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LANDSCAPE OF STRESS: DOES DROUGHT PREVAIL OVER ANTHROPOGENIC

ACTIVITY IN INFLUENCING CORTISOL LEVELS AND FITNESS IN THE

PACIFIC FISHER?

by

Jennifer R. Kordosky

A thesis submitted in partial fulfillment of the requirements for the degree

of

MASTER OF SCIENCE

in

Wildlife Biology

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2019

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ABSTRACT

Landscape of Stress: Does Drought Prevail over Anthropogenic Activity in Influencing Cortisol Levels and Fitness in the Pacific Fisher?

by

Jennifer R. Kordosky, Master of Science

Utah State University, 2019

Major Professor: Dr. Eric M. Gese Department: Wildland Resources

Climate change and anthropogenic modifications to the landscape are known to have effects on different wildlife species. The Pacific fisher (*Pekania pennanti*) is a threatened mesocarnivore in the Sierra Nevada Mountains of California. They occupy a relatively small geographic area in the Sierra National Forest, because of their preference for late-successional forest. Once found statewide, their numbers declined dramatically in the 1900s due to trapping, habitat fragmentation, and human development. More recently, drought induced by climate change may be stressing this isolated population. Cortisol levels can be used as an indication of an individual's physiological response to its environment. By collecting samples of fisher hair and measuring an individual's cortisol level, we examined the physiological stress state of fishers across a landscape of various human disturbances (housing density, road density, and silvicultural treatments), and drought response as measured by tree mortality in their home ranges in the central Sierra Nevada Mountains, California. We examined these metrics of human disturbances and drought within a fisher home range, measured within the 30, 60, and 95% isopleths. Our objectives were: 1) determine what various anthropogenic modifications (mainly silvicultural treatments), human activities (road and building density), and drought response (tree mortality) influence cortisol concentrations among individuals in this population, 2) determine which habitat characteristics fishers prefer and avoid in the core of their home ranges or areas of use, and 3) examine the relationship between cortisol levels and fitness components (body condition, survival, and litter size). Using model selection, we found that levels of tree mortality within a fisher's home range significantly influenced cortisol levels across all 3 home range estimators. The various metrics of human disturbances had a smaller effect on cortisol levels. We also found that survival rates were highest when cortisol levels were lowest in female fishers, with lower survival at medium and high cortisol levels. With the recent drought and subsequent tree mortality being >80% in some areas of our study, cortisol levels could continue to increase, potentially leading to decreased increments of fitness within individual fishers. Furthermore, continued tree mortality may lead to a decrease of late-successional forests that are preferred by fishers.

(129 pages)

PUBLIC ABSTRACT

Landscape of Stress: Does Drought Prevail over Anthropogenic Activity in Influencing Cortisol Levels and Fitness in the Pacific Fisher?

Jennifer R. Kordosky

Fishers (Pekania pennanti) are a species of concern in the Sierra Nevada Mountains of California. Cortisol is a glucocorticoid hormone released to mobilize energy in response to stress and has been used as an indication of an individual's physiological response to its environment. By collecting samples of fisher hair and measuring an individual's cortisol, we examined the physiological stress response of the animals to human disturbances (housing density, road density, habitat type, and silvicultural treatements) and drought (tree mortality) in their home ranges. Using AICc model selection, we found that levels of tree mortality within a fisher's home range significantly influenced cortisol levels. Various human disturbances had a smaller effect on cortisol levels. Furthermore, we examined the relationship between cortisol and fitness through the metrics of body condition, female kit counts, and survival. We found that females with low cortisol had significantly higher survival rates than females with medium and high cortisol. With the recent drought, bark beetle infestation and subsequent tree mortality being >80% in some areas of our study, cortisol levels could continue to increase, potentially leading to further decreased fitness within this fisher population. We also examined the difference in habitat selection between the core and entirety of the home ranges and found that fishers prefer late-successional forest in the core of their home ranges.

ACKNOWLEDGMENTS

I want to start by giving my sincerest thanks to Eric Gese for taking me on as a graduate student and helping me through this entire process. His depth of knowledge and experience has been incredibly valuable for my growth as a scientist. I would also like to thank Craig Thompson for initially coming up with this project and having the confidence in me to complete it. Susannah French and Lori Neuman-Lee have been irreplaceable in their knowledge of physiology. They have taught me so much, and I could not have completed this without their help and friendship. Patricia Terletzky helped me through the statistics, ArcGIS, and coding. She was a valuable resource throughout this process.

Along with the support of my committee members and colleagues at Utah State University, many people in the Kings River and Sugar Pine study areas worked tirelessly to gather data and help me succeed. Rebecca Green helped me with so many concepts and ideas that I cannot begin to thank her enough. The crew leader of the Kings River Project, Nathan Hebert, was amazing at motivating the crew to complete all the field tasks that needed to be done and collect as much data as possible. I would not have survived the summer of 2017 without the help of Jonathan Schneiderman who spent hours helping me with ArcGIS, R, and Access to properly format and gather all the data that I needed. This project would not have been feasible without the year round effort of the technicians to gather data: Tessa Smith, Bradly Nichols, Jordan Latter, Laura Van Vranken, Logan Moon, Jordan Heiman, Leah Kerschner, Robin Eng, Erin Wood, Chloe Burt, Brandon Swanson, and Emerson Paton. Without funding it is impossible to complete any research project. I would like to thank the Pacific Southwest Research Station, the United States Forest Service, the United States Department of Agriculture-National Wildlife Research Center, and Utah State University for the funding they have given me.

More than anything I would like to thank my friends and family. I would not have been able to succeed without their love and support. My undergraduate professor Jeff Duerr took me under his wing as a research student and taught me the basics of hormone research and proper laboratory technique. Without him, I would not have been given the opportunity to work on this project in the first place. My sister, Amy Kordosky, was always there to share the collective woes of graduate school. My best friend, Audrey Miller, gave constant perspective and helped me to stay focused and driven throughout this process. My grandparents fostered my love of nature and the outdoors from a young age, and I cannot thank them enough for this gift. Finally, my partner Charlie Beaudoin has supported me mentally, emotionally, and financially through this long process. Without his love and support, I would not have been able to succeed. Thanks to all my old friends and new friends that I have met along the way.

Jennifer R. Kordosky

Page

ABSTRACTiii
PUBLIC ABSTRACTv
ACKNOWLEDGMENTS vi
LIST OF TABLESx
LIST OF FIGURES xii
CHAPTER
1. INTRODUCTION1
LITERATURE CITED5
2. LANDSCAPE OF STRESS: DOES DROUGHT PREVAIL OVER ANTHROPOGENIC ACTIVITY IN INFLUENCING CORTISOL LEVELS AND FITNESS IN THE PACIFIC FISHER?
ABSTRACT
3. THE LINK BETWEEN CORTISOL AND FITNESS: HOW STRESS IS INFLUENCING THE FITNESS OF THE PACIFIC FISHER IN AN EVER-CHANGING FOREST
ABSTRACT

4.	CONCLUSIONS	
	LITERATURE CITED	

LIST OF TABLES

Table		Page
2.1.	Various metrics (mean \pm SD) of human disturbance, habitat, and drought response across the 30%, 60%, and 95% kernel estimates for areas of use for male fishers, Sierra National Forest, California, 2014-2016; significant differences between kernel estimators denoted with *.	54
2.2.	Various metrics (mean \pm SD) of human disturbance, habitat, and climate change across the 30%, 60%, and 95% kernel estimates for areas of home ranges of female fishers, Sierra National Forest, California, 2014-2016; significant differences between kernel estimators denoted with *.	55
2.3.	Top 10 ranked models for the relationship between individual cortisol levels and levels of various metrics of disturbance, habitat, and drought response within the 30% kernel home range of female fishers, Sierra Nevada Mountains, California, 2014-2016; models with >0.1 weight denoted by *	56
2.4.	Top 10 ranked models for the relationship between individual cortisol levels and levels of various metrics of disturbance, habitat, and drought response within the 60% kernel home range of female fishers, Sierra Nevada Mountains, California, 2014-2016; models with >0.1 weight denoted by *	57
2.5.	Top 10 ranked models for the relationship between individual cortisol levels and levels of various metrics of disturbance, habitat, and drought response within the 95% kernel home range of female fishers, Sierra Nevada Mountains, California, 2014-2016; models with >0.1 weight denoted by *	58
2.6.	Top 10 ranked models for the relationship between individual cortisol levels and levels of various metrics of disturbance, habitat, and drought response within the 30% kernel area of use for male fishers, Sierra Nevada Mountains, California, 2014-2016; models with >0.1 weight denoted by *	59
2.7.	Top 10 ranked models for the relationship between individual cortisol levels and levels of various metrics of disturbance, habitat, and drought response within the 60% kernel area of use for male fishers, Sierra Nevada Mountains, California,	

	2014-2016; models with >0.1 weight denoted by *
2.8.	Top 10 ranked models for the relationship between individual cortisol levels and levels of various metrics of disturbance, habitat, and drought response within the 95% kernel area of use for male fishers, Sierra Nevada Mountains, California,
	2014-2016; models with >0.1 weight denoted by *
3.1.	Annual survival rate of female and male fishers in the Kings River and Sugar Pine study areas, Sierra National Forest, California, 2014-2016; 95% confidence interval in parentheses
3.2.	Annual survival rates of male and female fishers as a function of their level of cortisol, Sierra National Forest, California, 2014-2016. Levels of cortisol were low (<0.12 pg/mg), medium (0.121-0.189 pg/mg), and high (>0.190 pg/mg); 95% confidence intervals in parentheses

xi

LIST OF FIGURES

Figure		Page
2.1.	Various metrics of landscape attributes within the 30, 60, and 95% areas of use for male fishers, including (A) building density (# buildings/km ²), B) percent open water, (C) percent low canopy cover, and (D) percent developed habitat type, Sierra National Forest, California, 2014-2016.	62
2.2.	Various metrics of landscape attributes within the 30, 60, and 95% kernel home ranges of female fishers, including (A) building density (# buildings/km ²), (B) percent conifer forest, (C) percent granite, and (D) percent hardwood, Sierra National Forest, California, 2014-2016	63
2.3.	Various metrics of landscape attributes within the 30, 60, and 95% kernel home ranges of female fishers, including (A) percent hardwood-conifer, (B) percent dense canopy cover, and (C) percent low canopy cover, Sierra National Forest, California, 2014-2016	64
2.4.	Relationship between cortisol levels in their hair and the amount of tree mortality in the 30%, 60%, and 95% kernel home ranges for individual female fishers, Sierra National Forest, California, 2014-2016	65
2.5.	Relationship between cortisol levels in their hair and the amount of tree mortality in the 30 and 60% areas of use for male fishers, Sierra National Forest, California, 2014-2016.	66
2.6.	Relationship between cortisol levels in their hair and the amount of tree mortality + road density in the 95% kernel areas of use for individual male fishers, Sierra National Forest, California, 2014-2016.	67
2.7.	Level of tree mortality (trees/acre) in 2015 and 2016, Sierra National Forest, California.	68
3.1.	Cortisol levels (pg/mg) among juvenile, subadult, and adult male fishers in the Sierra National Forest, California, 2014-2016.	97
3.2.	Cortisol levels (pg/mg) for fishers in 2014, 2015, and 2016 in the Sierra National Forest, California, 2014-2016.	98
3.3.	Cortisol levels (pg/mg) in fishers in the Kings River and Sugar Pine study areas, Sierra National Forest, California, 2014-2016.	99

3.4. The level of tree mortality during 2015 and 2016, Sierra National Forest,

	California, 2015-2016.	100
3.5.	Annual survival rates of female fishers as a function of their level of cortisol, Sierra National Forest, California, 2014-2016. Cortisol levels were low (<0.12 pg/mg), medium (0.121-0.189 pg/mg), and high (>0.190 pg/mg); error bars represent 95% confidence interval	101

xiii

CHAPTER 1

INTRODUCTION

Climate change and anthropogenic modifications to the landscape can have direct and indirect effects on wildlife populations (Wingfield 2008, Rangel-Negrin et al. 2009, Wingfield 2013). As climate change and anthropogenic alterations expand throughout the world, it becomes increasingly important to monitor threatened and endangered species that may be affected by the constantly changing environment (Hoegh-Guldberg et al. 2008). Climate change has been shown to increase the severity and frequency of droughts and insect outbreaks (Dale et al. 2001, Allen et al. 2010) with the potential to influence tree health. Extreme environmental factors such as temperature or reduced precipitation through climate change, can act as stressors that challenge physiological systems in animals (Gunderson and Stillman 2015) and can potentially decrease measures of fitness (Koolhaas et al. 1999, Mostl and Palme 2002, Bonier et al. 2009). Concurrently, anthropogenic landscape alterations can influence stress levels for many species (French et al. 2010). In the Sierra Nevada Mountains of California, a 4-year drought has drastically impacted the forest through tree mortality followed by increased fire activity and severity (Bart et al. 2016). In addition, anthropogenic changes to the forest landscape, such as expansion of developed areas, logging, prescribed burning, and restoration activities, have created a highly fragmented and constantly changing forest (Franklin and Fites-Kaufmann 1996, Heilman et al. 2002). The increase in human activity of this forest has led to the question of whether anthropogenic alteration or climate change through drought is directly affecting this native mammal community and creating a "landscape of stress" for animals living in this ecosystem.

Physiological stress in wildlife populations is commonly measured through cortisol concentrations (Davenport et al. 2006, Dantzer et al. 2014). Cortisol is a glucocorticoid hormone released to mobilize energy in response to stress (Boonstra 2013). The relationship between glucocorticoids and animal stress has been well established (Romero 2004, French et al. 2010, Neuman-Lee et al. 2017b), and has been successfully measured in numerous species, from garter snakes (Taricha granulosa; Neuman-Lee et al. 2017a) to polar bears (Ursus maritimus; Mislan et al. 2016, Neuman-Lee et al. 2017b). Wild populations exposed to constant environmental stressors can have prolonged elevations in levels of cortisol (Sheriff et al. 2011, Boonstra 2013, Wingfield 2013, Dantzer et al. 2014) resulting in decreased fitness (Tilbrook et al. 2000, Schneiderman et al. 2005, Glaser and Kiecolt-Glaser 2005, Blaustein et al. 2012, Crespi et al. 2013, Dantzer et al. 2013). Environmental stressors can be measured with a number of endocrine-related endpoints (McCormick and Romero 2017). Three different sample types are commonly used to measure cortisol in mammals: blood, scat, and hair (Sheriff et al. 2011, Dantzer et al. 2014, Narayan 2015). While cortisol in blood and scat are accurate measurements of acute stress, they are not suitable for measuring chronic stress or baseline stress (Narayan 2015). Cortisol in the blood is generally considered ephemeral and represents stress from the prior seconds to minutes, while cortisol in the scat may represent a stress response from the preceding hours or days (Narayan 2015). In contrast, cortisol in hair can indicate an accumulation of physiological responses over months depending on growth and molt cycles (Tallo-Parra et al. 2016), suggesting it could be used as a measure of chronic stress.

