF FECAL39GLUCOCORTICOID CONCENTRATIONS IN SPOTTED HYENAS39INTRODUCTION39METHODS44Study Site and Subjects44Fecal sample collection, extraction, and immunoassay of hormone concentrations46Statistical Analysis47Longitudinal analysis of juvenile fGC concentrations 1993-201448	Data Collection	16
Statistical Analysis18Independent Variables18Models of variation in neophobia, exploration and boldness.20Consistency across time and context22Relationship between personality traits and survival23RESULTS24Neophobia24Exploration25Boldness27Consistency across time and context29Relationship between personality traits and survival21Boldness27Consistency across time and context29Relationship between personality traits and survival31DISCUSSION32ACKNOWLEDGEMENTS38CHAPTER 3 SEX- AND AGE-SPECIFIC CORRELATES OF FECAL39GLUCOCORTICOID CONCENTRATIONS IN SPOTTED HYENAS39METHODS44Study Site and Subjects44Fecal sample collection, extraction, and immunoassay of hormone concentrations46Statistical Analysis47Longitudinal analysis of juvenile fGC concentrations 1993-201448	Data extraction	17
Independent Variables18Models of variation in neophobia, exploration and boldness20Consistency across time and context22Relationship between personality traits and survival23RESULTS24Neophobia24Exploration25Boldness27Consistency across time and context29Relationship between personality traits and survival31DISCUSSION32ACKNOWLEDGEMENTS38CHAPTER 3 SEX- AND AGE-SPECIFIC CORRELATES OF FECAL39GLUCOCORTICOID CONCENTRATIONS IN SPOTTED HYENAS39METHODS44Study Site and Subjects44Fecal sample collection, extraction, and immunoassay of hormone concentrations46Statistical Analysis47Longitudinal analysis of juvenile fGC concentrations 1993-201448	Statistical Analysis	18
Models of variation in neophobia, exploration and boldness.  20    Consistency across time and context  22    Relationship between personality traits and survival  23    RESULTS  24    Neophobia  24    Exploration  25    Boldness  27    Consistency across time and context  29    Relationship between personality traits and survival  31    DISCUSSION  32    ACKNOWLEDGEMENTS  38    CHAPTER 3 SEX- AND AGE-SPECIFIC CORRELATES OF FECAL  39    GLUCOCORTICOID CONCENTRATIONS IN SPOTTED HYENAS  39    METHODS  44    Study Site and Subjects  44    Fecal sample collection, extraction, and immunoassay of hormone concentrations  46    Statistical Analysis  47    Longitudinal analysis of juvenile fGC concentrations 1993-2014  48	Independent Variables	18
Consistency across time and context22Relationship between personality traits and survival23RESULTS24Neophobia24Exploration25Boldness27Consistency across time and context29Relationship between personality traits and survival31DISCUSSION32ACKNOWLEDGEMENTS38CHAPTER 3 SEX- AND AGE-SPECIFIC CORRELATES OF FECAL39GLUCOCORTICOID CONCENTRATIONS IN SPOTTED HYENAS39NTRODUCTION39METHODS44Study Site and Subjects44Fecal sample collection, extraction, and immunoassay of hormone concentrations46Statistical Analysis47Longitudinal analysis of juvenile fGC concentrations 1993-201448	Models of variation in neophobia, exploration and boldness	20
Relationship between personality traits and survival23RESULTS24Neophobia24Exploration25Boldness27Consistency across time and context29Relationship between personality traits and survival31DISCUSSION32ACKNOWLEDGEMENTS38CHAPTER 3 SEX- AND AGE-SPECIFIC CORRELATES OF FECALGLUCOCORTICOID CONCENTRATIONS IN SPOTTED HYENAS39INTRODUCTION39METHODS44Study Site and Subjects44Fecal sample collection, extraction, and immunoassay of hormone concentrations46Statistical Analysis47Longitudinal analysis of juvenile fGC concentrations 1993-201448	Consistency across time and context	22
RESULTS  24    Neophobia  24    Exploration  25    Boldness  27    Consistency across time and context  29    Relationship between personality traits and survival  31    DISCUSSION  32    ACKNOWLEDGEMENTS  38    CHAPTER 3 SEX- AND AGE-SPECIFIC CORRELATES OF FECAL  39    GLUCOCORTICOID CONCENTRATIONS IN SPOTTED HYENAS  39    INTRODUCTION  39    METHODS  44    Study Site and Subjects  44    Fecal sample collection, extraction, and immunoassay of hormone concentrations  46    Statistical Analysis  47    Longitudinal analysis of juvenile fGC concentrations 1993-2014  48	Relationship between personality traits and survival	23
Neophobia24Exploration25Boldness27Consistency across time and context29Relationship between personality traits and survival31DISCUSSION32ACKNOWLEDGEMENTS38CHAPTER 3 SEX- AND AGE-SPECIFIC CORRELATES OF FECAL39GLUCOCORTICOID CONCENTRATIONS IN SPOTTED HYENAS39INTRODUCTION39METHODS44Study Site and Subjects44Fecal sample collection, extraction, and immunoassay of hormone concentrations46Statistical Analysis47Longitudinal analysis of juvenile fGC concentrations 1993-201448	RESULTS	24
Exploration25Boldness27Consistency across time and context29Relationship between personality traits and survival31DISCUSSION32ACKNOWLEDGEMENTS38CHAPTER 3 SEX- AND AGE-SPECIFIC CORRELATES OF FECAL38GLUCOCORTICOID CONCENTRATIONS IN SPOTTED HYENAS39INTRODUCTION39METHODS44Study Site and Subjects44Fecal sample collection, extraction, and immunoassay of hormone concentrations46Statistical Analysis47Longitudinal analysis of juvenile fGC concentrations 1993-201448	Neophobia	24
Boldness27Consistency across time and context29Relationship between personality traits and survival31DISCUSSION32ACKNOWLEDGEMENTS38CHAPTER 3 SEX- AND AGE-SPECIFIC CORRELATES OF FECALGLUCOCORTICOID CONCENTRATIONS IN SPOTTED HYENAS39INTRODUCTION39METHODS44Study Site and Subjects44Fecal sample collection, extraction, and immunoassay of hormone concentrations46Statistical Analysis47Longitudinal analysis of juvenile fGC concentrations 1993-201448	Exploration	25
Consistency across time and context29Relationship between personality traits and survival31DISCUSSION32ACKNOWLEDGEMENTS32CHAPTER 3 SEX- AND AGE-SPECIFIC CORRELATES OF FECALGLUCOCORTICOID CONCENTRATIONS IN SPOTTED HYENAS39INTRODUCTION39METHODS44Study Site and Subjects44Fecal sample collection, extraction, and immunoassay of hormone concentrations46Statistical Analysis47Longitudinal analysis of juvenile fGC concentrations 1993-201448	Boldness	27
Relationship between personality traits and survival  .31    DISCUSSION  .32    ACKNOWLEDGEMENTS  .38    CHAPTER 3 SEX- AND AGE-SPECIFIC CORRELATES OF FECAL  .39    GLUCOCORTICOID CONCENTRATIONS IN SPOTTED HYENAS  .39    INTRODUCTION  .39    METHODS  .44    Study Site and Subjects  .44    Fecal sample collection, extraction, and immunoassay of hormone concentrations  .46    Statistical Analysis  .47    Longitudinal analysis of juvenile fGC concentrations 1993-2014  .48	Consistency across time and context	29
DISCUSSION	Relationship between personality traits and survival	
ACKNOWLEDGEMENTS	DISCUSSION	32
CHAPTER 3 SEX- AND AGE-SPECIFIC CORRELATES OF FECAL GLUCOCORTICOID CONCENTRATIONS IN SPOTTED HYENAS	ACKNOWLEDGEMENTS	
GLUCOCORTICOID CONCENTRATIONS IN SPOTTED HYENAS	CHAPTER 3 SEX- AND AGE-SPECIFIC CORRELATES OF FECAL	
INTRODUCTION  39    METHODS  44    Study Site and Subjects  44    Fecal sample collection, extraction, and immunoassay of hormone concentrations  46    Statistical Analysis  47    Longitudinal analysis of juvenile fGC concentrations 1993-2014  48	GLUCOCORTICOID CONCENTRATIONS IN SPOTTED HYENAS	
METHODS	INTRODUCTION	
Study Site and Subjects	METHODS	
Fecal sample collection, extraction, and immunoassay of hormone concentrations	Study Site and Subjects	
Statistical Analysis of juvenile fGC concentrations 1993-2014	Fecal sample collection, extraction, and immunoassay of hormone concentrations	46
Longitudinal analysis of juvenile fGC concentrations 1993-2014	Statistical Analysis	47
	Longitudinal analysis of juvenile fGC concentrations 1993-2014	48

Cross-sectional comparison of fGC concentrations 2009-2014	
Relationship between early life fGC concentrations and fitness	50
RESULTS	52
Longitudinal analysis of socio-ecological factors affeting juvenile fGC concentrations	52
Cross-sectional comparison of fGC concentrations in high- and low-disturbance areas	52
Juveniles	52
Lactating and Pregnant Females	55
Relationship between early life fGC concentrations and fitness	57
DISCUSSION	
CHAPTER 4 VARIATION IN MATERNAL BEHAVIOR AND	
MOTHER-OFFSPRING INTERACTIONS	64
INTRODUCTION	64
METHODS	69
Subjects & Study Populations	69
Data Collection	71
Statistical Analysis	72
Individual variability and repeatability of maternal behavior	75
Relationship between maternal behavior and offspring fitness	76
RESULTS	76
Models of time spent nursing, grooming, and in close proximity to offspring	76
Models of proximity maintenance	82
Individual variability and repeatability of maternal behavior	82
Relationship between maternal behavior and offspring fitness	85
DISCUSSION	85
CHAPTER 5 ANTHROPOGENIC EFFECTS ON MATERNAL	
BEHAVIOR IN SPOTTED HYENAS	91
INTRODUCTION	91
METHODS	97
Subjects & Study Populations	97
Data Collection	99
Maternal attendance at dens	99
Maternal behavior at dens	102
Statistical analysis of maternal den attendance and behavior	103
Maternal den attendance and offspring survivorship	105
RESULTS	106
Maternal attendance at dens	106
Maternal behavior at dens	108
Maternal den attendance and offspring survivorship	113
DISCUSSION	115
REFERENCES	

# LIST OF TABLES

Table 2.1. Results of a Cox proportional hazards model for variation in latency to contact the novel object (N=58). Asterisks indicate $P \le 0.051$
Table 2.2. Results of a logistic regression model for variation in exploration of the novel object (N=58). Asterisks indicate $P \le 0.051$
Table 2.3. Results of a logistic regression model for variation in boldness (N=60). Asterisks indicate $P \le 0.051$
Table 3.1. Factors explaining variation in fGC concentrations in juvenile spotted hyenas fromthe Talek West clan 1993-2014 (N=382 samples from 171 individuals). An asterisk indicates $P<0.05$
Table 3.2. Results of preliminary analysis of variation in fGC concentrations among juveniles,lactating, and pregnant females in the low disturbance area. An asterisk indicates $P < 0.05$
Table 4.1. Results of a zero-inflated negative binomial model explaining variation amongmothers in duration of nursing. An asterisk indicates $P < 0.05$
Table 4.2. Results of a zero-inflated negative binomial model explaining variation in time mothers spent in close proximity to their cubs. An asterisk indicates $P$ <0.05
Table 4.3. Results of a negative binomial model explaining variation in time mothers spentgrooming offspring. An asterisk indicates $P < 0.05$
Table 4.4. Results of a negative binomial model explaining variation in how often mothersapproached their cub. An asterisk indicates $P < 0.05$
Table 4.5. Results of a negative binomial model explaining variation in how often mothers moved more than 1 m from their cub. An asterisk indicates $P$ <0.05
Table 5.1. Results of zero-inflated negative binomial model explaining variation inmaternal den attendance. ** indicates $P < 0.05$ . * indicates $P < 0.1$
Table 5.2. Tukey pairwise comparisons of rates of nursing, grooming, and close proximity among mothers living in the three clans in the undisturbed area

Table 5.4. Results of a zero-inflated negative binomial model explaining variation in how often mothers approached their cubs to within 1m. \*\* indicates P < 0.05. \* indicates P < 0.1.....114

## LIST OF FIGURES

Figure 2.4. A logistic regression model for variation in exploration score indicated that: *a*) juveniles in high disturbance were significantly more exploratory than those in low disturbance (P=0.003) and *b*) high-ranking juveniles were significantly more exploratory than low-ranking juveniles (P=0.020; N=58).

Figure 4.2. The total number of Focal Animal Surveys (FAS) collected on mothers with cubs 10-15 months. Data are binned by the cub's age in months. Numbers above bars indicate the number of cubs observed in that age

category. .....77

#### CHAPTER 1

### GENERAL INTRODUCTION

Across the globe, there are few ecosystems that remain unaffected by human population growth, changes in land-use, or climate change. Species that appear best able to adjust to such changes are those expressing a great deal of phenotypic plasticity (Reed et al. 2010, Sih 2013). Numerous mechanisms of plasticity have been documented in mammalian systems, including those involving neural circuitry, endocrine function, behavior, immunological responses, and gene expression. Plasticity is often shaped by developmental processes (Lindström 1999, Bateson and Gluckman 2011), and mothers can play a significant role in shaping developmental plasticity through maternal effects, in which a mother's phenotype directly influences her offspring's phenotype (Bernardo 1996). In recent years, there has been great interest in the potential of maternal effects to be adaptive for offspring, whereby mothers transmit information to offspring about the environment that in turn allows offspring to maximize their survival or reproduction (Mousseau and Fox 1998, West-Eberhard 2005). The extent to which maternal effects serve an adaptive function for offspring is controversial (Marshall and Uller 2007). However, their potential to shape offspring phenotypes in situations of rapid environmental change is intriguing, because it could serve as a powerful mechanism of plasticity that facilitates species persistence in disturbed environments (Jablonka et al. 1995, Bernardo 1996).

In my dissertation, I aim to develop spotted hyenas as a model species for studying mammalian developmental plasticity in the context of environmental change. I test an hypothesis suggesting that the developmental trajectory of spotted hyenas is shaped by anthropogenic disturbance, and that spotted hyena mothers transmit information about

anthropogenic disturbance in their environment to their offspring via behavioral and/or physiological maternal effects. Spotted hyenas make an excellent species in which to examine *developmental flexibility* because they show an unusually protracted period of physical and behavioral development, and because mothers invest very heavily in a small number of offspring (Watts et al. 2009). Mothers give birth to 1 or 2 cubs and nurse cubs until approximately 14 months. Even after being weaned, offspring survival is dependent on maternal support at carcasses, where young hyenas must compete against adults for food despite the fact that the feeding apparatus of young spotted hyenas is not fully developed for eating meat and bone until 36 months of age (Tanner et al. 2009). Individuals do not reach full proficiency at hunting until roughly five years of age (Holekamp et al. 1997b).

Adult spotted hyenas show remarkable flexibility in many aspects of their biology and ecology. The most abundant large carnivores in sub-Saharan Africa, spotted hyenas exist, and often thrive, in a diverse range of habitats. Hyenas' *ecological flexibility* allows them to feed on multiple food sources (at least 43 types of prey in the Masai Mara alone; Cooper et al. 1999); hunt up to 95% of their own food at some sites but also exist as opportunistic scavengers and trash-eaters at others; and fill a bone-eating niche that no other animal can (Honer et al. 2002, Holekamp and Dloniak 2010). They also show pronounced *behavioral flexibility*, exhibiting the ability, for example, to follow migratory prey in seasonal patterns on "commuting" trips for up to 6 days or to shift their daily biorhythms in response to local human activity (Hofer and East 1993a, Kolowski et al. 2007). Spotted hyenas' impressive *physiological flexibility* affords them the ability to conceive and reproduce year round and survive exposure to many pathogens that compromise the immune systems of other carnivores, such as canine distemper and rabies (Holekamp et al. 1996, Flies et al. 2012). Finally, they show a great deal of *social flexibility* in that their fission-fusion social system allows them to adjust their group size and composition in order to take advantage of highly ephemeral carcasses and kills (Frank 1986a, Smith et al. 2008).

Each of these aspects of spotted hyenas' flexibility has likely played a role in allowing them to adjust to increasing anthropogenic disturbance over the past twenty-nine years at our study site in the Masai Mara National Reserve, Kenya (MMNR). In the territory of the Talek West clan, which lives along the border of the MMNR, the human population has grown exponentially since the late 1990s, resulting in increased wildlife-human conflict, and numbers of livestock being grazed within the reserve (Kolowski and Holekamp 2006, Holekamp and Dloniak 2010, Green et al. 2017). As human disturbance has increased, adult spotted hyenas have become more nocturnal, expanded their home ranges, and increased the distances they travel to hunt (Boydston et al. 2003b). Compared with hyenas living in pristine areas of the MMNR, hyenas in the disturbed Talek region show a greater preference for protective vegetation (Kolowski and Holekamp 2009), are more vigilant (Pangle and Holekamp 2010), shift their activity patterns to avoid interaction with humans (Kolowski et al. 2007), travel greater distances in the mid-day heat (Ikime 2015), and travel faster overall (Green 2015).

The marked flexibility that has been documented among adult hyenas raises questions regarding the development of such plasticity. When during a hyena's lifetime do we begin to see disturbance-related differences? How is plasticity shaped throughout the course of a hyena's development? What cues provide information to a developing hyena about anthropogenic disturbance in its environment? These questions are even more intriguing given that young spotted hyenas reside at a communal den, in an environment that is distinct from that

experienced by their mothers and other adults. Much of the research in my dissertation focuses on juveniles when they are residing at communal dens at approximately 1-9 months old. Each communal den is composed of a series of burrows and tunnels that protect hyenas from dangers present in the rest of the clan's territory (Boydston et al. 2006). However, the communal den is also a social focal point of the clan, and cubs emerge daily to interact with their mothers and other hyenas, who are directly experiencing those dangers (Holekamp and Smale 1998a). In species that are long-lived and slow-growing, like spotted hyenas, the ability to respond to strong maternal signals of anthropogenic disturbance, starting very early in life, while still inhabiting a relatively safe environment, may be critical to their success in disturbed environments.

### OVERVIEW OF CHAPTERS

Each of the remaining chapters of my dissertation helps to test the hypothesis that the developmental trajectory of spotted hyenas is shaped by anthropogenic disturbance, and that spotted hyena mothers transmit information about anthropogenic disturbance in their environment to their offspring via behavioral and/or physiological maternal effects. Throughout, I employ both longitudinal analyses of the hyena population that has been monitored since the MSU Hyena Project began in 1988, as well as cross-sectional analyses comparing hyenas in "high-disturbance" and "low-disturbance" areas of the MMNR. In each chapter, I also examine the influence of other socio-ecological factors on cub development, and whether anthropogenic disturbance interacts with these other factors to affect spotted hyena behavior or physiology. Finally, in each chapter, I test whether the juvenile or maternal trait of interest is significantly associated with offspring survivorship and/or longevity. When sample

size allows, I also test whether the relationship between these traits and offspring fitness differs in areas of high- and low- disturbance.

In Chapter 2, I examine variation in personality among juvenile spotted hyenas, and test the hypothesis that juvenile spotted hyenas, like birds and small mammals, show consistent differences in personality between high- and low-disturbance areas. I use field experiments to assess individual variation and consistency in neophobia, exploration, and boldness. I find that juveniles in the high-disturbance area are significantly less neophobic and more exploratory, but also less bold, than those in low-disturbance areas. Because most of the subjects tested were residing at the communal den, where cubs are not directly exposed to anthropogenic activity, these findings raise the question of what mechanisms mediate these behavioral differences. It suggests that disturbance-related differences in maternal behavior and/or physiology may be shaping these differences in hyenas, starting very early in life.

In Chapter 3, I inquire which anthropogenic and socio-ecological factors are associated with variation in juvenile fecal glucocorticoid (fGC) concentrations. I also test the hypothesis that disturbance-related differences in fGC, either among juveniles or their mothers, mediate the behavioral differences documented in Chapter 2. I do not find strong support for this hypothesis because, although juvenile females show higher fGC concentrations in highdisturbance than low-disturbance areas, there were no significant disturbance-related differences in fGC concentrations among juvenile males, lactating females, or pregnant females. This suggests that juvenile females may show a particular sensitivity to anthropogenic disturbance and that there may be important sex differences in how early stressors affect hyenas. However, it also suggests that stress physiology, either among juveniles themselves or their mothers, are

likely not a major mediator of behavioral differences between juveniles inhabiting high- and lowdisturbance areas. This suggests that disturbance-related differences in maternal behavior may be the more important factor.

In Chapter 4, I use archived focal animal surveys (FAS) to document naturally-occurring variation in maternal behavior among individuals living in the Talek West clan before anthropogenic disturbance was prevalent. This provides the first study documenting the degree to which spotted hyena mothers vary in basic measures of maternal care, such as nursing, grooming, and maintaining close proximity with offspring. Interestingly, I find that mothers show little consistent individual variation in the amount of time they spend nursing and in close proximity to cubs, or in how they maintain proximity with cubs. However, they show significant individual variation with respect to how much time they spend grooming cubs. Unlike most aspects of hyena behavior that have been studied to date, a female's social rank is not significantly associated with the amount of time she spends nursing, grooming, or in close proximity to offspring. My findings also indicate that a cub's litter size significantly shape its experience of maternal care.

Having investigated naturally-occurring variation in maternal behavior, in Chapter 5, I go on to ask how maternal behavior is influenced by anthropogenic disturbance and test the hypothesis that differences in maternal behavior between the high- and low-disturbance areas mediate differences in juvenile personality. First, I use spatial data from mothers fitted with GPS collars to compare rates of maternal attendance at communal dens in the high- and lowdisturbance areas. Second, to inquire whether mothers in high- and low-disturbance areas, when present at communal dens, behave differently towards their cubs, I conducted FAS on mother-

offspring pairs. Spotted hyena mothers in the high disturbance area showed significantly lower rates of den attendance than did mothers in low disturbance areas. Perhaps to compensate, mothers in the high- disturbance area spent a higher proportion of their time nursing their cubs than did mothers in low-disturbance areas. Mothers in the high-disturbance area also showed differences regarding the ways in which they interacted with their cubs, spending less time close to their cubs and more often leaving their cubs rather than staying close to them. Our findings suggest that, although spotted hyenas show a remarkable ability to persist in disturbed areas, such conditions present a challenge to mothers. Our findings also support the hypothesis that disturbance-related differences in maternal behavior are associated with differences in juvenile personality. The association between maternal behavior and offspring personality shows some consistency with results from studies of Old World Monkeys. In these primates, mothers who show a low investment maternal strategy or are more "rejecting" tend to have offspring that are less neophobic and more exploratory than do mothers who show high investment in offspring or are more "protective" (reviewed in Fairbanks, 1996).

All of the research presented in this dissertation was the result of collaboration. My analyses utilize data collected by the many research assistants and graduate students who have contributed to the daily monitoring of spotted hyenas in Kenya since 1988. Additionally, each of my chapters was prepared in manuscript form, with significant input from co-authors. Therefore, I use the first person plural, rather than the first person singular, throughout the remainder of this dissertation.

### CHAPTER 2

## HUMAN DISTURBANCE AFFECTS PERSONALITY DEVELOPMENT IN A WILD CARNIVORE

Julia R. Greenberg & Kay E. Holekamp. 2017. Human disturbance affects personality development in a wild carnivore. *Animal Behaviour* 132:303-312

### ABSTRACT

Human activity can dramatically affect personality traits in birds and small mammals. However, we know very little about how anthropogenic disturbance shapes personality in mammalian carnivores, and whether the personality traits that may be affected have fitness consequences in human-dominated landscapes. We adapted standard experiments commonly used to assess personality in captive animals to compare three personality traits in 72 wild juvenile spotted hyenas (Crocuta crocuta) living in either areas heavily disturbed by human activity or areas with low levels of disturbance. We examined neophobia, defined as the tendency to avoid unfamiliar things, exploration, defined as the number of different ways an individual interacts with an object, and boldness, defined as an individual's tendency to take risks. To assess neophobia and exploration, we measured individuals' responses to a novel object, and to assess boldness, we measured hyenas' propensity to enter a wire-mesh box to obtain food. Juvenile spotted hyenas living in low disturbance areas were significantly more neophobic and less exploratory than individuals living in high disturbance areas. This is consistent with results obtained with birds and small mammals; however, unlike these other taxa, hyenas living in low disturbance areas were bolder than individuals living in high disturbance areas. The expression of some of these personality traits was also affected by the subject's social rank and the presence of a litter-mate,

but not by subject age or sex. Of the three traits, only boldness predicted survival to adulthood; less bold individuals were significantly more likely to survive than bolder individuals, in both high disturbance and low disturbance habitats. We propose that behavioral or physiological maternal effects may be shaping offspring temperament differences related to disturbance.

Keywords: personality, temperament, anthropogenic disturbance, human-wildlife conflict, social rank, neophobia, spotted hyena, *Crocuta crocuta* 

### INTRODUCTION

Human activity has been found to affect the behavior of animals in a variety of ways. Some of the most pronounced effects have been documented on animals' personality or temperament traits, which are individual differences in behavior that are stable across contexts and over time (Réale et al. 2007, Miranda et al. 2013, Sol et al. 2013). Changes in personality traits due to anthropogenic disturbance can result from individual behavioral plasticity or micro-evolutionary changes (Miranda et al. 2013). Across numerous bird and small mammal species, individuals tend to show more neophilic, exploratory, aggressive, and bold personalities in urban than rural areas (Miranda et al. 2013, Sol et al. 2013). However, we know virtually nothing about whether or how human disturbance shapes personality in most other animals, including large mammalian carnivores.

There is a critical need to understand how human activity influences carnivore behavior and populations (Baker et al. 2008, Darrow and Shivik 2009). Worldwide, carnivores are increasingly living in close proximity to humans (Treves and Karanth 2003, Treves et al. 2006,

Bateman and Fleming 2012), resulting in more frequent predation on livestock, injury to humans, and retaliatory killing of carnivores (Baker et al. 2008, Ripple et al. 2014). Understanding whether variation in personality affects carnivore survival in disturbed landscapes can help us to predict the effects of human activity on carnivore populations. Consistent interindividual differences in behavior have been documented in a few wild adult carnivores (e.g., coyotes, Canis latrans: Harris and Knowlton 2001, Heffernan et al. 2007), but most studies have been conducted on captive individuals (maned wolves, Chrysocyon brachyurus: Silva and Azevedo 2013; gray wolves, Canis lupus: Fox 1972, MacDonald 1983, Moretti, Hentrup, Kotrschal, & Range, 2015; coyotes, Mettler and Shivik 2007; European mink, Mustela lutreola: Haage et al. 2013; American mink, Neovison vison: Noer et al. 2015; kowaris, Dasyuroides byrnei: Russell and Pearce 1971, Wynne and McLean 1999 or domestic animals (dogs, Canis familiaris: Draper 1995, Jones and Gosling 2005; cats, Felis catus: Durr and Smith 1997. Understanding variation in personality among wild carnivores may also help researchers and managers to discern whether certain individuals consistently display behavior patterns that put them at risk of conflict with humans, and to target such "problem animals" for intervention (Caro 1999, McDougall et al. 2006, Shivik 2006).

Few studies have assessed the fitness consequences of personality traits in areas disturbed by human activity (Archard and Braithwaite 2010), although a number of recent studies have found that personality traits may be heritable and affect fitness in undisturbed habitats (Dingemanse et al. 2002, Dall et al. 2004, Smith and Blumstein 2008, Nicolaus et al. 2012, Taylor et al. 2012, Kortet et al. 2014, Petelle et al. 2015, Yoshida et al. 2016). It has been hypothesized that, in disturbed areas, birds and small mammals with more neophilic,

exploratory, and bold personalities may have a fitness advantage, as these traits may allow them to exploit novel habitats, live at high population densities, and take advantage of new resources (Miranda et al. 2013). However, the fitness consequences of these personality traits in areas with human activity may be dramatically different for carnivores than for birds or small mammals, as such tendencies would likely bring carnivores into direct conflict with humans.

Understanding whether personality traits in juvenile carnivores predict survivorship may be particularly important because young animals may be especially likely to engage in risky behavior and end up in conflict with humans (Anderson 1981, Saberwal et al. 1994). Lack of hunting experience, tendency to prey on animals that are easy to kill, and poor body condition relative to adults, are all factors that might make juvenile carnivores prone to human-wildlife conflicts, such as depredation of livestock (Stirling and Latour 1978, Payne and Jameson 1984, Matlack and Evans 1992, Seidensticker and McDougal 1993, Caro 1994, Holekamp et al. 1997b, Litvaitis et al. 2017). Young dispersing males may be particularly prone to engage in such activities (Linnell et al. 1999).

Here we used an experimental approach to explore variation in three personality traits in wild juvenile spotted hyenas (*Crocuta crocuta*), and inquire whether these traits differ between hyenas reared in habitat heavily disturbed by anthropogenic activity and those reared in areas with very low anthropogenic activity. Specifically, we examined neophobia, defined as the tendency to avoid or fear unfamiliar things (Barnett 1958), exploration, defined as the number of different ways an individual interacts with an object (Sroges and Glickman 1966), and boldness, defined as an individual's tendency to take risks (Réale et al. 2007). We predicted that, if juvenile hyenas living in areas with high human disturbance behaved like "urbanized" birds and small

mammals (Miranda et al. 2013, Sol et al. 2013), they would be less neophobic, more exploratory, and bolder than juveniles in low disturbance areas. In addition to human disturbance, we explored the effects of sex and social rank on juvenile personality traits because these variables begin to shape hyena behavior early in life (Smale et al. 1995, Dloniak et al. 2006, Holekamp et al. 2013). We also explored the effects of age on expression of these personality traits because, even though all of our subjects were juveniles, neophobia, exploration, and boldness change in some species as individuals approach reproductive maturity (Kendal et al. 2005, Biondi et al. 2010). Finally, we tested for consistency in behavior across time and context, and inquired whether any of these personality traits predicted survival to reproductive maturity.

### METHODS

### Subjects & Study Populations

Study subjects were 72 juvenile spotted hyenas inhabiting two protected areas in the Mara-Serengeti ecosystem in southwestern Kenya. Fifty-eight of these juveniles were subjects in our tests of neophobia and exploration and 60 of them were subjects in our test of boldness; 45 participated in both tests. Roughly half of the subjects (59% for tests of neophobia and exploration; 50% for test of boldness) lived in three clans whose territories were located in The Mara Conservancy, a pristine area managed by a private non-profit organization that strictly prohibits cattle grazing and human presence except in tour vehicles. We will therefore refer to this area as "low disturbance." The remaining subjects were from a clan that has been continuously monitored since 1988, and lives just inside the border of the Masai Mara National Reserve (henceforth, "the Reserve"). We refer to this area as "high disturbance" because, since the late 1990s, there has been exponential human population growth along the border of the Reserve (Watts and Holekamp 2009), humans are active with their livestock both day and night inside the Reserve in this area, and direct conflict between hyenas and livestock is common both inside and outside the Reserve (Kolowski and Holekamp 2006). Since 2005, humans have been responsible for the majority of hyena deaths (through spearing, snaring, and poisoning) for which mortality sources can be determined (Holekamp and Dloniak 2010). Through historical analyses and comparative studies with populations living in more pristine areas, numerous behavioral effects of increased human activity have been documented in this population, including increased nocturnality, increased daily travel, lower rates of den attendance by mothers, active avoidance of livestock and herders, and a preference for areas with dense vegetative cover (Boydston et al. 2003b, Kolowski et al. 2007, Kolowski and Holekamp 2009).

Our subjects ranged in age from 50-463 days old (mean = 171 days, median = 153 days). Spotted hyenas do not reach reproductive maturity until after ~720 days of age, nor do they reach full morphological maturity before 1000 days of age. All subjects could be individually identified based on unique spot patterns and ear damage. Age was initially estimated when cubs were first observed, based on their appearance and size (Holekamp et al. 1996), and sex was determined based on the shape of the glans of the erect phallus (Frank et al. 1990). However, two subjects died before we could determine their sex and were excluded from our models.

Juveniles were assigned social ranks based on our observations of their mother's position in the clan's dominance hierarchy. Observations of each adult female's aggressive and submissive behaviors during dyadic agonistic interactions were used to construct the hierarchy, as described previously (Martin and Bateson 1988, Smale et al. 1993). Juveniles "inherit"

dominance ranks immediately below those of their mothers in a process of social learning that is not complete until at least 18 months of age (Holekamp and Smale 1993, Smale et al. 1993). Hyenas of both sexes retain their maternal ranks as long as they reside in the natal clan, which females do throughout their lives, but most males disperse to new clans at 2-5 years of age (Frank 1986a, Holekamp and Smale 1998a).

We conducted our tests on groups of individuals at communal dens, where juvenile hyenas live together and are rarely found alone. Spotted hyenas give birth to litters containing 1 or 2 cubs at isolated natal dens. However, when cubs are 3-4 weeks of age, mothers move them to a communal den, where they live for the next 7-12 months. The communal den is a complex of underground tunnels and chambers that cannot be entered by adults. However, juveniles emerge from the warmth and safety of the den daily to nurse from their mothers and socialize near the den with clan-mates (Holekamp and Smale 1998a, Holekamp and Dloniak 2010). Mothers select protective den sites (Boydston et al. 2006) such that hyenas in neither high nor low disturbance areas, experience direct exposure to humans or anthropogenic activity at their dens aside from occasional visitation by tour vehicles, to which subjects in both high and low disturbance areas are well habituated.

### Experimental Stimuli

We used a *novel object test* to measure neophobia and exploration and a *baited box test* to measure boldness. In the novel object test, we used one of four objects (Figure 2.1). Each object was approximately 0.3m tall when deployed. Novel objects were chosen that juveniles would be highly unlikely to encounter otherwise, even if they had begun to spend time away from the



Figure 2.1. The four objects used for the *novel object test* to measure neophobia and exploration. Each object was approximately 0.3m tall when deployed. The cooler, funnel, and stool were plastic, and the bucket was metal. Novel objects were chosen that juveniles would be highly unlikely to encounter otherwise.

communal den and even if they lived in areas with human activity. Because test sessions often involved multiple individuals, we varied the object being used to ensure that it was novel to the individuals being tested.

In the baited box test, we used a metal mesh box (70cm long x 40cm wide x 44cm high) with one open side large enough for cubs to enter (Figure 2.2). The box had no bottom surface except for a metal tray (20cm X 40cm) at the back end of the box where we could sprinkle a layer of powdered milk (15ml), which young hyenas prefer over other food rewards. Cubs could only reach the powdered milk to feed if they fully entered the box. Prior to baited box test sessions, we familiarized individuals with powdered milk by liberally distributing it around each active communal den on three separate occasions when no hyenas were present above ground. Although we did not systematically observe and record whether each subject consumed powdered milk prior to testing, we assumed that any juveniles actively using the den would be exposed to the scent and taste of milk on at least one of these three occasions.



Figure 2.2. The metal box used for the *baited box test* to measure boldness (70cm long x 40cm wide x 44cm high). The box had no bottom surface except for a metal tray (20cm X 40cm) at the back end of the box where we could sprinkle a layer of powdered milk (15ml). The box was open on one side, allowing a subject to walk in and fully enter the box in order to access the powdered milk at the back.

## Ethical Note

All subjects were free-living, and were thus free to choose whether or not to interact with the stimuli we presented. The study was conducted in compliance with Kenyan law and guidelines for work with mammals provided by the American Society of Mammalogists (Sikes et al. 2011) . The work was conducted under IACUC approval #05/14-087-00 from Michigan State University and Research Clearance #NACOSTI/P/14/ 2154/1323 from the Kenyan Commission on Science, Technology and Innovation.

## Data Collection

Experiments were conducted opportunistically during our two daily observation periods, in the

morning (0600-1000) and evening (1700-2000). Stimuli were deployed from our research vehicles, to which all subjects were well habituated. In both the novel object test and the baited box test, we placed the stimulus approximately 20m away from the den entrance. The stimulus was deployed either when no individuals were present above ground, or by positioning the research vehicle to block the view of any individuals present above ground. In the baited box test, the open side of the box was oriented towards the den entrance. All trials were videotaped from approximately 20m away from the stimulus, using a Sony HDD Handycam Super Steady Shot HDR-SR11 mounted on a tripod affixed to our research vehicle. Trials were terminated when no new individuals entered within a 10m radius of the stimulus for 5 minutes, or due to other logistical constraints (e.g., darkness or to prevent an individual from destroying an object).

### Data extraction

Behaviors were coded from the video footage using JWatcher 1.0 (Blumstein & Daniel, 2007). In each test session, any individual approaching within 10m of the stimulus was considered a subject, and we coded each subject's behavior for the entire time it was within 10m of the stimulus. This cut-off distance allowed us to obtain high-quality footage of subjects' interactions with the stimuli.

In the novel object test, we operationally defined neophobia as the number of minutes it took a subject to contact the object after it had come within 5m of the object (i.e. latency), as has been done in previous work assessing spotted hyenas' approaches to novel stimuli (Benson-Amram and Holekamp 2012). If a subject never contacted the object, its latency was calculated as the total time the subject was within 5m of the object during the trial. In the novel object test, we defined exploration as a subject's tendency to interact with the novel object in multiple different ways. We assigned each subject an "exploration score," ranging from 0-4, based on how many exploratory behaviors it exhibited. Subjects received a score of "0" if they came within 10m of the object but never contacted it. Subjects received a score of "1" if they contacted the object with their snout. Subjects received a score of "2" if they bit or licked the object in addition to contacting it with their snout. Subjects received a score of "3" if they displayed these two behaviors and, in addition, either pawed the object or picked it up. The majority of the subjects who received a score of "3" (seven of nine subjects) picked up the object but did not use their paws; nevertheless, we gave both behavioral combinations a score of "3." Subjects with a score of "4" displayed all four of these behaviors.

In the baited box test, we defined boldness as a subject's willingness to enter the box to feed on milk. We assigned subjects a "boldness score," ranging from 0-3. Subjects received a score of "0" if they came within 10m of the box but never contacted or entered it. Subjects received a score of "1" if they contacted the box but did not go inside. Subjects received a score of "2" if they entered the box partially, or entered the box fully but never attempted to feed. Subjects received a score of "3" if they entered the box completely and fed on the milk.

#### Statistical Analysis

#### Independent Variables

We first ran a Cox proportional hazard model to inquire whether there were differences among the three clans in the low disturbance area with respect to neophobia, and Kruskal-Wallis Rank Sum Tests to determine whether there were any significant differences in either boldness or exploration among the three clans in the low disturbance area. We then inquired whether neophobia, exploration and boldness were predicted by whether the subject lived in a high or low disturbance area, as well as by its age, sex and maternal rank. Age in days was included as a continuous variable. We assigned each subject the current social rank of its mother within the clan's dominance hierarchy on the day of testing. Ranks were standardized from -1 to 1, with the most dominant individual having a standardized rank of 1. None of our results changed if we calculated a subject's social rank relative only to the other individuals present in its specific test session. We also included time of day at testing (morning or evening) as a predictor variable.

We conducted the novel object and baited box tests on groups of individuals because juvenile hyenas are almost never found alone at the communal den, and therefore, we examined effects of several variables related to the social context of the testing situation. First, in our models for neophobia, exploration and boldness, we included as a variable the total number of subjects present in order to determine whether the presence of other hyenas facilitated or interfered with an individual's interaction with the stimulus. In a small percentage of test sessions (4 of 18 novel object sessions and 2 of 17 box test sessions), in addition to test subjects, an adult was present and within 10m of the stimulus at some point during the session. Although we did not analyze the behavior of these individuals as subjects, we included them when assessing the social context of the test session by increasing the number of subjects present to account for them. Second, we included as a binary variable whether or not the subject had a litter-mate who was also a subject during the test because, in other species, litter-mates may be less fearful or inhibited when together than when apart (Stöwe et al. 2006b, Bergmüller and Taborsky 2010, Hudson et al. 2011).

In our models for neophobia and boldness, we additionally included whether or not the subject had seen another individual contact the novel object or baited box before doing so itself or, if the subject did not contact the stimulus, whether it observed another subject contact the stimulus earlier during that test session. We did not include this as a variable in our model for exploration because we assumed that seeing another individual contact the novel object would be likelier to influence whether or not the subject contacted the object than to affect the diversity of behaviors emitted during interactions with the object, which was our dependent variable. Furthermore, because our measures of neophobia and exploration were assessed from the same novel object test, we chose to examine the effect of seeing another individual contact the novel object in our analysis of neophobia, where the main behavior of interest was a subject's willingness to approach and contact the object. However, our results did not change if we included this variable in our model for exploration.

### Models of variation in neophobia, exploration and boldness

In statistical analysis of neophobia, we modeled variation in latency to contact the novel object using a Cox proportional hazards model in the "survival" package in R. Our full model included the following predictor variables: age (in days), disturbance (high or low), social rank, sex, time of day (morning or evening), number of subjects present in the session, presence of a litter-mate, and whether the subject observed another individual contact the object. A Cox proportional hazards model, originally developed for survival analyses, provides a method to analyze right censored "time to event" data. This approach allowed us to analyze the responses of those subjects that never touched the object together with those that did, instead of performing two separate analyses or assigning "time out scores" to subjects that never contacted the object (Budaev 1997, Jahn-Eimermacher et al. 2011). Right-censored latencies for the subjects that never contacted the novel object were specified in the model. This model assumes no underlying distribution of the latency measure, but does assume that effects of predictor variables on the latency to respond are constant and additive. These assumptions were satisfied in our dataset.

In statistical analysis of exploration and boldness, we treated subjects' scores as ordered categorical variables and used the "ordinal" package in R to perform cumulative link models. These models, also known as proportional odds models or ordered regression models, assume intrinsic ordering in the levels of the response measure (in our case, from least bold or exploratory, to most bold or exploratory). Our full model for exploration included the following predictor variables: age (in days), disturbance (high or low), social rank, sex, time of day (morning or evening), number of subjects in the session, and presence of a litter-mate. Our model for boldness included these same predictor variables plus the binary variable of whether or not the subject observed another individual contact the box. In both models, we also included each subject's total trial time as a covariate to control for variation among subjects in trial duration. We calculated the odds ratio for each predictor variable from the output of the models. Preliminary analyses indicated no effect of the object type (Figure 1) on neophobia or exploration, so we did not include it as a factor in our analyses. All analyses were conducted in R (R version 3.3.1; Development Core Team, 2015). Parameter estimates were considered significant when  $P \le 0.051$ .
### Consistency across time and context

Due to the opportunistic nature of conducting these experiments, a subset of individuals participated twice in either the novel object test (N=14) or the baited box test (N=14). To examine individual consistency over time in neophobia, we looked exclusively at individuals who had participated in two novel object tests, each with a different object (N=14). We did not analyze data for subjects who participated in two novel object tests with the same object, as the stimulus was no longer novel to the subject in its second test. Because we had data points that were right-censored when an individual never contacted the object, we used an extension of the Cox proportional hazards model from our analysis of initial trials, described above. We incorporated a random effect of subject ID into a Cox proportional hazards model using a shared gamma frailty model (Wienke 2011). We also included exposure number (first or second) as a fixed effect, and any variables that had significant parameter estimates in our analysis of first trial responses. We then used Akaike's Information Criterion corrected for small sample sizes (AICc) to compare the fit of this frailty model to the fit of a Cox proportional hazards model that did not include subject identity. A smaller AICc value indicated a better fit. We also calculated a parameter estimate for the effect of subject identity using a likelihood ratio test.

To examine individual consistency over time in exploration and boldness, we again analyzed responses for only the 14 subjects that participated twice in a test. We used a repeated measures ANOVA, with the subject's exploration or boldness score as the response measure. We included as a predictor variable whether the trial was a subject's first or second exposure, as well as the predictor variables that had significant parameter estimates in our analysis of first trial responses. To estimate repeatability, defined as the proportion of total variance accounted for by

the subject's identity (Lessells and Boag 1987), we calculated intra-class correlation coefficients (ICC), and associated confidence intervals according to Wolak, Fairbairn, & Paulsen (2011) using the "ICC" package in R.

We examined consistency in neophobia across contexts for subjects who were tested in both a novel object test and a box test (N=45). To do this, we treated the box as a fifth novel stimulus and inquired whether there was a significant correlation between a subject's latency to approach a novel object and its latency to approach the box baited with food. We calculated each subject's latency to contact or enter the box, whichever came first, after coming within 5m of it. We then fit a shared gamma frailty model, similar to the one described above, modeling subject as a random effect, and including as fixed effects the type of test (novel object or box), exposure number (first or second), and variables that had significant parameter estimates in our analysis of neophobia in first trial responses. Using AICc, we compared this model to a model that did not incorporate subject identity, and calculated a parameter estimate for subject identity using a likelihood ratio test.

### Relationship between personality traits and survival

Using our demographic records, we determined whether each subject survived to reproductive maturity (2 years of age); all subjects could potentially have reached this age by the time this analysis was conducted. We then used three different generalized linear models (GLM) to determine whether performance in each of the temperament tests predicted the log odds of a subject surviving to reproductive maturity. We used the "glm" package in R and specified a binomial distribution. In our model for neophobia, latencies to contact the novel object were

included as a predictor variable and were right-censored for subjects that never contacted the object. In our analyses testing whether exploration or boldness predicted survival to maturity, exploration scores or boldness scores were included as predictor variables in the GLM model. In each of the three models, we also included maternal rank, sex, and level of disturbance (high or low) as predictor variables. We also included the age at testing as a covariate in each model, but it did not significantly correlate with survival, so we report the results of our models without controlling for age at testing. To further test whether the relationship between each of the three personality traits and survival was mediated by the degree of human disturbance, or the subject's sex, or social rank, we used AICc to compare the fit of our full models to models including interaction terms between each of these factors and the score on the personality test (latency to contact the object for neophobia, exploration score or boldness score).

### RESULTS

Preliminary analysis indicated that subjects did not differ among the three clans in the lowdisturbance area in their neophobia (Wald Test=2.68, df=3, P=0.44), exploration (H(2)=4.75, P= 0.09), or boldness (H(2)=3.77, P = 0.15). In subsequent analysis, we therefore treated all individuals from clans in low-disturbance areas together as we compared them to juveniles in the clan living in the high disturbance area.

## Neophobia

Fifty-eight juveniles participated in the novel object test across 18 test sessions, and 69% (40 of 58) of these individuals contacted the object. The Cox proportional hazards model was

significant (Wald Test=26.2, df=8, N=58, P<0.001), indicating that subjects in the high disturbance area were significantly less neophobic than subjects in low disturbance areas (P=0.051; Table 1; Figure 2.3a). The model also indicated that high-ranking juveniles were less neophobic than low-ranking juveniles (P=0.002; Figure 2.3b), and that juveniles with a littermate present were less neophobic than other juveniles (P=0.032). Subjects were also less neophobic in evening than in morning sessions (P=0.011), and in sessions where more hyenas were present (P=0.043). Surprisingly, subjects were significantly more neophobic if they saw another individual contact the object earlier in the session (P=0.036).

### Exploration

Exploration scores in the novel object test varied from 0-4 (N=58). Eighteen subjects received a score of 0 (31%), 13 subjects received a score of 1 (22%), 7 subjects received a score of 2 (12%), 9 subjects received a score of 3 (16%), and 11 subjects received a score of 4 (19%)

	Hazard ratio (exp(beta))	Coefficient	Std. Error	Z	Р
Age	1.00	0.0002	0.002	0.12	0.936
Disturbance	1.96	0.67	0.35	1.95	0.051*
Social Rank	2.78	1.02	0.33	3.07	0.002*
Sex	1.21	0.19	0.36	0.54	0.597
Time of day	2.96	1.08	0.43	2.53	0.011*
# Subjects in session	1.16	0.15	0.08	2.02	0.043*
Litter-mate present	2.36	0.86	0.40	2.15	0.032*
Observe another	0.39	-0.95	0.45	-2.10	0.036*
subject make contact					

Table 2.1. Results of a Cox proportional hazards model for variation in latency to contact the novel object (N=58). Asterisks indicate  $P \le 0.051$ .



Figure 2.3. A Cox proportional hazards model indicated that: *a*) juveniles in high disturbance were significantly less neophobic than subjects in low disturbance (P=0.05). *b*) social rank was inversely related to latency to contact the object (P=0.002). Social rank was analyzed statistically as a continuous variable but is shown here by dividing the clan's hierarchy into three dominance classes (the high rank category includes ranks 1.00 to 0.33, the middle rank category includes ranks 0.33 to -0.33, and the low rank category includes ranks -0.33 to -1.00).

(Figure 2.4). The logistic regression model indicated significant effects of disturbance and rank on exploration behavior (Table 2.2). Specifically, individuals in the high disturbance area were significantly more exploratory than individuals in the low disturbance area (P=0.003; Figure 2.4a), and high-ranking individuals were more exploratory than low-ranking individuals (P=0.020; Figure 2.4b). There was a significant positive association between exploration and trial duration (P=0.0005), and subjects were significantly more exploratory in the evenings than mornings (P=0.006).

# Boldness

Sixty juveniles participated in the boldness box test, and boldness scores varied from 0-3. Nineteen subjects received a score of 0 (31%), 10 subjects received a score of 1 (17%), 10 subjects received a score of 2 (17%), and 21 subjects received a score of 3 (35%). The logistic regression model indicated that subjects in low disturbance areas were significantly more bold

	Odds Ratio	Coefficient	Std. Error	Z	Р
Trial duration	1.22	0.20	0.06	3.49	0.0005*
Age	1.00	0.001	0.003	0.36	0.718
Disturbance	6.37	1.85	0.63	2.95	0.003*
Social Rank	2.93	1.08	0.46	2.32	0.020*
Sex	1.87	0.62	0.58	1.08	0.282
Time of day	5.62	1.72	0.63	2.73	0.006*
# Subjects in session	1.21	0.19	0.13	1.52	0.129
Litter-mate present	1.70	0.53	0.60	0.89	0.375





Figure 2.4. A logistic regression model for variation in exploration score indicated that: *a*) juveniles in high disturbance were significantly more exploratory than those in low disturbance (P=0.003) and *b*) high-ranking juveniles were significantly more exploratory than low-ranking juveniles (P=0.020; N=58).

than individuals in high disturbance areas (P=0.019; Table 2.3; Figure 2.5). There was a significant positive association between boldness and trial duration (P=0.0006), and a trend for females to be bolder than males (P=0.066), but we observed no effects of rank on boldness.

### Consistency across time and context

For neophobia, our frailty model containing the random effect of subject ID was a significantly better fit than the model without subject ID as a factor, indicating significant repeatability in subjects' neophobia across trials ( $^2$ =10.99, P=0.006). Subjects' latency to contact a novel object did not change significantly across exposures (Hazard ratio = 0.94, P = 0.92). For exploration, the repeated measures ANOVA similarly indicated that subjects' exploratory behavior did not change significantly across exposures, as indicated by a non-significant within-subjects parameter

	Odds Ratio	Coefficient	Std. Error	Z	Р
Trial duration	1.15	0.15	0.04	3.44	0.0006*
Age	0.99	-0.01	0.005	-1.40	0.161
Disturbance	0.22	-1.50	0.64	-2.34	0.019*
Social Rank	0.74	-0.35	0.46	-0.77	0.440
Sex	0.28	-1.31	0.71	-1.84	0.066
Time of day	1.05	-0.02	0.88	-0.03	0.980
# Subjects in session	0.97	-0.01	0.12	-0.12	0.910
Litter-mate present	1.35	0.36	0.57	0.63	0.529
Observe another	2.45	0.79	0.87	0.91	0.365
subject enter or					
contact box					

Table 2.3. Results of a logistic regression model for variation in boldness (N=60). Asterisks indicate  $P \le 0.051$ .



Figure 2.5. A logistic regression model for variation in boldness score indicated that juveniles in high disturbance were less bold than juveniles in low disturbance (P=0.019; N=60).

estimate for exposure number ( $F_{i,11} = 0.258$ , P = 0.62). However, the repeatability estimate for exploration was also non-significant (r = 0.20, CI= -0.34, 0.64). For boldness, the repeated measures ANOVA indicated that subjects' boldness did not change significantly across repeated trials, as indicated by a non-significant within-subjects parameter estimate for exposure number ( $F_{i,12} = 0.222$ , P = 0.424). The repeatability estimate for boldness was significant (r = 0.78, CI= 0.45, 0.92). Thus, juvenile spotted hyenas appear to show consistency over time in their neophobia and boldness, but not in their degree of exploration.

In our assessment of consistency in neophobia in two different contexts, namely a subject's latency to contact the novel object and latency to contact or enter the baited box, our

frailty model with subject ID as a random effect was a significantly better fit than our model without subject ID as a factor ( $^2$ =28.1, P=0.002). This indicates significant individual consistency in subjects' neophobia when encountering novel stimuli that offer a food reward and those that do not. Subjects had significantly shorter latencies to approach the stimulus on the second test they received than on their first test (Hazard ratio = 2.33, P= 0.001) regardless of whether it was the novel object test or baited box test (Hazard ratio = 1.20, P= 0.513).

#### Relationship between personality traits and survival

Of the 58 subjects in the novel object test, 39 (67%) survived to reproductive maturity. Latency to contact the object (B=1.20, P=0.22) did not predict survival to maturity, but juveniles in low disturbance were more likely to survive than juveniles in high disturbance (B=0.18, P=0.018), and juveniles of high rank were more likely to survive than juveniles of low rank (B=4.59, P=0.02). Sex did not predict survival (B=0.61, P=0.49). Exploration score also failed to predict survival to reproductive maturity (B=0.91, P=0.70), but again subjects in low disturbance were significantly more likely to survive than subjects in high disturbance (B=0.18, P=0.02), and survival was significantly positively correlated with social rank (B=4.34, P=0.015). Sex did not predict survival to reproductive maturity (B=0.65, P=0.54). Models that included interactions between the temperament traits and the other predictor variables did not fit the data significantly better than our model without interactions.

Of the 60 subjects tested in the baited box test, 40 (67%) survived to reproductive maturity. Boldness affected survival similarly in both disturbed and undisturbed areas. Subjects who were less bold were significantly more likely to survive (B=0.49, P=0.05), and a model that

included boldness was a significantly better fit than one that did not (likelihood ratio test,  $^{2}$ =-4.51, P=0.034). In this sample, individuals in low disturbance were again significantly more likely to survive than individuals in high disturbance (B=0.075, P=0.004). Higher ranking individuals were also more likely to survive than lower ranking individuals (B=3.91, P=0.03), but sex did not predict survival to reproductive maturity (B=0.47, P=0.36). Models that included interactions between boldness and the other predictor variables failed to fit the data significantly better than our model without interactions.

## DISCUSSION

Our findings are consistent with the growing literature demonstrating the profound influence that human disturbance can have on personality traits in wild populations, and permit comparisons with other taxa that have been more thoroughly studied. We found significant effects of human disturbance on all three of the juvenile personality traits we measured in spotted hyenas. Patterns in neophobia and exploration were similar to those documented in comparisons of urban and rural individuals among birds and small mammals (Smith and Blumstein 2008, Miranda et al. 2013, Sol et al. 2013); juvenile hyenas in low disturbance areas were significantly more neophobic and less exploratory than juveniles in high disturbance areas. However, in contrast to these other taxa, juvenile spotted hyenas in low disturbance areas were significantly more bold than those in high disturbance areas.

Our results also demonstrate that personality traits, although often challenging to measure in lone individuals in the field, can be effectively assessed in a group context. Conducting personality tests in group contexts rather than in isolation enhances ecological

validity for individuals who are always in a social group (Drea 2006), and better indicates how personality traits are actually expressed in free-living animals than testing subjects alone (Webster and Ward 2010). We were able to account for the effects of social context by measuring situational variables and including them in our statistical models. Neophobia and boldness, but not exploration, showed significant repeatability within the juvenile period. We also found evidence for consistency in neophobia across contexts. Among juveniles that participated in both the novel object and baited box tests, latencies to contact the stimuli were highly consistent.

In addition to human disturbance, maternal rank emerged as an important factor shaping juvenile personality traits in spotted hyenas. The relationship between social rank and personality varies among species, and our finding that higher-ranking juveniles were less neophobic and more exploratory than low ranking juveniles is consistent with some previous studies (Mettler and Shivik 2007, David et al. 2011), but not others (Gómez-Laplaza 2002, Fox et al. 2009, An et al. 2011). That there was still a significant effect of rank when we calculated subjects' ranks relative only to those of the other subjects present in a specific test session suggests that the influence of rank was not dependent on the immediate social context of the test; low-ranking individuals were more neophobic and less exploratory even when they happened to be among the highest ranking subjects present in a particular test session. Maternal rank, therefore, appears to shape personality traits from a young age, as it does offspring growth, aggression, and play(Hofer and East 2003, Dloniak et al. 2006, Holekamp et al. 2013, Lewin et al. 2017).

It is perhaps surprising that we found no relationship between maternal rank and

boldness, as high-ranking individuals could potentially have dominated access to the powdered milk in the test box by attacking lower ranking subjects. Yoshida et al. (2016) found that rank and boldness, as measured by individuals' closest approach to lions (Panthera leo) during lionhyena interactions, were significantly positively related in females but not males. Our results might be due to the fact that juveniles are still in the process of learning which conspecifics they can appropriately dominate or displace from food. Additionally, although a low-ranking juvenile subject may have had to wait for a higher-ranking subject to enter the box and feed first, when the higher-ranking animal left the box, the scent of milk likely still attracted lower- ranking subjects, which could still scrounge for remaining milk and thus receive the highest boldness score. It seems unlikely that juveniles in low disturbance areas are bolder than those in high disturbance areas because they are more hungry, as has been found in other studies (Biro and Booth 2009, Chapman et al. 2010). If hunger were primarily driving the expression of boldness in our test, we would expect lower-ranking individuals to be bolder than higher-ranking individuals, who are generally better fed. We would also expect individuals to be less bold in trials conducted in the morning than in the evening, when juveniles typically have not nursed since the morning, so they should be more highly motivated to reach the milk in our test apparatus. However, neither time of day nor rank significantly predicted boldness.