Fishers (*Pekania pennanti*) are mesocarnivores in the mustelid family that depend largely on old-growth forest for hunting, denning, and shelter (Douglas and Strickland 1987, Thompson et al. 2011, Zielinski et. al. 2013) and could serve as a bioindicator species giving insight to the qualitative health of their ecosystem (Roemer et al. 2009). The historic range of fishers in California had been described as a continuous arc from the Coast Range eastward to the southern Cascades, then south through the Sierra Nevada Mountains (Grinnell et al. 1937). However, due to fur trapping, forest fragmentation, and habitat loss, fishers now "persist at a very low density" in the northern Sierras (Schempf and White 1977). Fisher populations in California declined in the 1940s and they were listed as a species of concern when fur trapping reduced their population close to extinction (Hall 1942). Concerns for their population size resulted in a ban on trapping in 1946 (Lewis and Zielinski 1996) from which fishers have never fully recovered. For these reasons, fishers were petitioned for listing under the U.S. Endangered Species Act in 1990 (Long et al. 2014). Currently the southern Sierra Nevada population is small at <300 adults (Spencer et al. 2011, Tucker et al. 2017) and has been genetically isolated for many years (Knaus et al. 2011, Tucker et al. 2012, Tucker et al. 2017). Presently, factors potentially limiting recovery of the Pacific fisher include climate change and anthropogenic modifications to the landscape (U.S. Fish and Wildlife Service 2014).

Concerns over persistently low population numbers and conflict between fisher population persistence and fuel reduction efforts on national forests led to the initiation of two long-term studies in 2007 in the Sierra Nevada Mountains of California to investigate the general ecology of fishers and monitor population fluctuations (Thompson et al. 2011, Zielinski et al. 2013, Wengert et al. 2014). Over 9 years, these studies radio-collared and monitored >250 fishers to determine habitat use, survival and reproduction (Thompson et al. 2011). Results of these studies have shown that fishers prefer late-successional forests of dense tree cover within multi-layered old growth stands (Purcell et al. 2009, Thompson et al. 2011) suggesting that alteration of the landscape through human disturbance, management activities, habitat fragmentation, and climate change could negatively influence this isolated population of fishers. Despite these predictions, previous studies have shown that management activities influence fishers, but do not cause them to re-orient their home ranges (Zielinski et al. 2013).

Continuously high cortisol levels (i.e., chronic stress) have been shown to influence animal fitness by negatively affecting reproductive abilities or output (Tilbrook et al. 2000, Love and Williams 2008, Ebensperger et al. 2013), increasing vulnerability to different diseases (Schneiderman et al. 2005, Glaser and Kiecolt-Glaser 2005, Blaustein et al. 2012), and reducing immune function (Bonier et al. 2009, Crespi et al. 2013, Dantzer et al. 2013). Furthermore, decreased immune function has the potential to lead to decreased survival rates (Romero et al. 2009). However, higher cortisol levels alone are not always linked to decreased fitness (Bonier et al. 2009). Fitness must be examined along with cortisol in order to determine whether cortisol levels are influencing the health of the individual. A few common fitness components are clutch size and body mass to length ratio (Jakob et al. 1996, Godfray et al. 1991). Survival can also be used as a measure of fitness (Gaillard et al. 2000) as individuals with decreased fitness may have increased difficulty persisting on a landscape.

This study examines the relationship between environmental disturbances and physiological stress among the Pacific fisher. Our objectives were: 1) determine what various anthropogenic modifications (mainly silvicultural treatments), human activities (road and building density), and drought response (tree mortality) influence cortisol concentrations among individuals in this population, 2) determine which habitat characteristics fishers prefer and avoid in the core of their home ranges or areas of use, and 3) examine the relationship between individual cortisol levels and measures of individual fitness components to determine the effects of chronic stress on fitness. We measured all metrics of anthropogenic factors and drought response across 3 spatial scales (30, 60, 95% isopleths) of home-range use for each individual. The results of this study may demonstrate how various factors are contributing to a "landscape of stress" and how cortisol levels may be useful to monitor the physiological stress response of a species of concern.

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CHAPTER 2

LANDSCAPE OF STRESS: DOES DROUGHT PREVAIL OVER ANTHROPOGENIC ACTIVITY IN INFLUENCING CORTISOL LEVELS IN THE PACIFIC FISHER?

ABSTRACT

Climate change and anthropogenic modifications to the landscape are known to have effects on different wildlife species, including the Pacific fisher (Pekania pennanti). Fishers are a threatened mesocarnivore in the Sierra Nevada Mountains of California. They occupy a relatively small geographic area in the Sierra National Forest, because of their preference to late-successional forest. Once found statewide, their numbers declined dramatically in the 1900s due to trapping, habitat fragmentation, and human development. More recently, drought induced by climate change may be stressing this isolated population. Cortisol levels can be used as an indication of an individual's physiological response to its environment. By collecting samples of fisher hair and measuring an individual's cortisol level, we examined the physiological stress state of fishers across a landscape of various human disturbances (housing density, road density, and silvicultural treatments), and drought response as measured by tree mortality in their home ranges in the central Sierra Nevada Mountains, California. We examined these metrics of human disturbances and drought within a fisher home range, measured within the 30, 60, and 95% isopleths. Our objectives were: 1) determine what various anthropogenic modifications (mainly silvicultural treatments), human activities (road and building density), and drought response (tree mortality) influence cortisol concentrations among individuals in this population, and 2) determine which habitat characteristics

fishers prefer and avoid in the core of their home ranges or areas of use. Using model selection, we found that levels of tree mortality within a fisher's home range significantly influenced cortisol levels across all 3 home range estimators. The various metrics of human disturbances had a smaller effect on cortisol levels. With the recent drought and subsequent tree mortality being >80% in some areas of our study, cortisol levels could continue to increase, potentially leading to decreased increments of fitness within individual fishers. Furthermore, continued tree mortality may lead to a decrease of late-successional forests that are preferred by fishers.

INTRODUCTION

Climate change and anthropogenic modifications to the landscape can have direct and indirect effects on wildlife populations (Wingfield 2008, Rangel-Negrin et al. 2009, Wingfield 2013). As climate change and anthropogenic alterations expand throughout the world, it becomes increasingly important to monitor threatened and endangered species that may be affected by the constantly changing environment (Hoegh-Guldberg et al. 2008). Climate change has been shown to increase the severity and frequency of droughts and insect outbreaks (Dale et al. 2001, Allen et al. 2010) with the potential to influence tree health. Extreme environmental factors such as temperature or reduced precipitation through climate change, can act as stressors that challenge physiological systems in animals (Gunderson and Stillman 2015) and can potentially decrease measures of fitness (Koolhaas et al. 1999, Mostl and Palme 2002, Bonier et al. 2009). Concurrently, anthropogenic landscape alterations can influence stress levels for many species (French et al. 2010). In the Sierra Nevada Mountains of California, a 4-year drought has drastically impacted the forest through tree mortality followed by increased fire activity and severity (Bart et al. 2016). In addition, anthropogenic changes to the forest landscape, such as expansion of developed areas, logging, prescribed burning, and restoration activities, have created a highly fragmented and constantly changing forest (Franklin and Fites-Kaufmann 1996, Heilman et al. 2002). The increase in human activity of this forest has led to the question of whether anthropogenic alteration or climate change through drought is directly affecting the native mammal community and creating a "landscape of stress" for animals living in this ecosystem.

Physiological stress in wildlife populations is commonly measured through cortisol concentrations (Davenport et al. 2006, Dantzer et al. 2014). Cortisol is a glucocorticoid hormone released to mobilize energy in response to stress (Boonstra 2013). The relationship between glucocorticoids and animal stress has been well established (Romero 2004, French et al. 2010, Neuman-Lee et al. 2017b), and has been successfully measured in numerous species, from garter snakes (*Taricha granulosa*; Neuman-Lee et al. 2017a) to polar bears (Ursus maritimus; Mislan et al. 2016, Neuman-Lee et al. 2017b). Wild populations exposed to constant environmental stressors can have prolonged elevations in levels of cortisol (Sheriff et al. 2011, Boonstra 2013, Wingfield 2013, Dantzer et al. 2014) resulting in depressed reproductive abilities (Tilbrook et al. 2000), reduced immune function (Crespi et al. 2013, Dantzer et al. 2013), and increased vulnerability to different diseases (Schneiderman et al. 2005, Glaser and Kiecolt-Glaser 2005, Blaustein et al. 2012). Environmental stressors can be measured with a number of endocrine-related endpoints (McCormick and Romero 2017). Three different sample types are commonly used to measure cortisol in mammals: blood, scat, and hair (Sheriff

et al. 2011, Dantzer et al. 2014, Narayan 2015). While cortisol in blood and scat are accurate measurements of acute stress, they are not suitable for measuring chronic stress or baseline stress (Narayan 2015). Cortisol in the blood is generally considered ephemeral and represents stress from the prior seconds to minutes, while cortisol in the scat may represent a stress response from the preceding hours or days (Narayan 2015). In contrast, cortisol in hair can indicate an accumulation of physiological responses over months depending on growth and molt cycles (Tallo-Parra et al. 2016), suggesting it could be used as a measure of chronic stress.

Fishers (Pekania pennanti) are mesocarnivores in the mustelid family that depend largely on old-growth forest for hunting, denning, and shelter (Douglas and Strickland 1987, Thompson et al. 2011, Zielinski et. al. 2013) and could serve as a bioindicator species giving insight to the qualitative health of their ecosystem (Roemer et al. 2009). The historic range of fishers in California had been described as a continuous arc from the Coast Range eastward to the southern Cascades, then south through the Sierra Nevada Mountains (Grinnell et al. 1937). However, due to fur trapping, forest fragmentation, and habitat loss, fishers now "persist at a very low density" in the northern Sierras (Schempf and White 1977). Fisher populations in California declined in the 1940s and they were listed as a species of concern when fur trapping reduced their population close to extinction (Hall 1942). Concerns for their population size resulted in a ban on trapping in 1946 (Lewis and Zielinski 1996) from which fishers have never fully recovered. For these reasons, fishers were petitioned for listing under the U.S. Endangered Species Act in 1990 (Long et al. 2014). Currently the southern Sierra Nevada population is small at <300 adults (Spencer et al. 2011, Tucker et al. 2017) and has been genetically isolated for

many years (Knaus et al. 2011, Tucker et al. 2012, Tucker et al. 2017). Presently, factors potentially limiting recovery of the Pacific fisher include climate change and anthropogenic modifications to the landscape (U.S. Fish and Wildlife Service 2014).

Concerns over persistently low population numbers and conflict between fisher population persistence and fuel reduction efforts on national forests led to the initiation of two long-term studies in 2007 in the Sierra Nevada Mountains of California to investigate the general ecology of fishers and monitor population fluctuations (Thompson et al. 2011, Zielinski et al. 2013, Wengert et al. 2014). Over 9 years, these studies radio-collared and monitored >250 fishers to determine habitat use, survival and reproduction (Thompson et al. 2011). Results of these studies have shown that fishers prefer late-successional forests of dense tree cover within multi-layered old growth stands (Purcell et al. 2009, Thompson et al. 2011) suggesting that alteration of the landscape through human disturbance, management activities, habitat fragmentation, and climate change could negatively influence this isolated population of fishers. Despite these predictions, previous studies have shown that management activities influence fishers, but do not cause them to re-orient their home ranges (Zielinski et al. 2013).

This study examines the relationship between environmental disturbances and physiological stress among the Pacific fisher. Our objectives were: 1) determine what various anthropogenic modifications (mainly silvicultural treatments), human activities (road and building density), and drought response (tree mortality) influence cortisol concentrations among individuals in this population, and 2) determine which habitat characteristics fishers prefer and avoid in the core of their home ranges or areas of use. We measured all metrics of anthropogenic factors and drought response across 3 spatial scales (30, 60, 95% isopleths) of home-range use for each individual. The results of this study may demonstrate how various factors are contributing to a "landscape of stress" and how cortisol levels may be useful to monitor the physiological stress response of a species of concern.

METHODS AND MATERIALS

Study Area

This study was conducted in the Sugar Pine (SP) and Kings River (KR) study areas located in the Sierra Nevada Mountains of central California. The SP study area was located near Oakhurst, California, and was approximately 800-km² in size, while the 450-km² KR study area was located near Shaver Lake, California. The KR study area was located approximately 11 km SE of the SP study area. During the summer, precipitation was very rare in this region. Temperatures averaged highs of 23°C and lows of 9°C in the summer months

(https://weather.com/weather/monthly/l/Shaver+Lake+CA+93664:4:US). During the winter months snow accumulation was typical throughout the Sierra Nevada Mountains, with the majority of both study areas having a layer of snow from November-April of each year. During these winter months temperatures averaged highs of 7°C and lows of -4°C (https://weather.com/weather/monthly/l/Shaver+Lake+CA+93664:4:US).

Both study areas were similar in elevation from 915 to 2,385 m (Purcell et al. 2009). Lower elevations were dominated by montane hardwood-conifer forest (Allen-Diaz et al. 2007) consisting primarily of ponderosa pine (*Pinus ponderosa*), manzanita (*Arcostaphylos spp.*), California black oak (*Quercus kelloggii*), and canyon live oak (*Q*. *chrysolepis*) with a dense shrub cover understory (Allen-Diaz et al. 2007, Keeley and Davis 2007). The dominant shrubs were manzanita, white thorn (*Ceanothus leucodermis*), and bear clover (*Chamaebatia foliolosa*). Mid-elevations consisted of mixed conifer habitats, dominated by white fir (*Abes concolor*), ponderosa pine, sugar pine (*P. lambertiana*), and California black oak (Fites-Kaufman et al. 2007). The highest elevation areas were primarily non-vegetated granite outcrops with little shrub cover and a few trees, mostly sugar pine, Jeffery pine (*P. jeffreyi*), and white fir (Fites-Kaufman et al. 2007). The combination of an extended 4-year (2012-2015) drought and infestation of pine beetle (*Dendroctonus ponderosae*) has resulted in extensive tree mortality of ponderosa pine and other coniferous species with large areas of dead or dying mature trees with tree mortality numbers reaching hundreds of dead trees per km² (Young et al. 2017).