Neophobia was the only trait significantly affected by the presence and behavior of conspecifics. Subjects were less neophobic when more subjects were present in the test session, which is consistent with the behavior of hyenas solving a puzzle box problem (Benson-Amram and Holekamp 2012), the important role of social facilitation in hyena social behavior (Glickman et al. 1997), and results of novel object tests administered to other species (Cadieu et

al. 1995, Moscovice and Snowdon 2006, Webster et al. 2007, Moretti et al. 2015). The presence of a hyena litter-mate, in particular, significantly facilitated approach to the novel object, suggesting that litter-mates may have especially strong effects on juvenile hyenas' confidence and propensity to explore new things.

Interestingly, individuals were more neophobic if they saw another individual contact the novel object, which is consistent with findings that a conspecific's presence or behavior can inhibit approach to novel objects (Ryer and Olla 1991, Brown and Laland 2002, Stöwe et al. 2006a). In our test, this inhibitory effect may have been due to the tendency of juvenile hyenas to startle or become skittish when others do. Juveniles are strongly influenced by the behavior of their peers and it is common to see many cubs run into the communal den after a single cub startles. In our test, subjects often backed away or startled after contacting the novel object for the first time, so it seems possible that viewing this may have either inhibited other subjects from approaching at all or slowed their approach.

Notably, our study is one of only a few to directly relate personality traits to fitness in a wild mammal (Archard and Braithwaite 2010). We found that boldness, but not neophobia or exploration, predicted survival to adulthood, with less bold individuals showing enhanced survivorship. These findings are consistent with a meta-analysis by Smith and Blumstein (2008), demonstrating that boldness consistently shows a significant negative correlation with survival across species. Furthermore, we found a negative relationship between boldness and juvenile survivorship regardless of disturbance, suggesting that the benefits of showing restraint in the face of risk accrue regardless of the level of human activity.

Differences in juvenile survivorship and predation pressure in the high and low

disturbance areas may be shaping the differences we observed in juvenile boldness. When looking at hyenas of all ages, Yoshida et al. (2016) found evidence for stabilizing selection with regard to boldness during lion-hyena interactions, with reduced longevity for individuals who were either highly prone to take risks or who completely avoided them. However, we found that juveniles in high disturbance were significantly less bold than juveniles in low disturbance. Although surprising, this is likely due to decreased predation by lions in the high disturbance area, where there has been a significant decline in lion densities and where juvenile survivorship was significantly greater than in the low disturbance area from 2009-2013 (Green 2015). This suggests that a reduction in predation pressure may be relaxing the selection pressure against shy individuals in the high disturbance area.

Several questions should be answered by future research to make our results of the most use to applied carnivore conservation and the emerging field of conservation behavior (Greggor et al. 2016). First, we must determine whether a juvenile's performance in our personality experiments predicts its behavior and success in disturbed landscapes, particularly its likelihood of leaving protected areas, attacking livestock, or being killed by humans. Second, to determine whether juvenile personality traits remain stable into adulthood, we could measure these personality traits in individuals several times across the lifespan. We cannot assume stability in these traits beyond the juvenile period, as juvenile personality traits may represent adaptations specific to immature life-stages (Bell and Stamps 2004, Sinn et al. 2008, Stamps and Groothuis 2010, Petelle et al. 2013).

Third, given our findings regarding boldness, disturbance, and juvenile survivorship, it would be interesting to explore whether hyena personality traits have fitness trade-offs over the lifetime of an individual, and whether such trade-offs differ in areas of high and low disturbance. In their meta-analysis, Smith and Blumstein (2008) found that, although bold individuals showed significantly lower survival, they also had higher reproductive success than less bold individuals. To test for such a trade-off in spotted hyenas, we could relate boldness in the juveniles tested here to their future reproductive success. Furthermore, this would allow us to explore whether the fitness costs and benefits of boldness differ in areas of high and low disturbance, and whether disturbance may be acting as a selective pressure on the life-history strategies of individuals inhabiting these areas.

Overall, our study not only reveals significant behavioral differences between animals in disturbed and pristine areas, but also raises important questions regarding how animals achieve behavioral adjustments in response to anthropogenic disturbance. Juvenile spotted hyenas in both high and low disturbance areas are remarkably well-buffered from direct human disturbance at their communal dens. Even juveniles living in areas that are generally disturbed by people, are not directly bothered by people while they live at the den, and in fact, they experience virtually no direct exposure at their dens to any anthropogenic activity, including livestock grazing. Therefore, it seems unlikely that human disturbance is directly shaping the behavior of juveniles residing at the communal den. Nevertheless, the juveniles tested here showed marked disturbance-related behavioral variation very early in life. We are currently investigating the hypothesis that, such behavioral differences are shaped indirectly, via maternal effects, as the behavior and stress physiology of adult female spotted hyenas can be greatly affected by human disturbance (Boydston et al. 2003b, Kolowski and Holekamp 2009, Van Meter et al. 2009). In many species, variation in maternal care or hormone exposure in utero

shapes offspring personality, providing a potentially adaptive signal of environmental quality to offspring (Maestripieri and Mateo 2009, Dantzer et al. 2013). In species that are long-lived and slow-growing, like spotted hyenas, it may be that responding early in life to strong maternal signals of disturbance is critical to persistence in changing environments.

#### ACKNOWLEDGEMENTS

We thank the Kenyan National Council for Science and Technology, the Narok County Council, the Kenya Wildlife Service, and the Mara Conservancy for permission to conduct field research with hyenas. We thank Wes Binder, David Green, Lily Johnson-Ulrich, Wilson Kilong, Charles Kolodziejski, Kevin McCormick, Jenna Parker, and Benson Pion for assistance in the field. We also thank Andrew Dennhardt of MSU's Center for Statistical Training and Consulting for guidance with statistical analysis and Lily Johnson-Ulrich for consultation about video coding. Finally, we thank Tom Getty, Joe Lonstein, Laura Smale, and two anonymous reviewers for helpful comments on earlier drafts of the manuscript. This work was supported by NSF Grants OISE 1556407 and DEB 1353110 to KEH, and by a NSF Graduate Research Fellowship to JRG. JRG was also supported by fellowships from the College of Natural Sciences and the program in Ecology, Evolutionary Biology, and Behavior at Michigan State University. This work was made possible by support from the BEACON Center for the Study of Evolution in Action, funded by NSF grant OIA 0939454.

### CHAPTER 3

# SEX- AND AGE-SPECIFIC CORRELATES OF FECAL GLUCOCORTICOID CONCENTRATIONS IN SPOTTED HYENAS

Julia R. Greenberg, Stephanie M. Dloniak, Page E. Van Meter, Jeffrey A. French, Jacinta C. Beehner, & Kay E. Holekamp

### INTRODUCTION

Stress can have dramatic effects on an animal's morphology, physiological functioning, and behavior, and stress experienced early in life often has particularly profound effects on the animal's adult phenotype and fitness (Lindström 1999, Tung et al. 2016). Glucocortioids (GCs; e.g. cortisol, corticosterone) are metabolic hormones that serve multiple physiological functions. Circulating GCs support metabolic processes, mediate glucose availability, and maintain body mass (Hau et al. 2016). GCs are often referred to as "stress hormones" due to their role in responses mediated by the hypothalamic-pituitary-adrenal (HPA) axis to environmental or physiological perturbations. GCs released in response to a stressor stimulate gluconeogenesis, inhibit energy storage, and promote cardiovascular activity (Sapolsky et al. 2000). However, continual exposure to an acute stressor can result in chronic activation of the HPA axis, dysregution of the negative feedback mechanisms that minimize long-term exposure to GCs (McEwen and Wingfield 2003), and negative effects on the animal's immune function (Hoffman et al. 2011), cardiovascular system (Sapolsky and Share 1994), and reproduction (Sheriff et al. 2009).

Exposure to early stress can shape a variety of phenotypic traits that may last into adulthood. For example, individual humans who are exposed to chronic early stressors, such as low-socioeconomic status or abuse, are likelier than unexposed individuals to suffer from chronic disease as adults (Barker et al. 2002, O'Rand and Hamil-Luker 2005, Kittleson et al. 2006, Gluckman et al. 2008). Early in life, glucocorticoids also shape the development of neuroendocrine pathways associated with emotion regulation and personality. In laboratory rodents and primates, individuals who experience an early stressful environment are often less neophobic, more exploratory, and less risk-averse (i.e. "bold") than those who do not (Caldji et al. 1998, Carere et al. 2003, Parker and Buckmaster 2004, Martins et al. 2007, Parker et al. 2007, Guenther et al. 2014). Finally, due to their role in resource allocation, early exposure to elevated glucocorticoids can affect offspring growth (Dantzer et al. 2013), and life-history trade-offs (McGlothlin and Ketterson 2008, Crespi et al. 2012, Sheriff and Love 2013). For example, individuals who experience early stressful environments sometimes show accelerated lifehistories, reaching menarche earlier than peers who do not experience early stress (Maestripieri 2005, Cameron et al. 2008a).

Maternal stress, either during pregnancy or lactation, can also shape phenotypic traits in offspring. For example, in non-human primates, prenatal stress is associated with offspring impairments in learning, attention, and neuromotor ability (Schneider et al. 1999), and in humans, stress experienced prenatally has been identified as a risk factor for attention deficit and anxiety disorders (Bergh et al. 2004, Huizink et al. 2004). During the post-natal period, ingestion of GCs in a mother's milk can also shape offspring phenotype. For example, in rhesus macaques, mothers who produce high concentrations of GCs in their milk have sons who are more cautious and nervous than those who produce milk containing lower GC concentrations (Hinde et al. 2015). Maternal stress during lactation can also shape offspring traits indirectly by

affecting maternal behavior. For example, in several free-ranging primate species, GC concentrations in lactating mothers are associated with their protectiveness of offspring (Bahr et al. 1998, Nguyen et al. 2008, Maestripieri et al. 2009). These maternal behaviors, in turn, can influence the pace at which offspring achieve independence, their exploratory tendencies, their sociability, and even their own parenting behavior when they become adults (Fairbanks 1996, Bardi and Huffman 2002, Maestripieri 2005).

Despite the well-documented role played by GCs in influencing behavior and physiology, few studies of free-living animal populations have tested whether early stress is associated with fitness. Although a variety of early-life factors have been found to correlate negatively with fitness, including high population density, low resource availability, or sub-optimal maternal condition (Descamps et al. 2008, Rodel et al. 2009, Hamel et al. 2010, Tung et al. 2016), few studies have specifically associated particular environmental conditions with fGC concentrations in juveniles themselves to confirm that such conditions are experienced as physiological stressors. Furthermore, although early stress is often assumed to be associated with fitness costs, it could actually be adaptive if early stress prepares offspring for their environment later in life (Monaghan 2008, Beery and Francis 2011, Champagne 2011). For instance, individuals that experience high early stress are often more reactive to environmental circumstances and exhibit greater behavioral flexibility, traits that may be beneficial in an unpredictable environment (Sheriff and Love 2013, Crino and Breuner 2015).

Human activity is an increasingly common stressor in the lives of wild animals, as indicated by individuals' concentrations of glucocorticoid stress hormone (reviewed in (Busch and Hayward 2009, Dantzer et al. 2014). However, few studies of anthropogenic stress and its

consequences have focused on juveniles to inquire whether anthropogenic stressors experienced early in life are associated with phenotypic traits later in life, or whether early stress has longterm fitness consequences. Here our primary goal was to determine whether anthropogenic disturbance affects GC concentrations in free-living juvenile spotted hyenas (*Crocuta crocuta*) or their mothers. We measured GC concentrations in feces (fGC), which provide an integrated measure of GCs released in the body over approximately 24 hours (Dloniak 2004). We tested a hypothesis suggesting that fGC concentrations, either in juveniles themselves or in their mothers during pregnancy or lactation, differ between animals exposed to anthropogenic disturbance and those living in undisturbed areas.

Previous research showed that human disturbance is associated with elevated fGC concentrations in free-living adult spotted hyenas and that pastoralist activity in particular, rather than tourism, is associated with elevated fGC concentrations. Van Meter et al. (Van Meter et al. 2009) compared fGC concentrations of adult spotted hyenas living in four territories that varied with respect to lion densities, rainfall, tourist activity, and pastoralism. Adults that lived on the border of the Masai Mara National Reserve in Kenya were exposed to the most pastoralist activity and had the highest fGC concentrations. Furthermore, a twelve-year longitudinal analysis of one clan exposed to human disturbance indicated that fGC concentrations among adult males had significantly increased over time as the neighboring human population expanded and livestock grazing increased (Van Meter et al. 2009). This finding is consistent with a recent meta-analysis showing that males are particularly sensitive to the stressor of human disturbance (Dantzer et al. 2014).

In the current study, we first used a longitudinal analysis to test whether a variety of

environmental and social factors, all examined previously in adult hyenas, correlate with fGC concentrations among juveniles. We inquired whether fGC concentrations in juveniles increased between 1993 and 2014, as human disturbance in our study area increased. We also inquired whether juvenile fGC concentrations were associated with prey density or clan size, as resource competition and population density have been found to correlate positively with fGC concentrations in other species (Harper and Austad 2000, Foley et al. 2001, Ethan Pride 2005, Beehner and McCann 2008, Dantzer et al. 2013). We also assessed the effects of a juvenile's sex and dominance relationship to its litter-mate, because these factors were found to correlate with fGC concentrations in a Tanzanian population of spotted hyenas (Benhaiem et al. 2013). We tested whether a juvenile's social rank was associated with fGC concentrations. Across species, the relationship between rank and fGC concentrations is variable and appears to be mediated by environmental conditions, the stability of the social hierarchy, the breeding system, how individuals maintain their rank, and whether subordinate individuals have opportunities for social support (Creel 2001, Abbott et al. 2003, Goymann and Wingfield 2004).

After completing our longitudinal analysis to identify salient predictors of fGC concentrations, we compared fGC concentrations between hyenas living in disturbed and undisturbed areas of a single wildlife reserve from 2009-2014. Specifically, we used a cross-sectional analysis to test the hypothesis that stress hormone concentrations, either in juveniles themselves or in their mothers during pregnancy or lactation, differ between animals exposed to anthropogenic disturbance and those living in pristine areas of the Mara-Serengeti ecosystem in eastern Africa. This hypothesis predicts that juveniles in high-disturbance areas should have significantly higher fGC concentrations than juveniles in low disturbance areas. If maternal

stress is affecting offspring phenotypes, then pregnant or lactating females in high-disturbance areas should have significantly higher fGC concentrations than those in low disturbance areas. Finally, we tested whether early life fGC concentrations, regardless of the causes of its variation, predict survivorship or longevity in spotted hyenas.

#### METHODS

#### Study Site and Subjects

Study subjects were spotted hyenas inhabiting two protected areas in southwestern Kenya; one area was undisturbed, whereas the other was highly disturbed by anthropogenic activity. Data from the "low disturbance" area were collected from hyenas belonging to three clans inhabiting the pristine Mara Conservancy since monitoring began there in 2008. The Mara Conservancy is managed by a private non-profit organization that strictly prohibits cattle grazing or any human activity except wildlife viewing from tour vehicles. Data collected from hyenas inhabiting a highly-disturbed area were those taken from the Talek West clan, which lives along the northern border of the Masai Mara National Reserve (henceforth, the Reserve), near the burgeoning town of Talek. This clan has been monitored closely and continuously since 1988. Since the late 1990s, there has been exponential human population growth along the border of the Reserve (Watts and Holekamp 2009), humans are active with their livestock both day and night inside the Reserve in this area, and direct conflict between hyenas and livestock is common both inside and outside the Reserve (Kolowski and Holekamp 2006). Since 2005, humans have been responsible for the majority of hyena deaths (through spearing, snaring, and poisoning) for which mortality sources could be determined (Holekamp and Dloniak 2010). Numerous

behavioral effects of increased human activity have been documented in this population, including increased nocturnality, increased daily travel, lower rates of den attendance by mothers, active avoidance of livestock, and a preference for areas with dense vegetative cover (Boydston et al. 2003a, Kolowski et al. 2007, Kolowski and Holekamp 2009).

All subjects were individually identified based on their unique spot patterns and ear damage. Age was initially estimated when cubs were first observed above ground, based on their appearance and size (Holekamp et al. 1996), and sex was determined based on the shape of the glans of the erect phallus (Frank et al. 1990). Spotted hyenas usually give birth to litters of 1 or 2 cubs. Shortly after the birth of twin litters, litter-mates fight for priority of access to the mother's teats for nursing and, within the first month of life, establish a consistent dominance relationship that typically remains intact as long as both litter-mates remain in the natal clan. Here each cub born in a twin litter could therefore be assigned an "intra-litter rank" based on observations of aggressive and appeasement behavior between litter-mates (Smale et al. 1995, Holekamp and Smale 1998a). Individuals were considered juveniles until they reached two years of age. A female's first parturition could be determined reliably because her pseudopenis tears when she gives birth to her first litter, and leaves an unambiguous stripe of pink scar tissue on the posterior surface of the phallus (Frank and Glickman 1994, Holekamp et al. 1996).

The reproductive state of adult females was continuously monitored to determine periods of pregnancy and lactation, as described by Holekamp et al. (1996). The gestation period for spotted hyenas is 110 days (Kruuk 1972). Conception dates were determined by back-calculating from a cub's date of birth, and based on observations of matings, or on sighting a fresh tear in a phallus, which indicated parturition. To delineate lactation intervals for adult

females, we calculated weaning dates for juveniles based on observations of nursing conflicts and observations of cubs subsequently seen with their mother when no nursing occurred (Holekamp et al. 1996).

Spotted hyenas live in fission-fusion societies of 5-130 individuals (Holekamp et al. 2015) that are structured according to a strict matrilineal hierarchy (Frank 1986b). The social rank of adult females here was calculated on a yearly basis based on outcomes of dyadic agonistic interactions (Martin and Bateson 1988, Smale et al. 1993). Juveniles "inherit" dominance ranks immediately below those of their mothers in a process of social learning that is not complete until at least 18 months of age (Holekamp and Smale 1993, Smale et al. 1993). We therefore assigned each juvenile the social rank of its mother. Hyenas of both sexes retain their maternal ranks as long as they reside in the natal clan, which females do throughout their lives, but most males disperse to new clans at 2-5 years of age (Boydston et al 2005; Smale et al. 1997). Each of the clans analyzed in the cross-sectional analysis experienced at least one period of instability in the social hierarchy during the period in which they were compared (2009-2014), when the alpha female died or was in the process of being overtaken by lower ranking females.

### Fecal sample collection, extraction, and immunoassay of hormone concentrations

We measured excreted hormone concentrations by assaying fecal samples collected from individuals in four different clans. Fecal samples were collected whenever a known hyena was observed defecating during our routine morning (0600-1000h) and evening (1600-2000h) observation sessions. Samples were stored in liquid nitrogen, and carried to Michigan State University on dry ice for storage in -80° freezers. An ethanol extraction procedure was used that has been described previously (Dloniak 2004, Dloniak et al. 2004, 2006, Van Meter et al. 2008, 2009). Samples were assayed in duplicate using a corticosterone radioimmunoassay kit (ImmuChem Double Antibody Corticosterone <sup>125</sup>I RIA kit, MP Biomedicals), and were diluted to a 1:20 concentration with RIA buffer supplied with the commercial kit. The detection range for the assay was 10-1200 ng/g. Any samples that had fGC concentrations below 10 ng/g were assigned the lower detection limit as their value. An analytical validation was performed for use of this assay with hyena fecal samples and the assay demonstrated accuracy, precision, and linearity (Dloniak, unpublished; Greenberg et al. in prep). For our longitudinal analysis, the intra-assay coefficient of variation was 4.66  $\pm$  2.32 and the inter-assay coefficient of variation was 10.27% (N=39 assays). For our cross-sectional analysis, the intra-assay coefficient of variation was 4.28  $\pm$  0.98 and the inter-assay coefficient of variation was 10.73% (N=22 assays).

#### Statistical Analysis

We modeled variation in fGC concentrations using generalized linear mixed models (GLMM) with the "lmer" package in R, and used the package "multcomp" to perform Tukey's post-hoc tests for pairwise comparisons. fGC concentrations were log transformed to better approximate a normal distribution. We used Variance Inflation Factors (VIF) to assess multi-colinearity of predictor variables in all of our models. No variable had a VIF above 3, indicating that there was no significant multi-colinearity among variables in our models. To assess potential outliers or data points with high leverage, we calculated Cook's Distances, used visual inspection of graphs, and compared results with and without inclusion of these data points.

### Longitudinal analysis of juvenile fGC concentrations 1993-2014

To determine which ecological and social variables predicted fGC concentrations in juveniles, we analyzed samples collected in the Talek West clan from 1993-2014. Because we had 1-8 samples per individual hyena, we included subject ID as a random effect. To control for the time of day at which the sample was collected, we included a binary variable, morning or evening, to indicate during which observation session the sample was collected. Age (in months), sex, and intra-litter rank were included as fixed effects. Clan size was included as a fixed effect and calculated as the number of breeding females present in the clan during the year in which the sample was collected. To determine whether fGC concentrations changed over the course of the study, we included study year as a fixed effect. Following Van Meter et al. (2009), prey density was calculated as the average density of prey counted within 100m of three 4-km transect lines, run biweekly, during the month before the sample was collected.

To explore whether a juvenile's rank mediated the effect of socio-ecological variables on fGC concentrations, we tested our model containing no interaction terms against models that contained two-way interactions between rank and each of our other variables. To compare the fit of models, we used Akaike's Information Criterion corrected for small sample sizes (AICc), with the lowest AICc score indicating the best model fit. We used likelihood ratio tests to obtain a parameter estimate for the best-fitting model.

## Cross-sectional comparison of fGC concentrations 2009-2014

Our cross-sectional analysis inquired whether juveniles, pregnant females, or lactating females in the highly disturbed Talek region showed significantly higher fGC concentrations than individuals in the low disturbance area. We first conducted preliminary analysis of fGC concentrations to determine which socio-ecological variables to include in our cross-sectional analysis. Because previous analyses have explored the socio-ecological correlates of fGC concentrations in the high-disturbance area (Van Meter et al. 2009), we tested whether the same or different variables as in those earlier analyses were associated with fGC concentrations among hyenas in the low disturbance area. In preliminary analysis of juvenile fGC concentrations in the low disturbance area, we tested the same variables that we examined in our longitudinal analysis of the Talek region; these were time of day, age, sex, clan size, prey, maternal rank, and litter size.

In our preliminary analyses of pregnant and lactating females, we separately modeled fGC concentrations among females in each of these two reproductive states, because in previous studies, reproductive state was a significant predictor of fGC concentrations (Goymann et al. 2001, Van Meter et al. 2009). In our preliminary analysis of fGC concentrations among lactating females in the low disturbance area, we replicated Van Meter et al.'s (2009) analysis by testing whether the following variables significantly predicted fGC concentrations: social rank, prey density, rainfall (mm), tourism (high or low), and social instability (occurring or not occurring). Social rank and prey density were calculated as described above for analysis of juvenile samples. Daily rainfall (mm) was measured using a plastic rain gauge, and we included as a variable in our models the daily mean rainfall during the 30 days prior to the collection date of each fecal sample. Following Van Meter et al (2009), we treated tourism as a binary variable; samples collected June-October and December were considered "high tourism season," and samples collected in the remaining six months of each year were considered "low tourism season."

Social instability was a binary fixed factor indicating whether the subject's clan was experiencing social upheaval at the time of sample collection.

In our preliminary analysis of fGC concentrations among pregnant females in the low disturbance area, we could not include all these variables in our model due to our small sample size. We therefore only tested the effects of variables that we hypothesized were most likely to correlate with fGC concentrations, namely, time of day, day of gestation, social rank, prey density, and whether or not social instability was occurring. Finally, Van Meter et al. (2009) tested whether fGC concentrations were elevated due to recent presence of lions, although this was found to have no effect on ffGC concentrations. We did not test for such an effect among lactating or pregnant females in our low disturbance area because none of our samples were collected when lions had recently interacted with our subjects.

In our cross-sectional comparison of fGC concentrations between high-and lowdisturbance areas, we separately modeled fGC concentration for juveniles, pregnant females, and lactating females. We included disturbance as a binary fixed factor (high versus low), time of day as a binary fixed factor (morning or evening), and subject ID and year as random effects, as well as any variables determined to be significant in our preliminary analyses. To examine whether the association between fGC concentrations and disturbance was mediated by other socioecological factors, we also compared each of our models to models that contained a two-way interaction with disturbance and each of the other predictor variables.

### Relationship between early life fGC concentrations and fitness

We focused our analysis on the relationship between early life fGC concentration and fitness of

hyenas in the Talek clan, as we have monitored and collected fecal samples from this clan for 21 years (1993-2014). Our preliminary analysis indicated that fGC concentrations were significantly correlated with age for individuals younger than one year, but that age was not significant if we restricted the analysis to individuals 6-12 months of age. The time of day in which the sample was collected was also not a significant predictor of fGC concentrations for individuals 6-12 months. Therefore, we used samples collected from individuals in this age range to relate fGC concentrations to our fitness measures. When we had more than one fecal sample for an individual during this 6 month time period, we averaged the sample values.

We used our demographic records to determine whether individuals survived to reproductive maturity and to calculate their longevity. For longevity, we restricted our analysis to females in the Talek clan, as males can often not be monitored after they disperse from their natal clan. We included all females who died within the course of the study (1988-2014), and for which we had at least one fecal sample during her first 6-12 months life. Using the glmer package in R, we used a GLMM with a binomial distribution to test whether an individual's fGC concentrations during this period of early life was associated with its likelihood of surviving to 2 years. Using the lmer package in R, we used a linear mixed-effects model to test whether an individual's fGC concentrations during this period of early life were associated with its longevity (in days). In both models, we log-transformed the fGC value and in our longevity model, we also log-transformed longevity. We also included each model, as a fixed effect, the social rank of the female's mother in the year the female was born, because social rank has been found to predict numerous measures of fitness in spotted hyenas (Holekamp et al. 1996, Swanson et al. 2011). Additionally, we included the female's birth year as a random effect to account for

particularly high or low mortality in certain years due to time-specific environmental conditions.

### RESULTS

# Longitudinal analysis of socio-ecological factors affecting juvenile fGC concentrations

Our longitudinal analysis of juvenile fGC concentrations included 382 samples collected from 171 juveniles in the Talek clan between 1993 and 2014. Our model indicated significant effects of time of day, age, sex, and prey density on fGC concentrations (Table 3.1). Specifically, juvenile fGC concentrations were higher in the morning than in the evening (P<0.001), higher in females than in males (P=0.032), decreased with age as individuals approached the end of their second year of life (P<0.001; Figure 3.1), and were positively correlated with prey density (P=0.034). None of our models containing two-way interactions between rank and our other predictor variables fit the data significantly better than the models containing no interaction terms.

Because sex was a significant predictor of fGC concentrations, we next analyzed fGC concentrations in juvenile males and females in separate models including the same predictor variables as in the prior model. Females, but not males, showed a significant positive association between fGC concentrations and prey density (females  $\beta$ =0.14, SE=0.07, *P*= 0.051; males  $\beta$ =0.08, SE=0.07, *P*= 0.26).

#### Cross-sectional comparison of fGC concentrations in high- and low-disturbance areas

### Juveniles

Preliminary analysis of juveniles in the low disturbance area (Table 3.2) showed that fGC concentrations significantly declined as individuals matured (P<0.001), that juveniles had

	Estimate	Std. Error	Р
(Intercept)	4.09	0.22	< 0.001
Study year	0.02	0.01	0.163
Time of day	-0.36	0.10	<0.001*
Age	-0.37	0.05	<0.001*
Sex	-0.25	0.12	0.032*
Clan Size	-0.13	0.08	0.096
<b>Prey Density</b>	0.11	0.05	0.034*
Maternal Rank	-0.06	0.10	0.552
Litter Size	-0.07	0.13	0.583

Table 3.1. Factors explaining variation in fGC concentrations in juvenile spotted hyenas from the Talek West clan 1993-2014 (N=382 samples from 171 individuals). An asterisk indicates P < 0.05



Figure 3.1. A longitudinal analysis of juvenile fGC concentrations in the Talek West clan 1993-2014 indicated that fGC concentrations significantly decrease with age among juveniles of both sexes, and that juvenile females have significantly higher fGC concentrations than do juvenile males. Only samples collected in the morning are shown (N=188 samples).

	Estimate	Std. Error	Р
Juveniles			
(Intercept)	4.20	0.28	< 0.001
Time of day	-1.01	0.21	<0.001*
Age	-0.48	0.12	<0.001*
Sex	0.48	0.23	0.043*
Maternal Rank	-0.03	0.20	0.90
Prey Density	-0.13	0.08	0.13
Litter Size	-0.07	0.23	0.78
Lactating Females			
(Intercept)	4.8	0.24	< 0.001
Time of day	-0.41	0.22	0.07
Rank	0.27	0.19	0.16
Prey Density	-0.09	0.11	0.41
Social Instability	0.53	0.41	0.21
Rainfall	-0.01	0.06	0.85
Tourism Season	-0.26	0.24	0.27
Pregnant Females			
(Intercept)	4.35	0.54	< 0.001
Time of day	-0.87	0.52	0.12
Rank	0.17	0.41	0.68
Prey Density	-0.28	0.32	0.41
Social Instability	1.22	0.6	0.07
Day of gestation	0.01	0.01	0.14

Table 3.2. Results of preliminary analysis of variation in fGC concentrations among juveniles, lactating, and pregnant females in the low disturbance area. An asterisk indicates P<0.05

significantly higher fGC concentrations in the mornings than in the evenings (P<0.001), and that males had significantly higher fGC concentrations than did females (P=0.043). These variables were also significant correlates of juvenile fGC concentrations in our longitudinal analysis of the Talek West clan (although with the direction of the sex effect in the opposite direction), so we included them here in our cross-sectional analysis for juveniles. Although prey density was not a significant predictor of juvenile fGC concentrations in the low disturbance area, we included it as a fixed effect in our cross-sectional analysis because it had a significant parameter estimate in our longitudinal analysis of juvenile females the high-disturbance area.

Our cross-sectional analysis of juvenile fGC concentrations included 194 samples collected from 123 juveniles (49 juveniles from the low-disturbance area and 74 juveniles from the high-disturbance area). Our model indicated that fGC concentrations were significantly higher for samples collected in the morning than in the evening ( $\beta$ =-0.35, SE=0.14, P = 0.011), and were significantly negatively correlated with age ( $\beta$ =-0.34, SE=0.07, P < 0.001). No other variables were significant predictors of juvenile fGC concentrations (disturbance,  $\beta = 0.18$ , SE=0.16, P = 0.256; sex,  $\beta = -0.12$ , SE=0.15, P = 0.442; prev density,  $\beta = 0.00$ , SE=0.00, P = 0.442; prev density,  $\beta = 0.00$ , SE=0.00, P = 0.442; prev density,  $\beta = 0.00$ , SE=0.00, P = 0.442; prev density,  $\beta = 0.00$ , SE=0.00, P = 0.442; prev density,  $\beta = 0.00$ , SE=0.00, P = 0.442; prev density,  $\beta = 0.00$ , SE=0.00, P = 0.442; prev density,  $\beta = 0.00$ , SE=0.00, P = 0.442; prev density,  $\beta = 0.00$ , SE=0.00, P = 0.442; prev density,  $\beta = 0.00$ , SE=0.00, P = 0.442; prev density,  $\beta = 0.00$ , SE=0.00, P = 0.442; prev density,  $\beta = 0.00$ , SE=0.00, P = 0.442; prev density,  $\beta = 0.00$ , SE=0.00, P = 0.442; prev density,  $\beta = 0.00$ , SE=0.00, P = 0.442; prev density,  $\beta = 0.00$ , SE=0.00, P = 0.442; prev density,  $\beta = 0.00$ , SE=0.00, P = 0.442; prev density,  $\beta = 0.00$ , SE=0.00, P = 0.442; prev density,  $\beta = 0.00$ , SE=0.00, P = 0.442; prev density,  $\beta = 0.00$ ; SE=0.00, P = 0.442; prev density,  $\beta = 0.00$ ; SE=0.00, P = 0.442; prev density,  $\beta = 0.00$ ; SE=0.00, P = 0.442; prev density,  $\beta = 0.00$ ; SE=0.00, P = 0.442; prev density,  $\beta = 0.00$ ; SE=0.00, P = 0.442; prev density,  $\beta = 0.00$ ; SE=0.00, P = 0.442; prev density,  $\beta = 0.00$ ; SE=0.00, P = 0.442; prev density,  $\beta = 0.00$ ; SE=0.00, P = 0.442; prev density,  $\beta = 0.00$ ; SE=0.00, P = 0.442; prev density,  $\beta = 0.00$ ; SE=0.00, P = 0.442; prev density,  $\beta = 0.00$ ; SE=0.00, P = 0.442; prev density,  $\beta = 0.00$ ; SE=0.00, P = 0.442; prev density,  $\beta = 0.00$ ; SE=0.00, P = 0.442; prev density,  $\beta = 0.00$ ; SE=0.00, P = 0.442; prev density,  $\beta = 0.00$ ; SE=0.00, P = 0.442; prev density,  $\beta = 0.00$ ; SE=0.00, P = 0.442; prev density,  $\beta = 0.00$ ; SE=0.00, P = 0.00; SE=0.00, P = 0.0.767). However, the model containing an interaction between disturbance and sex was our best fitting model and a significantly better fit than the model without interactions  $(^{2}(1) = 9.65, P =$ 0.002). We therefore analyzed males and females separately. Juvenile females in the highdisturbance area showed significantly higher fGC concentrations than did those in the low disturbance area ( $\beta$ =0.68, SE=0.24, P=0.007), but fGC concentrations did not vary with disturbance among juvenile males ( $\beta$ =-0.24, SE=0.19, P=0.223; Figure 3.2). Among juveniles of both sexes, age (females  $\beta$ = -0.22, SE=0.11, P=0.040; males  $\beta$ = -0.43, SE=0.09, P<0.001) and time of day (females  $\beta$ = -0.54, SE=0.21, P=0.013; males  $\beta$ = -0.28, SE=0.17, P=0.11) were again significant predictors of fGC concentrations. There was no significant association between prey density and fGC concentrations among either males ( $\beta$ =0.00, SE=0.00, P=0.374) or females  $(\beta = 0.00, SE = 0.00, P = 0.230).$ 

### Lactating and Pregnant Females

In our preliminary analysis of fGC concentrations among pregnant (N=11 samples from 10



Figure 3.2. Residual juvenile fGC concentrations in high- and low-disturbance areas after controlling for time of day and age at sample collection. N=55 females (82 samples) and 67 males (112 samples). An asterisk indicates p < .05.

individuals) and lactating (N=70 samples from 29 individuals) females in the low disturbance area, social rank, prey density, and social instability were not significantly correlated with fGC concentrations (Table 3.2). Among lactating females, rainfall and tourism were also not significant predictors of fGC concentrations. Therefore, in our cross sectional analyses for pregnant and lactating females, we did not include any of these variables as fixed effects. Although day of gestation was not significantly correlated with fGC concentration among pregnant females in low disturbance areas (P=0.14), we included day of gestation as a continuous fixed effect in our cross-sectional model because previous hyena studies have found that fGC concentrations significantly increase over the course of gestation (Goymann et al. 2001, Van Meter et al. 2009). Our cross-sectional analysis of fGC concentrations among pregnant females included 42 samples collected from 31 females. Disturbance level did not significantly predict fGC concentrations ( $\beta$ =-0.11, SE=0.27, *P*=0.68). fGC concentrations significantly increased over the course of gestation ( $\beta$ =0.44, SE=0.12, *P*<0.001), but the time of sample collection (morning or evening) was not associated with fGC concentration ( $\beta$ =-0.30, SE=0.26, *P*=0.27). Neither our model containing an interaction between disturbance and social rank nor our model containing an interaction.

Our cross-sectional analysis of fGC concentrations among lactating females included 191 samples collected from 65 females. Samples collected in the morning had significantly higher fGC concentrations than samples collected in the evening ( $\beta$ =-0.46, SE=0.14, *P*=0.002), but disturbance level did not significantly predict fGC concentrations ( $\beta$ =-0.21, SE=0.18, *P*=0.24). Neither our model containing an interaction between disturbance and social rank nor our model containing an interaction between disturbance and prey density fit the data significantly better than our model containing no interaction term.

### Relationship between early life fGC concentrations and fitness

Seventy-nine individuals in the Talek West clan met the criteria for inclusion in our analysis of whether early life fGC concentrations predicted the likelihood of surviving to reproductive maturity. There was not a significant association between early life fGC concentration and survivorship ( $\beta$ =-0.67, SE=0.41, *P*=0.104). There was a postive trend between rank and survivorship ( $\beta$ =1.00, SE=0.60, *P*=0.074).
Thirty-one Talek West females met the criteria for inclusion in our analysis of the relationship between juvenile fGC concentrations and longevity. Longevity in this sample ranged from 1.07-14.81 years. There was a trend for females with higher fGC concentrations in early life to have lower longevity ( $\beta$ =-0.24, SE=0.14, *P*=0.089) and the association was weaker when we removed one data point from an individual that had a relatively high fGC concentration and a short lifespan ( $\beta$ =-0.19, SE=0.15, *P*=0.20). The mother's rank in the year the juvenile was born was also a significant predictor of longevity ( $\beta$ =0.72, SE=0.20, *P*= 0.001).

#### DISCUSSION

Our study indicates important sex- and age-specific effects of ecological and anthropogenic variables on stress hormone concentrations in a wild carnivore. Juvenile females had significantly higher fGC concentrations than juvenile males, with fGC concentrations significantly decreasing in both sexes as they approached reproductive maturity. Furthermore, a juvenile's sex mediated the effects of environmental variables on stress hormone concentrations. The fGC concentrations of female juveniles, but not of male juveniles or adult females, were affected by human disturbance and prey density. Overall, these results suggest that females may be more sensitive to environmental stressors than males and are consistent with studies of laboratory rats showing that females show greater HPA reactivity than males from birth (Patchev et al. 1999, Romeo 2010). This finding has important implications for studying effects of early stress in wild populations because it suggests that wild spotted hyenas, like laboratory rodents, may also show sex differences in the long-term behavioral and physiological effects of early stress (McCormick et al. 1995, Reznikov et al. 2001, Papaioannou et al. 2002, Park et al.

2003).

Our cross-sectional analysis indicated sex and age-specific effects of human disturbance on fGC concentrations. Juvenile females had higher fGC concentrations in the highdisturbance area than in the low disturbance area. However, fGC concentrations among juvenile males, pregnant females, and lactating females did not differ significantly between highand low-disturbance areas. This is surprising given that, across species, adult males show a greater increase in stress hormone concentrations due to human disturbance than do adult females (Dantzer et al. 2014). However, these results are again consistent with findings from laboratory rats that the response of the HPA axis to environmental events shows age-related variation (Jacobson-Pick and Richter-Levin 2010, Romeo 2010). Our findings demonstrate that individuals of different ages and sexes within a population may show different sensitivities to anthropogenic disturbance and suggest the importance of looking for such effects when studying the effects of disturbance on stress physiology.

Our longitudinal analysis provided further evidence that the stress physiology of juvenile females may be particularly sensitive to environmental variables because juvenile females, but not males, showed a significant positive association between prey density and fGC concentrations. A previous study at our site found no relationship between prey density and fGC concentrations among adult hyenas of both sexes (Van Meter et al. 2009), nor did we find a significant relationship between prey density and fGC concentrations in our preliminary analysis of adult females in the low-disturbance area (Figure 2). In the current study, it is perhaps surprising that juvenile females showed a significant positive, not negative, association between prey density and fGC concentrations. Spotted hyenas are found in larger groups when prey

densities are high than when prey is scarce. Perhaps it is larger group sizes, and not high prey per se, that is stressful for juvenile females. We did not see a significant association between prey density and fGC concentrations among juvenile females in our cross-sectional analysis, where maximum prey densities tended to be around 400-500 animals/meter less than in our longitudinal analysis. This suggests that samples collected during times of very high prey density were primarily driving the effect in our longitudinal analysis and that this situation may only serve as a stressor for juvenile females when prey density is very high.

Our results regarding fGC concentrations in adult females differ from previous findings, which were that adults in the highly disturbed Talek area had significantly higher fGC concentrations than adults in low disturbance regions (Van Meter et al. 2009). However, the authors of this previous study sampled from different low disturbance areas than we did, and fGC concentrations from individuals in low disturbance clans in the current study appear to be higher than those from individuals in the low disturbance clans studied by Van Meter et al. (2009). We suspect this might be due to an unusually high frequency of social instability in the low disturbance clans studied here compared to those studied by Van Meter et al (2009). We used a binary variable to indicate social instability due to sudden events, such as the death of an alpha female. However, we are currently exploring several continuous measures of group stability that better reflect more subtle social dynamics, and that might reveal associations with fGC concentrations.

Another possible explanation for why we found no significant effect of disturbance on fGC concentrations among pregnant and lactating females in the current study is that hyenas in the high-disturbance Talek area may be undergoing long-term adjustments in their HPA activity

in response to the chronic stress of pastoralism that has been increasing since the late 1990s. Chronically stressed animals may show attenuation in HPA activity due to habituation, desensitization, or HPA axis exhaustion. If hyenas living in the high-disturbance Talek area have been chronically stressed and attenuation has occurred, we would not necessarily expect fGC concentrations to differ between individuals sampled in high- and low-disturbance areas. Changes in HPA activity due to chronic stress are particularly difficult to predict and detect in wild populations (Cyr and Michael Romero 2007, Cyr and Romero 2009, Dickens and Romero 2013). However, we are currently exploring this hypothesis by investigating whether changes in fGC concentrations indicate attenuation among hyenas who have been repeatedly sampled and who have lived in the Talek area as pastoralism has increased.

The data from our study suggest that differences in circulating GC concentrations among mothers or juveniles are likely not mediating the behavioral differences we previously documented between juvenile hyenas living in high- and low-disturbance areas. Juveniles in the high- and low-disturbance areas studied here display consistent differences in personality traits, with juveniles in high-disturbance areas being less neophobic, more exploratory, and less bold than juveniles in low disturbance areas (Greenberg and Holekamp 2017). It remains possible that stress physiology plays a role in mediating these behavioral differences, but that a different measure of physiological stress is functionally related to personality traits. For example, biomarkers of stress in the autonomic system have been found to relate to personality traits and offspring stress physiology in humans (Ponirakis et al. 1998, Nater and Rohleder 2009). Additionally, numerous studies have found a significant relationship between personality and HPA axis reactivity, or how quickly and to what degree GCs are released in response to a

stressor (Oswald et al. 2006, Schoech et al. 2011, Baugh et al. 2012). In future research, we hope to compare HPA axis reactivity of spotted hyenas in high- and low-disturbance areas by performing ACTH challenges during routine immobilizations, and collecting serial blood samples to be assayed for GC concentrations.

Examination of other aspects of HPA functionality, and not simply baseline fGC concentrations, should also be conducted to further explore the relationship between stress physiology and fitness in this species. Although the associations between early life fGC concentrations and the fitness measures we investigated were in the predicted directions, the associations were not statistically significant. Our study, along with several other recent studies and meta-analyses, therefore challenge the assumptions that baseline stress hormone concentrations are negatively associated with fitness (Bonier et al. 2009a, 2009b), and that elevations of stress hormone concentrations due to anthropogenic disturbance in particular are associated with fitness costs (Dantzer et al. 2014). This recent work, along with the current study, therefore raise important questions regarding the role of non-invasive monitoring of stress hormone concentrations for wildlife management and conservation. However, as other researchers have argued, measures like survivorship and longevity may be related to other aspects of HPA sensitivity or feedback, rather than the crude measure of glucocorticoids excreted in feces (Romero and Wikelski 2010, Breuner et al. 2012).

In conclusion, the data from our current study allow us to rule out an hypothesis suggesting that maternal or offspring stress physiology shape personality differences observed between high- and low-disturbance areas. We are currently testing an alternative hypothesis that maternal behavior influences these juvenile personality differences. Previous research in the

high-disturbance Talek area has shown that, as human activity has increased, mothers spend less time at communal dens (Kolowski et al. 2007), and preferentially select dens in thicketed areas, which can be costly when this means they must travel farther to hunt and avoid livestock than females in undisturbed areas (Boydston et al. 2003a, Kolowski and Holekamp 2009). Research in other species, both in captivity and the wild, has indicated that changes in environmental quality or predictability can shape maternal investment strategies and maternal care styles, resulting in subsequent changes in juvenile traits, such as personality (Rosenblum and Paully 1984, Andrews and Rosenblum 1991, Fairbanks 1996). We are testing this alternative hypothesis by comparing maternal den attendance and maternal care in areas of high- and lowdisturbance.

#### CHAPTER 4

# VARIATION IN MATERNAL BEHAVIOR AND MOTHER-OFFSPRING INTERACTIONS

Julia R. Greenberg, Zach M. Laubach, Shannon Carvey, Kaycee Morra, Laura Smale, & Kay E. Holekamp

### INTRODUCTION

Observers of mammalian behavior have long noted that mothers often vary in the way they care for offspring (Hinde 1976, Altmann 1980, Clutton-Brock 1991), and that such variation seems to be consistent across generations (Fairbanks 1989, Berman 1990, Maestripieri and Wallen 2003). In recent years, scientists have demonstrated that laboratory rodents show "maternal styles," revealing natural variation in the way they groom and nurse pups that is consistent over development and across multiple litters (Liu et al. 1997). This variation in maternal behavior affects offspring personality, stress reactivity, reproductive strategies, and sociality (Moore 1984, 1992, Coplan et al. 1996, Caldji et al. 1998, Francis et al. 1999, Cameron et al. 2008b, Stevens et al. 2009, Andrews and Rosenblum 2010). Maternal styles in this laboratory model system are transmitted across generations via epigenetic mechanisms, and a female's early experience of maternal care shapes her own reproductive strategies and patterns of maternal behavior towards her own pups (Francis, Diorio, & Liu, 1999).

To understand the ecological and evolutionary factors shaping and maintaining such variation, we need a better understanding of maternal styles in natural systems. Maternal styles have been documented in some free-living animals (yellow baboon Nguyen et al. 2008, red squirrel Dantzer et al. 2011, grey seal Twiss et al. 2012, gray-cheeked mangabeys Arlet et al. 2016). However, it remains unclear how widespread they are in nature, where variation in ecological and social conditions is far greater than in the laboratory (Stewart and Mcadam 2014). Additionally, determining whether maternal styles are directly related to offspring fitness would shed light on whether the epigenetic mechanisms documented in the laboratory are also likely to occur in nature.

The causes and consequences of variation in maternal behavior have been most thoroughly studied in Old World Monkeys (reviewed in Fairbanks 1996). As infants mature, mothers generally spend less time in contact with offspring, and offspring become more responsible for mediating changes in contact and proximity with their mothers, using their mothers as a "secure base" from which to explore their environment (Hinde and Atkinson 1970, Nash 1978). However, beyond this ontogenetic trend, mothers often show marked variation in how much time they spend in contact with offspring, to what degree they initiate contact with and departures from, offspring, and how often they groom offspring. Highly protective mothers spend a relatively large proportion of their time in contact with offspring, frequently initiate contact with their offspring, and restrain offsprings' attempts to break contact. Highly rejecting mothers spend a relatively small proportion of their time in contact with offspring, often reject offspring attempts to make contact, and show high rates of breaking contact with offspring (Fairbanks 1996). In some species, a third dimension of maternal behavior emerges, sometimes referred to as *warmth*, which is positivity correlated with maternal grooming and cradling of offspring (pigtail macaques, Maestripieri 1998).

Although a large proportion of the variation in maternal behavior among cercopithecines is due to individual variation, maternal styles may also be shaped by the degree to which a

mother's physical or social environment is challenging. For example, mothers that are resourcelimited, live in unpredictable environments, or experience extreme environmental events tend to be more rejecting of offspring than those living in more favorable environments (Hauser and Fairbanks 1988, Andrews and Rosenblum 1991, Fairbanks and McGuire 1995, Lycett et al. 1998). Such effects may be mediated by effects of the environment on maternal condition. On the other hand, if the mother experiences a challenging social environment that poses risks to offspring, such as aggression, harassment, or kidnapping, mothers tend to be more protective (rhesus macaque Maestripieri 1995, 2001). This is consistent with the findings of some primate studies that low-ranking mothers, who experience more social challenges, are more protective than high-ranking mothers (savannah baboon Altmann 1980, rhesus macaque Berman 1990, vellow baboon Wasser and Wasser 1995). However, in other primate studies, the opposite relationship between rank and maternal behavior has been found (Schino et al. 1995, Bentley-Condit 2003). It is also unclear how the degree of environmental or social risk to young relates to the common finding that primiparous mothers tend to be more protective than multiparous mothers (Tanaka 1989, Maestripieri 1998). Instead, differences in maternal behavior due to parity have largely been interpreted as the result of maternal experience.

Across species, mothers may also behave differently towards offspring based on offspring traits. For example, maternal investment is predicted to be greater in the sex that shows more variation in reproductive success (Trivers 1972, 1974, Smith 1980, Clutton-Brock et al. 1981). This may explain why male offspring sometimes nurse at higher rates than females (Lee and Moss 1986), initiate contact with their mothers more often than female offspring (Nguyen et al. 2012), or receive more grooming from mothers than do female offspring (Moore

1984, 1992). Additionally, an individual's litter size may mediate mother-offspring relationships not only because an individual competes with siblings for maternal resources (Hudson and Trillmich 2008, Hudson et al. 2011), but also because mothers may behave differently towards some members of a litter than toward others (Mendl 1988). For example, even among same-sex siblings, mothers groom some rat pups significantly more frequently than their siblings (Cavigelli and McClintock 2003, Ragan et al. 2011, 2016).

In this study, we explored natural variation in maternal behavior and its fitness consequences for offspring in a free-living population of spotted hyenas. In terms of their basic developmental biology and life-history, spotted hyenas show both similarities and differences to the primates and rodents that have been the focus of most earlier studies of maternal behavior. Understanding patterns of maternal variation in this wild carnivore can therefore help us to better understand the ecological and evolutionary factors shaping variation in maternal care across species. Spotted hyenas show a degree of maternal investment and a protracted period of offspring growth and development that is more typical of primates than other carnivores (Watts et al. 2009). Mothers give birth to small litters (1-2 cubs) at isolated natal dens and cubs do not wean until an average of 14 months of age at our site (Holekamp et al. 1996, Holekamp and Dloniak 2010). A mother's presence and defense remain critical for offspring survival even after weaning, as the feeding apparatus of young spotted hyenas is not fully developed for eating meat and bone until 36 months of age (Tanner et al. 2009), and they do not reach full proficiency at hunting until roughly five years of age (Holekamp et al. 1997b).

Whereas patterns of maternal investment among spotted hyenas are similar to many primates, young spotted hyenas spend much less time with their mothers than do primates, and

instead reside at a communal den that is more similar to the nests utilized by rodents. When cubs are approximately 3-4 weeks of age, mothers move cubs from the natal den to communal dens where they live for the next 7-12 months. The communal den is composed of a series of burrows and tunnels that protect young hyenas from dangers present in the rest of the clan's territory (Boydston et al. 2006). Hyena cubs emerge from the den to nurse from their mothers and socialize with other hyenas. Whereas primate mothers carry their offspring for many months, young hyenas only have the opportunity to interact with their mothers when mothers visit the communal den; this can occur at intervals ranging from a few hours, as at our study site, to 3-4 days at other sites (Hofer & East, 1993b). Furthermore, whereas juvenile primates typically treat their mothers as a "secure base" from which to explore their environment and flee to her during times of danger, the communal den may also serve this function for young hyenas.

Spotted hyenas live in matrilineal societies nearly identical in size and structure to those of many Old World Monkey species; however unlike those primate species, female spotted hyenas are dominant to males and therefore primate and hyena mothers may vary in their treatment of male and female offspring. Juvenile hyenas "inherit" dominance ranks immediately below those of their mothers in a process of social learning that is not complete until at least 18 months of age (Holekamp & Smale 1993; Smale et al. 1993). Hyenas of both sexes retain their maternal ranks as long as they reside in the natal clan, which females do throughout their lives, but most males disperse to new clans at 2-5 years of age (Boydston et al 2005; Smale et al. 1997; Höner et al. 2007). High-ranking spotted hyena mothers intervene on behalf of their offspring in aggressive interactions more often and more effectively than do low-ranking mothers (Engh et al. 2000) To examine variation in maternal behavior, we first used focal animal surveys (FAS) on mother-cub dyads to determine the proportion of time that mothers nursed their cub(s), were in close proximity to their cub(s), groomed their cub (s), and to what degree the mother initiated being close to cub(s). To determine sources of this variation, we examined the effects of a mother's parity and social rank, cub age, cub sex, litter size, and external factors such as time of day and prey density. Next, we explored whether spotted hyena mothers show consistent individual variation in their maternal behavior, and to what extent variation in maternal behavior is due to these consistent individual differences. Finally, we inquired whether measures of maternal behavior predicted offspring survivorship to reproductive maturity.

### METHODS

### Subjects & Study Populations

Study subjects lived in a single large clan of spotted hyenas inhabiting the Talek region of the Masai Mara National Reserve (MMNR) in southwestern Kenya during two different time periods, 1988-1991 and 1993-1996. Individuals could be identified by their unique spot patterns and ear damage. Subjects were juveniles who had not yet been weaned, and their mothers. Age was initially estimated when cubs were first observed, based on their appearance and size (Holekamp et al. 1996), and sex was determined based on the shape of the glans of the erect phallus (Frank et al. 1990). The date on which a cub was last seen was assigned as its death date because female hyenas spend their entire lives in their natal clan and male hyenas do not typically disperse before two years of age (Smale et al. 1997, Holekamp and Smale 1998a). In litters of two cubs, we determined the cub's intra-litter rank based on nursing position and

aggressive and submissive interactions between the siblings. Spotted hyena mothers have only two teats, and once the dominance relationship between litter-mates has been established, typically by 4 weeks of age, a cub's position while nursing consistently varies with its intra-litter rank. As shown in Figure 4.1, the dominant cub typically nurses while lying against its mother's stomach, whereas the subordinate sibling typically nurses while strattled between its mother's hind legs.

Spotted hyenas live in clans of up to 130 individuals (Holekamp et al. 2015) that are structured according to a strict matrilineal hierarchy (Frank 1986). Here the social ranks of adult females were calculated on a yearly basis, based on outcomes of dyadic agonistic interactions, and were used to construct a dominance hierarchy each year (Martin & Bateson 1988; Smale et



Figure 4.1. Photograph of a spotted hyena mother nursing two cubs. As is typical, the dominant member of the litter is laying up against its mother's stomach while nursing, whereas the subordinate litter-mate is strattled between her legs. Photo courtesy of Lily Johnson-Ulrich.

al. 1993). Ranks were standardized from -1 to 1, with the most dominant individual having a rank of 1.

The reproductive state and parity of adult females was continuously monitored as described by Holekamp et al. (1996). A female's first parturition could be determined reliably because a female's pseudopenis tears when she gives birth to her first litter, leaving an unambiguous stripe of pink scar tissue on the posterior side of the phallus (Frank and Glickman 1994, Holekamp et al. 1996).

# Data Collection

From 1988-1991, we conducted focal animal surveys (FAS) on every juvenile in the clan approximately every two weeks. From 1993-1996, we collected FAS data on adult females opportunistically, making every attempt to balance the dataset with regards to the mother's social rank, parity, and litter size. FAS were conducted during our routine daily morning (0600-1000h) and evening (1600-2000h) observation periods. We followed the focal subject, either the cub or the adult female, for 30 minutes or until the subject had been out of sight for 5 minutes. During FAS, we recorded all affiliative interactions between individuals, such as, sniffing, grooming, playing, and ritualitized "greetings" in which two individual lift a leg to allow the other to sniff their genital region). We also recorded all aggressive interactions, ranging from displacements to chasing and biting, counterattacks, and coalitionary attacks. Lastly, we recorded all instances in which the focal subject approached or was approached by another individual to within 1m.

We extracted data on maternal behavior from FAS on cubs in which the cub's mother

was present, and from FAS on adult females in which one or more of her dependent cubs was present. If a female had a litter of two cubs and both were present during a FAS conducted on her, we extracted two different sets of FAS data, one for each cub. From each FAS, we extracted the number of minutes in which the mother engaged in three maternal behaviors: nursing, grooming the cub, or being in close proximity to the cub (within 1m or in contact). We then calculated the total duration of each behavior in each FAS. For the time that a mother spent in close proximity to the cub, we excluded any minutes when the mother was nursing her cub, as nursing necessitates contact with the cub. Additionally, whenever the mother-cub pair moved in or out of close proximity, we recorded which individual initiated the change as an "event" (e.g. cub approach mother, mother approach cub, cub leave mother, or mother leave cub). In some cases, both the mother and cub simultaneously approached one another (0.4% of all approaches) or left one another (0.3% of all departures), or we could not determine which individual approached (5% of all approaches) or departed (2% of all departures). However, because these cases represented a small percentage of all proximity changes, and because we were primarily interested in whether the mother or cub was primarily responsible for proximity changes, we excluded these cases from analysis.

### Statistical Analysis

We used generalized linear mixed models to model variation in each of the three maternal behaviors of interest (nursing, grooming, and time spent in close proximity to the cub), and our event measures that indicated proximity maintenance. All analyses were conducted using the glmmADMB package in R (R version 3.3.1; Development Core Team, 2015), which allows for the incorporation of both random effects and zero-inflated predictor variables. We used the multicomp package to perform post-hoc tests with a Tukey correction for pairwise comparisons. For our models involving durations of maternal behaviors, we used the number of minutes in which the mother engaged in each behavior during a particular FAS as our response measure. To model variation in how often mothers approached their offspring, we limited our analysis to sessions in which either the mother or the cub approached the other at least once during the FAS. We used the number of times a mother approached her cub in the FAS as our response measure and included the total number of approaches by either mother or cub in the FAS as a predictor variable. To determine the extent to which mothers left their cubs, we limited our analysis to sessions in which the mother and cub left one another at least once, used the number of times a mother left her cub within a FAS as our response measure, and included the total count of times the mother or cub left one another as a predictor variable. To account for the fact that our FAS varied in the amount of time when both a mother and her cub were present, we included as a logged offset the number of minutes in which the mother and cub were both present during the FAS in each model. We logged the offset value as this is the standard way to incorporate offsets in glmmADMB and helps to scale the offset to be proportional to the predictor variable. We excluded from all analyses any FAS in which the mother and cub were present together for less than 5 min. In each model, we also included the mother's ID as a random effect.