Trapping and Monitoring

For three trapping seasons since the fall of 2014 (2014-2015, 2015-2016, 2016-2017), hair samples from both male and female fishers have been collected from the KR and SP study areas during capture and radio-collaring operations. Trapping typically occurred from September through March to minimize black bear (*Ursus americanus*) disturbance and to prevent separating fisher females from newborn kits in the spring. Fishers were trapped with live-traps (81.28 x 25.4 x 30.48 cm, original series model 108, Tomahawk Live Trap, Hazelhurst, WI) (Green et al. 2018) with wooden cubby shelters (Wilbert 1992, Seglund 1995). Traps were covered with natural materials for cover and baited with chicken and bait lure (Hawbaker's Fisher Lure, Hawbaker and Sons, Fort Loudon, PA, or Fisher Red Lure, Proline Lures, Indianapolis, IN) (Green et at. 2018). Distance lures (Caven's Gusto, Minnesota Trapline Products, Pennock, MN or Outreach Call Lure, Proline Lures, Indianapolis, IN) (Green et al. 2018) were also used to draw animals in from farther distances. We handled fishers using a canvas sleeve and metal handling cone (Seglund 1995). Animals were sedated with an intramuscular injection of ketamine hydrochloride (22.5 mg/kg) mixed with Diazapam or Midazolam (0.125 mg/kg), with dosages based on sex and estimated age to estimate body mass. We collected hair samples when shaving the hair in preparation for a blood draw from the jugular vein. Hair samples were stored individually at room temperature in sealed paper envelopes until analysis.

During processing, we measured body length (cm), weight (kg), tail length (cm), teeth length (mm), and reproductive status through teat condition and testicular size. Fishers were fitted with a VHF radio-collar weighing 31g (Holohil model mI-2M, Holohil Systems Ltd., Carp, Ontario, Canada) with a handmade breakaway (Thompson et al. 2011). They were also injected with a Passive Integrated Transponder (Biomark, Boise, ID) for future identification and tracking (Green et al. 2018). Animals were captured and handled under authorization of the United States Forest Service with permits from the California Department of Fish and Wildlife with appropriate institutional animal care and use committee (IACUC) review and approval.

Home Range Determination

During each trapping season (fall 2014 to spring 2017), one hair sample was collected from each captured fisher. Fishers replace their fur from September through early November (Douglas and Strickland 1987), therefore accumulation of cortisol in the hair occurs during this time period. Thus, to associate the level of cortisol in a hair sample with the appropriate time period when cortisol was laid down in the hair, we used the annual home range for each fisher for the year prior to hair collection. Annual home ranges were determined by relocating animals, which included a combination of radiotelemetry locations, rest sites, flight locations, and den sites (Green et al. 2018). The error for these locations varied but were distributed similarly among all fishers and therefore any bias was weighted equally among all individuals. The main difference was male fishers use a larger spatial area and do not use den sites, while females raise the young independently. Due to these spatial and behavioral differences, we conducted separate analyses for males and females.

Rest sites were defined as a single structure in which an individual was located (i.e., tree, snag, burrow). Rest areas were defined as an area (within 50 m) in which an individual was located, but could not be narrowed down to one specific structure (Green et al. 2018). Locations (triangulations, rest sites, and rest areas) of female fishers were obtained approximately every 3 days, with rest site locations and rest areas were collected opportunistically when searching an area. Den locations were added into the data set once for every 3 days a female was at a den; this was based on how often female fishers are typically relocated, and these locations were subsampled as to not skew the home range estimators towards these den sites.

We used R (adehabitatHR package; version 3.3.0) in R studio (version 1.0.136, R Development Core Team, 2016)) and ArcGIS 10.5 (ESRI, Redlands, CA, USA) (Harris et al. 2008) to determine annual home ranges with 30%, 60%, and 95% kernel estimators (Worton 1989). An area-observation curve (Laver and Kelly 2008) was calculated for each female and male fisher to determine the number of points needed to accurately encompass their home range. We determined that 25-30 points adequately described a female fisher's home range. In contrast, the area-observation curves for the males did not reach an asymptote. Therefore, the areas the males inhabited were defined as "areas of use" as opposed to home ranges.

Human Disturbances

Previous studies have shown that habitat fragmentation and human activity can increase stress levels in animals (Rangel-Negrin et al. 2009, French et al. 2010), therefore we examined the influence of various human disturbances on cortisol levels in fishers. Broadly, these disturbances were related to either human activities (e.g., housing and road density) or management activities (i.e., silvicultural practices such as logging, thinning). Many silvicultural activities can have a significant effect on fisher habitat structure (Zielinski et al. 2013) and can alter forest structure from their preferred habitat type of late-successional forest. In contrast, human activities generally increase habitat fragmentation. To relate levels of human disturbances within an individual's home range with the associated cortisol level in their hair sample, we compiled several metrics of human disturbance.

Data layers showing where various silvicultural treatments (i.e., logging, thinning, burning, regeneration) had occurred were obtained for both study areas over the 3 years of sampling from the Forest Service Activity Tracking System (FACTS) database through the U.S. Department of Agriculture

(https://data.fs.usda.gov/geodata/edw/datasets.php). These records of management actions since November 2013 (the year before this study began) were used to examine the relationship between management activity and fisher cortisol levels. Zielinski et al. (2013) defines which management activities impact fisher habitat. Of these impactful activities, 6 occurred within both study areas and included: (1) thinning of natural fuels, (2) commercial thinning, (3) full planting without concurrent site preparation, (4) fill-in re-planting without concurrent site preparation, (5) individual tree release and weeding, and (6) pre-commercial thinning of individual or selected trees. The area of each of these management activities (m²) was calculated for each female home range or male area of use, and standardized to m² of activity per km² of home range or area of use to determine the proportion of land each activity covered for each home range during each year. We used m² for the area of management activities instead of km² because some management activity areas were very small. Though some management activities may have occurred before the year in question, they still persist on the landscape having a continual presence and effect on the forest.

For the human activities, locations of buildings and roads were obtained for both study areas over the 3 years of the study from the U.S. Forest Service. We examined the relationship between cortisol levels and the density of buildings (# buildings/km²) and roads within a female fisher's home range or a male fisher's area of use at each kernel level (i.e., 30, 60, and 95% kernels). Some limitations were found with the building density layer, as not all private property buildings were accounted for. However, there was limited private land with buildings, thus we concluded their influence was minimal. We divided total road length (m) contained within each home range or area of use by home range size (km²) to determine the density of roads in home ranges or areas of use. We used m for road length instead of km because some roads were <1km.

Habitat Characteristics

Rangel-Negrin et al. (2009) showed that habitat fragmentation and landscape variation can alter cortisol levels in mammals. We examined 2 habitat characteristics (canopy cover, habitat type) for both study areas over the 3 years as a measure of habitat quality for fishers. We obtained canopy cover and habitat type data from the Land Fire database through the U.S. Forest Service (USFS; 2014 U.S. Department of Interior, Geological Survey). We delineated 8 habitat types: conifer forest, hardwood forest, mixed hardwood-conifer forest, granite, water, developed land, shrubland, and sparse cover. Shrubland consisted of areas that were shrub dominated where fishers would have cover from predation risk and access to small mammals. Sparse cover referred to herbaceous ground cover where prey may be abundant but cover from predation was limited. We measured habitat type as the percentage of land occupied per home range or area of use at each kernel estimator.

In late-successional forest, dense canopy cover has previously been characterized as $\geq 60\%$ canopy cover (Thompson et al. 2011). Therefore, canopy cover was divided into 3 classes: $\geq 60\%$ (dense), 40-59% (moderate), and $\leq 40\%$ (low). We calculated the percentage of each canopy coverage category (i.e., dense, moderate, low) for each home range or area of use at each kernel estimator.

Tree Mortality

Previous studies have shown that climate change can influence cortisol in mammals (Jenssen 2006, Gunderson and Stillman 2015). Tree mortality due to drought and beetle infestation is a symptom of climate change (Dale et al. 2001, Allen et al. 2010) that is present in the Sierra Nevada Mountains. As global temperatures continue to rise as they are expected to (Hansen et al. 2006), drought severity and frequency will continue to increase (Dale et al. 2001, Allen et al. 2010), possibly leading to the further decline of trees and the forest.

Both study areas were impacted by the 4-year (2012-2015) drought with subsequent high levels of tree mortality due to drought and bark beetle infestation in later years of the study. Tree mortality was considered a measurement of climate change because it was the direct result of the infestation of bark beetles and drought stress (Young et al. 2017), and climate change is increasing the severity and frequency of droughts (Dale et al. 2001, Allen et al. 2010). We obtained tree mortality data from the USDA R5 Remote Sensing Lab

(https://www.fs.usda.gov/detail/catreemortality/toolkit/?cid=fseprd498067) and

intersected this data layer with the home ranges and areas of use using Arc GIS. Tree mortality was measured as the number of dead trees per acre. Although acres are not a measurement that is typically used in the scientific literature, we used acres as this is a measurement foresters generally use and we wanted this information to be applicable to them. Data on levels of tree mortality were used for the years 2015 and 2016 (tree mortality had not yet occurred previously in 2014) to examine any influence of tree mortality on cortisol levels of individual fishers. Our prediction was that animals with higher levels of tree mortality in their home range or area of use would exhibit higher cortisol levels. We also predicted that tree mortality would be higher within the 95% kernel estimator than at the 30% kernel estimator as the core area would be the area in which fishers seek refuge in the least disturbed habitat.

Sample Extractions and Radioimmunoassay

We analyzed the hair samples collected from the fishers at Utah State University. Hair samples were washed three times with high performance liquid chromatography (HPLC)-grade methanol to remove any dirt and waste from the hair. After the samples were air dried, they were weighed and placed in a vial with a 7-mm steel grinding ball (Restch ball mill 201MM, Retsch GmbH, Haan, Germany), and ground into a fine homogenous powder (Davenport et al. 2006); samples were ground for 20 minutes at 30Hz. Balls and vials were cleaned thoroughly with ethanol and dried between samples. The ground hair was weighed and transferred to an Eppendorf tube. We added 3mL of methanol to the sample, vortexed for 1 minute, placed samples on a slow vortexer for 24 hours. Next, we centrifuged for 10 minutes at 2000 rpm, and 1.5 mL of supernatant was removed and placed in a clean glass tube. Samples were dried under a stream of nitrogen and resuspended in an assay dilutant (Neuman-Lee et al. 2017b). Samples were stored in assay dilutent until analysis (Martin and Réale 2008).

We assayed hair samples using a radioimmunoassay (RIA) in duplicate for cortisol 3 antibody (Fitzgerald 20-CR50) (French et al. 2010). For each sample, we used an aliquot of the re-suspended fractions to correct final sample concentration value for individual recoveries following extraction. The intra-assay variation was 4.0%. All assayed samples fell within the standard curve for the assay (i.e., were detectable values).

Statistical Analysis

Analysis of variance (ANOVA) was used to determine whether measures of disturbance were significantly different between the different kernel home range estimators (i.e., 30%, 60%, 95%). We used R (version 3.3.0) in R studio (version 1.0.136)

(R Development Core Team, 2016)) for statistical inference and data analysis. Any variables that had a p-value ≤ 0.1 were considered to be significantly different between the 30, 60, and 95% kernel home ranges or areas of use. This significance level was chosen because it is a standard p-value cutoff although not as strong as p < 0.05 (Figueiredo Filho et al. 2013) and biological significance could be lost if we used a p-value <0.05. The proportion of thinning of natural fuels, full planting without concurrent site preparation, and fill-in re-planting without concurrent site preparation data in male areas of use were not normally distributed, but a log transformation normalized the data, while a square root transformation normalized the proportion of pre-commercial thinning of individual or selected trees data. The proportion of granite, thinning of natural fuels, commercial thinning, and fill-in re-planting without concurrent site preparation in female home ranges were not normal, but a log transformation normalized the data.

We used generalized linear models (GLM) with normal distribution followed by Akaike Information Criterion (AIC) model selection (Burnham and Anderson 2002) to determine which measurements of anthropogenic modification and drought response, as well as the combination of these factors, influenced cortisol levels in fishers at each of the home range estimators (30, 60, 95% kernel). Vegetation cover and habitat type were analyzed as univariate models in their respective categories (i.e. low, medium, and high canopy cover) in a phased modeling approach (Symonds and Moussalli 2011, Anderson-Cook et al. 2015). We compared all univariate models and all possible combinations of GLMs for the six management activities conducted on the study sites to determine if the presence of multiple activities in a home range had an additive effect on cortisol levels. Two activities were restorative and four were destructive to the habitat, meaning their

additive outcome may be a null effect. By looking at all possible combinations, we were able to determine what additive effects existed and were significant to influencing cortisol levels. Also, following the phased modeling approach, the top model from the "management activities" category was moved to the global model for further analysis. The phased modeling approach was used to create a more rigorous and thoughtful approach to the analysis instead of incorporating every model into the final model. Within the phased approach, the top model from each group (% canopy cover, % habitat type, and management activity) was then moved to the global model for further analysis. Road density, building density, and tree mortality, were then added to the global model for the final model analysis. Any variables that were correlated (p < 0.05) through the cor.test function in program R were not included in the same model. In the global model, all possible combinations were analyzed. Tree mortality data was only present for 2015 and 2016, so we only used these two years for analysis in the AICc model selection for tree mortality. Male and female fishers were analyzed separately because of biologically different life histories (Moors 1980, Kilpatrick and Rego 1994).

RESULTS

Over the three winter trapping seasons of 2014, 2015, and 2016, 23 male fishers (10 from KR, 13 from SP) and 41 female fishers (22 from KR, 19 from SP) were captured with some individuals being captured multiple years for 32 male samples collected (19 from KR, 13 from SP) and 68 female samples collected (44 from KR, 24 from SP). For each year, only one sample per fisher was used in the analyses (i.e.,

multiple captures were only represented once each year). However, some animals were captured every year of the study for a total of 3 hair samples.

Metrics of Human Disturbance and Climate Change

For male fishers, we found significant differences in building density, percent developed habitat, percent open water, and percent low canopy cover among the 30, 60, and 95% kernel home ranges (Table 2.1). All other variables measured were not significantly different among the 3 kernel estimators (Table 2.1). All of the significant variables increased as the home range kernel estimator increased suggesting that males were avoiding developed habitat with low canopy cover within the core of their areas of use (i.e., the core areas appeared to represent refugia with the best habitat and least amount of human disturbance).

For females, several landscape variables were significantly different among the 30, 60, and 95% kernel estimators, including building density, percent conifer forest, percent developed habitat, percent granite, percent hardwood forest, percent hardwood-conifer forest, percent low canopy cover, and percent dense canopy cover (Table 2.2). The other landscape variables showed no significant difference among the different home range kernels (Table 2.2). Percent conifer forest and percent dense canopy cover decreased as the home range kernel size increased (Figs. 2.2B, 2.3B). Building density, percent developed habitat, percent granite, percent hardwood, percent hardwood-conifer, and percent low canopy cover all increased as home range kernel size increased (Figs. 2.2A, 2.2C, 2.2D, 2.3A, 2.3C), suggesting that female fishers were selecting against developed areas with low canopy cover and sparse forest in the core of their home ranges.

Influence of Human Disturbance and Drought Response on Cortisol Levels in Female Fishers

From the AICc model selection, we used ad hoc criteria. Although 68 samples were collected from female fishers, only 40 female samples were analyzed for the relationship between tree mortality and cortisol due to lack of tree mortality data for 2014. We constructed 63 models (but used 36 models after correlated variables were removed) examining the influence of various anthropogenic disturbances and metrics of drought response within the 30% kernel home range estimator on cortisol levels of individual female fishers. We found that for female fishers, a model containing only tree mortality had the greatest impact on cortisol (Table 2.3) with increasing cortisol levels in home ranges related to increasing tree mortality (Fig. 2.4). A second model containing % developed and tree mortality added an additional 11.2% of the weight, while a third model containing road density and tree mortality added an additional 10.2% of the weight, with the three top-ranked models carrying 47.6% of the model weight (Table 2.3). Although these models were over the threshold of a weight ≥ 0.1 , tree mortality was carrying these models and the other variables were uninformative (Arnold 2010). Tree mortality was present in all of the top-performing models, with models containing tree mortality having a combined weight of 98.4%. All other model variables were determined to have no influence on cortisol levels of female fishers within the 30% kernel home range (Table 2.3).

Within the 60% kernel home range estimator, 63 models were constructed (48 models were used after correlated variables were removed) from the 40 female samples and we found that the model containing only tree mortality was the top-ranked model for influencing cortisol levels in female fishers (Table 2.4) with cortisol levels increasing as

tree mortality increased (Fig. 2.4). The second-ranked model containing percent hardwood-conifer and tree mortality added additional weight, with the two top-ranked models carrying 39.4% of the model weight (Table 2.4). Although these models were over the threshold of a weight ≥ 0.1 , tree mortality was carrying these models and the other variables were uninformative (Arnold 2010). Tree mortality was again present in all of the top-performing models, with models containing tree mortality having a combined weight of 90.9%. All other model variables were determined to have no influence on cortisol levels of female fisher within the 60% kernel home range (Table 2.4).

Within the 95% kernel home range estimator, 19 models were constructed after removing models with correlated variables from the initial 63, and the top-ranked model contained only tree mortality as influencing cortisol levels of female fishers (Table 2.5) with cortisol levels increasing with increased tree mortality (Fig. 2.4). Three additional models containing road density and tree mortality, pre-commercial thinning of individual or selected trees and tree mortality, and building density and tree mortality also had >0.1 weight, with the four top-ranked models carrying 91.4% of the model weight; tree mortality was in each of the five top-ranked models (Table 2.5). Although these models were over the threshold of a weight \geq 0.1, tree mortality was carrying these models and the other variables were uninformative (Arnold 2010). Tree mortality was present in all but one of the top-performing models, with models containing tree mortality having a combined weight of 95.8%. All other model variables were determined to have no influence on cortisol levels of female fisher within the 95% kernel home range (Table 2.5).

Influence of Human Disturbance and Drought Response on Cortisol Levels in Male Fishers

Although 32 samples were collected from male fishers, only 20 male samples were analyzed for the relationship between tree mortality and cortisol due to lack of tree mortality data for 2014. We constructed 63 models (but only used 48 models after correlated variables were removed) examining the influence of various anthropogenic disturbances and metrics of climate change within the 30% kernel area of use estimator on cortisol levels of individual male fishers. We found that for male fishers, the topranked model contained only tree mortality (Table 2.6) with cortisol levels increasing in areas with increased tree mortality (Fig. 2.5). Two other models containing percent low canopy cover and tree mortality, and percent sparse cover and tree mortality also had >0.1 weight, with the 3 top-ranked models carrying 45.5% of the model weight (Table 2.6). Although these models were over the threshold of a weight ≥ 0.1 , tree mortality was carrying these models and the other variables were uninformative (Arnold 2010). Tree mortality was present in all of the top-performing models, with models containing tree mortality having a combined weight of 86.7%. All other model variables were determined to have no influence on cortisol levels of male fishers within the 30% kernel area of use (Table 2.6).

Within the 60% kernel area of use estimator, 63 models were constructed (40 models were used after correlated variables were removed) from the 20 hair samples and we found that the top-ranked model contained only tree mortality (Table 2.7) with cortisol levels increasing as tree mortality increased (Fig. 2.5). Three other models containing pre-commercial thinning strip and tree mortality, sparse cover and tree mortality, and % low canopy cover and tree mortality also had >0.1 weight, with the 4

top-ranked models carrying 65.7% of the model weight (Table 2.7). Although these models were over the threshold of a weight ≥ 0.1 , tree mortality was carrying these models and the other variables were uninformative (Arnold 2010). Tree mortality was present in all of the top-performing models, with models containing tree mortality having a combined weight of 99.3%. All other model variables were determined to have no influence on cortisol levels of male fishers within the 60% kernel area of use (Table 2.7).

Within the 95% kernel area of use estimator, 26 models were constructed after removing models with correlated variables from the initial 63, and we found that the top-ranked model contained the variables of tree mortality and road density (Table 2.8) with cortisol levels increasing as tree mortality and road density increased (Fig. 2.6). A second-ranked model containing road density and building density and tree mortality added an additional 25% of the weight, with the 2 top-ranked models carrying 58% of the model weight (Table 2.8). All other model variables were determined to have no influence on cortisol levels of male fisher within the 95% kernel area of use (Table 2.8).

DISCUSSION

Consistent with other studies (Douglas and Strickland 1987, Thompson et al. 2011, Zielinski et. al. 2013, Sauder and Rachlow 2014), we found that fishers preferred late-successional forest. However, we found fishers preferred higher quantities of latesuccessional forest in the core of their home ranges or areas of use. This partially contradicts the study by Sauder and Rachlow (2015) which reported fishers in the Rocky Mountains preferred heterogeneity in the landscape with edge and high canopy cover in the cores of their home ranges. However, the presence of snowshoe hares (*Lepus* *americanus*) in the Rocky Mountain region, a preferred prey species for fisher were available, may drive this difference. Snowshoe hares prefer late stand-initiation forest structure with dense conifer cover (Holbrook et al. 2016), and the presence of this habitat type within a mature forest matrix may drive fisher habitat selection in that region. Comparatively, snowshoe hares are not present in the Sierra Nevada. Instead, fishers in the Sierra Nevada have a more diverse diet, relying on smaller-bodied prey more frequently found in older multi-story forests (Zielinski et al. 1999).

When comparing the 30, 60, and 95% kernel home range estimates for female fishers, a significant difference was found in building density, percent conifer forest, percent granite, percent hardwood forest, percent hardwood-conifer forest, percent low canopy cover, and percent dense canopy cover. Although the amounts of percent granite and percent hardwood-conifer forest were significantly different between kernel estimators, we deemed them not biologically relevant because they occur at low levels in the females' home ranges. Building density, percent hardwood forest, and percent low canopy cover all were found in higher amounts with increased home range size. In contrast, percent conifer forest and percent dense canopy cover decreased when home range size was increased. These results indicate that late-successional, dense coniferous forest was more abundant in the core of females' home ranges.

When comparing the 30, 60, and 95% kernel areas of use for male fishers, a significant difference was found in building density, percent developed habitat, percent open water, and percent low canopy cover. We deemed percent open water as not biologically relevant in terms of area use because this includes lakes and ponds, which are not used by fishers. Male fishers had smaller amounts of developed areas and low

canopy cover in the core of their areas of use supporting our predictions that male fishers preferred late-successional forest in the core of their areas of use.

Continuously high cortisol levels (i.e., chronic stress) has been shown to influence animal fitness by negatively affecting reproductive capability and output (Tilbrook et al. 2000, Love and Williams 2008, Ebensperger et al. 2013), increasing vulnerability to different diseases (Schneiderman et al. 2005, Glaser and Kiecolt-Glaser 2005, Blaustein et al. 2012), and reducing immune function (Bonier et al. 2009, Crespi et al. 2013, Dantzer et al. 2013). Furthermore, decreased immune function has the potential to lead to decreased survival rates (Romero et al. 2009). While many studies have shown climate change can have direct and indirect effects on wildlife populations (Wingfield 2008, Rangel-Negrin et al. 2009, Wingfield 2013), this is the first study that has observed increased cortisol levels of fishers in areas of increased tree mortality.

Tree mortality due to drought and bark beetle infestation in the Sierra Nevada Mountains is a good metric of climate change because climate change is known to increase the severity of droughts and insect infestations (Dale et al. 2001, Allen et al. 2010). These effects were seen on this forest during the drought of 2012-2015. For female fishers across all three home range estimators, increasing tree mortality was the most important variable influencing cortisol levels (Fig. 2.4). These factors suggest drought response measured via subsequent tree mortality are most likely the principle driver creating a "landscape of stress" for female fishers in the Sierra Nevada Mountains of California. Levels of tree mortality have increased in the Sierra Nevada Mountains due to the recent 4-year drought, as well as the increase of bark beetles attacking the already struggling trees (Young et al 2017). Although the winter of 2016-2017 experienced greater than average levels of precipitation, the high levels of tree mortality are irreversible (Young et al. 2017). If tree mortality continues to increase over the next few years, and as tree fall increases, the amount of late-successional forest fishers prefer will continue to decrease (van Mantgem et al. 2009). Extreme environmental factors, such as increased tree mortality, could lead to a continued rise in cortisol levels, or maintain steadily high levels, and increase the occurrence of chronic stress in these animals (Gunderson and Stillman 2015), thereby possibly leading to decreased fitness among female fishers (Koolhaas et al. 1999, Mostl and Palme 2002, Bonier et al. 2009). Kotler (1984) showed that loss of canopy cover can lead to an increase in exposure to predation. Furthermore, decreased canopy cover due to tree mortality may also increase the outside temperature or reproductive den temperature due to lack of shade. Kilpatrick and Rego (1994) reported fishers selected rest sites in part due to thermoregulation. Increased predation risk and/or increased temperature could be contributing to an increase in female cortisol levels through tree mortality.

For male fishers in their 30 and 60% kernel areas of use, we again found the highest ranked model showed tree mortality having the greatest influence on cortisol levels and cortisol levels increasing with increased tree mortality (Fig. 2.5). As with the female fishers, tree mortality as a consequence of drought is also driving the landscape of stress for this cohort of the fisher population. Within the 95% kernel estimator for male fishers, we found the combined effect of tree mortality and road density had the greatest influence on cortisol levels, with the same relationship of increased tree mortality and increasing cortisol levels (Fig. 2.6). The additive effect of road density being related to increased cortisol levels follows the biological life history of male fishers. Large

carnivores, such as mountain lions (*Puma concolor*), frequently use roads to hunt (Van Dyke et al. 1986). Furthermore, Whittington et al. (2011) found that predator-prey interactions increase near roads. Given male fishers' widespread movement, increased road density may lead to increased encounter rates with the top predator (mountain lions), which could lead to increased cortisol levels in male fishers and potentially decreased survival. Lack of familiarity with the landscape can also lead to stress (Johnson et al. 2018). During the breeding season male fishers move widely in search of females and suffer increased mortality as a result (Gabriel et al. 2015). Male fishers may be less familiar with the habitat in the fringes of their areas of use, making road density in these regions a greater stressor.

Although increasing tree mortality was related to increasing cortisol levels, fishers do not appear to be selecting against tree mortality in the core of their home ranges and areas of use possibly because areas without high levels of tree mortality are scarce on the landscape. The sharp increase of tree mortality has also developed very quickly over the last few years, giving individuals little time to adapt or acclimate (Levine 2000). Further research should continue to monitor this population as the forest continues to change.

These results show that male and female fishers would have higher amounts of late-successional forest in the core of their home ranges and areas of use. While previous studies have shown events such as climate change can influence cortisol levels (Gunderson and Stillman 2015, French et al. 2010), we observed a relationship between higher amounts of tree mortality and higher cortisol levels. The link between these two analyses suggests that if tree mortality continues to increase in the future (Fig. 2.7), fishers will have less access to late-successional forest which provides both cover and food. This could lead to increased cortisol levels with possible subsequent effects on fitness.

Our results suggest that fishers in this study area are not being negatively influenced by management actions. This supports other studies which have found that animals can adapt and maybe even benefit from anthropogenic modifications (Arnould et al. 2015). Sweitzer et al. (2015) suggested that managers should actively maintain quality fisher habitat around reproductive dens in an attempt to decrease mortality rates of reproductive females and kits. Levine (2000) showed environmental stressors that are controllable or predictable may not be perceived as stressful, so no stress response may occur. This could explain why anthropogenic modifications to the landscape are having a lesser effect on cortisol levels among fishers than tree mortality through climate change in this population. Management actions and development are more easily avoided than the widespread tree mortality. The areas impacted by human activity and silvicultural practices are generally very selective in placement and small in area, while tree mortality is occurring across the entire landscape. Furthermore, Zielinski et al. (2013) showed that fishers may tolerate small amounts of management on the landscape. Tree mortality is severe in both study areas, with mortality of Ponderosa pines exceeding $\geq 80\%$ in some areas (Young et al. 2017). The effects of drought are unavoidable amongst individual fishers, whereas anthropogenic modifications in low density and small patches allow the animals to concentrate their activity (i.e., their core areas of use) to areas less impacted by human activities and anthropogenic modifications. Although we found no relationship between the management activities and cortisol, a relationship could potentially develop with increased salvage logging occurring due to the high levels of tree mortality.

We concluded that the drought response as mediated through tree mortality is creating a landscape of stress within this isolated fisher population. With anticipated increases in drought severity with climate change, this landscape of stress will likely continue to persist and expand. Measures of cortisol concentrations in hair samples from captured fishers appeared to be a viable technique for measuring physiological stress and could prove useful for other species of concern. By understanding how climate change is influencing this sensitive species, managers may be able to take action in preserving the late-successional forest that fishers inhabit. This study is the first to show the link between tree mortality and physiological stress in fishers. How these responses effect increments of fitness in this fisher population remains to be investigated.

MANAGEMENT IMPLICATIONS

This study was consistent with other studies indicating that small amounts of management activity had little to no influence on fishers, given appropriate conservation of fine-scale habitat features (Zielinski et al. 2013). However, this may not be true for large amounts of management activity. Further studies in other regions should examine the relationship between fisher stress responses (i.e., cortisol concentrations) and large-scale management activities.

Over the winter of 2016-2017, this region received greater than average precipitation (snowfall), ending the 4-year drought. Although tree mortality in the Sierra Nevada Mountains is irreversible (Young et al. 2017), this increase in precipitation may lead to a decrease of cortisol levels, although the high levels of tree mortality will remain and canopy cover will decline (van Mantgem et al. 2009). Future research is required to determine the relationship between the ending of the drought and future cortisol levels.