In all models, we included several ecological, social, and demographic variables as predictor variables. As binary fixed effects, we included the time of day (morning or evening), and the mother's parity (primiparous or multiparous), and litter size (i.e. whether the focal cub

did or did not have a living litter-mate alive on the day of the FAS). As continuous fixed effects, we included the cub's age, the mother's social rank (standardized from -1 to 1, as described above), and prey density. Prey density was calculated as the average number of prey counted within 100m of three 4-km prey transects sampled within two-weeks of the FAS. For the first three months of the study in 1988, we did not have data from prey transects and we therefore used the values averaged from the other years of the study during the corresponding two-week intervals. Cub's age and prey density were scaled and centered in our analyses. If models indicated a significant effect of whether the cub had a litter-mate or not, we then restricted our dataset to include only cubs that had a living litter-mate, and tested whether a cub's intra-litter rank (dominant or subordinate) was a significant predictor of the maternal behavior of interest.

In all models except our model for nursing, we also included a random effect of study year. We removed this random effect in our models for nursing in order for our models to converge. Our diagnostics suggested that this term was problematic, as random effects with relatively few levels can sometimes cause non-convergence especially when they have low variance, as was the case for this term. There are several suggestions regarding what to do in this situation, including removing the term from the model and fitting the model with the term as a fixed effect instead of a random effect (Barr, D.J., Levy, R., Scheeperes, C., Tily 2014). We did both of these and our results did not differ when using these alternative approaches. Because we were mainly interested in controlling for year-to-year variation in our models of maternal behavior, we report the results of our nursing model that does not include study year as a factor.

For each maternal behavior, we assessed the fit of four types of count models; a negative

binomial model, a poisson model, a zero-inflated negative binomial model, and a zero-inflated poisson model. We then compared the fit of the four models using Akaike's Information Criterion corrected for small sample sizes (AICc). A smaller AICc value indicated a better fit, and we report the results from the best-fitting model for each maternal behavior.

glmmADMB is a relatively new R package, and model diagnostic methods are still being developed. We therefore focused our evaluation of model fit on the residual deviance. We also evaluated overdispersion using the theta value provided in the model outputs. We used Variance Inflation Factors (VIF) to assess multi-colinearity of predictor variables in all of our models. No variable had a VIF above 3, indicating that there was no significant multi-colinearity among variables in our models.

#### Individual variability and repeatability of maternal behavior

We assessed the contribution of individual variation among mothers to our models of nursing, close proximity, and grooming durations, as well as our models of proximity maintenance in two ways. First, for each of these models we determined whether the random effect of maternal ID significantly improved the fit of our models. We used a likelihood ratio test to compare each model to a model that did not include the mother's ID as a random effect, and calculated a parameter estimate for the mother's identity. Second, we calculated the proportion of variance explained by the random effect of maternal ID by dividing the proportion of variance explained by maternal ID by the total residual variance of the model. This offers a measure of repeatability in maternal behavior (Lessells and Boag 1987, Dantzer et al. 2011).

# Relationship between maternal behavior and offspring fitness

To determine whether an individual's experience of maternal behavior in early life predicted it likelihood of surviving to adulthood, we first used our demographic records to determine whether each cub survived to reproductive maturity (2 years of age). We calculated the proportion of time that a cub nursed, was groomed by its mother, or was in close proximity to its mother from 3-6 months of age. To control for differences in behavior based on time of day, we only included evening sessions. We chose this time interval in order to maximize the number of individuals we could include and we only included individuals that had multiple evening observations in each of the three months. In three separate general linear models, we then modeled the likelihood of surviving (Y/N) to reproductive maturity as a function of the proportion of FAS time a cub spent nursing, being groomed by its mother, or in close proximity to its mother. We used the "glm" package in R and specified a binomial response measure.

# RESULTS

Our data on mothers with cubs 0-15 months included a total of 1075 FAS collected on 34 mothers and 122 cubs (Figure 4.2). An average of 8.83 FAS (range 1-32) were conducted on each mother-offspring pair. Figure 4.3 shows the average percent of the time that a mother and cub were both present in a FAS during which the mother was nursing her cub (a), in close proximity to her cub (b), and grooming her cub (c).

# Models of time spent nursing, grooming, and in close proximity to offspring

A zero-inflated negative binomial model best described variation in nursing among mothers



Figure 4.2. The total number of Focal Animal Surveys (FAS) collected on mothers with cubs 10-15 months. Data are binned by the cub's age in months. Numbers above bars indicate the number of cubs observed in that age category.

(Table 4.1). Mothers spent a significantly greater proportion of their time nursing as cubs matured, and in the evening than in the morning. There was a trend for primiparous mothers to nurse their cubs more than did multiparous females. Mothers of twin litters spent a greater proportion of their time nursing than did mothers of singletons. A model including only cubs who had a living litter-mate indicated no significant effect of intra-litter rank on the proportion of time a mother spent nursing (B = -0.04, SE = 0.05, P = 0.50).

A zero inflated negative binomial model best explained variation in how much time mothers spent in close proximity to their cubs (Table 4.2). Mothers spent a significantly lower proportion of their time close to cubs as the cubs matured, and a significantly greater proportion of their time in close proximity in the mornings than in the evenings. Mothers spent a



Figure 4.3. Developmental changes in three maternal behaviors among spotted hyena mothers with cubs 0-15 months old. Out of the total minutes a mother and cub were both present in an FAS, figures show the proportion of time a mother spent a) nursing the cub; b) grooming the cub and; c) in close proximity to the cub. Labels indicate the number of FAS averaged for each month of development.

	Odds Ratio	Estimate	Std. Error	Р
(Intercept)	0.54	-0.62	0.06	< 0.001
Cub Age	1.16	0.14	0.03	<0.001*
Cub Sex	0.94	-0.06	0.05	0.221
Time of Day	1.20	0.18	0.05	<0.001*
Maternal Rank	1.00	0.00	0.05	0.964
Parity	0.88	-0.12	0.06	0.057
Litter Size	1.18	0.16	0.06	0.005*
Prey Density	1.01	0.01	0.02	0.598

Table 4.1. Results of a zero-inflated negative binomial model explaining variation among mothers in duration of nursing. An asterisk indicates P < 0.05.

	Odds Ratio	Estimate	Std. Error	Р
(Intercept)	0.42	-0.88	0.13	< 0.001
Cub Age	0.88	-0.13	0.04	0.001*
Time of Day	0.72	-0.33	0.07	<0.001*
Cub Sex	1.19	0.17	0.07	0.020*
Maternal Rank	1.04	0.04	0.08	0.573
Parity	0.91	-0.10	0.12	0.405
Litter Size	0.73	-0.31	0.08	<0.001*
Prey Density	0.95	-0.05	0.04	0.169

Table 4.2. Results of a zero-inflated negative binomial model explaining variation in time mothers spent in close proximity to their cubs. An asterisk indicates P<0.05.

significantly greater proportion of their time in close proximity to male than female cubs, and a significantly greater proportion of their time in close proximity to the focal cub if it was a singleton than if it had a litter-mate. A model including only cubs who had a litter-mate indicated that a cub's intra-litter rank was not significantly associated with the proportion of time a mother spent in close proximity to the cub (B = 0.05, SE = 0.08, P = 0.54).

Because our analysis indicated a significant effect of cub sex on how much time a mother spent in close proximity to the cub, we stratified the dataset according to cub sex and ran the model separately for male and female cubs. We included the same offset, random effects, and independent variables, but excluded cub sex as a variable. Mothers of males cubs spent a greater proportion of their time in close proximity to the focal cub if it was a singleton than if it had a litter-mate (B = -0.48, SE = 0.10, P < 0.001). A model including only male cubs that had a litter-mate indicated that a cub's intra-litter rank was not associated with the proportion of time a mother spent in close proximity to the cub (B = 0.11, SE = 0.14, P = 0.42). Among mothers of female cubs, there was no significant effect of litter size on the proportion of time a mother spent in close proximity to the cub (B = 0.04, SE = 0.16, P = 0.80).

A negative binomial model best described variation in grooming among mothers (Table 4.3). Mothers spent a significantly lower proportion of their time grooming cubs as the cubs matured and spent a significantly greater proportion of their time grooming cubs in the evening than in the morning. Multiparous mothers spent a significantly greater proportion of their time grooming cubs than did primiparous mothers. Mothers spent a significantly greater proportion of their time grooming the focal cub when the cub was a singleton than when it had a littermate. A model including only cubs who had a litter-mate indicated that a cub's intra-litter rank

	Odds Ratio	Estimate	Std. Error	Р
(Intercept)	0.02	-3.96	0.31	< 0.001
Cub Age	0.41	-0.90	0.12	<0.001*
Time of Day	1.79	0.58	0.21	0.005*
Cub Sex	1.23	0.21	0.22	0.341
Maternal Rank	0.68	-0.39	0.29	0.179
Parity	0.48	-0.74	0.36	0.041*
Litter Size	0.39	-0.93	0.25	<0.001*
Prey Density	0.88	0.12	0.11	0.254

Table 4.3. Results of a negative binomial model explaining variation in time mothers spent grooming offspring. An asterisk indicates P < 0.05.

was not significantly associated with the proportion of time a mother spent grooming it (B = -0.36, SE = 0.25, P = 0.16).

Because our analysis indicated a significant effect of maternal parity on how often a mother groomed her offspring, we stratified the dataset according to parity and ran the model separately for primiparous and multiparous mothers. We included the same offset, random effects, and independent variables, but excluded parity as a predictor variable. Results suggest that variation among multiparous mothers was driving the effect of litter size on maternal grooming because there was a significant effect of litter size on the proportion of time multiparous mothers spent grooming their cubs, but this effect was not observed among primiparous mothers. Multiparous mothers spent a significantly greater proportion of their time grooming the focal cub when the cub was a singleton than when it had a litter-mate (B = -0.94, SE = 0.27, *P* = 0.001), but there was no significant effect of a cub's intra-litter rank on grooming (B = -0.29, SE = 0.26, *P* = 0.268). Grooming by primiparous mothers did not vary with litter size (B = -0.82, SE = 0.66, *P* = 0.214).

### Models of proximity maintenance

Our model of variation in how often a mother approached her cub included 541 surveys collected from 34 mothers and 116 cubs (Figure 4.4). Each mother-cub pair had an average of 5.92 FAS (range: 1-22). Our best fitting model was a negative binomial model (Table 4.4). The total number of approaches within a session was a significant predictor of the number of times mothers approached their cub and mothers approached singletons significantly more often than cubs that had a litter-mate. A model including only cubs who had a litter-mate indicated that a cub's intra-litter rank was not significantly associated with the number of times a mother approached her cub (B = -0.24, SE = 0.21, P = 0.25).

Our model for variation in how often a mother left her cub included 712 surveys collected from 34 mothers and 115 cubs (Figure 4.5). Each mother-cub pair had an average of 6.21 FAS sessions (range: 1-23). Our best fitting model was a negative binomial model (Table 4.5). Mothers left their cubs significantly more as cubs matured and the total number of departure events within a session was also a significant predictor of the number of times mothers left their cub.

#### Individual variability and repeatability of maternal behavior

In regard to nursing, the model that included maternal ID was not a significantly better fit than the model without maternal ID as a random effect. In fact, our model without maternal ID was a significantly better fit than our model with maternal ID (likelihood ratio test,  $^2$ =4.88, *P* =0.027), and variation among mothers explained only a small proportion (0.17%) of the residual variance in the model. In regard to time spent in close proximity, the model that included



Figure 4.4. Developmental changes in the percentage of approaches to within 1m that were initiated by the mother. Labels indicate the number of FAS averaged for each month of development.

	Odds Ratio	Estimate	Std. Error	Р
(Intercept)	0.01	-4.32	0.26	< 0.001
Total # of				
approaches	1.18	0.16	0.03	<0.001*
Cub Age	1.08	0.08	0.09	0.386
Time of Day	0.92	-0.08	0.17	0.620
Sex	0.99	-0.01	0.18	0.960
Maternal Rank	1.07	0.07	0.17	0.684
Parity	1.28	0.25	0.27	0.349
Litter Size	0.38	-0.96	0.18	<0.001*
Prey Density	1.04	0.04	0.09	0.671

Table 4.4. Results of a negative binomial model explaining variation in how often mothers approached their cub. An asterisk indicates P < 0.05.



Figure 4.5. Developmental changes in the percentage of departures to further than 1m apart that were initiated by the mother. Labels indicate the number of FAS averaged for each month of development.

	Odds Ratio	Estimate	Std. Error	Р
(Intercept)	0.02	-3.86	0.19	< 0.001
Total # of leaves	1.09	0.09	0.01	<0.001*
Cub Age	1.40	0.34	0.05	<0.001*
Time of Day	1.00	0.00	0.10	0.973
Cub Sex	0.94	-0.06	0.10	0.556
Maternal Rank	0.84	-0.17	0.10	0.095
Parity	1.04	0.04	0.17	0.797
Litter Size	0.93	-0.07	0.11	0.505
Prey Density	0.96	-0.05	0.06	0.441

Table 4.5. Results of a negative binomial model explaining variation in how often mothers moved more than 1 m from their cub. An asterisk indicates P<0.05.

maternal ID was not a significantly better fit than the model without maternal ID as a random effect (likelihood ratio test,  ${}^2$ =2.28, P=0.131), and variation among mothers explained only 2% of the residual variance of the model. However, in regard to grooming, the model including maternal ID was a significantly better fit than the model without maternal ID as a random effect (likelihood ratio test,  ${}^2$ =9.86, P=0.002), and variation among mothers explained 48% of the residual variance of the model (Figure 4.6). Neither model for proximity maintenance was a better fit when including maternal ID as a random effect (approaches by mother:  ${}^2$ =1.61, P=0.20; departures by mother:  ${}^2$ =1.62, P=0.20). Maternal ID explained 5% of the residual variance of the model for maternal approaches and 2% for maternal departures from the cub.

### Relationship between maternal behavior and offspring fitness

Only nine individuals met our criteria for inclusion in the analysis of whether maternal behaviors predict offspring survivorship. Neither the proportion of time a mother spent nursing her cubs, time she was in close proximity to her cubs, nor the proportion of time she spent grooming her cubs significantly predicted a cub's likelihood of surviving to reproductive maturity (nursing, B = 7.87, SE = 7.41, P = 0.29; close proximity, B = 9.79, SE = 9.24, P = 0.29; grooming B = 4.43, SE = 34.91, P = 0.90).

#### DISCUSSION

Our study documents natural variation in maternal behavior among free-living carnivores, and adds to a growing body of literature elucidating the factors that shape intra-specific variation in maternal behavior. Compared to the Old World Monkey and rodent species that have been



Figure 4.6. Individual variation in the proportion of a mother's time spent nursing, grooming, and in close proximity to cubs. Y-axis is the best linear unbiased predictor (BLUP) for each of the 34 mothers calculated from our models for proportion of time spent in each of the three maternal behaviors.

most thoroughly studied, spotted hyenas showed both important similarities and differences in their patterns of maternal behavior. Spotted hyena mothers showed significant and consistent inter-individual variation in how much time they spent grooming cubs, suggesting that, like laboratory rodents, the degree to which a mother grooms her cubs is a component of maternal styles in spotted hyenas.

Unlike Old World Monkeys, spotted hyenas did not show significant and consistent inter-individual variation in how much time they spent close to cubs and how they mediated proximity with their cubs. It is possible that this difference stems from the fact that cercopithecines, unlike spotted hyenas, carry their young for extended periods of time and that young can physically cling to mothers. Additionally, while juveniles of these primate species often flee to their mothers in times of danger, or use their mother as a secure base from which to explore their environments, spotted hyena cubs often utilize the communal den as a secure base, and this could mean that changes in proximity between mother and offspring do not reflect maternal protectiveness or offspring attachment as meaningfully in hyenas as monkeys. Future research should investigate other aspects of maternal behavior that may contribute to individual maternal styles. For example, the degree to which a mother is aggressive (Dwyer and Lawrence 2000), or playful (Blehar et al. 1977) towards offspring can vary consistently and significantly among mothers of other species, and it would be interesting to look at this in spotted hyenas.

Interestingly, a female's social rank was not a significant predictor of any of the maternal behaviors we measured. This is in contrast to most other aspects of spotted hyena behavior that have been studied at our field site, including patterns of aggression (Dloniak et al. 2006), space

use (Boydston et al. 2003a), personality (Yoshida et al. 2016), and social bond formation (Holekamp et al. 1997a, Smith et al. 2007). Spotted hyenas live in a more flexible fission-fusion society than cercopithecines, and previous studies have found that low-ranking mothers are able to avoid threatening social situations by visiting the den when few high-ranking females are present (White 2007). Furthermore, even within cercopithecines, some studies have found that rank and maternal care are not significantly associated (Schino et al. 1995, Bentley-Condit 2003), or that rank and maternal behaviors are significantly related among male but not female offspring (Schino et al. 1999). Our finding suggests that rank-related maternal effects on cub growth and development are not mediated by how much time mothers spend nursing, grooming, or in close proximity to their offspring, suggesting that these rank effects are due solely to differences in maternal condition (Hofer and East 2003), gestational hormones (Dloniak et al. 2006), and aggressive defense of offspring (Engh et al. 2000).

Our study also indicated that a spotted hyena's experience of maternal care is profoundly influenced by litter size. Mothers spent a significantly greater proportion of their time grooming, or in close proximity to a cub if it was a singleton than if it had a litter-mate. However, mothers of twin litters did not bias their behavior towards either the dominant or subordinate cub for either behavior. This suggests that mothers of twin litters divide their maternal care between litter-mates. Regarding nursing, mothers spent a significantly greater proportion of their time nursing when they had twin litters as opposed to singletons. Again, there was no difference in the proportion of time mothers spent nursing dominant versus subordinate litter-mates, which is surprising given previous findings that subordinate litter-mates sustain more nursing interruptions due to sibling aggression (Benhaiem et al. 2012), and are often considerably smaller

than their dominant litter-mates (Holekamp and Dloniak 2009). This suggests that, at least in our dataset, such nursing interruptions were brief and subordinates were able to offset their effects. It also suggests that, although dominant litter-mates are often larger than subordinate litter-mates, this pattern is not due to differences in durations of nursing, but perhaps differences due to nursing position (for example, differences in milk let-down from different teats or differences in the degree of body contact with the mother). In addition to a cub's litter size, its sex was also a significant predictor how much time it spent in close proximity to its mother. Mothers appeared to bias this aspect of their maternal care towards male singletons. Studies in other species have found similar evidence for sex-biased maternal care in some behavioral measures, but not others (Schino et al. 1999, Dettmer et al. 2015, King and Goldizen 2016)

Contrary to findings among Old World Monkeys, we found that primiparous mothers showed behaviors indicative of less protectiveness than multiparous mothers. Primiparous spotted hyena mothers spent a significantly lower proportion of their time grooming cubs than did multiparous mothers. And unlike multiparous mothers, who groomed singletons more than members of twin litters, primiparous mothers groomed cubs equally regardless of litter size. These differences could be due either to primiparous mothers having less experience rearing offspring or being in poorer condition than multiparous mothers. Consistent with studies on Old World Monkeys, there was a trend for primiparous mothers to nurse their cubs more than multiparous mothers, suggesting that primiparous mothers may attempt to compensate for deficits in milk quality or nursing ability.

Our study raises questions regarding how variation in maternal behavior influences

offspring and how it is maintained within this population. Future research could determine whether offspring of "high-grooming" and "low-grooming" spotted hyena mothers show consistent differences in personality or stress reactivity, as they do in laboratory rodents. Given that we found notable differences in how mothers behave towards singletons versus members of twin litters, it would also be interesting to inquire whether individuals show personality differences into adulthood based on their litter status. If maternal behaviors such as grooming and spending time close to offspring influence offspring traits as they do in cercopithecines, we would predict that singletons would be more neophobic, fearful, and less exploratory than individuals who were in twin litters (Fairbanks 1993, Schino et al. 2001). Finally, our study raises the question of whether the natural variation in grooming among spotted hyena mothers is transmitted across generations through epigenetic mechanisms, as it is among laboratory rodents. Future research should examine cross-generational consistency in these maternal behaviors.

#### CHAPTER 5

### ANTHROPOGENIC EFFECTS ON MATERNAL BEHAVIOR IN SPOTTED HYENAS

Julia R. Greenberg, Zach M. Laubach, & Kay E. Holekamp

# INTRODUCTION

Maternal effects, whereby a mother influences her offspring phenotype independently of offspring genotype, can profoundly affect offspring fitness, physiology, and behavior (Mousseau and Fox 1998). Mothers alter their patterns of maternal care in response to environmental challenges or altered resource availability (Ono et al. 1987, Hauser and Fairbanks 1988, Lee et al. 1991, Burkanov et al. 2011). Such changes can, in turn, affect offspring growth, physiology, behavior, or survivorship (Rosenblum and Paully 1984, Coplan et al. 2001, Stevens and Boness 2003, Arroyo and Razin 2005, Gibbs et al. 2010, Champagne 2011, French et al. 2011, Johnson et al. 2012, Almasi et al. 2015). Maternal effects can therefore be an important mechanism through which organisms respond flexibly to changing conditions in their environment (Jablonka et al. 1995, Räsänen and Kruuk 2007).

Anthropogenic disturbance affects the behavior of a range of wildlife species (reviewed in Blumstein, Fernández-Juricic, Zollner, & Garity, 2005, Miranda, Schielzeth, Sonntag, & Partecke, 2013, Sol, Lapiedra, & González-Lagos, 2013), and therefore has the potential to affect offspring through behavioral maternal effects. Among mammalian carnivores, which are threatened worldwide (Ripple et al. 2014), human activity has both lethal and sub-lethal effects on behavior. Compared to carnivores in undisturbed areas, carnivores in disturbed areas are often more nocturnal (Ciucci et al. 1997), actively avoid humans (Riley et al. 2003, Gosselink et al. 2007), show shifts in habitat preference and diet (reviewed in (Bateman and Fleming 2012), and changes in home range size (Gehrt et al. 2009). Anthropogenic disturbance may also shape variation in carnivore social tolerance, social structure (Boyd et al. 1995; Macdonald, Courtenay, Forbes, & Mathews, 1999), and personality (Greenberg and Holekamp 2017). Despite the potential for disturbance-induced maternal effects on the behavior of offspring, most studies of disturbance-related maternal effects in carnivores have focused on offspring survivorship. In several species, studies have shown that disturbance may influence maternal behavior and offspring development without negatively affecting offspring survival (Amstrup 1994, Engelhard et al. 2002, Stevens and Boness 2003, James and Myfanwy 2013).

Here we inquired whether anthropogenic activity shapes maternal behavior among freeliving spotted hyenas, and tested an hypothesis suggesting that disturbance-related maternal effects influence offspring behavior. Maternal effects have the potential to profoundly affect offspring fitness and behavior in this species because spotted hyena mothers show an unusually protracted period of maternal investment in comparison to most other mammalian carnivores (Watts et al. 2009). Spotted hyenas give birth to litters containing 1 or 2 cubs at isolated natal dens, and move cubs to a communal den when they are 3-4 weeks of age. There they reside for the next 7-14 months (Holekamp & Dloniak 2010), emerging from the den to nurse from their mothers and socialize with clan-mates (Holekamp & Smale, 1998). Mothers must significantly alter their space use to visit the communal den (Hofer and East 1993a, Boydston et al. 2003a), from which they effectively become central place foragers while their cubs reside there. Maternal presence at dens is critical for cubs' physical and social development. In the Serengeti, maternal attendance rates at dens are significantly positively correlated with cub growth rates, which in turn predict cub survivorship to reproductive maturity (Hofer and East 1993a). Additionally, it is at the communal den that cubs learn their dominance ranks. Spotted hyenas live in fission-fusion social groups, called clans, that are structured according to a matrilineal dominance hierarchy (Frank 1986a). Maternal presence at dens is critical for the process of rank acquisition, as mothers reinforce cub's appropriate aggressive behavior and intervene on behalf of their offspring (Holekamp and Smale 1993, Engh et al. 2000).

Spotted hyenas also show remarkable flexibility in the face of anthropogenic disturbance (Holekamp and Dloniak 2010). Where we conducted our study, in Kenya's Masai Mara National Reserve (MMNR), the human population along the borders of the reserve has grown exponentially since the late 1990s, resulting in increased numbers of livestock grazing both day and night inside the protected area (Green et al. in press). Direct conflict between hyenas and livestock is common (Kolowski and Holekamp, 2006), resulting in high rates of hyenas being speared, snared, and poisoned (Holekamp and Dloniak, 2010). Since 2005, humans have been responsible for the majority of hyena deaths for which mortality sources could be determined (Holekamp and Dloniak 2010). In addition to these lethal effects, increased human activity along the borders of the MMNR has also had indirect effects on the behavior of adult spotted hyenas. As human disturbance has increased, individuals have become more nocturnal, expanded their home ranges, and increased the distances they travel to hunt (Boydston et al. 2003b). Compared with hyenas living in pristine areas of the MMNR, hyenas in the disturbed area show a greater preference for protective vegetation (Kolowski and Holekamp 2009), are more vigilant (Pangle and Holekamp 2010), shift their activity patterns to avoid interaction with humans (Kolowski et al. 2007), travel greater distances in the mid-day heat (Ikime 2015), and
travel faster overall (Green 2015).

In a recent study, Greenberg and Holekamp (2017) found that behavioral differences between juvenile hyenas living in high- and low-disturbance areas emerge early in life, when cubs reside at communal dens, despite experiencing virtually no direct exposure to human disturbance at dens. Using field experiments, they found that juveniles in high- and lowdisturbance areas showed consistent differences in personality traits, specifically, neophobia, exploration, and boldness. When exposed to a novel object, juveniles in the high-disturbance area were significantly less neophobic and more exploratory than juveniles in the lowdisturbance area. When exposed to a box they could enter to feed on a reward, juveniles in the high-disturbance area were less bold than juveniles in the low-disturbance area. Greenberg and Holekamp proposed that such behavioral differences might be shaped indirectly, by differences in maternal behavior between high- and low-disturbance areas. For example, among Old World Monkeys, which show many similarities to spotted hyenas in their maternal investment patterns, life-histories, and social behavior, the offspring of mothers who are the most "rejecting" or "laissez-faire" in their mothering style tend to be least neophobic and most exploratory (Andrews and Rosenblum 1991, Fairbanks 1996). Moreover, female primates are less attentive and protective of their offspring in more challenging or unpredictable environments (Andrews and Rosenblum 1991). The degree to which mothers are "protective" versus "rejecting" has been assessed in numerous primate species by measuring how much time a mother spends close to offspring (in contact or within 1m) and how often a mother approaches and leaves her offspring from this close proximity range. However, in other taxanomic groups, such as rodents, it is mothers who are more protective, as measured by rates of grooming and the use of particular

nursing positions, that tend to have offspring who are less afraid to approach and explore new aspects of their environment (Caldji et al. 1998).

Despite the many effects of human disturbance previously documented on the behavior of spotted hyenas, Green et al. (2017) recently found that survivorship of juvenile hyenas was actually greater in a highly disturbed area than in low-disturbance areas with the Masai Mara National Reserve, Kenya, and as a result, their study clan in the disturbed area grew to be the largest on record at 130 individuals. They demonstrated that this surprising demographic pattern was likely due, at least in part, to a decline in lion densities in the high-disturbance area, presumably also due to anthropogenic activity, and an increase in the hyenas' depredation of livestock. However, it is also possible that differential maternal behavior in high- and lowdisturbance areas contributes to the success of juveniles in the disturbed habitat.

Previous studies using VHF telemetry and direct observation indicated that anthropogenic disturbance negatively affects maternal den attendance among spotted hyenas living in a highly disturbed area of the MMNR. As human disturbance has increased, adult females have been found significantly farther from the communal den (Boydston et al. 2003b), and they arrive at communal dens later in the evening and leave them earlier in the morning than in undisturbed areas (Kolowski et al. 2007). However, these studies were limited by the coarse scale of the spatial data and the difficulty of obtaining data during the night.

Here we inquired how maternal behavior is shaped by human disturbance and tested the hypothesis that differences in maternal behavior mediate the disturbance-related patterns in juvenile personality at this site. If this hypothesis is correct, then we expected to see unambiguous differences in maternal den attendance and behavior between high- and low-

95

disturbance areas. First, we compared maternal attendance rates at communal dens in high- and low- disturbance areas of the MMNR using spatial data from spotted hyena mothers fitted with GPS collars transmitting hourly locational data. We also examined the effects of offspring age, litter size, prey density, and social rank on maternal den attendance rates. We predicted that maternal den attendance would significantly decrease as cubs matured, and that mothers with litters containing two cubs would show higher den attendance rates than mothers with singletons. We also predicted that den attendance would be positively correlated with local prey density, as has been found in other populations (Hofer and East 1993a), because spotted hyenas travel significantly greater distances from the den when prey are scarce than when they are abundant (Boydston, Kapheim, & Szykman, 2003; Hofer & East, 1993; Kolowski & Holekamp, 2009). Previous research on space use at our study site has shown that, among females with dendwelling cubs, there is no rank-related variation in how far females are found from the communal den (Boydston et al. 2003a). However, we suspected that low-ranking mothers might have lower attendance rates than high-ranking mothers in areas affected by human disturbance because low-ranking mothers bear a higher cost of den visitation (White 2007); therefore, we also assessed the effects of maternal social rank on den attendance.