For fishers, tree mortality due to drought was found to be the leading variable influencing chronic stress. Future research should focus on how cortisol levels change as more dead trees begin to fall, further decreasing canopy cover, as well as potential effects of climate change on drought severity and subsequent tree mortality in this ecoregion. Furthermore, a study on small mammal abundance and diversity would be beneficial in determining the health of the ecosystem as many animals rely on small mammals as prey (Pearce and Venier 2005). With increased tree mortality, seed and nut production could be reduced and may create a trophic cascade effect triggering population declines within small mammal populations and upwards to the larger carnivores. Concurrently, the loss of ponderosa pines frees competition for the oak trees which may allow them to flourish. This alone may be able to sustain the small mammal populations. Further research is needed to determine the effect of the high tree mortality on the small mammal community.

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TABLES AND FIGURES

Table 2.1. Various metrics (mean \pm SD) of human disturbance, habitat, and drought response across the 30%, 60%, and 95% kernel estimates for areas of use for male fishers, Sierra National Forest, California, 2014-2016; significant differences between kernel estimators denoted with *.

Model	30%	60%	95%	F	Р
Building density	0.74±1.94	1.84±3.41	2.38±2.85	2.84	0.064*
Road density	1799.49±836.38	1968.52±634.86	2000.89±601.97	0.76	0.468
Tree mortality	17.09±15.98	19.08±13.72	16.93±12.76	0.14	0.868
% conifer	82.54±9.37	81.14±8.61	78.84±6.43	1.65	0.198
% developed habitat	0.05±0.21	0.30±0.60	0.58±0.74	7.13	0.001*
% granite	0.22±0.69	0.21±0.48	0.43±0.76	1.19	0.308
% hardwood	8.56±9.06	9.41±8.25	9.71±5.27	0.19	0.825
% hardwood-conifer	0.01±0.01	0.01±0.02	0.01 ± 0.01	0.70	0.498
% open water	0.005±0.016	0.005±0.010	0.315±0.699	6.27	0.003*
% shrub	5.95±3.08	6.28±2.70	7.22±2.19	1.92	0.152
% sparse	2.67±3.12	2.65±2.44	2.87±2.31	0.07	0.931
% low canopy cover	15.15±10.36	17.10±9.39	20.86±9.48	2.84	0.064*
% moderate canopy cover	47.41±14.30	50.38±11.25	50.11±6.57	0.69	0.502
% dense canopy cover	37.38±20.75	32.19±16.81	28.41±13.31	2.19	0.118
Thinning of natural fuels	324.70±1836.78	277.29±996.97	260.41±873.52	0.02	0.580
commercial thinning	0	117.94±465.72	216.74±694.43	1.62	0.204
Full planting without concurrent site preparation	327.98±1855.36	115.38±652.68	73.26±275.28	0.45	0.522
Fill-in re-planting without concurrent site preparation	312.25±1458.72	2202.18±9819.23	1015.82±3681.14	0.78	0.847
Individual tree release and weeding	4019.53±18946.43	7422.403±19067.60	6055.05±13019.12	0.32	0.730
Pre-commercial thinning of individual or selected trees	6774.51±23456.50	3800.99±9487.33	4032.48±10586.07	0.35	0.873

Table 2.2. Various metrics (mean \pm SD) of human disturbance, habitat, and climate change across the 30%, 60%, and 95% kernel estimates for areas of home ranges of female fishers, Sierra National Forest, California, 2014-2016; significant differences between kernel estimators denoted with *.

Model	30%	60%	95%	F	Р
Building density	0.18±0.56	0.18±0.48	0.54±0.79	7.29	0.001*
Road density	1781.66±1231.21	1904.60±883.65	2052.79±633.99	1.41	0.246
Tree mortality	22.11±24.98	20.96±22.13	20.48±21.47	0.05	0.948
% conifer	85.18±10.33	84.33±8.89	81.01±8.58	3.88	0.022*
% developed habitat	0.06±0.49	0.10±0.46	0.17±0.45	1.07	0.345
% granite	$0.00{\pm}0.00$	0.01 ± 0.06	0.04 ± 0.18	4.53	0.012*
% hardwood	8.21±8.00	8.73±7.37	11.11±7.75	2.77	0.065*
% hardwood-conifer	0.004 ± 0.019	0.007 ± 0.014	0.011±0.012	3.22	0.042*
% open water	0.003 ± 0.023	0.003 ± 0.019	0.006 ± 0.017	0.74	0.480
% shrub	5.43±5.12	5.43±3.27	5.89±2.39	0.34	0.709
% sparse	1.11±1.83	1.39±2.26	1.76±2.24	1.62	0.200
% low canopy cover	8.44±7.36	9.78±7.85	13.80±9.42	7.89	0.001*
% moderate canopy cover	46.63±19.27	47.41±15.29	51.13±10.81	1.66	0.193
% dense canopy cover	44.84±22.99	42.68±20.21	34.88±16.15	4.75	0.010*
Thinning of natural fuels	972.36±8077.04	58.29±484.21	174.70±1081.03	0.22	0.805
Commercial thinning	1290.37±10444.44	650.49±3802.57	530.09±2909.24	0.12	0.890
Full planting without concurrent site preparation	547.16±4197.12	808.57±4553.94	737.67±2331.11	0.09	0.917
Fill-in re-planting without concurrent site preparation	2432.91±20209.25	1804.27±14927.96	1558.67±11169.53	0.16	0.855
Individual tree release and weeding	2594.57±20234.10	4627.10±26780.50	5779.54±22229.25	0.33	0.718
Pre-commercial thinning of individual or selected trees	6084.77±30953.58	6840.82±26237.06	4318.11±13268.35	0.19	0.827

Table 2.3. Top 10 ranked models for the relationship between individual cortisol levels and levels of various metrics of disturbance, habitat, and drought response within the 30% kernel home range of female fishers, Sierra Nevada Mountains, California, 2014-2016; models with >0.1 weight denoted by *.

Model	dAICc	weight	p-value
Tree mortality *	0.0	0.262	0.001
% developed + tree mortality *	1.7	0.112	% developed- 0.403
			tree mortality- 0.002
Road density + tree mortality *	1.9	0.102	road density- 0.462
			tree mortality- 0.002
Thinning of natural fuels + tree mortality	2.3	0.083	thinning of natural fuels- 0.692
			tree mortality- 0.002
% dense canopy cover + tree mortality	2.3	0.082	% dense canopy cover- 0.708
			tree mortality- 0.002
Building density + tree mortality	2.5	0.076	building density- 0.985
			tree mortality- 0.002
Road density + % developed + tree mortality	4.0	0.035	road density- 0.595
			% developed - 0.509
			tree mortality- 0.002
% developed + thinning of natural fuels + tree	4.2	0.033	% developed- 0.414
mortality			thinning of natural fuels- 0.708
			tree mortality- 0.002
% dense canopy cover + % developed + tree	4.3	0.031	% dense canopy cover- 0.830
mortality			% developed- 0.442
			tree mortality- 0.003
Road density + thinning of natural fuels + tree	4.3	0.030	road density- 0.466
mortality			thinning of natural fuels – 0.691
			tree mortality- 0.002

Table 2.4. Top 10 ranked models for the relationship between individual cortisol levels and levels of various metrics of disturbance, habitat, and drought response within the 60% kernel home range of female fishers, Sierra Nevada Mountains, California, 2014-2016; models with >0.1 weight denoted by *.

Model	dAICc	weight	p-value
Tree mortality *	0.0	0.210	0.002
% hardwood_conifer + tree mortality *	0.3	0.184	% hardwood conifer- 0.156
			tree mortality- 0.010
Road density + tree mortality	1.9	0.083	road density- 0.453
			tree mortality- 0.006
Building density + tree mortality	2.0	0.075	building density- 0.532
			tree mortality- 0.003
Road density + % hardwood_conifer + tree	2.5	0.062	road density- 0.534
mortality			% hardwood conifer- 0.180
			tree mortality- 0.019
Thinning of natural fuels + tree mortality	2.5	0.062	thinning of natural fuels- 0.889
			tree mortality- 0.003
Building density + % hardwood_conifer + tree	2.6	0.059	building density- 0.586
mortality			% hardwood conifer- 0.171
			tree mortality- 0.013
% hardwood_conifer + thinning of natural fuels +	2.9	0.050	% hardwood conifer- 0.163
tree mortality			thinning of natural fuels- 964
			tree mortality- 0.011
Road density + building density + tree mortality	4.2	0.025	road density - 0.529
			building density- 0.633
			tree mortality- 0.007
Road density + thinning of natural fuels + tree	4.5	0.023	road density- 0.456
mortality			thinning of natural fuels- 0.872
			tree mortality- 0.007

Table 2.5. Top 10 ranked models for the relationship between individual cortisol levels and levels of various metrics of disturbance, habitat, and drought response within the 95% kernel home range of female fishers, Sierra Nevada Mountains, California, 2014-2016; models with >0.1 weight denoted by *.

Model	dAICc	weight	p-value
Tree mortality *	0.0	0.444	0.001
Road density + tree mortality *	1.9	0.175	road density- 0.456
			tree mortality- 0.001
Pre-commercial thinning of individual or selected trees + tree mortality *	2.0	0.163	pre-commercial thinning of individual or selected trees - 0.511
			tree mortality- 0.002
Building density + tree mortality *	2.4	0.132	building density- 0.843
			tree mortality- 0.001
Building density + pre-commercial thinning of	4.6	0.045	building density- 0.825
individual or selected trees + tree mortality			pre-commercial thinning of individual or selected trees- 0.512
			tree mortality- 0.002
% hardwood	7.2	0.012	% hardwood- 0.050
% hardwood + pre-commercial thinning of	8.5	0.006	% hardwood- 0.048
individual or selected trees			pre-commercial thinning of individual or selected trees - 0.291
Null	9.0	0.005	
Road density + % hardwood	9.7	0.004	road density- 0.855
			% hardwood- 0.058
% moderate canopy cover	10.0	0.003	% moderate canopy cover- 0.270

Table 2.6. Top 10 ranked models for the relationship between individual cortisol levels and levels of various metrics of disturbance, habitat, and drought response within the 30% kernel area of use for male fishers, Sierra Nevada Mountains, California, 2014-2016; models with >0.1 weight denoted by *.

Model	dAICc	weight	Р
Tree mortality *	0.0	0.171	0.019
% low canopy cover + tree mortality *	0.2	0.153	% low canopy cover- 0.120
			tree mortality- 0.012
% sparse + tree mortality *	0.5	0.131	% sparse- 0.141
			tree mortality- 0.017
Full planting without concurrent site preparation + tree mortality	2.1	0.061	full planting without concurrent site preparation- 0.546
			tree mortality- 0.022
% low canopy cover + full planting without	2.7	0.044	% low canopy cover- 0.096
concurrent site preparation + tree mortality			full planting without concurrent site preparation - 0.359
			tree mortality- 0.013
Building density + tree mortality	2.8	0.043	building density- 0.625
			tree mortality- 0.023
Road density + tree mortality	2.9	0.041	road density- 0.812
			tree mortality- 0.025
Building density + % low canopy cover + tree	3.1	0.036	building density- 0.488
mortality			% low canopy cover- 0.111
			tree mortality- 0.015
Null	3.2	0.034	
Building density + % sparse + tree mortality	3.5	0.029	building density- 0.515
			% sparse- 0.135
			tree mortality- 0.021

Table 2.7. Top 10 ranked models for the relationship between individual cortisol levels and levels of various metrics of disturbance, habitat, and drought response within the 60% kernel area of use for male fishers, Sierra Nevada Mountains, California, 2014-2016; models with >0.1 weight denoted by *.

Model	dAICc	weight	p-value		
Tree mortality *	0.0	0.264	0.0004		
Pre-commercial thinning of individual or selected trees + tree mortality *	1.2	0.147	pre-commercial thinning of individual or selected trees - 0.200 tree mortality- 0.002		
% sparse + tree mortality *	1.2	0.144	% sparse- 0.205 tree mortality- 00009.		
% low canopy cover + tree mortality *	1.9	0.102	% low canopy cover- 0.307 tree mortality- 0.001		
Building density + tree mortality	2.7	0.068	building density- 0.542 tree mortality- 0.0004		
Road density + tree mortality	23.1	0.057	road density- 0.769 tree mortality- 0.0005		
Building density + pre- commercial thinning of individual or selected trees + tree mortality	4.2	0.032	building density- 0.502 pre-commercial thinning of individual or selected trees- 0.200 tree mortality- 0.002		
% low canopy cover + pre-commercial thinning of individual or selected trees + tree mortality	4.2	0.032	% low canopy cover- 0.504 pre-commercial thinning of individual or selected trees- 0.316 tree mortality- 0.003		
Road density + pre- commercial thinning of individual or selected trees + tree mortality	4.4	0.029	road density- 0.586 pre-commercial thinning of individual or selected trees- 0.185 tree mortality- 0.002		
Building density + % sparse + tree mortality	4.6	0.027	building density- 0.667 % sparse- 0.246 tree mortality- 0.001		

Table 2.8. Top 10 ranked models for the relationship between individual cortisol levels and levels of various metrics of disturbance, habitat, and drought response within the 95% kernel area of use for male fishers, Sierra Nevada Mountains, California, 2014-2016; models with >0.1 weight denoted by *.

Model	dAICc	weight	p-value
Road density + tree mortality*	0.0	0.330	road density- 0.013
			tree mortality- 0.007
Road density + building density + tree	0.6	0.250	road density- 0.011
mortality *			building density- 0.126
			tree mortality- 0.003
Road density + % sparse + tree mortality	2.6	0.092	road density- 0.017
			% sparse- 0.374
			tree mortality- 0.025
Road density + building density + % sparse +	2.9	0.080	road density- 0.014
tree mortality			building density- 0.093
			% sparse- 0.240
			tree mortality- 0.010
Road density + full planting without	4.1	0.044	road density- 0.002
concurrent site preparation			full planting without concurrent site preparation - 0.049
Tree mortality	4.3	0.040	tree mortality- 0.001
% dense canopy cover + tree mortality	5.1	0.026	% dense canopy cover- 0.168
			tree mortality- 0.005
Building density + tree mortality	5.2	0.025	building density- 0.177
			tree mortality- 0.0006
Road density + % sparse	5.5	0.022	road density- 0.005
			% sparse- 0.097
Road density	5.6	0.020	road density- 0.002

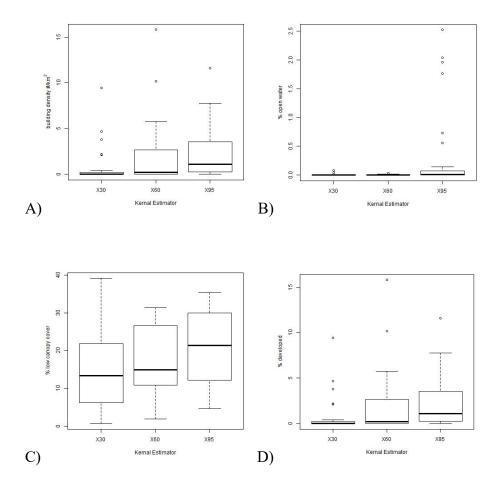


Figure 2.1. Various metrics of landscape attributes within the 30, 60, and 95% areas of use for male fishers, including (A) building density (# buildings/km²), B) percent open water, (C) percent low canopy cover, and (D) percent developed habitat type, Sierra National Forest, California, 2014-2016.

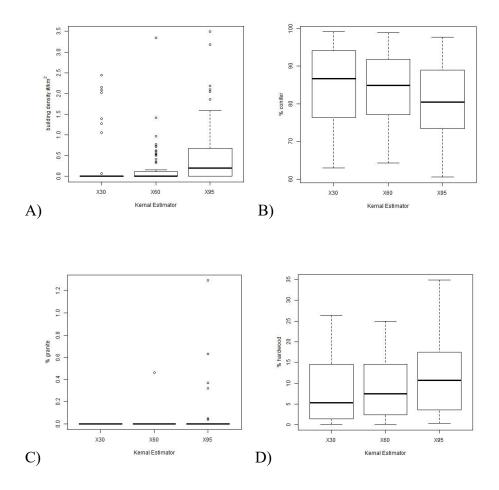
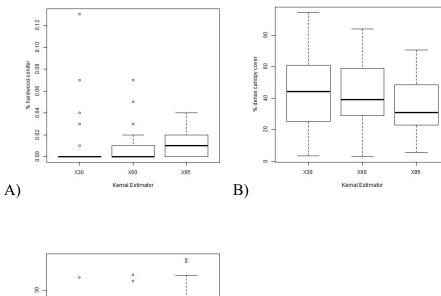


Figure 2.2. Various metrics of landscape attributes within the 30, 60, and 95% kernel home ranges of female fishers, including (A) building density (# buildings/km²), (B) percent conifer forest, (C) percent granite, and (D) percent hardwood, Sierra National Forest, California, 2014-2016.



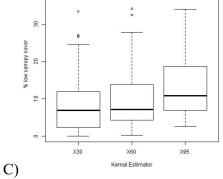


Figure 2.3. Various metrics of landscape attributes within the 30, 60, and 95% kernel home ranges of female fishers, including (A) percent hardwood-conifer, (B) percent dense canopy cover, and (C) percent low canopy cover, Sierra National Forest, California, 2014-2016.

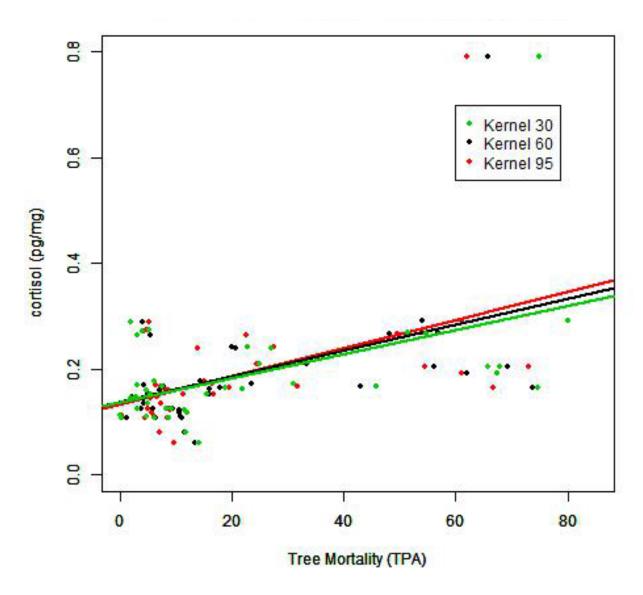


Figure 2.4. Relationship between cortisol levels in their hair and the amount of tree mortality in the 30%, 60%, and 95% kernel home ranges for individual female fishers, Sierra National Forest, California, 2014-2016.

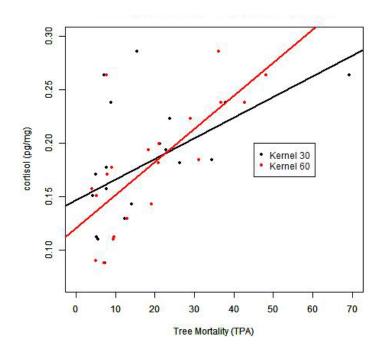


Figure 2.5. Relationship between cortisol levels in their hair and the amount of tree mortality in the 30 and 60% areas of use for male fishers, Sierra National Forest, California, 2014-2016.

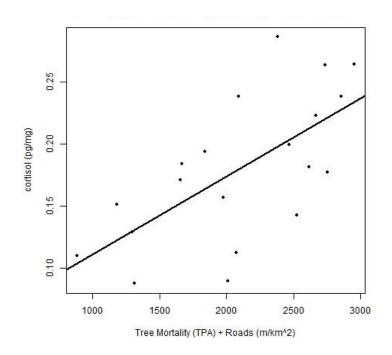


Figure 2.6. Relationship between cortisol levels in their hair and the amount of tree mortality + road density in the 95% kernel areas of use for individual male fishers, Sierra National Forest, California, 2014-2016.

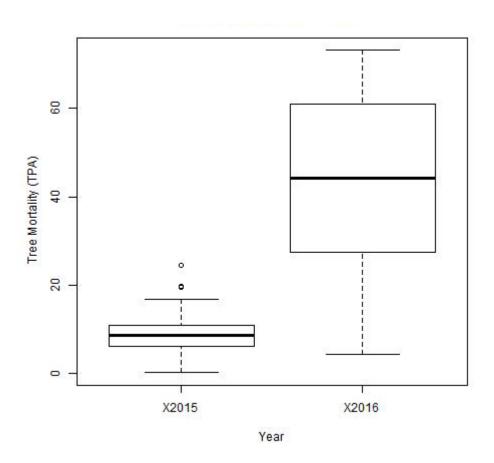


Figure 2.7. Level of tree mortality (trees/acre) in 2015 and 2016, Sierra National Forest, California.

CHAPTER 3

THE LINK BETWEEN CORTISOL AND FITNESS: HOW PHYSIOLOGICAL STRESS IS INFLUENCING THE FITNESS OF THE PACIFIC FISHER IN AN EVER-CHANGING FOREST

ABSTRACT

Changes in the environment can both directly and indirectly affect a species. Fishers (Pekania pennanti) are a threatened mesocarnivore in the Sierra Nevada Mountains of California. They prefer dense late-successional forest and occupy a relatively small geographic area in the Sierra National Forest. Once found statewide, their numbers declined dramatically in the 1900s due to trapping, habitat fragmentation, and human development. More recently, climate change induced drought is likely causing increased physiological stress to this isolated population. Cortisol is released to mobilize energy in response to stress and has been used as an indicator of an individual's physiological response to its environment. By collecting samples of fisher hair and measuring an individual's cortisol level, we examined the relationship between cortisol levels and fitness components (body condition, survival, and litter size) among the fisher population in the central Sierra Nevada Mountains, California. We also examined the influence of sex and age class on cortisol levels. We found that survival rates were highest when cortisol levels were lowest in female fishers, with lower survival at medium and high cortisol levels. We found no significant relationship between cortisol levels and body condition, male survival, and female kit counts. How climate change and increased severity of drought with subsequent tree mortality will impact this fisher population

remains to be seen. Previously, we found that increased tree mortality within an individual's home range was related to increased cortisol levels among Pacific fishers. With the recent drought and subsequent tree mortality being >80% in some areas of our study, cortisol levels could continue to increase, potentially leading to decreased female fitness within this fisher population and imperiling this isolated population.

INTRODUCTION

Continuously high cortisol levels (i.e., chronic stress) have been shown to influence animal fitness by negatively affecting reproductive abilities or output (Tilbrook et al. 2000, Love and Williams 2008, Ebensperger et al. 2013), increasing vulnerability to different diseases (Schneiderman et al. 2005, Glaser and Kiecolt-Glaser 2005, Blaustein et al. 2012), and reducing immune function (Bonier et al. 2009, Crespi et al. 2013, Dantzer et al. 2013). Furthermore, decreased immune function has the potential to lead to decreased survival rates (Romero et al. 2009). However, higher cortisol levels alone are not always linked to decreased fitness (Bonier et al. 2009). Fitness must be examined along with cortisol in order to determine whether cortisol levels are influencing the health of the individual. A few common fitness components are clutch size and body mass to length ratio (Jakob et al. 1996, Godfray et al. 1991). Survival can also be used as a measure of fitness (Gaillard et al. 2000) as individuals with decreased fitness may have increased difficulty persisting on a landscape.

Physiological stress amongst individuals in a wildlife population is commonly measured through cortisol concentrations (Davenport et al. 2006, Dantzer et al. 2014). Cortisol is a glucocorticoid hormone released to mobilize energy in response to stress (Boonstra 2013). The relationship between glucocorticoids and animal stress has been well established (Romero et al. 2009, French et al. 2010, Neuman-Lee et al. 2017), and has been successfully measured in numerous species, from chipmunks (*Tamias striatus*; Martin and Reale 2008) to grizzly bears (*Ursus arctos*; Macbeth et al. 2010).

Fishers (*Pekania pennanti*) are mesocarnivores in the mustelid family that depend largely on old-growth forest for hunting, denning, and shelter (Douglas and Strickland 1987, Thompson et al. 2011, Zielinski et. al. 2013) and could serve as a bioindicator giving insight to the qualitative health of their ecosystem (Roemer et al. 2009). The historic range of fishers in California was a continuous arc from the Coast Range eastward to the southern Cascades, then south through the Sierra Nevada Mountains (Grinnell et al. 1937). However, due to fur trapping, forest fragmentation, and habitat loss, fishers "persist at a very low density" in the Sierras (Schempf and White 1977). Fisher populations in California declined in the 1940s and were subsequently listed as a species of concern (Hall 1942). Concerns for their population size resulted in a ban on trapping in 1946 (Lewis and Zielinski 1996). However, despite 70 years of protection the population has never fully recovered (U.S. Fish and Wildlife Service 2014). For these reasons, fishers were first petitioned for listing under the U.S. Endangered Species Act in 1990 (Long et al. 2014). Presently, factors potentially limiting recovery of the Pacific fisher include climate change, habitat loss, illegal poisoning, and anthropogenic modifications to the landscape (U.S. Fish and Wildlife Service 2014).

Concerns over persistently low population numbers led to the initiation of two long-term research studies in the Sierra Nevada Mountains of California to investigate the general ecology of fishers and monitor their response to habitat change (Thompson et al. 2011, Zielinski et al. 2013, Wengert et al. 2014). Over 9 years, these two studies captured, radio-collared, and monitored >250 individual fishers (Thompson et al. 2011, Purcell et al. 2018).

The objective of this study was to examine the relationship between individual cortisol levels and fitness, to determine the effects of chronic stress on fitness. We collected hair samples from captured fishers during three trapping seasons (2014-2015, 2015-2016, 2016-2017), and examined whether the cortisol levels measured for each individual fisher was related to fitness metrics. Cortisol in hair can indicate an accumulation of physiological responses over months (Tallo-Parra et al. 2016), suggesting it could be used as a measure of chronic stress. Our prediction was that individuals with high levels of cortisol would have decreased fitness (body condition, survival, reproduction) when compared to individuals with lower cortisol levels.

METHODS AND MATERIALS

Study Area

This study was conducted in the Sugar Pine (SP) and Kings River (KR) study areas located in the Sierra Nevada Mountains of central California. The SP study area was located near Oakhurst, California, and was approximately 800-km² in size, while the 450-km² KR study area was located near Shaver Lake, California. The KR study area was located approximately 11km SE of the SP study area. During the summer, precipitation was very rare in this region. Temperatures averaged highs of 23°C and lows of 9°C in the summer months

(https://weather.com/weather/monthly/l/Shaver+Lake+CA+93664:4:US). During the

winter months' snow accumulation was typical throughout the Sierra Nevada Mountains, with the majority of both study areas having a layer of snow from November-April of each year. During these winter months temperatures averaged highs of 7°C and lows of - 4°C (https://weather.com/weather/monthly/l/Shaver+Lake+CA+93664:4:US).

Both study areas were similar in elevation from 915 to 2,385 m (Purcell et al. 2009). Lower elevations were dominated by montane hardwood-conifer forest (Allen-Diaz et al. 2007), consisting primarily of ponderosa pine (Pinus ponderosa), manzanita (Arcostaphylos spp.), California black oak (Quercus kelloggii), and canyon live oak (Q. chrysolepis) with a dense shrub cover understory (Allen-Diaz et al. 2007, Keeley and Davis 2007). The dominant shrubs were manzanita, white thorn (Ceanothus leucodermis), and bear clover (Chamaebatia foliolosa). Mid-elevations consisted of mixed conifer habitats, dominated by white fir (Abes concolor), ponderosa pine, sugar pine (P. lambertiana), and California black oak (Fites-Kaufman et al. 2007). The highest elevation areas were primarily non-vegetated granite outcrops with little shrub cover and a few trees, mostly sugar pine, Jeffery pine (P. jeffrevi), and white fir (Fites-Kaufman et al. 2007). The combination of an extended 4-year (2012-2015) drought and infestation of pine beetle (Dendroctonus ponderosae) resulted in extensive tree mortality of ponderosa pine and other coniferous species with large areas of dead or dying mature trees and tree mortality numbers reaching into hundreds of dead trees per km² (Young et al. 2017).

Trapping and Monitoring

Since fall of 2014, hair samples from both male and female fishers have been collected from the KR and SP study areas during capture and radio-collaring operations. Trapping typically occurred from September through March to avoid black bears (*Ursus*

americanus) disturbing traps and to prevent separating females from newborn kits in the spring. Fishers were trapped with live-traps (81.28 x 25.4 x 30.48 cm, original series model 108, Tomahawk Live Trap, Hazelhurst, WI) (Green et al. 2018) with wooden cubby shelters (Wilbert 1992, Seglund 1995). Traps were covered with natural materials for cover and baited with chicken and bait lure (Hawbaker's Fisher Lure, Hawbaker and Sons, Fort Loudon, PA, or Fisher Red Lure, Proline Lures, Indianapolis, IN) (Green et al. 2018). Distance lures (Caven's Gusto, Minnesota Trapline Products, Pennock, MN, or Outreach Call Lure, Proline Lures, Indianapolis, IN) were also used to draw animals in from farther distances (Green et al. 2018). We handled fishers using a canvas sleeve and metal handling cone (Seglund 1995). Animals were sedated with an intramuscular injection of ketamine hydrochloride (22.5mg/kg) mixed with Diazapam or Midazolam (0.125 mg/kg) based on sex and age estimates. We collected hair samples when shaving the hair in preparation for a blood draw from the jugular vein. Hair samples were stored individually at room temperature in sealed paper envelopes until processing in the lab.