To compare how mothers in high- and low-disturbance areas interacted with offspring at communal dens, we conducted Focal Animal Surveys (FAS) on mother-offspring pairs. Specifically, we determined the proportion of a mother's FAS time when she was present at a den with her cubs that she spent nursing her cubs, in close proximity to her cubs, or grooming her cubs. We also determined to what extent mothers were responsible for maintaining close proximity with their cubs. In a previous study (Greenberg et al. Ch.4), we analyzed naturally occurring variation among spotted hyena mothers during a time period in which there was no human disturbance. We found that mothers significantly altered their participation in these activities based on cub age, litter size and time of day, but that social rank and prey density did not appear to shape these maternal behaviors.

Finally, because we found that human disturbance does affect some aspects of maternal behavior, we next inquired whether these maternal behaviors predict offspring survivorship and whether it might also help explain the differences that have been documented in juvenile survivorship in the high- and low- disturbance area. Greenberg et al. (chapter 4) previously found that variation in how much time a mother spent nursing, grooming, or in close proximity to offspring did not significantly predict either juvenile survivorship to reproductive maturity or longevity. We therefore focused our analysis on whether maternal den attendance significantly predicted offspring survivorship to reproductive maturity.

#### METHODS

## Subjects & Study Populations

Study subjects were spotted hyenas inhabiting two areas within the Mara-Serengeti ecosystem in southwestern Kenya. As described above, one area was undisturbed, whereas the other was highly disturbed by anthropogenic activity (Figure 5.1). Hyenas living in the high-disturbance area were members of the Talek West clan, which lives along the Northern border of the Masai Mara National Reserve, near the burgeoning town of Talek. This clan has been continuously monitored on a daily basis since 1988. Hyenas living in the low-disturbance area were members of three clans living in the pristine Mara Conservancy. The Mara Conservancy is managed by a

97



Figure 5.1. Map of the territories of the four study clans monitored in the Masai Mara National Reserve, Kenya. Study clans were Talek West (TW), Serena North (SN), Serena South (SS), and Happy Zebra (HZ). The three clan in the shaded area are located in the Mara Conservancy. Star indicates the town of Talek.

private non-profit organization that strictly prohibits cattle grazing or any human activity except wildlife viewing from tour vehicles or hot-air balloons. These clans have been monitored daily since 2008.

Spotted hyenas live in fission-fusion societies, each containing up to 130 individuals, and each structured according to a strict matrilineal hierarchy (Frank 1986). Females are dominant to males, which disperse from their natal clans at 2-5 years of age, and assume the lowest possible ranks in the new clans into which they immigrate (Holekamp and Smale 1998b, van Horn et al. 2003). The social rank of adult females was calculated here on a yearly basis based on outcomes of dyadic agonistic interactions used to construct an annual hierarchy for each clan (Martin & Bateson 1988; Smale et al. 1993). To account for variation in clan size, ranks were standardized from -1 to 1, with the most dominant individual having a standardized rank of 1.

Age was initially estimated when cubs were first observed, based on their appearance and size (Holekamp et al. 1996), and sex was determined based on the shape of the glans of the erect phallus (Frank et al. 1990). The reproductive state of adult females was continuously monitored to determine periods of pregnancy and lactation, as described by Holekamp et al (1996). The gestation period for spotted hyenas is 110 days (Kruuk, 1972). Conception dates were determined by back-calculating from a cub's date of birth, and based on observations of matings and fresh tears in females' phalluses, indicating recent parturition.

## Data Collection

### Maternal attendance at dens

GPS radio collars (Vectronic Aerospace, Berlin, Germany) were deployed in 2012 on 18 sexually mature adult females. Of these, 10 females were members of two clans in the low-disturbance area (5 from the Serena North clan and 5 from the Serena South clan), and 8 were members of the Talek West clan, located in the high-disturbance area. In the low-disturbance area, 7 of the collared females were high ranking and 3 were low ranking. In the high-disturbance area, 4 of the collared females were high ranking and 4 were low ranking. To fit females with collars, we anesthetized hyenas using Telazol (6.5 mg/kg) administered in a plastic dart fired from a CO<sub>2</sub> powered rifle (Telinject Inc., Saugus, California). Collars were programmed to record GPS locations hourly from 1600 h to 1000 h, and also once at 1300 h, for a total of 20 location fixes per 24 hour period.

For each female, we defined periods in which she had a litter residing at the communal den. We defined the start date for each period as the day on which the mother moved her litter to the communal den from the natal den. It was not always possible to confirm the mother's presence at a natal den in the field because mothers often used dens in areas that were difficult for our field team to access, and were often wary of humans during this period such that they sometimes left the den when they saw our research vehicle approach. There was also often a lag time in when we received GPS fixes due to spotty cell tower coverage. We identified the natal den used by each mother by inspecting her GPS locations and looking for high concentrations of points approximately one month prior to the cubs' date of birth (see Figure 5.2). Then, using a mother's GPS locations along with our field notes, we determined the date on which each mother brought her cub(s) to the active communal den.



Figure 5.2. Map of one female's GPS locations while she was at a natal den, showing a high concentration of points in close proximity to the natal den. Rings indicate 10m and 25m from the natal den. Approximately 50% of her total GPS points for the one-month period were within 10m of the natal den.

We defined the end date of the period during which a mother had den-dwelling cubs as the date on which her cubs were eight months of age, which is the average age at which cubs become independent of the den in the MMNR (Holekamp and Dloniak 2010). Not all 18 collared females had location fixes for the entire period during which their cubs lived at the communal den because collars were sometimes deployed when females already had den-dwelling litters, and some collars malfunctioned before the end of the cub's den-dwelling period. If the mother or cub died before the date on which cubs reached 8 months of age, we defined the end date as the day on which the mother or cub was last seen alive.

We determined which communal den was actively being used by each clan at any given time using archived behavioral records. Spotted hyenas may re-use dens within their territories, and on average, change communal dens monthly (Boydston et al. 2006). During our routine observation sessions, we typically visited the communal den being actively used by each clan twice per day. Dens here were defined as being actively used when cubs were observed entering and exiting the den entrance. If more than one den hole was being used within 200m, we characterized the entire area as a "den complex." In some cases, a clan was actively using two dens that were more than 200m apart, or were clearly in transition as mothers moved their cubs from one den to another. In these cases, we considered both dens to be actively used. If we were unable to identify the active communal den via direct observation in the field, we determined which den was being used by inspecting the GPS points of females with dendwelling cubs during that period. This was typically the case only for dens in areas that were impossible for us to access due to rocks or dense vegetation.

We used QGIS to create a 100m buffer around each communal den. We then calculated

the proportion of each female's total fixes that occurred within 100m of the active den during the time period defined by the above start and end dates. In the case of den complexes, we defined the mother as present at the den if her location fix was within 100m of any of the den holes included in the complex.

## Maternal behavior at dens

During our routine morning (0600-1000h) and evening (1600-2000h) observation periods, we conducted focal animal surveys (FAS) on mother-cub pairs. We initiated a FAS when a mother and cub were both present. We followed the pair for 30 minutes or until either the mother or cub had been out of site for five minutes. During a FAS, we recorded all minutes during which a mother was nursing, grooming, or in close proximity (within 1m or in contact) to the cub. Additionally, whenever the mother-cub pair moved in or out of close proximity, we recorded which individual initiated the change as an "event" (e.g. cub approach mother, mother approach cub, cub leave mother, or mother leave cub). FAS were conducted opportunistically, but we made every attempt to balance the dataset with regards to the mother's social rank, mother's parity, and litter size. Although we had no den attendance (GPS) data from primiparous mothers, we conducted FAS on primiparous mothers in addition to parous mothers. We also conducted FAS on mother-cubs pairs in three study clans in the low-disturbance area, rather than two. That is, in addition to collecting FAS data from the Serena North (SN) and Serena South (SS) clans, we also collected them from mother-infant pairs in the Happy Zebra (HZ) clan.

## Statistical analysis of maternal den attendance and behavior

We used the glmmADMB package in R (R version 3.3.1; Development Core Team, 2015) to model both variation in maternal attendance at dens and maternal behaviors during FAS. The glmmADMB package allows for the incorporation of both random effects and zero-inflated predictor variables. For maternal den attendance, we modeled the number of points during each 24 hour period when a mother was at the den while she had den-dependent cubs as our response measure, and her total number of points for that 24 hr period (a maximum of 20 fixes) as a logged-offset. We compared the fit of four types of count models; a negative binomial model, a poisson model, a zero-inflated negative binomial model, and a zero-inflated poisson model. The model with the lowest Akaike's Information Criterion corrected for small sample sizes (AICc) was chosen as our best fitting model. In each of these candidate models, we included the mother's ID as a random effect. As fixed effects, we included the cub's age as a continuous measure, the disturbance level (high or low), the mother's social rank as a continuous variable, the number of cubs the mother had alive at the den on that day (1 or 2), and prey density. Prey density was calculated as the average density of prey (including livestock) counted within 100m of multiple prey transects run bi-monthly within the territorial boundaries of our study clans, during each two-week interval in which maternal behavior data were collected. Prey density and cub age were centered and scaled for analyses.

We next asked whether disturbance mediated the relationship between other variables and den attendance. To do this, we tested for a significant two-way interaction between disturbance and each of our other variables of interest (litter size, maternal rank, and prey density) by performing a likelihood ratio test between our model without interactions and each of our models that included an interaction.

To model variation in maternal behavior, we calculated the total number of minutes during which mother and cub were both present in each FAS as well as the number of FAS minutes in which the mother was nursing her cub, in close proximity her cub, and grooming her cub. For the number of minutes a mother spent in close proximity to her cub, we excluded any minutes during which she was nursing the cub because she would necessarily be in contact with the cub while nursing. We then constructed a separate model for each of these three maternal behaviors, with the time spent engaged in the behavior as the response measure and the total time mother and cub were present together as a logged offset. In each model, we included the mother's ID as a random effect, and disturbance as a fixed effect (high-disturbance or lowdisturbance). We also included the following fixed effects: time of day (morning or evening), litter size (singleton or twins), cub sex, maternal rank, maternal parity (primiparous or multiparous), and prey density. We again compared the fit of count models with four different distributions (negative binomial, poisson, zero-inflated negative binomial, and zero-inflated poisson), and report the model with the lowest AICc score. We excluded from analysis any FAS in which the mother and cub were present together for less than 5 min, and only included FAS in which the cub was less than 9 months old, the average age at which cubs begin to spend the majority of their time away from the den in our study area.

We used our data on whether the mother or cub initiated changes in proximity to model the extent to which mothers were responsible for maintaining proximity with their cubs. To model how often mothers approached their offspring, we limited our analysis to sessions in which either the mother or the cub had approached the other at least once during the FAS. We

104

used the number of times a mother approached her cub in the FAS as our response measure and included the total number of approaches by either mother or cub in the FAS as a predictor variable. To determine the extent to which mothers left their cubs, we limited our analysis to sessions in which the mother and cub left one another at least once, used the number of times a mother left her cub within a FAS as our response measure, and included the total count of times the mother or cub left one another as a predictor variable. For each of our maternal behaviors, we again asked whether disturbance mediated the relationship between the behavior and our other variables. To do this, we tested for a significant two-way interaction between disturbance and each of our other variables of interest (litter size, maternal rank, cub sex, maternal parity, and prey density) by performing a likelihood ratio test between our model without interactions and each of our models that included an interaction. We included mother ID as a random effect and cub age and time of day as fixed effects in each model.

glmmADMB is a relatively new R package and model diagnostic methods are still being developed. We therefore focused our evaluation of model fit on the residual deviance. We also evaluated overdispersion using the theta values provided in model outputs. We used Variance Inflation Factors (VIF) to assess multi-colinearity of predictor variables in all of our models. No variable had a VIF above 3, indicating that there was no significant multi-colinearity among variables in our models.

# Maternal den attendance and offspring survivorship

We used our demographic records to determine whether the offspring of mothers fitted with GPS collars survived to reproductive maturity, which occurs at 24 months of age in spotted hyenas. All youngsters could have reached this age by the time our analysis was conducted.\_For each juvenile, we calculated the proportion of its mothers' location fixes that were at the den when the cub was 30-120 days old, or roughly the first three months in which a cub resided at the communal den. We excluded from analysis any juveniles whose mothers' collars did not transmit at least 10 location fixes during each of the three months.

#### RESULTS

#### Maternal attendance at dens

Preliminary analysis showed that, within the low disturbance area, hyenas in the two clans did not show significantly different den attendance rates (zero inflated negative binomial model, B=0.070, SE=0.187, P=0.707). Therefore, we did not include clan as a factor in subsequent analyses of spatial data.

Our analysis included a total of 40,317 points, with an average of 14.39 points per female per day. The dataset included an average of 4.94 months of data from each mother per litter (range 0.57-8.26 months). A zero-inflated negative binomial distribution best fit our den attendance data (Table 5.1). Den attendance was significantly higher among mothers in lowthan high-disturbance areas (P = 0.002; Figure 5.3). Den attendance significantly decreased as cubs matured (P < 0.001; Figure 5.3), high-ranking mothers had significantly lower den attendance than low-ranking mothers (P = 0.024), and we also found a significant positive association between maternal attendance and prey density (P < 0.001). There was a trend for mothers to show higher den attendance when they had twins than singletons (P = 0.057).

We observed a marginally significant interaction between disturbance and litter size  $\binom{2}{1}$ 

		т ·	0.1 E	D
	Odds Katio	Estimate	Std. Error	P
(Intercept)	0.351	-1.048	0.103	< 0.001
Cub Age	0.765	-0.267	0.019	<0.001**
Disturbance	0.625	-0.470	0.151	0.002**
Social Rank	0.804	-0.218	0.096	0.024**
Prey Density	1.077	0.074	0.013	<0.001**
Litter Size	1.096	0.092	0.048	0.057 *

Table 5.1. Results of zero-inflated negative binomial model explaining variation in maternal den attendance. \*\* indicates P < 0.05. \* indicates P < 0.1.



Figure 5.3. Plot showing mean maternal den attendance as cubs mature in low- and highdisturbance areas. Data points represent monthly means of mothers' daily den attendance (the proportion of mothers' total points that were within 100m of the communal den).

= 2.96, P = 0.085). Looking at mothers of singletons versus twins separately indicated that the magnitude of the disturbance effect was greater among mothers of singletons (odds ratio = 0.722, B = -0.326, SE = 0.139, P = 0.019) than of twin litters (odds ratio = 0.536, B = -0.623, SE = 0.212, P = 0.003). A likelihood ratio test indicated a significant interaction between disturbance and prey density (<sup>2</sup>(1) = 34.72, P < 0.001). However, when we removed data points from one out-lying two-week interval with high prey densities in one clan's territory, this effect was no longer significant (<sup>2</sup>(1) = 2.68, = 0.102). The interaction between disturbance and rank was not significant (<sup>2</sup>(1) = 1.3, P = 0.254).

### Maternal behavior at dens

Our analysis included a total of 333 FAS collected on 43 mothers (24 from low disturbance areas; 19 from the high disturbance area) and 62 cubs (33 from low disturbance areas; 29 from the high disturbance area). An average of 5.32 FAS (range 1-16) were conducted on each mother-offspring pair. Preliminary analysis showed that, within the undisturbed area, hyenas in the three study clans did not show significantly different rates of nursing, grooming, or being in close proximity to offspring (Table 5.2). Therefore, we did not include clan as a factor in subsequent analyses.

A zero-inflated negative binomial model best explained variation in nursing among mothers (Table 5.3a). Mothers in the high-disturbance area spent significantly more of their time during FAS nursing their cubs than did mothers in the low-disturbance areaa (P < 0.001; Figure 5.4a). Mothers also nursed their cub significantly more as the cub matured (P < 0.001), and significantly less as prey density increased (P < 0.029). There were non-significant trends



Figure 5.4. Plot showing mean proportion of time mothers spent a) nursing and b) in close proximity to cubs as cubs mature in low- and high-disturbance areas. Data points represent monthly means of mothers' behavior.

	Estimate	Std. Error	Р
Nursing			
North - Happy Zebra	-0.02	0.15	0.98
South - Happy Zebra	-0.08	0.20	0.91
North - South	-0.06	0.25	0.97
Close Proximity			
North - Happy Zebra	-0.12	0.29	0.91
South - Happy Zebra	0.15	0.37	0.91
North - South	0.27	0.48	0.84
Grooming			
North - Happy Zebra	-0.30	0.57	0.85
South - Happy Zebra	-0.06	0.71	1.00
North - South	0.25	0.94	0.96

Table 5.2. Tukey pairwise comparisons of rates of nursing, grooming, and close proximity among mothers living in the three clans in the undisturbed area.

for high-ranking mothers to spend more time nursing cubs than did low-ranking mothers (P < 0.060), and mothers of female cubs to spend more time nursing than did mothers of male cubs (P < 0.071). Likelihood ratio tests comparing models for nursing with and without interaction terms indicated a significant interaction between disturbance and maternal rank ( $^2(1) = 5.2$ , P = 0.023). Looking at high-ranking mothers and low-ranking mothers separately indicated that, although mothers of all ranks spent more FAS time nursing in high- than low-disturbance areas, the magnitude of the disturbance effect was larger among low-ranking mothers (odds ratio = 1.36, B = 0.306, SE = 0.077, P < 0.001). All other interactions were non-significant (disturbance X cub sex ( $^2(1) = 0.52$ , P = 0.471; disturbance X litter size ( $^2(1) = 0.4$ , P = 0.527; disturbance X litter size (( $^2(1) = 0.4$ ).

	Odds Ratio	Estimate	Std. Error	Р
A. Nursing				
(Intercept)	0.679	-0.39	0.07	< 0.001
Cub age	1.136	0.13	0.03	<0.001**
Time of day	1.053	0.05	0.05	0.340
Disturbance	1.278	0.25	0.05	<0.001**
Litter Size	0.993	-0.01	0.05	0.895
Cub sex	0.909	-0.10	0.05	0.071*
Maternal rank	0.926	-0.08	0.04	0.060*
Maternal parity	1.102	0.10	0.06	0.116
Prey density	1.000	0.00	0.00	0.029**
B. Close Proximity				
(Intercept)	0.255	-1.37	0.21	< 0.001
Cub age	0.687	-0.38	0.07	<0.001**
Time of day	0.775	-0.26	0.13	0.055 *
Disturbance	0.554	-0.59	0.15	<0.001**
Litter Size	0.855	-0.16	0.16	0.331
Cub sex	1.282	0.25	0.17	0.133
Maternal rank	1.017	0.02	0.12	0.886
Maternal parity	1.169	0.16	0.19	0.418
Prey density	1.000	0.00	0.00	0.991
C. Grooming				
(Intercept)	0.087	-2.44	0.40	< 0.001
Cub age	0.600	-0.51	0.12	<0.001**
Time of day	1.226	0.20	0.25	0.419
Disturbance	1.243	0.22	0.34	0.520
Litter Size	0.379	-0.97	0.34	0.004**
Cub sex	0.596	-0.52	0.34	0.131
Maternal rank	0.653	-0.43	0.25	0.094*
Maternal parity	0.699	-0.36	0.43	0.404
Prev density	1.000	0.00	0.00	0.152

Table 5.3. Results of models explaining variation in how much FAS time mothers spent: a) nursing cubs (zero-inflated negative binomial model), b) in close proximity to cubs (negative binomial model), and c) grooming cubs (negative binomial model). \*\* indicates P < 0.05. \* indicates P < 0.1.

= 0.4, P = 0.527; disturbance X maternal parity (<sup>2</sup>(1) = 0.12, P = 0.729; disturbance X prey density (<sup>2</sup>(1) = 0.06, P = 0.807).

A negative binomial model best explained variation in how often mothers were in close proximity to their cub (s; Table 5.3b). Mothers in the high-disturbance area spent significantly less FAS time in close proximity than did mothers in the low-disturbance area (P < 0.001; Figure 5.4b). Mothers also spent significantly less time in close proximity to cubs as they matured (P < 0.001), and mothers tended to spend more time in close proximity to cubs in the mornings than in the evenings (P < 0.055).

Likelihood ratio tests comparing models for FAS time spent in close proximity to cubs with and without interaction terms indicated a significant interaction between disturbance and maternal parity ( $^{2}(1) = 4.77$ , P = 0.029). Looking at primiparous and multiparous mothers separately indicated that the magnitude of the disturbance effect was greater among multiparous mothers (odds ratio = 0.66, B = -0.41, SE = 0.15, P = 0.006) than primiparous mothers (odds ratio = 0.29, B = -1.25, SE = 0.43, P = 0.004). There was also a marginally significant interaction between disturbance and maternal rank ( $^{2}(1) = 3.13$ , P = 0.077). Looking at highranking and low-ranking mothers separately indicated that the disturbance effect was significant among high-ranking mothers (odds ratio = 0.79, B = -0.65, SE = 0.20, P = 0.001), but not lowranking mothers (odds ratio = 0.52, B = -0.24, SE = 0.29, P = 0.406). All other interactions were non-significant (disturbance X prey density ( $^{2}(1) = 0.50$ , P = 0.481; disturbance X cub sex  $^{2}$ (1) = 0.14, P = 0.710; disturbance X litter size ( $^{2}(1) = -0.22$ , P = 0.99).

A negative binomial model best explained variation in grooming among mothers (Table 5.3c). There was no significant difference between high- and low-disturbance areas with respect

to how much FAS time mothers spent grooming their cubs (P = 0.520). Mothers spent significantly less time grooming cubs as they matured (P < 0.001), and significantly more time grooming singletons than cubs in twin litters (P = 0.004). There was a non-significant trend for low-ranking mothers to spend more time grooming cubs than high-ranking mothers (P = 0.094). Likelihood ratio tests indicated that no interactions with disturbance were significant (disturbance X prey density <sup>2</sup>(1) = 1.52, P = 0.218; disturbance X maternal parity <sup>2</sup>(1) = 0.69, P= 0.407; disturbance X maternal rank <sup>2</sup>(1) = 0.62, P = 0.432; disturbance X litter size <sup>2</sup>(1) = 0.49, P = 0.499; disturbance X cub sex <sup>2</sup>(1) = 0.10, P = 0.747).

A zero-inflated negative binomial model based on 160 FAS best explained variation in how often a mother approached her cub. Only the total number of approaches in the survey significantly predicted how often mothers approached cubs (Table 5.4). A poisson model that included 156 surveys best explained variation in how often a mother left her cub (Table 5.5). Mothers in the high-disturbance area left their cubs significantly more often than did mothers in the low-disturbance area (P < 0.001), and mothers of females showed higher rates of leaving their cubs than did mothers of males (P = 0.053). Due to the small size of these datasets, we did not test for significant interactions between disturbance and our other predictor variables.

## Maternal den attendance and offspring survivorship

Twenty-one individuals met our criteria for inclusion in the analysis. Of these 21 individuals, 4 did not survive to reproductive maturity (3 in the high-disturbance area and 1 in the low-disturbance area). Maternal attendance at dens did not significantly predict offspring survivorship (B = 9.43, SE = 7.46, P = 0.206).

	Odds Ratio	Estimate	Std. Error	Р
(Intercept)	0.018	-4.05	0.47	< 0.001
Total number of				
approaches	1.132	0.12	0.04	<0.001**
Cub age	1.252	0.23	0.15	0.146
Time of day	0.995	-0.01	0.23	0.984
Disturbance	1.296	0.26	0.31	0.404
Litter size	1.183	0.17	0.31	0.585
Cub sex	0.985	-0.02	0.32	0.961
Maternal rank	1.099	0.09	0.23	0.683
Maternal parity	1.032	0.03	0.47	0.947
Prey density	1.165	0.15	0.13	0.231

Table 5.4. Results of a zero-inflated negative binomial model explaining variation in how often mothers approached their cubs to within 1m. \*\* indicates P < 0.05. \* indicates P < 0.1.

	Odds Ratio	Estimate	Std. Error	Р
(Intercept)	0.024	-3.74	0.30	< 0.001
Total number of				
departures	1.086	0.08	0.09	0.003**
Cub age	1.559	0.44	0.10	<0.001**
Time of day	1.155	0.14	0.17	0.398
Disturbance	2.978	1.09	0.22	<0.001**
Litter size	0.879	-0.13	0.23	0.568
Cub sex	0.673	-0.40	0.21	0.053**
Maternal rank	0.783	-0.25	0.17	0.143
Maternal parity	1.003	0.003	0.28	0.991
Prey density	0.893	-0.11	0.10	0.272

Table 5.5. Results of a poisson model explaining variation in how often mothers left their cubs. \*\* indicates P < 0.05. \* indicates P < 0.1.

### DISCUSSION

Our study demonstrates that, although spotted hyenas show a remarkable ability to persist in areas with human-wildlife conflict and disturbance, such conditions present a challenge to mothers, who show different patterns of den attendance and maternal care in high and low disturbance areas. When den attendance rates were averaged over the time period during which cubs resided at communal dens, we found that mothers in high-disturbance areas had den attendance rates 12% lower than mothers in low-disturbance areas. Previous studies have demonstrated that hyenas living in disturbed areas have larger home ranges and travel farther to hunt than hyenas in undisturbed areas (Boydston et al. 2003b). Although this behavioral change may allow hyenas to effectively hunt while avoiding confrontation with humans, our results likely demonstrate a cost to this strategy. In the high-disturbance area, mothers spent a greater proportion of their time at the den nursing their cubs than did mothers in the low-disturbance areas. Mothers in the high-disturbance area also showed differences in the ways in which they interacted with their cubs, spending less time close to their cubs and more often leaving their cubs than mothers in the low-disturbance area. Although our maternal den attendance data were collected throughout the 24-hr cycle, we could not conduct FAS in total darkness. Therefore, we cannot rule out the possibility that anthropogenic disturbance affected the circadian pattern of when mothers engage in the behaviors we studied rather than the overall amount of time that mothers spent engaging in these activities.

Taken together, our findings are consistent with the hypothesis that maternal behavior is mediating the disturbance-related differences in juvenile personality documented by (Greenberg and Holekamp 2017). Here, we found that, in the area where spotted hyena cubs are less

115

neophobic and more exploratory, mothers also show low rates of attendance at dens, and, when present, spend less time close to offspring and leave their cubs at higher rates. In other species, such a pattern of maternal care has sometimes been interpreted as a "low investment" maternal strategy (Hauser and Fairbanks 1988, Champagne 2011, Fairbanks and Hinde 2012). In the lowdisturbance area on the other hand, mothers are able to invest more, showing higher rates of den attendance and time spent near cubs, and as predicted, cubs in this area are more neophobic and less exploratory than cubs in the high-disturbance area.

A recent study documenting a trade-off in the quality and quantity of spotted hyena milk may shed light on our results regarding effects of disturbance and rank on maternal behavior. We found that mothers in high-disturbance areas, who otherwise show low investment in offspring, spent a greater proportion of their time nursing than mothers in the low-disturbance area. This may allow mothers in high-disturbance areas to compensate for their lower den attendance rates and is consistent with the finding that spotted hyena mothers in the Serengeti who visit the den less often also engage in longer nursing bouts (Hofer et al. 2016). However, a mother's time spent nursing does not take into account variation in milk quality and can be a surprisingly poor indicator of actual milk intake by offspring (Cameron 1998). Hofer et al. (2016) analyzed the nutritional content of spotted hyena milk and weighed cubs to measure milk intake. They found a trade-off between milk quality and quantity; mothers transferred high quantities of low-quality milk or small quantities of high-quality milk. Therefore, it would be interesting to determine whether the consumption of more livestock by hyenas in the highdisturbance (Green et al. 2017) affects the nutritional quality of hyena milk. If consumption of livestock causes the milk of mothers in the high-disturbance area to be of lower quality than the

milk of mothers in the low-disturbance area, this might explain our finding that mothers in the high-disturbance area spent a greater proportion of their time nursing than did mothers in the low-disturbance area.

Examining variation in milk quality as it relates to a mother's social rank could also help to shed light on our surprising finding that high-ranking mothers showed lower den attendance rates than low-ranking mothers. We predict that high-ranking females, who have priority access to food, would have more nutritious milk than low-ranking females, who are typically in poorer condition. If this is the case, low-ranking mothers may provide their offspring with a relatively large volume of low-quality milk during frequent visits to the den whereas high-ranking mothers may provide offspring with a lower volume of high-quality milk at less frequent visits to the den.

Our findings also demonstrate that anthropogenic disturbance may interact with effects of other socio-ecological variables on maternal behavior. High-ranking and more experienced mothers appear better able to adopt flexible maternal strategies in response to disturbance than mothers who are low-ranking or inexperienced, and typically also in poorer condition. Whereas high-ranking mothers spent significantly less time close to cubs in high- than low-disturbance areas, this measure did not differ for low-ranking mothers between low- and high-disturbance areas. One interpretation of this finding is that high-ranking mothers can afford to be less protective of their offspring in high-disturbance areas, whereas low-ranking mothers, who experience a more threatening social environment, cannot. Similarly, whereas all mothers in high-disturbance areas spent less time close to cubs than did mothers in low-disturbance, the magnitude of this disturbance effect was greater among multiparous mothers than among primiparous mothers. Again, it might be that maintaining close proximity with offspring is

117

particularly important for inexperienced mothers, who cannot alter their behavior as flexibly in response to disturbance. Although we do not know whether these maternal behaviors take time away from other important activities, our findings suggest that disturbance may disproportionately affect certain individuals who are less able to flexibly alter their maternal behavior.