During animal handling, we measured body length (cm), weight (kg), tail length (cm), teeth length (mm), and reproductive status through teat condition and testicular size. Animals were assigned one of three age classes: juvenile (<1 year old), subadult (1-2 years old), adult (>2 years old). Juveniles had sharp molars, no sagittal crest, and female teats showed no sign of prior reproduction. Subadults had slightly rounded molars, a partially developed sagittal crest, and female teats also showed no sign of prior reproduction. Adults had rounded or flattened molars, a defined sagittal crest, and female teats showed signs of prior reproduction (brown, larger teats) (Green et al. 2018).

Fishers were fitted with a 31 g radio-collar (Model mI-2M, Holohil Systems Ltd., Carp, Ontario, Canada) with a handmade breakaway (Thompson et al. 2011), and marked with a Passive Integrated Transponder (PIT tag; Biomark, Boise, ID) for future identification and tracking (Green et al. 2018). Animals were captured and handled under authorization of the United States Forest Service with permits from the California Department of Fish and Wildlife with appropriate institutional animal care and use committee (IACUC) review and approval.

Body Mass Index

We calculated a body mass index (BMI) for each trapped individual and paired this with the hair sample collected at the time of capture. We measured body length (in cm) at capture from the tip of the nose along the back of the spine to the base of the tail. Body mass was also measured at capture and recorded in kg. The BMI was determined as the ratio of body mass (kg)/body length (cm).

Survival rates

During this study, fishers were monitored through radio telemetry. KR animals were predominately monitored through ground telemetry, while SP animals were predominately monitored through aerial telemetry. This difference was due to the different objectives between these studies. The long-standing goal of the KR project has been to focus on habitat selection and management implications, while the objectives of the SP project have been to focus on survival, cause of death, and occupancy.

When a radio-collar remained inactive for an 8-hour period, the collar turned on a rapid signal indicating a stationary collar. When this mortality signal was detected, we

immediately located and recovered the collar to determine whether the signal was from a dropped collar or a mortality. The survival intervals were measured from when the individual was captured and radio-collared until September 15 of the year following their capture. September 15 was chosen as the end-point for the survival interval each year because this is when new hair begins to grow in and it is close to the beginning of the next trapping season (Douglas and Strickland 1987). However, for animals captured during the 2016-2017 trapping season, survival was only measured until May 1, 2017 (project monitoring ended). For 2014, survival was measured from the capture date of the winter of 2014-2015 through September 15, 2015. Interval survival rates and 95% confidence intervals were calculated using the program MICROMORT (Heisey and Fuller 1985) for each year, and for males and females separately. To examine the influence of cortisol on survival rates, animals were divided into three groups of cortisol levels (low: <0.12 pg/mg, medium: 0.121-0.189 pg/mg, high: >0.190 pg/mg). These cortisol measurement cutoffs were chosen by dividing the animals into thirds based on the cortisol levels.

Kit Counts

Denning season occurred between April and June. Female fishers give birth almost exclusively in tree cavities (Green et al. 2018), termed the natal den, then periodically move the kits to new (maternal) dens in different structures as the kits age. By mid-June kits are highly mobile and the family group is moving on a near-daily basis. Once the natal den tree was identified, remote cameras were set up to monitor the tree until the female moved her kits to the next den tree; these photos were used to determine kit counts. Females used up to 5 den trees a season, and cameras were set up at most dens opportunistically for the purpose of tracking litter sizes and kit survival. Den trees were climbed after the female moved her kits to document any kit mortality or abandonment. The female and kits were monitored as consistently and non-intrusively as possible throughout the denning season. To investigate the influence of cortisol levels on reproduction, we examined the relationship between cortisol levels and the kit count during the denning season before the hair sample was collected, and the kit count the year after the hair sample was collected. This allowed us to determine whether the number of kits a female produced influenced her cortisol levels, or whether cortisol levels influenced the number of kits the female produced the following year.

Sample Extractions and Radioimmunoassays

Hair samples collected from fishers in the field were analyzed at the French Lab at Utah State University. Hair samples were washed three times with HPLC (high performance liquid chromatography)-grade methanol to remove any dirt and waste from the hair. After the samples were air dried, they were weighed and placed in a vial (Restch ball mill 201MM, Retsch GmbH, Haan, Germany) with a 7-mm steel grinding ball, and ground into a fine powder (Davenport et al. 2006); samples were ground for 20 minutes at 30Hz. Balls and vials were cleaned thoroughly with ethanol and dried between samples. The ground hair was weighed (weights ranged from 21.1 to 254.3 mg depending on hair availability) and transferred to a centrifuge tube. We added 3mL of methanol to the sample, vortexed for 1 minute, placed samples on a slow vortexer for 24 hours, then spun for 10 minutes at 2000 rpm, and 1.5 mL of supernatant was removed and placed in a clean glass tube. Samples were dried under a stream of nitrogen and resuspended in an assay dilutant (Neuman-Lee et al. 2017). Samples were stored in assay dilutent until analysis (Martin and Réale 2008).

We assayed hair samples using a radioimmunoassay (RIA) in duplicate for cortisol 3 antibody (Fitzgerald 20-CR50) (French et al. 2017). For each sample, we used an aliquot of the re-suspended fractions to correct final sample concentration value for individual recoveries following extraction. The intra-assay variation was 4.0%. All assayed samples fell within the standard curve for the assay (i.e., were detectable values).

Statistical Analysis

We used a Student's t-test to determine differences between sexes and study areas on levels of cortisol for individual fishers. We used linear regression to examine the relationship between cortisol and BMI. We used analysis of variance (ANOVA) to examine the relationship between cortisol levels and the factors of age class and year for male and female fishers, as well as the number of kits produced for the year before, and the year after the cortisol sample was collected from the individual female fisher.

RESULTS

Over the three winter trapping seasons of 2014, 2015, and 2016, 23 male fishers (10 from KR, 13 from SP) and 41 female fishers (22 from KR, 19 from SP) were captured with some individual being captured multiple years for 32 male samples collected (19 from KR, 13 from SP) and 68 female samples collected (44 from KR, 24 from SP). For each year, only one sample per fisher was used in the analyses (i.e., multiple captures were only represented once each year). However, some animals were captured every year of the study for a total of 3 hair samples.

Influence of sex, age class, year, study area, and BMI on cortisol levels

We examined the influence of sex and age class on cortisol levels using samples from 32 male and 68 female samples. We found cortisol levels did not differ between male and female fishers (t = -1.3384, df = 131, P = 0.183). Among female fishers, there were no differences in cortisol levels among juveniles, subadults, and adults (F = 0.6392, df = 2, 77, P = 0.531). Among male fishers, cortisol levels did vary among age classes (F = 5.658, df = 2, 46, P = 0.006) with juvenile males having the highest cortisol levels, followed by the adults, and subadults having the lowest cortisol levels (Fig. 3.1).

We found cortisol levels differed significantly among years (F = 25.96, df = 2, 130, P < 0.001) with cortisol levels being similar in the first 2 years of sampling (2014 = 0.145 ± 0.063 pg/mg; 2015 = 0.155 ± 0.051 pg/mg), then increasing in the third year (2016 = 0.266 ± 0.119 pg/mg; Fig. 3.2). In addition, cortisol levels varied between the study areas (t = -3.1245, df = 130.99, P = 0.002) with cortisol levels being higher in the KR study area (0.188 ± 0.099 pg/mg) compared to the SP study area (0.146 ± 0.056 pg/mg; Fig. 3.3).

We examined the influence of BMI on cortisol levels using samples from the males (n = 32) and females (n = 68). We found no relationship between cortisol levels and individual BMI estimates (F = 0.356, df = 1, 117, P = 0.552).

Relationship between kits produced and cortisol levels

We examined if the number of kits produced influenced the subsequent cortisol level of an individual female fisher using samples from 37 females where kit counts were obtained. We found no difference in cortisol levels among individual females after they produced 0, 1, 2, or 3 kits (F = 0.354, df = 3, 33, P = 0.786). In these study areas, it has

been found that the mean litter size for fishers is 1.57 kits (Green et al. 2018). Similarly, we examined if cortisol levels influenced the subsequent litter size using samples from 54 females where kit counts were obtained. We found no significant difference in cortisol levels and whether they subsequently produced 0, 1, 2, or 3 kits (F = 1.251, df = 3, 50, P = 0.301).

Influence of cortisol on fisher survival rates

Among male fishers, annual survival rates were generally increasing over time (Table 3.1), however, there was no significant differences amongst the years (all z-tests had P > 0.30). When examining the relationship between cortisol level and annual survival rates (Table 2.2), we found males with the highest cortisol level had the lowest annual survival and males with low cortisol levels had the highest survival, but there was no significant difference in survival rate among low, medium, and high levels of cortisol (all z-tests had P > 0.17).

Among female fishers, annual survival showed an increasing trend over the 3 years of sampling (Table 3.1), however, there was no significant difference among years (all z-tests had P > 0.26). When examining the relationship of the level of cortisol and annual survival rates (Table 3.2), we found females with low cortisol levels had a significantly higher annual survival rate than females with medium (z = 1.56, P = 0.06) and high (z = 1.44, P = 0.07) levels of cortisol; females with medium and high levels of cortisol did not have significantly different survival rates (z = 0.27, P = 0.39).

DISCUSSION

Many studies have shown that chronic stress (resulting in continuously high levels of cortisol) can have a negative influence on individual fitness (Tilbrook et al. 2000, Schneiderman et al. 2005, Bonier et al. 2009), but this is the first study to measure this relationship in fishers. During this study, cortisol levels increased over the 3 years of sampling. Over these 3 years, there was a severe drought throughout both the SP and KR study areas. Tree mortality increased during the 3 years (Fig. 3.4) with some areas experiencing >80% tree mortality (Young et al. 2017). We previously found that tree mortality was the greatest environmental variable causing increased cortisol levels among fishers with individuals living in areas of greater tree mortality having higher cortisol levels (Chapter 1). This leads to the idea that our fitness results may become more drastic in the near future if tree mortality continues to increase and decreases the amount of latesuccessional forest that fishers prefer (Thompson et al. 2011). Tree mortality was a direct result of the drought. Climate change will likely increase drought severity and insect infestations in the future (Dale et al. 2001, Allen et al. 2010), therefore it will be important to continue to monitor this population over the following years to document whether this pattern continues.

Many studies have found significant differences in cortisol levels between sexes (Kirschbaum et al. 1992, Touma and Palme 2005, Myers et al. 2010, Kershaw and Hall 2016) and age classes (Robbins and Czekala 1997, and Myers et al. 2010). However, our study was consistent with Macbeth et al. (2010) which found no significant differences in cortisol levels between males and females, or between the female age classes. Although no difference was seen between female age classes, significant differences in cortisol

levels were measured between male age classes. Among male fishers, juveniles had higher cortisol levels than subadults and adults which may reflect the biology and lifestyle of these animals. In the late summer, juveniles disperse and attempt to find and establish their own home range. During this time, they suffer significantly higher mortality than other male age classes (Sweitzer et al. 2015). Adult males are territorial, and may pose a threat to juvenile males seeking to establish a home range as has been seen in other mammal species (Le Boeuf 1974, McLean 1983, Pusey and Packer 1987). In contrast, subadults have established home ranges where they are more familiar with their surroundings, yet are too young to mate, so they do not spend the spring leaving their home ranges in pursuit of adult females. Adult males had intermediate cortisol levels, possibly because of their familiarity with their established home ranges and their large body size, while also spending the spring leaving their home ranges in search of adult females for breeding. Gabriel et al. (2015) found that depredation of fishers is highest in the spring when male fishers leave their home ranges in search of a mate. Thus, adult males may experience more stress than subadult males because of their breeding activity. Furthermore, Bonnet et al. (1999) documented a pattern of high juvenile (due to dispersal) and adult (during breeding season) mortality with low subadult mortality in snakes which is consistent with our male age class findings.

A significant difference was found between cortisol levels for fishers sampled in the KR study area and the SP study area, with the KR population having higher cortisol levels. This could be due to observed differences in the amount of tree mortality documented in the SP and KR study areas (Chapter 1). In the SP study area, home ranges (95% kernel) for males had an average of 10.45 dead trees per acre and female ranges had an average of 7.5 dead trees per acre. In contrast, in the KR study area, male ranges had an average of 19.71 dead trees per acre, and female ranges had an average of 26.04 dead trees per acre. The large difference in tree mortality between the two study areas is most likely the key variable influencing the significantly different levels of cortisol in these two study populations. We suggest further research to determine why tree mortality is so much lower in the SP study area. Topographic and microclimatic differences between these 2 study sites is likely influencing different rates of beetle infection and subsequent tree mortality.

Chronic stress has also been shown to increase an individual's vulnerability to different diseases (Schneiderman et al. 2005, Glaser and Kiecolt-Glaser 2005, Blaustein et al. 2012), therefore possibly influencing survival. We found that female fishers with low cortisol levels had higher annual survival rates than females with medium and high cortisol levels (Fig. 3.5). Although females with high cortisol levels had a slightly higher survival rate than females with medium cortisol levels, these two groups did not have significantly different survival rates. Decreased survival in female fishers could greatly influence the future of this small, isolated population.

Although the body mass to length ratio can be used as a measure of fitness (Jakob et al. 1996), no significant relationship was observed between cortisol levels and BMI in this population. Consistent with other studies (Jakob et al. 1996, Godfray et al. 1991), we found no relationship between cortisol and body condition. There was also no significant relationship between cortisol and kit production, even though female kit count numbers varied between 1-3 during any given year. However, the continued effects of increasing tree mortality and reductions in the small mammal prey base will undoubtedly affect body condition and reproduction at some point.

With the loss of trees and decreased cone production, small mammal populations will likely decline during the following years (Hansson 1979). Numerous studies have shown that prey declines can lead to changes in reproduction and reduce body condition in carnivores (Ward and Krebs 1985, O'Donoghue et al. 1997). If the small mammal prey base declines, this isolated fisher population could be adversely impacted. Furthermore, if the amount of late-successional forest continues to decrease with increased tree fall (van Mantgem et al. 2009), these animals will have less cover to avoid predation, and fewer trees to use for shelter and dens. Climate change is continuing to increase the average temperatures of this region as well (Stewart et al. 2004). With all of these factors at play, it is increasingly important to monitor this isolated fisher population moving forward. If cortisol levels continue to increase, we may begin to see greater impacts of physiological stress on the fitness and health of individuals in this population.