Whereas our results support the hypothesis that maternal behavior is mediating differences in juvenile personality, they do not support the hypothesis that these differences are driving Green et al.'s (2017) finding that juvenile survivorship is higher in the high-disturbance area than in the low-disturbance area. First, den attendance and the maternal behaviors we monitored (Chapter 1) were not significantly correlated with offspring survival. Second, mothers in high-disturbance areas showed lower, not higher, rates of den attendance and time spent close to cubs than mothers in low-disturbance areas. Therefore an increase in maternal investment cannot explain higher survivorship in the high- than low-disturbance areas. This result provides support for a recent hypothesis suggesting that spotted hyenas are able to persist in areas of high anthropogenic disturbance as long as competition with lions is reduced and prey densities are high (M'soka et al. 2016). That spotted hyena mothers are able to maintain high cub survivorship in disturbed areas even with significantly reduced investment in cubs suggests that these ecological factors alone may predict which areas are habitable for spotted hyenas in landscapes increasingly affected by human activity.

Our study raises important questions regarding the role of maternal effects in shaping population responses to anthropogenic disturbance and the consequences of these maternal effects for mothers and offspring living in disturbed areas. Numerous researchers have hypothesized that a low investment maternal strategy, such as the strategy that appears to be adopted by spotted hyena mothers in high disturbance areas, could provide a signal to offspring of a challenging or unpredictable adult environment, leading offspring to develop traits that will be adaptive in these environments, such as a fast life-history and enhanced risk-taking behavior. However, it remains controversial how often maternal effects function as adaptive signals of environmental quality, allowing offspring to optimize their fitness, and some researchers have argued that selection more often acts on maternal effects to maximize a female's fitness across her lifespan (Marshall and Uller 2007). Our results indicate that the maternal behaviors we studied are not associated with offspring survivorship. Similarly, Greenberg and Holekamp (2017) did not find evidence that the juvenile personality traits observed in the high-disturbance area conferred any type of survivorship benefit. Future research should assess whether there are longer-term impacts of these disturbance-related changes in maternal and offspring behavior on offspring longevity, reproductive success, or life-history. However, it is also possible that any potential effects of disturbance-related changes in maternal behavior on offspring are masked by the effects of other changes in the area, such as the decline in lion densities, and increased depredation of livestock by hyenas.

REFERENCES

#### REFERENCES

- Abbott, D. H., E. B. Keverne, F. B. Bercovitch, C. A. Shively, S. P. Mendoza, W. Saltzman, C. T. Snowdon, T. E. Ziegler, M. Banjevic, J. Garland T, and R. M. Sapolsky. 2003. Are subordinates always stressed? a comparative analysis of rank differences in cortisol levels among primates. Hormones and Behavior 43:67–82.
- Almasi, B., P. Béziers, A. Roulin, and L. Jenni. 2015. Agricultural land use and human presence around breeding sites increase stress-hormone levels and decrease body mass in barn owl nestlings:89–101.
- Altmann, J. 1980. Baboons Mothers and Infants. The University of Chicago Press, Chicago.
- Amstrup, S. C. 1994. Human Disturbances of Denning Polar Bears in Alaska 46:246-250.
- An, Y. S., B. Kriengwatana, A. E. Newman, E. A. MacDougall-Shackleton, and S. A. MacDougall-Shackleton. 2011. Social rank, neophobia and observational learning in blackcapped chickadees. Behaviour 148:55–69.
- Anderson, J. L. 1981. The re-establishment and management of a lion (Panthera leo) population in Zululand, South Africa. Biological Conservation 19:107–117.
- Andrews, M. W., and L. A. Rosenblum. 1991. Attachment in monkey infants raised in variableand low-demand environments. Child Development 62:686–693.
- Andrews, M. W., and L. A. Rosenblum. 2010. The Development of Affiliative and Agonistic Social Patterns in Differentially Reared Monkeys. Child Development:1–8.
- Archard, G. A., and V. A. Braithwaite. 2010. The importance of wild populations in studies of animal temperament. Journal of Zoology 281:149–160.
- Arlet, M. E., L. Veromann, R. Mand, and A. Lemasson. 2016. Call Rates of Mothers Change With Maternal Experience and With Infant Characteristics in Free-Ranging Gray-Cheeked Mangabeys. American journal of primatology 991:983–991.
- Arroyo, B., and M. Razin. 2005. Effect of human activities on bearded vulture behaviour and breeding success in the French Pyrenees 8:0–8.
- Bahr, N. I., C. R. Pryce, M. Döbeli, and R. D. Martin. 1998. Evidence from urinary cortisol that maternal behavior is related to stress in gorillas. Physiology & Behavior 64:429–437.
- Baker, P. J., L. Boitani, S. Harris, G. Saunders, and P. C. L. White. 2008. Terrestrial carnivores and human food production: impact and management. Mammal Review 38:123–166.

- Bardi, M., and M. A. Huffman. 2002. Effects of Maternal Style on Infant Behavior in Japanese Macaques (Macaca fuscata). Developmental Psychobiology 41:364–372.
- Barker, D. J. P., J. G. Eriksson, T. Forsén, and C. Osmond. 2002. Fetal origins of adult disease: strength of effects and biological basis. International Journal of Epidemiology 31:1235– 1239.
- Barnett, S. A. 1958. Experiments on "neophobia" in wild and laboratory rats. British Journal of Psychology 49:195–201.
- Barr, D.J., Levy, R., Scheeperes, C., Tily, H. J. 2014. Random effects structure for confirmatory hypothesis testing: Keep it maximal. Journal of Memory and Language 68:1–43.
- Bateman, P. W., and P. A. Fleming. 2012. Big city life: carnivores in urban environments. Journal of Zoology 287:1–23.
- Bateson, P., and P. D. Gluckman. 2011. Plasticity, Robustness, Development and Evolution. Cambridge University Press, New York, NY.
- Baugh, A. T., S. V Schaper, M. Hau, J. F. Cockrem, and P. De Goede. 2012. Corticosterone responses differ between lines of great tits (Parus major) selected for divergent personalities. General and Comparative Endocrinology 175:488–494.
- Beehner, J., and C. McCann. 2008. Seasonal and altitudinal effects on glucocorticoid metabolites in a wild primate (Theropithecus gelada). Physiology & Behavior 95:508–514.
- Beery, A. K., and D. D. Francis. 2011. Adaptive significance of natural variations in maternal care in rats: A translational perspective. Neuroscience & Biobehavioral Reviews 35:1552– 1561.
- Bell, A. M., and J. A. Stamps. 2004. Development of behavioural differences between individuals and populations of sticklebacks, Gasterosteus aculeatus. Animal Behaviour 68:1339–1348.
- Benhaiem, S., H. Hofer, M. Dehnhard, J. Helms, and M. L. East. 2013. Sibling competition and hunger increase allostatic load in spotted hyaenas. Biology Letters 9:20130040.
- Benhaiem, S., H. Hofer, S. Kramer-Schadt, E. Brunner, and M. L. East. 2012. Sibling rivalry: training effects, emergence of dominance and incomplete control. Proceedings of the Royal Society B: Biological Sciences 279:3727–3735.
- Benson-Amram, S., and K. E. Holekamp. 2012. Innovative problem solving by wild spotted hyenas. Proceedings of the Royal Society B: Biological Sciences 279:4087–4095.
- Bentley-Condit, V. K. 2003. Sex Differences in Captive Olive Baboon Behavior During the First Fourteen Days of Life. International Journal of Primatology 24:1093–1112.

- Bergh, V. Den, B. R. H. Van Den Bergh, and A. Marcoen. 2004. High antenatal maternal anxiety is related to ADHD symptoms, externalizing problems, and anxiety in 8- and 9-year-olds High Antenatal Maternal Anxiety Is Related to ADHD Symptoms, Externalizing Problems, and Anxiety in 8- and 9-Year-Olds. Child Development 75:1085–1097.
- Bergmüller, R., and M. Taborsky. 2010. Animal personality due to social niche specialisation. Trends in Ecology & Evolution 25:504–511.
- Berman, C. M. 1990. Consistency in Maternal-Behavior Within Families of Free-Ranging Rhesus-Monkeys - an Extension of the Concept of Maternal Style. American Journal of Primatology 22:159–169.
- Bernardo, J. 1996. Maternal Effects in Animal Ecology. American Zoologist 36:83-105.
- Biondi, L. M., M. S. Bó, and A. I. Vassallo, 2010. Inter-individual and age differences in exploration, neophobia and problem-solving ability in a Neotropical raptor (Milvago chimango). Animal Cognition 13:701–710.
- Biro, P. A., and D. J. Booth. 2009. Extreme boldness precedes starvation mortality in six-lined trumpeter (Pelates sexlineatus ). Hydrobiologia 635:395–398.
- Blehar, M. C., A. F. Lieberman, and M. D. S. Ainsworth. 1977. Early Face-to-Face Interaction and Its Relation to Later Infant-Mother Attachment. Child Development 48:182–194.
- Blumstein, D. T., E. Fernández-Juricic, P. A. Zollner, and S. C. Garity. 2005. Inter-specific variation in avian responses to human disturbance. Journal of Applied Ecology 42:943–953.
- Bonier, F., P. R. Martin, and I. T. Moore. 2009a. Do baseline glucocorticoids predict fitness? Trends in Ecology & Evolution 24:634–642.
- Bonier, F., I. T. Moore, P. R. Martin, and R. J. Robertson. 2009b. The relationship between fitness and baseline glucocorticoids in a passerine bird. General and Comparative Endocrinology 163:208–213.
- Boydston, E. E., K. M. Kapheim, and K. E. Holekamp. 2006. Patterns of den occupation by the spotted hyaena (Crocuta crocuta). Journal of African Ecology 44:77–86.
- Boydston, E., K. Kapheim, and M. Szykman. 2003a. Individual variation in space use by female spotted hyenas. Journal of Mammalogy 84:1006–1018.
- Boydston, E., K. Kapheim, H. Watts, M. Szykman, and K. E. Holekamp. 2003b. Altered behaviour in spotted hyenas associated with increased human activity. Animal Conservation 6:207–219.
- Breuner, C. W., B. Delehanty, and R. Boonstra. 2012. Evaluating stress in natural populations of

vertebrates: total CORT is not good enough. Functional Ecology 27:24-36.

- Brown, C., and K. N. Laland. 2002. Social learning of a novel avoidance task in the guppy: conformity and social release. Animal Behaviour 64:41–47.
- Budaev, S. V. 1997. The statistical analysis of behavioural latency measures. International Society for Comparative Psychology Newsletter 14:1–4.
- Burkanov, V., E. Gurarie, A. Altukhov, E. Mamaev, P. Permyakov, A. Trukhin, J. Waite, and T. Gelatt. 2011. Environmental and biological factors influencing maternal attendance patterns of Steller sea lions (Eumetopias jubatus) in Russia. Journal of Mammalogy 92:352–366.
- 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.
- Cadieu, J. C., N. Cadieu, and J. Lauga. 1995. Local enhancement and seed choice in the juvenile canary , Serinus canarius. Animal Behaviour 50:793–800.
- Caldji, C., B. Tannenbaum, S. Sharma, D. Francis, P. M. Plotsky, and M. J. Meaney. 1998. Maternal care during infancy regulates the development of neural systems mediating the expression of fearfulness in the rat. Proceedings of the National Academy of Sciences of the United States of America 95:5335–5340.
- Cameron, E. Z. 1998. Is suckling behaviour a useful predictor of milk intake? A review. Animal Behaviour 56:521–532.
- Cameron, N., A. Del Corpo, J. Diorio, K. McAllister, S. Sharma, and M. J. Meaney. 2008a. Maternal Programming of Sexual Behavior and Hypothalamic-Pituitary-Gonadal Function in the Female Rat. PLoS ONE 3:e2210.
- Cameron, N. M., D. Shahrokh, A. Del Corpo, S. K. Dhir, M. Szyf, F. A. Champagne, and M. J. Meaney. 2008b. Epigenetic Programming of Phenotypic Variations in Reproductive Strategies in the Rat Through Maternal Care. Journal of Neuroendocrinology 20:795–801.
- Carere, C., T. G. G. Groothuis, E. Möstl, S. Daan, and J. M. Koolhaas. 2003. Fecal corticosteroids in a territorial bird selected for different personalities: daily rhythm and the response to social stress. Hormones and Behavior 43:540–548.
- Caro, T. 1994. Cheetahs of the Serengeti Plains: Group living in an asocial species. University of Chicago Press, Chicago.
- Caro, T. 1999. The behaviour-conservation interface. Trends in Ecology & Evolution. 14:366-369.

- Cavigelli, S. A., and M. K. McClintock. 2003. Fear of novelty in infant rats predicts adult corticosterone dynamics and an early death. Proceedings of the National Academy of Sciences 100:16131–16136.
- Champagne, F. A. 2011. Maternal imprints and the origins of variation. Hormones and Behavior 60:4–11.
- Chapman, B. B., L. J. Morrell, J. Krause, C. Biology, and I. Fisheries. 2010. Unpredictability in food supply during early life influences boldness in fish. Behavioral Ecology 21:501–506.
- Ciucci, P., L. Boitani, F. Francisci, and G. Andreoli. 1997. Home range, activity and movements of a wolf pack in central Italy. Journal of Zoology 243:803–819.
- Clutton-Brock, T. H. 1991. The evolution of parental care. Princeton University Press, Princeton, N.J.
- Clutton-Brock, T. H., S. D. Albon, and F. E. Guinness. 1981. Parental investment in male and female offspring in polygynous mammals. Nature 289:487–489.
- Cooper, S. M., K. E. Holekamp, and L. Smale. 1999. A seasonal feast: long-term analysis of feeding behaviour in the spotted hyaena (Crocuta crocuta). African Journal of Ecology 37:149–160.
- Coplan, J. D., E. L. Smith, M. Altemus, B. A. Scharf, M. J. Owens, C. B. Nemeroff, J. M. Gorman, and L. A. Rosenblum. 2001. Variable foraging demand rearing: sustained elevations in cisternal cerebrospinal fluid corticotropin-releasing factor concentrations in adult primates. Biological psychiatry 50:200–204.
- Coplan, J. R., M. W. Andrews, L. A. Rosenblum, M. . Owens, S. Friedman, J. M. Gorman, and C. B. Nemeroff. 1996. Persistent elevations of cerebrospinal fluid concentrations of corticotropin-releasing factor in adult nonhuman primates exposed to early-life stressors: Implications for the pathophysiology of mood and anxiety disorders. PNAS 93:1619–1623.
- Creel, S. 2001. Social dominance and stress hormones. Trends in Ecology & Evolution 16:491–497.
- Crespi, E. J., T. D. Williams, T. S. Jessop, and B. Delehanty. 2012. Life history and the ecology of stress: how do glucocorticoid hormones influence life-history variation in animals? Functional Ecology 27:93–106.
- Crino, O. L., and C. W. Breuner. 2015. Developmental stress: evidence for positive phenotypic and fitness effects in birds. Journal of Ornithology:1–10.
- Cyr, N. E., and L. Michael Romero. 2007. Chronic stress in free-living European starlings reduces corticosterone concentrations and reproductive success. General and Comparative

Endocrinology 151:82-89.

- Cyr, N. E., and L. M. Romero. 2009. Identifying hormonal habituation in field studies of stress. General and Comparative Endocrinology 161:295–303.
- Dall, S. R. X., A. I. Houston, and J. M. McNamara. 2004. The behavioural ecology of personality: consistent individual differences from an adaptive perspective. Ecology Letters 7:734–739.
- Dantzer, B., Q. E. Fletcher, R. Boonstra, and M. J. Sheriff. 2014. Measures of physiological stress: a transparent or opaque window into the status, management and conservation of species? Conservation Physiology 2:1–18.
- Dantzer, B., A. G. Mcadam, R. Palme, M. M. Humphries, S. Boutin, and R. Boonstra. 2011. Maternal androgens and behaviour in free-ranging North American red squirrels. Animal Behaviour 81:469–479.
- Dantzer, B., A. E. M. Newman, R. Boonstra, R. Palme, S. Boutin, M. M. Humphries, and A. G. McAdam. 2013. Density Triggers Maternal Hormones That Increase Adaptive Offspring Growth in a Wild Mammal. Science 340:1215–1217.
- Darrow, P. A., and J. A. Shivik. 2009. Bold, shy, and persistent: Variable coyote response to light and sound stimuli. Applied Animal Behaviour Science 116:82–87.
- David, M., Y. Auclair, and F. Cézilly. 2011. Personality predicts social dominance in female zebra fi nches , Taeniopygia guttata , in a feeding context. Animal Behaviour 81:219–224.
- Descamps, S., S. Boutin, D. Berteaux, A. G. McAdam, and J.-M. Gaillard. 2008. Cohort effects in red squirrels: the influence of density, food abundance and temperature on future survival and reproductive success. Journal of Animal Ecology 77:305–314.
- Dettmer, A. M., S. S. K. Kaburu, K. L. Byers, and A. M. Murphy. 2015. First-Time Rhesus Monkey Mothers , and Mothers of Sons , Preferentially Engage in Face-to-Face Interactions With Their Infants:1–9.
- Dickens, M. J., and L. M. Romero. 2013. A consensus endocrine profile for chronically stressed wild animals does not exist. General and Comparative Endocrinology 191:177–189.
- Dingemanse, N., C. Both, P. Drent, and K. Van Oers. 2002. Repeatability and heritability of exploratory behaviour in great tits from the wild. Animal Behaviour 64:929–938.
- Dloniak, S. M. 2004. Socioendocrinology of Spotted Hyenas. Michigan State University.
- Dloniak, S. M., J. A. French, and K. E. Holekamp. 2006. Rank-related maternal effects of androgens on behaviour in wild spotted hyaenas. Nature 440:1190–1193.

- Dloniak, S. M., J. A. French, N. J. Place, M. L. Weldele, S. E. Glickman, and K. E. Holekamp. 2004. Non-invasive monitoring of fecal androgens in spotted hyenas (Crocuta crocuta). General and Comparative Endocrinology 135:51–61.
- Draper, T. W. 1995. Canine analogs of human personality factors. The Journal of general psychology 122:241–252.
- Drea, C. M. 2006. Studying primate learning in group contexts: Tests of social foraging, response to novelty, and cooperative problem solving. Methods 38:162–177.
- Durr, R., and C. Smith. 1997. Individual differences and their relation to social structure in domestic cats. Journal of Comparative Psychology 111:412–418.
- Dwyer, C. M., and A. . Lawrence. 2000. Maternal Behaviour in Domestic Sheep (Ovis aries): Constancy and Change with Maternal Experience. Behaviour 137:1391–1413.
- Engelhard, G. H., A. N. J. Baarspul, M. Broekman, J. C. S. Creuwels, and P. J. H. Reijnders. 2002. Human disturbance, nursing behaviour, and lactational pup growth in a declining southern elephant seal (Mirounga leonina) population 1886:1876–1886.
- Engh, A. L., K. Esch, L. Smale, and K. E. Holekamp. 2000. Mechanisms of maternal rank "inheritance" in the spotted hyaena, Crocuta crocuta. Animal Behaviour 60:323–332.
- Ethan Pride, R. 2005. High faecal glucocorticoid levels predict mortality in ring-tailed lemurs (Lemur catta). Biology Letters 1:60–63.
- Fairbanks, L. 1993. Maternal protectiveness and response to the unfamiliar in vervet monkeys. American Journal of Primatology 30:119–129.
- Fairbanks, L. A. 1989. Early experience and cross-generational continuity of mother-infant contact in vervet monkeys. Developmental psychobiology 22:669–681.
- Fairbanks, L. A. 1996. Individual Differences in Maternal Style:: Causes and Consequences for Mothers and offspring. Advances in the Study of Behavior 25:579–611.
- Fairbanks, L. A., and K. Hinde. 2012. Behavioral Response of Mothers and Infants to Variation in Maternal Condition: Adaptation, Compensation, and Resilience. Pages 281–302Building babies. Springer New York, New York, NY.
- Fairbanks, L. A., and M. T. McGuire. 1995. Maternal condition and the quality of maternal care in vervet monkeys. Behaviour 132:733–754.
- Ficetola, G. F., R. Sacchi, S. Scali, A. Gentilli, F. De Bernardi, and P. Galeotti. 2007. Vertebrates respond differently to human disturbance: implications for the use of a focal species approach. Acta Oecologica 31:109–118.

- Flies, A. S., C. K. Grant, L. S. Mansfield, E. J. Smith, M. L. Weldele, and K. E. Holekamp. 2012. Development of a hyena immunology toolbox. Veterinary Immunology and Immunopathology 145:110–119.
- Foley, C. A. H., S. Papageorge, and S. K. Wasser. 2001. Noninvasive Stress and Reproductive Measures of Social and Ecological Pressures in Free-Ranging African Elephants. Conservation Biology 15:1134–1142.
- Fox, M. W. 1972. Socio-ecological implications of individual differences in wolf litters: a developmental and evolutionary perspective. Behaviour 41:298–313.
- Fox, R. A., L. D. Ladage, T. C. Roth, and V. V Pravosudov. 2009. Behavioural profile predicts dominance status in mountain chickadees, Poecile gambeli. Animal Behaviour 77:1441– 1448.
- Francis, D., J. Diorio, and D. Liu. 1999. Nongenomic Transmission Across Generations of Maternal Behavior and Stress Responses in the Rat. Science 286:1155–1158.
- Frank, L. 1986a. Social organization of the spotted hyaena (Crocuta crocuta). I. Demography. Animal Behaviour 34:1500–1509.
- Frank, L. 1986b. Social organization of the spotted hyaena Crocuta crocuta. II. Dominance and reproduction. Animal Behaviour 34:1510–1527.
- Frank, L. G., and S. E. Glickman. 1994. Giving birth through a penile clitoris: parturition and dystocia in the spotted hyena (Crocuta crocuta). J. Zool. Lond. 234:659–690.
- Frank, L. G., S. E. Glickman, and I. Powch. 1990. Sexual dimorphism in the spotted hyaena (Crocuta crocuta). Journal of Zoology 221:308–313.
- French, S. S., M. Gonzales-Suarez, J. K. Young, S. Durham, and L. R. Gerber. 2011. Human Disturbance Influences Reproductive Success and Growth Rate in California Sea Lions (Zalophus californianus). PLoS ONE 6:e17686.
- Gehrt, S. D., C. Anchor, and L. a. White. 2009. Home Range and Landscape Use of Coyotes in a Metropolitan Landscape: Conflict or Coexistence? Journal of Mammalogy 90:1045–1057.
- Gibbs, M., C. J. Breuker, and H. Van Dyck. 2010. Flight during oviposition reduces maternal egg provisioning and influences offspring development in Pararge aegeria (L.). Physiological entomology 35:29–39.
- Glickman, S., C. Zabel, S. Yoerg, M. Weldele, C. M. Drea, and L. G. Frank. 1997. Social Facilitation, Affiliation, and Dominance in the Social Life of Spotted Hyenas. Annals of the New York Academy of Sciences 807:175–184.

- Gluckman, P. D., M. A. Hanson, C. Cooper, and K. L. Thornburg. 2008. Effect of in utero and early-life conditions on adult health and disease. New England Journal of Medicine 359:61– 73.
- Gómez-Laplaza, L. M. 2002. Social status and investigatory behaviour in the angelfish (Pterophyllum scalare). Behaviour 139:1469–1490.
- Gosselink, T. E., T. R. Van Deelen, R. E. Warner, and P. C. Mankin. 2007. Survival and Cause-Specific Mortality of Red Foxes in Agricultural and Urban Areas of Illinois Survival and Cause-Specific Mortality of Red Foxes in Agricultural and Urban Areas of Illinois. Journal of Wildlife Management 71:1862–1873.
- Goymann, W., M. L. East, B. Wachter, O. P. Honer, E. Mostl, T. J. Van't Holf, and H. Hofer. 2001. Social, state-dependent and environmental modulation of faecal corticosteroid levels in free-ranging female spotted hyenas. Proceedings of the Royal Society B: Biological Sciences 268:2453–2459.
- Goymann, W., and J. C. Wingfield. 2004. Allostatic load, social status and stress hormones: the costs of social status matter. Animal Behaviour 67:591–602.
- Green, D. S. 2015. Anthropogenic disturbance, ecological change, and wildlife conservation at the edge of the mara-serengeti ecosystem. Michigan State University.
- Green, D. S., L. Johnson-Ulrich, H. E. Couraud, and K. E. Holekamp. 2017. Anthropogenic disturbance induces opposing population trends in spotted hyenas and African lions. Biodiversity and Conservation:1–19.
- Greenberg, J. R., and K. E. Holekamp. 2017. Human disturbance affects personality development in a wild carnivore. Animal Behaviour 132:303–312.
- Greggor, A. L., O. Berger-tal, D. T. Blumstein, L. Angeloni, C. Bessa-gomes, B. F. Blackwell, C. Cassady, S. Clair, K. Crooks, S. De Silva, E. Fernández-juricic, S. Z. Goldenberg, S. L. Mesnick, M. Owen, C. J. Price, D. Saltz, C. J. Schell, A. V Suarez, R. R. Swaisgood, C. S. Winchell, and W. J. Sutherland. 2016. Research Priorities from Animal Behaviour for Maximising Conservation Progress. Trends in Ecology & Evolution 31:953–964.
- Guenther, A., M. A. Finkemeier, and F. Trillmich. 2014. The ontogeny of personality in the wild guinea pig. Animal Behaviour 90:131–139.
- Haage, M., U. A. Bergvall, T. Maran, K. Kiik, and A. Angerbjörn. 2013. Situation and context impacts the expression of personality: The influence of breeding season and test context. Behavioural Processes 100:103–109.
- Hamel, S., S. D. Côté, and M. Festa-Bianchet. 2010. Maternal characteristics and environment affect the costs of reproduction in female mountain goats. Ecology 91:2034–2043.
- Harper, J. M., and S. N. Austad. 2000. Fecal glucocorticoids: a noninvasive method of measuring adrenal activity in wild and captive rodents. Physiological and Biochemical Zoology 73:12–22.
- Harris, C. E., and F. F. Knowlton. 2001. Differential responses of coyotes to novel stimuli in familiar and unfamiliar settings. Canadian Journal of Zoology 79:2005–2013.
- Hau, M., S. Casagrande, J. Q. Ouyang, and A. T. Baugh. 2016. Glucocorticoid-Mediated Phenotypes in Vertebrates: Multilevel Variation and Evolution. Advances in the Study of Behavior 48:1–75.
- Hauser, M., and L. Fairbanks. 1988. Mother-offspring conflict in vervet monkeys: variation in response to ecological conditions. Animal Behaviour 36:802–813.
- Heffernan, D. J., W. F. Andelt, and J. A. Shivik. 2007. Coyote Investigative Behavior Following Removal of Novel Stimuli. Journal of Wildlife Management 71:587–593.
- Hinde, K., A. L. Skibiel, A. B. Foster, L. Del Rosso, S. P. Mendoza, and J. P. Capitanio. 2015. Cortisol in mother's milk across lactation reflects maternal life history and predicts infant temperament. Behavioral Ecology 26:269–281.
- Hinde, R. A. 1976. On Describing Relationships. Journal of Child Psychology and Psychiatry 17:1–19.
- Hinde, R. A., and S. Atkinson. 1970. Assessing the roles of social partners in maintaining mutual proximity, as exemplified by mother-infant relations in rhesus monkeys. Animal Behaviour 18:169-176.
- Hofer, H., S. Benhaiem, W. Golla, and M. L. East. 2016. Trade-offs in lactation and milk intake by competing siblings in a fluctuating environment. Behavioral Ecology 27:1567–1578.
- Hofer, H., and M. L. East. 1993a. The commuting system of Serengeti spotted hyaenas: how a predator copes with migratory prey. III. Attendance and maternal care. Animal Behaviour:1–15.
- Hofer, H., and M. L. East. 1993b. The commuting system fo Serengeti spotted hyaenas: how a predator copes with migratory prey. I. Social organization. Animal Behaviour 46:547–557.
- Hofer, H., and M. L. East. 2003. Behavioral processes and costs of co-existence in female spotted hyenas: a life history perspective. Evolutionary Ecology 17:315–331.
- Hoffman, C. L., J. P. Higham, M. Heistermann, C. L. Coe, B. J. Prendergast, and D. Maestripieri. 2011. Immune function and HPA axis activity in free-ranging rhesus macaques. Physiology & Behavior 104:507–514.

- Holekamp, K. E., S. M. Cooper, C. I. Katona, N. A. Berry, L. G. Frank, and L. Smale. 1997a. Patterns of Association among Female Spotted Hyenas (Crocuta crocuta). Journal of Mammalogy 78:55–64.
- Holekamp, K. E., B. Dantzer, G. Stricker, K. C. S. Yoshida, and S. Benson-Amram. 2015. Brains, brawn and sociality: a hyaena's tale. Animal Behaviour 103:237–248.
- Holekamp, K. E., and S. M. Dloniak. 2009. Maternal Effects in Fissiped Carnivores. Pages 227– 255*in* D. Maestripieri and J. M. Mateo, editors.Maternal Effects in Mammals. The University of Chicago Press, Chicago.
- Holekamp, K. E., and S. M. Dloniak. 2010. Intraspecific Variation in the Behavioral Ecology of a Tropical Carnivore, the Spotted Hyena. Advances in the Study of Behavior 42:189–229.
- Holekamp, K. E., and L. Smale. 1993. Ontogeny of dominance in free-living spotted hyaenas: juvenile rank relations with other immature individuals. Animal Behaviour 46:451–466.
- Holekamp, K. E., and L. Smale. 1998a. Behavioral development in the Spotted Hyena. BioScience 48:997–1005.
- Holekamp, K. E., and L. Smale. 1998b. Dispersal Status Influences Hormones and Behavior in the Male Spotted Hyena. Hormones and Behavior 33:205–216.
- Holekamp, K. E., L. Smale, R. Berg, and S. M. Cooper. 1997b. Hunting rates and hunting success in the spotted hyena (Crocuta crocuta). Journal of Zoology 242:1–15.
- Holekamp, K. E., L. Smale, and M. Szykman. 1996. Rank and reproduction in the female spotted hyaena. Reproduction 108:229–237.
- Holekamp, K. E., E. M. Swanson, and P. E. Van Meter. 2013. Developmental constraints on behavioural flexibility. Philosophical Transactions of the Royal Society B: Biological Sciences 368:20120350.
- Honer, O. P., B. Wachter, M. L. East, and H. Hofer. 2002. The response of spotted hyaenas to long-term changes in prey populations: functional response and interspecific kleptoparasitism. Journal of Animal Ecology 71:236-246.
- van Horn, R. C., T. L. McElhinny, and K. E. Holekamp. 2003. Age estimation and dispersal in the spotted hyena (Crocuta crocuta). Journal of Mammalogy 84:1019–1030.
- Hudson, R., A. Bautista, V. Reyes-Meza, J. M. Montor, and H. G. Rödel. 2011. The effect of siblings on early development: A potential contributor to personality differences in mammals. Developmental Psychobiology 53:564–574.

Hudson, R., and F. Trillmich. 2008. Sibling competition and cooperation in mammals:

challenges, developments and prospects. Behavioral Ecology and Sociobiology 62:299-307.

- Huizink, A. C., E. J. H. Mulder, and J. K. Buitelaar. 2004. Prenatal stress and risk for psychopathology: specific effects or induction of general susceptibility? Psychological bulletin 130:115.
- Ikime, T. 2015. Diurnal movement patterns, habitat use and energy cost of locomotion by spotted hyenas (Crocuta crocuta) in the Masai Mara National Reserve, Kenya. Michigan State University.
- Jablonka, E., B. Oborny, I. Molnar, E. Kisdi, J. Hofbauer, and T. Czaran. 1995. The adaptive advantage of phenotypic memory in changing environments. Philosophical Transactions of the Royal Society of London B: Biological Sciences 350:133–141.
- Jacobson-Pick, S., and G. Richter-Levin. 2010. Differential impact of juvenile stress and corticosterone in juvenility and in adulthood , in male and female rats. Behavioural Brain Research 214:268–276.
- Jahn-Eimermacher, A., I. Lasarzik, and J. Raber. 2011. Statistical analysis of latency outcomes in behavioral experiments. Behavioural Brain Research 221:271–275.
- James, H., and C. Myfanwy. 2013. Individual differences in maternal behaviour in the grey seal (Halichoerus grypus) and the impact of disturbance at Donna Nook. Durham University.
- Johnson, J. C., P. J. Trubl, and L. S. Miles. 2012. Black widows in an urban desert: city-living compromises spider fecundity and egg investment despite urban prey abundance. The American Midland Naturalist 168:333–340.
- Jones, A. C., and S. D. Gosling. 2005. Temperament and personality in dogs (Canis familiaris): A review and evaluation of past research. Applied Animal Behaviour Science 95:1–53.
- Kendal, R. L., R. L. Coe, K. N. Laland, and P. Biology. 2005. Age Differences in Neophilia, Exploration, and Innovation in Family Groups of Callitrichid Monkeys. American Journal of Primatology 66:167–188.
- King, W. J., and A. W. Goldizen. 2016. Few sex effects in the ontogeny of mother-offspring relationships in eastern grey kangaroos. Animal Behaviour 113:59–67.
- Kittleson, M. M., L. A. Meoni, N.-Y. Wang, A. Y. Chu, D. E. Ford, and M. J. Klag. 2006. Association of childhood socioeconomic status with subsequent coronary heart disease in physicians. Archives of internal medicine 166:2356–2361.
- Kolowski, J., and K. Holekamp. 2009. Ecological and anthropogenic influences on space use by spotted hyaenas. Journal of Zoology 277:23–36.

- Kolowski, J., D. Katan, and K. Theis. 2007. Daily patterns of activity in the spotted hyena. Journal of Mammology 88:1017–1028.
- Kolowski, J. M., and K. E. Holekamp. 2006. Spatial, temporal, and physical characteristics of livestock depredations by large carnivores along a Kenyan reserve border. Biological Conservation 128:529–541.
- Kortet, R., A. Vainikka, M. Janhunen, J. Piironen, and P. Hyvärinen. 2014. Behavioral variation shows heritability in juvenile brown trout Salmo trutta. Behavioral Ecology and Sociobiology 68:927–934.
- Kruuk, H. 1972. The spotted hyaena. University of Chicago Press, Chicago.
- Lee, P. C., P. Majluf, and I. J. Gordon. 1991. Growth, weaning and maternal investment from a comparative perspective. Journal of Zoology 225:99–114.
- Lee, P. C., and C. J. Moss. 1986. Early maternal investment in male and female African elephant calves. Behavioral Ecology and Sociobiology 18:353–361.
- Lessells, C., and P. T. Boag. 1987. Unrepeatable repeatabilities. The Auk 104:116–121.
- Lewin, N., E. M. Swanson, B. L. Williams, and K. E. Holekamp. 2017. Juvenile concentrations of IGF-1 predict life-history trade-offs in a wild mammal. Functional Ecology 31:894–902.
- Lindström, J. 1999. Early development and fitness in birds and mammals. Trends in Ecology & Evolution 14:343–348.
- Linnell, J. D. C., J. Odden, M. E. Smith, R. Aanes, and J. E. Swenson. 1999. Large Carnivores That Kill Livestock: Do "Problem Individuals" Really Exist? Wildlife Society Bulletin 27:698–705.
- Litvaitis, J. A., A. G. Clark, and J. H. Hunt. 2017. Prey selection and fat deposits of bobcats (Felis rufus) during autumn and winter in Maine. Journal of Mammalogy 67:389–392.
- Liu, D., J. Diorio, B. Tannenbaum, C. Caldji, and D. Francis. 1997. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. Science 277:1659–1662.
- Lycett, J. E., P. Henzi, and L. Barrett. 1998. Maternal investment in mountain baboons and the hypothesis of reduced care. Behavioral Ecology and Sociobiology 42:49–56.
- M'soka, J., S. Creel, M. S. Becker, and E. Droge. 2016. Spotted hyaena survival and density in a lion depleted ecosystem : The effects of prey availability , humans and competition between large carnivores in African savannahs. Biological Conservation 201:348–355.

Macdonald, D. W., O. Courtenay, S. Forbes, and F. Mathews. 1999. The red fox (Vulpes vulpes)

in Saudi Arabia: loose-knit groupings in the absence of territoriality. Journal of Zoology 249:383–391.

- MacDonald, K. 1983. Stability of individual differences in a litter of wolf cubs (Canis lupus). Journal of Comparative Psychology 97:99–106.
- Maestripieri, D. 1995. Assessment of Danger to Themselves and Their Infants by Rhesus Macaque (Macaca mulatto) Mothers 109:416–420.
- Maestripieri, D. 1998. Social and demographic influences on mothering style in pigtail macaques. Ethology 104:379-385.
- Maestripieri, D. 2001. Intraspecific Variability in Parenting Styles of Rhesus Macaques (Macaca mulatta): The Role of the Social Environment. Ethology 107:237–248.
- Maestripieri, D. 2005. Early experience affects the intergenerational transmission of infant abuse in rhesus monkeys. Proceedings of the National Academy of Sciences of the United States of America 102:9726–9729.
- Maestripieri, D., C. L. Hoffman, G. M. Anderson, C. S. Carter, and J. D. Higley. 2009. Motherinfant interactions in free-ranging rhesus macaques: Relationships between physiological and behavioral variables. Physiology & Behavior 96:613–619.
- Maestripieri, D., and J. M. Mateo. 2009. Maternal effects in mammals. University of Chicago Press, Chicago.
- Maestripieri, D., and K. Wallen. 2003. Infant abuse runs in families of group-living pigtail macaques:1–7.
- Marshall, D. J., and T. Uller. 2007. When is a maternal effect adaptive? Oikos 116:1957–1963.
- Martin, T., and P. Bateson. 1988. Measuring Behavior: an Introductory Guide. Cambridge University Press, Cambridge.
- Martins, T., M. Roberts, I. Giblin, R. Huxham, and M. Evans. 2007. Speed of exploration and risk-taking behavior are linked to corticosterone titres in zebra finches. Hormones and Behavior 52:445–453.
- Matlack, C. R., and A. J. Evans. 1992. Diet and condition of bobcats, Lynx rufus, in Nova Scotia during autumn and winter. Canadian Journal of Zoology 70:1114–1119.
- McCormick, C. M., J. W. Smythe, S. Sharma, and M. J. Meaney. 1995. Sex-specific effects of prenatal stress on hypothalamic-pituitary-adrenal responses to stress and brain glucocorticoid receptor density in adult rats. Brain research. Developmental brain research 84:55–61.

- McDougall, P. T., D. Réale, D. Sol, and S. M. Reader. 2006. Wildlife conservation and animal temperament: causes and consequences of evolutionary change for captive, reintroduced, and wild populations. Animal Conservation 9:39–48.
- McEwen, B. S., and J. C. Wingfield. 2003. The concept of allostasis in biology and biomedicine. Hormones and Behavior 43:2–15.
- McGlothlin, J. W., and E. D. Ketterson. 2008. Hormone-mediated suites as adaptations and evolutionary constraints. Philosophical Transactions of the Royal Society of London B: Biological Sciences 363:1611–1620.
- Mendl, M. 1988. The effects of litter size variation on mother-offspring relationships and behavioural and physical development in several mammalian species (principally rodents). Journal of Zoology 215:15–34.
- Van Meter, P. E., J. A. French, K. Bidali, M. L. Weldele, J. L. Brown, and K. E. Holekamp. 2008. Non-invasive measurement of fecal estrogens in the spotted hyena (Crocuta crocuta). General and Comparative Endocrinology 155:464–471.
- Van Meter, P. E., J. A. French, S. M. Dloniak, H. E. Watts, J. M. Kolowski, and K. E. Holekamp. 2009. Fecal glucocorticoids reflect socio-ecological and anthropogenic stressors in the lives of wild spotted hyenas. Hormones and Behavior 55:329–337.
- Mettler, A. E., and J. A. Shivik. 2007. Dominance and neophobia in coyote (Canis latrans) breeding pairs. Applied Animal Behaviour Science 102:85–94.
- Miranda, A. C., H. Schielzeth, T. Sonntag, and J. Partecke. 2013. Urbanization and its effects on personality traits: a result of microevolution or phenotypic plasticity? Global Change Biology 19:2634–2644.
- Monaghan, P. 2008. Early growth conditions, phenotypic development and environmental change. Philosophical Transactions of the Royal Society B: Biological Sciences 363:1635–1645.
- Moore, C. L. 1984. Maternal contributions to the development of masculine sexual behavior in laboratory rats. Developmental Psychobiology 17:347–356.
- Moore, C. L. 1992. The role of maternal stimulation in the development of sexual behavior and its neural basis. Annals of the New York Academy of Sciences 662:160–77.
- Moretti, L., M. Hentrup, K. Kotrschal, and F. Range. 2015. The influence of relationships on neophobia and exploration in wolves and dogs. Animal Behaviour 107:159–173.
- Moscovice, L. R., and C. T. Snowdon. 2006. The role of social context and individual experience in novel task acquisition in cottontop tamarins, Saguinus oedipus. Animal Behaviour

71:933–943.

- Mousseau, T., and C. Fox. 1998. The adaptive significance of maternal effects. Trends in Ecology & Evolution 13:403–407.
- Nash, L. T. 1978. The development of the mother-infant relationship in wild baboons (Papio anubis). Animal Behaviour 26:746–759.
- Nater, U. M., and N. Rohleder. 2009. Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system : Current state of research. Psychoneuroendocrinology 34:486–496.
- Nguyen, N., L. Gesquiere, S. C. Alberts, and J. Altmann. 2012. Sex differences in the motherneonate relationship in wild baboons: social, experiential and hormonal correlates. Animal Behaviour 83:891–903.
- Nguyen, N., L. R. Gesquiere, E. O. Wango, S. C. Alberts, and J. Altmann. 2008. Late pregnancy glucocorticoid levels predict responsiveness in wild baboon mothers (Papio cynocephalus). Animal Behaviour 75:1747–1756.
- Nicolaus, M., J. M. Tinbergen, K. M. Bouwman, S. P. M. Michler, R. Ubels, C. Both, B. Kempenaers, and N. J. Dingemanse. 2012. Experimental evidence for adaptive personalities in a wild passerine bird. Proceedings of the Royal Society B: Biological Sciences 279:4885–4892.
- Noer, C. L., E. K. Needham, A.-S. Wiese, T. J. S. Balsby, and T. Dabelsteen. 2015. Context Matters: Multiple Novelty Tests Reveal Different Aspects of Shyness-Boldness in Farmed American Mink (Neovison vison). PLoS ONE 10:e0130474.
- Nussey, D., and A. Wilson. 2007. The evolutionary ecology of individual phenotypic plasticity in wild populations. Journal of Evolutionary Biology 20:831–844.
- O'Rand, A. M., and J. Hamil-Luker. 2005. Processes of cumulative adversity: Childhood disadvantage and increased risk of heart attack across the life course. The Journals of Gerontology Series B: Psychological Sciences and Social Sciences 60:S117–S124.
- Ono, K. A., D. J. Boness, and O. T. Oftedal. 1987. The effect of a natural environmental disturbance on maternal investment and pup behavior in the California sea lion. Behavioral Ecology and Sociobiology 21:109–118.
- Oswald, L. M., P. Zandi, G. Nestadt, J. B. Potash, A. E. Kalaydjian, and G. S. Wand. 2006. Relationship between cortisol responses to stress and personality. Neuropsychopharmacology 31:1583–1591.

Pangle, W. M., and K. E. Holekamp. 2010. Lethal and nonlethal anthropogenic effects on

spotted hyenas in the Masai Mara National Reserve. Journal of Mammalogy 91:154-164.

- Papaioannou, A., K. Gerozissis, A. Prokopiou, S. Bolaris, and F. Stylianopoulou. 2002. Sex differences in the effects of neonatal handling on the animal's response to stress and the vulnerability for depressive behaviour. Behavioural brain research 129:131–139.
- Park, M. K., T. A. Hoang, J. D. Belluzzi, and F. M. Leslie. 2003. Gender specific effect of neonatal handling on stress reactivity of adolescent rats. Journal of neuroendocrinology 15:289–295.
- Parker, K. ., K. . Rainwater, C. . Buckmaster, A. F. Schatzberg, S. E. Lindley, and D. M. Lyons. 2007. Early life stress and novelty seeking behavior in adolescent monkeys. Psychoneuroendocrinology 32:785–792.
- Parker, K., and C. Buckmaster. 2004. Prospective investigation of stress inoculation in young monkeys. Archives of General Psychiatry 61:933–941.
- Patchev, V. K., S. Hayashi, C. Orikasa, and O. F. X. Almeida. 1999. Ontogeny of gender-specific responsiveness to stress and glucocorticoids in the rat and its determination by the neonatal gonadal steroid environment. Stress 3:41–54.
- Payne, S. F., and R. J. Jameson. 1984. Early behavioral development of the sea otter, Enhydra lutris. Journal of Mammalogy 65:527–531.
- Petelle, M. B., J. G. A. Martin, and D. T. Blumstein. 2015. Heritability and genetic correlations of personality traits in a wild population of yellow-bellied marmots (Marmota flaviventris). Journal of Evolutionary Biology 28:1840–1848.
- Petelle, M. B., D. E. McCoy, V. Alejandro, J. G. A. Martin, and D. T. Blumstein. 2013. Development of boldness and docility in yellow-bellied marmots. Animal Behaviour:1–8.
- Ponirakis, A., E. J. Susman, and C. A. Stifter. 1998. Negative Emotionality and Cortisol during Adolescent Pregnancy and Its Effects on Infant Health and Autonomic Nervous System Reactivity. Developmental Psychobiology 33:163–174.
- Ragan, C. M., K. M. Harding, and J. S. Lonstein. 2016. Hormones and Behavior Associations among within-litter differences in early mothering received and later emotional behaviors, mothering, and cortical tryptophan hydroxylase-2 expression in female laboratory rats. Hormones and Behavior 77:62–71.
- Ragan, C. M., E. Loken, C. A. Stifter, and S. A. Cavigelli. 2011. Within-litter variance in early rat pup-mother interactions and adult offspring responses to novelty. Developmental Psychobiology 54:1–8.
- Räsänen, K., and L. E. B. Kruuk. 2007. Maternal effects and evolution at ecological time-scales.

Functional Ecology 21:408–421.

- Réale, D., S. M. Reader, D. Sol, P. T. McDougall, and N. J. Dingemanse. 2007. Integrating animal temperament within ecology and evolution. Biological Reviews 82:291–318.
- Reed, T. E., R. S. Waples, D. E. Schindler, J. J. Hard, and M. T. Kinnison. 2010. Phenotypic plasticity and population viability: the importance of environmental predictability. Proceedings of the Royal Society B: Biological Sciences 277:3391–3400.
- Reznikov, A. G., N. D. Nosenko, L. V. Tarasenko, P. V. Sinitsyn, and L. I. Polyakova. 2001. Early and long-term neuroendocrine effects of prenatal stress in male and female rats. Neuroscience and behavioral physiology 31:1–5.
- Riley, S. P. D., R. M. Sauvajot, T. K. Fuller, E. C. York, D. A. Kamradt, C. Bromley, and R. Wayne. 2003. Effects of Urbanisation and Habitat Fragmentation on Bobcats and Coyotes in Southern California. Conservation Biology 17:566–576.
- Ripple, W. J., J. A. Estes, R. L. Beschta, C. C. Wilmers, E. G. Ritchie, M. Hebblewhite, J. Berger, B. Elmhagen, M. Letnic, M. P. Nelson, O. J. Schmitz, D. W. Smith, A. D. Wallach, and A. J. Wirsing. 2014. Status and Ecological Effects of the World's Largest Carnivores. Science 343:1241484.
- Rodel, H. G., D. von Holst, and C. Kraus. 2009. Family Legacies: Short- and Long-Term Fitness Consequences of Early-Life Conditions in Female European Rabbits. Journal of Animal Ecology 78:789–797.
- Romeo, R. D. 2010. Frontiers in Neuroendocrinology Pubertal maturation and programming of hypothalamic pituitary adrenal reactivity. Frontiers in Neuroendocrinology 31:232–240.
- Romero, L. M., and M. Wikelski. 2010. Stress physiology as a predictor of survival in Galapagos marine iguanas:3157–3162.
- Rosenblum, L. A., and G. S. Paully. 1984. The effects of varying environmental demands on maternal and infant behavior. Child Development 55:305–314.
- Russell, E. ., and G. A. Pearce. 1971. Exploration of novel objects by marsupials. Behaviour 40:312–322.
- Ryer, C. H., and B. L. Olla. 1991. Information transfer and the facilitation and inhibition of feeding in a schooling fish. Environmental Biology of Fishes 30:317–323.
- Saberwal, V. K., J. P. Gibbs, R. Chellam, and A. J. . Johnsingh. 1994. Lion-human Conflict in the Gir Forest. Conservation Biology 8:501–507.
- 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.

- Sapolsky, R., and L. . Share. 1994. Rank-related differences in cardiovascular function among wild baboons: Role of sensitivity to glucocorticoids. American Journal of Primatology 32:261–275.
- Schino, G., R. Cozzolino, and A. Troisi. 1999. Social Rank and Sex-Biased Maternal Investment in Captive Japanese Macaques: Behavioural and Reproductive Data. Folia Primatologica 70:254–263.
- Schino, G., F. R. D'Amato, and A. Troisi. 1995. Mother infant relationships in Japanese macaques: sources of inter-individual variation. Animal Behaviour 49:151–158.
- Schino, G., A. Troisi, and C. Psichiatria. 2001. Early Maternal Rejection and Later Social Anxiety in Juvenile and Adult Japanese Macaques. Developmental Psychobiology 38:186–190.
- Schneider, M. L., E. C. Roughton, and A. J. Koehler. 1999. Growth and Development Following Prenatal Stress Exposure in Primates: An Examination of Ontogenetic Vulnerability. Child Development 70:263–274.
- Schoech, S. J., M. A. Rensel, and R. S. Heiss. 2011. Short- and long-term effect of developmental corticosterone exposure on avian physiology, behavioral phenotype, cognition, and fitness: A review. Current Zoology 4:514–530.
- Seidensticker, J., and C. McDougal. 1993. Tiger predatory behaviour, ecology and conservation. Symp. Zool. Soc. Lond. 65:105–125.
- Sheriff, M. J., C. J. Krebs, and R. Boonstra. 2009. The sensitive hare: sublethal effects of predator stress on reproduction in snowshoe hares. Journal of Animal Ecology 78:1249–1258.
- Sheriff, M. J., and O. P. Love. 2013. Determining the adaptive potential of maternal stress. Ecology Letters 16:271–280.
- Shivik, J. . 2006. Tools for the Edge: What's New for Conserving Carnivores. Bioscience 56:253–259.
- Sih, A. 2013. Understanding variation in behavioural responses to human-induced rapid environmental change: a conceptual overview. Animal Behaviour 85:1077–1088.
- Sikes, R. S., and W. L. Gannon. 2011. Guidelines of the American Society of Mammalogists for the use of wild mammals in research. Journal of Mammalogy 92:235–253.
- Silva, V. S., and C. Azevedo. 2013. Evaluating personality traits of captive maned wolves,

Chrysocyon brachyurus (Illiger, 1815) (Mammalia: Canidae), for conservation purposes. Lundiana 11:35–41.

- Sinn, D. L., S. D. Gosling, and N. A. Moltschaniwskyj. 2008. Development of shy/bold behaviour in squid: context-specific phenotypes associated with developmental plasticity. Animal Behaviour 75:433–442.
- Smale, L., L. G. Frank, and K. E. Holekamp. 1993. Ontogeny of dominance in free-living spotted hyaenas: juvenile rank relations with adult females and immigrant males. Animal Behaviour 46:467–477.
- Smale, L., K. E. Holekamp, M. Weldele, L. G. Frank, and S. E. Glickman. 1995. Competition and cooperation between litter-mates in the spotted hyaena, Crocuta crocuta. Animal Behaviour 50:671–682.
- Smale, L., S. Nunes, and K. E. Holekamp. 1997. Sexually dimorphic dispersal in mammals: patterns, causes, and consequences. Advances in the Study of Behavior 26:181–250.
- Smith, B. R., and D. T. Blumstein. 2008. Fitness consequences of personality: a meta-analysis. Behavioral Ecology 19:448–455.
- Smith, J. E., J. M. Kolowski, K. E. Graham, S. E. Dawes, and K. E. Holekamp. 2008. Social and ecological determinants of fission–fusion dynamics in the spotted hyaena. Animal Behaviour 76:619–636.
- Smith, J. E., S. K. Memenis, and K. E. Holekamp. 2007. Rank-related partner choice in the fission fusion society of the spotted hyena (Crocuta crocuta):753–765.
- Smith, J. M. 1980. A new theory of sexual investment. Behavioral ecology and sociobiology 7:247–251.
- Sol, D., O. Lapiedra, and C. González-Lagos. 2013. Behavioural adjustments for a life in the city. Animal Behaviour 85:1101–1112.
- Sroges, R. W., and S. E. Glickman. 1966. Curiosity in Zoo Animals. Behaviour 26:151-187.
- Stamps, J., and T. G. G. Groothuis. 2010. The development of animal personality: relevance, concepts and perspectives. Biological Reviews 85:301–325.
- Stevens, H. E., J. F. Leckman, J. D. Coplan, and S. J. Suomi. 2009. Risk and Resilience. Journal of the American Academy of Child & Adolescent Psychiatry 48:114–127.
- Stevens, M. A., and D. J. Boness. 2003. Influences of habitat features and human disturbance on use of breeding sites by a declining population of southern fur seals (Arctocephalus australis):145–152.

- Stewart, F. E. C., and A. G. Mcadam. 2014. Seasonal plasticity of maternal behaviour in Peromyscus maniculatus 151:1641–1662.
- Stirling, I., and P. B. Latour. 1978. Comparative hunting abilities of polar bear cubs of different ages. Canadian Journal of Zoology 56:1768–1772.
- Stöwe, M., T. Bugnyar, and B. Heinrich. 2006a. Effects of group size on approach to novel objects in ravens (Corvus corax). Ethology 112:1079–1088.
- Stöwe, M., T. Bugnyar, M. Loretto, and C. Schloegl. 2006b. Novel object exploration in ravens (Corvus corax): effects of social relationships. Behavioural Processes 73:68–75.
- Swanson, E. M., I. Dworkin, and K. E. Holekamp. 2011. Lifetime selection on a hypoallometric size trait in the spotted hyena. Proceedings of the Royal Society of London B: Biological Sciences 278:3277–3285.
- Tanaka, I. 1989. Variability in the Development of Mother-infant Relationships Among Freeranging Japanese Macaques. Primates 30:477–491.
- Tanner, J. B., M. L. Zelditch, B. L. Lundrigan, and K. E. Holekamp. 2009. Ontogenetic change in skull morphology and mechanical advantage in the spotted hyena (Crocuta crocuta). Journal of Morphology 271:353–365.
- Taylor, R. W., A. K. Boon, B. Dantzer, D. Réale, M. M. Humphries, S. Boutin, J. C. Gorrell, D.
  W. Coltman, and A. G. McAdam. 2012. Low heritabilities, but genetic and maternal correlations between red squirrel behaviours. Journal of Evolutionary Biology 25:614–624.
- Treves, A., and K. U. Karanth. 2003. Human-Carnivore Conflict and Perspectives on Carnivore Management Worldwide. Conservation Biology 17:1491–1499.
- Treves, A., R. B. Wallace, L. Naughton-Treves, and A. Morales. 2006. Co-Managing Human– Wildlife Conflicts: A Review. Human Dimensions of Wildlife 11:383–396.
- Trivers, R. L. 1972. Parental investment and sexual selection. Pages 136–179 in Sexual Selection & the Descent of Man, Aldine de Gruyter, New York.
- Trivers, R. L. 1974. Parent-Offspring Conflict. Integrative and Comparative Biology 14:249-264.
- Tung, J., E. A. Archie, J. Altmann, and S. C. Alberts. 2016. Cumulative early life adversity predicts longevity in wild baboons. Nature Communications 7:11181.
- Twiss, S. D., C. Cairns, R. M. Culloch, S. A. Richards, and P. P. Pomeroy. 2012. Variation in Female Grey Seal (Halichoerus grypus) Reproductive Performance Correlates to Proactive-Reactive Behavioural Types PLoS ONE 7:28–30.
- Wasser, L. M., and S. K. Wasser. 1995. Environmental Variation and Developmental Rate

Among Free Ranging Yellow Baboons (Papio cynocephalus). American Journal of Primatology 35:15–30.

- Watts, H. E., J. B. Tanner, B. L. Lundrigan, and K. E. Holekamp. 2009. Post-weaning maternal effects and the evolution of female dominance in the spotted hyena. Proceedings of the Royal Society B: Biological Sciences 276:2291–2298.
- Watts, H., and K. Holekamp. 2009. Ecological determinants of survival and reproduction in the spotted hyena. Journal of Mammalogy 90:461–471.
- Webster, M. M., and A. J. W. Ward. 2010. Personality and social context. Biological Reviews 86:759–773.
- Webster, M. M., A. J. W. Ward, and P. J. B. Hart. 2007. Boldness is influenced by social context in threespine sticklebacks (Gasterosteus aculeatus). Behaviour 144:351–371.
- West-Eberhard, M. J. 2005. Phenotypic accommodation: adaptive innovation due to developmental plasticity. Journal of Experimental Zoology Part B: Molecular and Developmental Evolution 304B:610–618.
- White, P. A. 2007. Costs and strategies of communal den use vary by rank for spotted hyaenas, Crocuta crocuta. Animal Behaviour 73:149–156.
- Wienke, A. 2011. Frailty models in survival analysis. CRC Press, Boca Raton, FL.
- Wolak, M. E., D. J. Fairbairn, and Y. R. Paulsen. 2011. Guidelines for estimating repeatability. Methods in Ecology and Evolution 3:129–137.
- Wynne, C. D. L., and I. G. McLean. 1999. The comparative psychology of marsupials. Australian Journal of Psychology 51:111–116.
- Yoshida, K. C. S., P. E. Van Meter, and K. E. Holekamp. 2016. Variation among free-living spotted hyenas in three personality traits. Behaviour 153:1665–1722.