MANAGEMENT IMPLICATIONS

Fishers fill an important niche in this forest ecosystem as mesocarnivores and bioindicators of ecosystem health making them an important species to study to understand overall forest health. Although the only significant impact we found between chronic stress (i.e., high cortisol levels) and the fitness components was higher survival among females with low cortisol levels, other relationships could develop in the future. With tree mortality having the greatest impact on fisher cortisol levels (see Chapter 1), and tree mortality continuing to increase in this region (Fig. 3.5), survival rates could continue to decline. If cortisol levels continue to increase in response to tree mortality and associated habitat changes, we could see an increase in the degree to which cortisol influences female survival rates, as well as impacts to body condition and other fitness components.

Tree mortality in the Sierra Nevada Mountains is irreversible (Young et al. 2017), however the end of the drought may lead to a decrease in cortisol levels as fishers adapt to the altered environment and find alternate resources. Further monitoring of this fisher population will be required to determine whether cortisol levels return to a lower level. How quickly forest regeneration occurs will possibly dictate the future well-being of this small isolated fisher population. Future research should focus on how cortisol levels change as more dead trees begin to fall, further decreasing canopy cover. Furthermore, a study on small mammal abundance and diversity would be beneficial in determining the health of the ecosystem as many animals rely on small mammals as prey (Pearce and Venier 2005). With the large increase in tree mortality, seed and nut production could be reduced and may create a cascading effect triggering population declines within small mammal populations and upwards to the larger carnivores. Concurrently, the loss of ponderosa pines frees competition for the oak trees and may allow them to flourish. This alone may be able to sustain the small mammal populations. Further research is needed to determine the effect of the high tree mortality percentage on the small mammal populations. Increased tree mortality could lead to a massive decrease in species diversity and abundance in the Sierra Nevada Mountains of California.

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TABLES AND FIGURES

Table 3.1. Annual survival rates of female and male fishers in the Kings River and Sugar Pine study areas, Sierra National Forest, California, 2014-2016; 95% confidence interval in parentheses.

	2014	2015	2016
Females	0.809 (0.672-0.974)	0.871 (0.746-1.000)	0.910 (0.756-1.000)
Males	0.731 (0.538-0.994)	0.772 (0.576-1.000)	0.877 (0.677-1.000)

Table 3.2. Annual survival rates of male and female fishers as a function of their level of cortisol, Sierra National Forest, California, 2014-2016. Levels of cortisol were low (<0.12 pg/mg), medium (0.121-0.189 pg/mg), and high (>0.190 pg/mg); 95% confidence intervals in parentheses.

Sex	Low	Medium	High
Males	0.809 (0.603-1.000)	0.742 (0.553-0.994)	0.706 (0.435-1.000)
Females	0.940 (0.832-1.000)	0.783 (0.631-0.970)	0.817 (0.650-1.000)

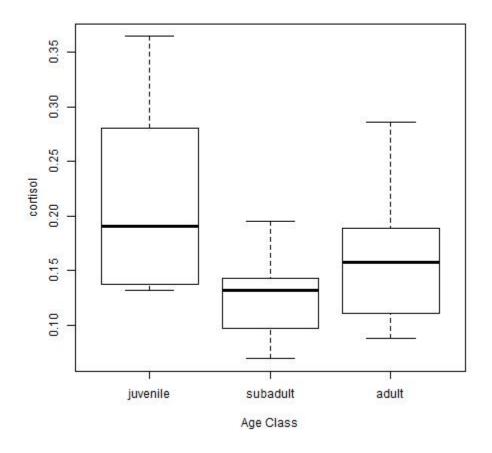


Figure 3.1. Cortisol levels (pg/mg) among juvenile, subadult, and adult male fishers in the Sierra National Forest, California, 2014-2016.

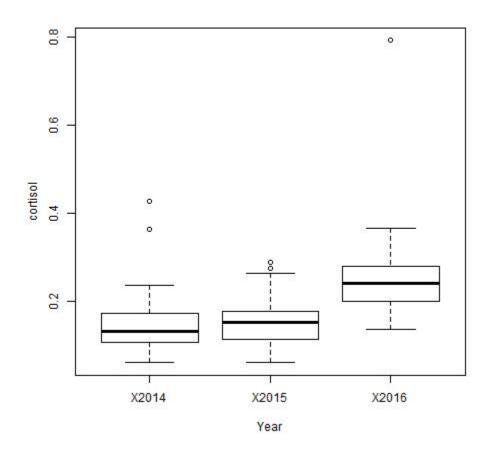


Figure 3.2. Cortisol levels (pg/mg) for fishers in 2014, 2015, and 2016 in the Sierra National Forest, California, 2014-2016.

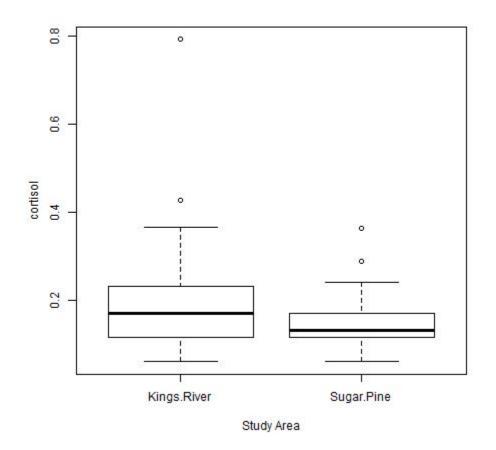


Figure 3.3. Cortisol levels (pg/mg) in fishers in the Kings River and Sugar Pine study areas, Sierra National Forest, California, 2014-2016.

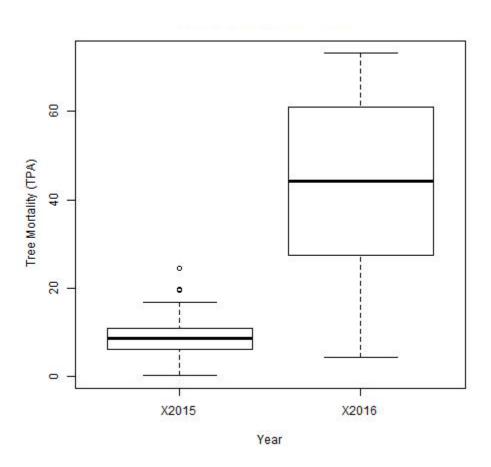
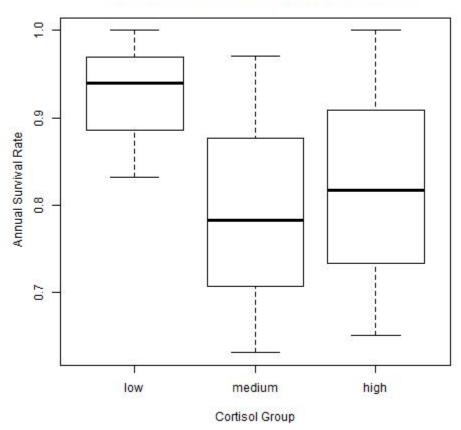


Figure 3.4. The level of tree mortality during 2015 and 2016, Sierra National Forest, California, 2015-2016.



Female Cortisol and Survival Relationship

Figure 3.5. Annual survival rates of female fishers as a function of their level of cortisol, Sierra National Forest, California, 2014-2016. Cortisol levels were low (<0.12 pg/mg), medium (0.121-0.189 pg/mg), and high (>0.190 pg/mg); error bars represent 95% confidence interval.

CHAPTER 4

CONCLUSIONS

Continuously high cortisol levels (i.e., chronic stress) have been shown to influence animal fitness by negatively affecting reproductive capability and output (Tilbrook et al. 2000, Love and Williams 2008, Ebensperger et al. 2013), increasing vulnerability to different diseases (Schneiderman et al. 2005, Glaser and Kiecolt-Glaser 2005, Blaustein et al. 2012), and reducing immune function (Bonier et al. 2009, Crespi et al. 2013, Dantzer et al. 2013). Furthermore, decreased immune function has the potential to lead to decreased survival rates (Romero et al. 2009). Because of the importance of cortisol, we examined how home range disturbance through anthropogenic modification and drought response influenced cortisol levels in individual fishers.

Consistent with other studies (Douglas and Strickland 1987, Thompson et al. 2011, Zielinski et. al. 2013, Sauder and Rachlow 2014), we found that fishers preferred late-successional forest. However, we found fishers preferred higher quantities of late-successional forest in the core of their home ranges or areas of use.

When comparing the 30, 60, and 95% kernel home range estimates for female fishers, a significant difference was found in building density, percent conifer forest, percent granite, percent hardwood forest, percent hardwood-conifer forest, percent low canopy cover, and percent dense canopy cover. Building density, percent hardwood forest, and percent low canopy cover all were found in higher amounts with increased home range size. In contrast, percent conifer forest and percent dense canopy cover decreased when home range size was increased. These results indicate that latesuccessional, dense coniferous forest was more abundant in the core of females' home ranges.

When comparing the 30, 60, and 95% kernel areas of use for male fishers, a significant difference was found in building density, percent developed habitat, percent open water, and percent low canopy cover. Male fishers had smaller amounts of developed areas and low canopy cover in the core of their areas of use supporting our predictions that male fishers preferred late-successional forest in the core of their areas of use.

While many studies have shown climate change can have direct and indirect effects on wildlife populations (Wingfield 2008, Rangel-Negrin et al. 2009, Wingfield 2013), this is the first study that has observed increased cortisol levels of fishers in areas of increased tree mortality. Tree mortality due to drought and bark beetle infestation in the Sierra Nevada Mountains is a good metric of climate change because climate change is known to increase the severity of droughts and insect infestations (Dale et al. 2001, Allen et al. 2010). These effects were seen on this forest during the drought of 2012-2015. For female fishers across all three home range estimators, increasing tree mortality was the most important variable influencing cortisol levels (see Fig. 2.4). These factors suggest drought response measured via subsequent tree mortality are most likely the principle driver creating a "landscape of stress" for female fishers in the Sierra Nevada Mountains of California. Levels of tree mortality have increased in the Sierra Nevada Mountains due to the recent 4-year drought, as well as the increase of bark beetles attacking the already struggling trees (Young et al 2017). Although the winter of 2016-2017 experienced greater than average levels of precipitation, the high levels of tree

mortality are irreversible (Young et al. 2017). If tree mortality continues to increase over the next few years, and as tree fall increases, the amount of late-successional forest fishers prefer will continue to decrease (van Mantgem et al. 2009). Extreme environmental factors, such as increased tree mortality, could lead to a continued rise in cortisol levels, or maintain steadily high levels, and increase the occurrence of chronic stress in these animals (Gunderson and Stillman 2015), thereby possibly leading to decreased fitness among female fishers (Koolhaas et al. 1999, Mostl and Palme 2002, Bonier et al. 2009). Kotler (1984) showed that loss of canopy cover can lead to an increase in exposure to predation. Furthermore, decreased canopy cover due to tree mortality may also increase the outside temperature or reproductive den temperature due to lack of shade. Kilpatrick and Rego (1994) reported fishers selected rest sites in part due to thermoregulation. Increased predation risk and/or increased temperature could be contributing to an increase in female cortisol levels through tree mortality.

For male fishers in their 30 and 60% kernel areas of use, we again found the highest ranked model showed tree mortality having the greatest influence on cortisol levels and cortisol levels increasing with increased tree mortality (see Fig. 2.5). As with the female fishers, tree mortality as a consequence of drought is also driving the landscape of stress for this cohort of the fisher population. Within the 95% kernel estimator for male fishers, we found the combined effect of tree mortality and road density had the greatest influence on cortisol levels, with the same relationship of increased tree mortality and increasing cortisol levels (see Fig. 2.6). The additive effect of road density being related to increased cortisol levels follows the biological life history of male fishers. Large carnivores, such as mountain lions (*Puma concolor*), frequently use

roads to hunt (Van Dyke et al. 1986). Furthermore, Whittington et al. (2011) found that predator-prey interactions increase near roads. Given male fishers' widespread movement, increased road density may lead to increased encounter rates with the top predator (mountain lions), which could lead to increased cortisol levels in male fishers and potentially decreased survival. Lack of familiarity with the landscape can also lead to stress (Johnson et al. 2018). During the breeding season male fishers move widely in search of females and suffer increased mortality as a result (Gabriel et al. 2015). Male fishers may be less familiar with the habitat in the fringes of their areas of use, making road density in these regions a greater stressor.

Although increasing tree mortality was related to increasing cortisol levels, fishers do not appear to be selecting against tree mortality in the core of their home ranges and areas of use possibly because areas without high levels of tree mortality are scarce on the landscape. The sharp increase of tree mortality has also developed very quickly over the last few years, giving individuals little time to adapt or acclimate (Levine 2000). Further research should continue to monitor this population as the forest continues to change.

These results show that male and female fishers would have higher amounts of late-successional forest in the core of their home ranges and areas of use. While previous studies have shown events such as climate change can influence cortisol levels (Gunderson and Stillman 2015, French et al. 2010), we observed a relationship between higher amounts of tree mortality and higher cortisol levels. The link between these two analyses suggests that if tree mortality continues to increase in the future (see Fig. 2.7), fishers will have less access to late-successional forest which provides both cover and food. This could lead to increased cortisol levels with possible subsequent effects on fitness.

Our results suggest that fishers in this study area are not being negatively influenced by management actions. This supports other studies which have found that animals can adapt and maybe even benefit from anthropogenic modifications (Arnould et al. 2015). Sweitzer et al. (2015) suggested that managers should actively maintain quality fisher habitat around reproductive dens in an attempt to decrease mortality rates of reproductive females and kits. Levine (2000) showed environmental stressors that are controllable or predictable may not be perceived as stressful, so no stress response may occur. This could explain why anthropogenic modifications to the landscape are having a lesser effect on cortisol levels among fishers than tree mortality through climate change in this population. Management actions and development are more easily avoided than the widespread tree mortality. The areas impacted by human activity and silvicultural practices are generally very selective in placement and small in area, while tree mortality is occurring across the entire landscape. Furthermore, Zielinski et al. (2013) showed that fishers may tolerate small amounts of management on the landscape. Tree mortality is severe in both study areas, with mortality of Ponderosa pines exceeding $\geq 80\%$ in some areas (Young et al. 2017). The effects of drought are unavoidable amongst individual fishers, whereas anthropogenic modifications in low density and small patches allow the animals to concentrate their activity (i.e., their core areas of use) to areas less impacted by human activities and anthropogenic modifications. Although we found no relationship between the management activities and cortisol, a relationship could potentially develop with increased salvage logging occurring due to the high levels of tree mortality.

Many studies have found significant differences in cortisol levels between sexes (Kirschbaum et al. 1992, Touma and Palme 2005, Myers et al. 2010, Kershaw and Hall 2016) and age classes (Robbins and Czekala 1997, and Myers et al. 2010). However, our study was consistent with Macbeth et al. (2010) which found no significant differences in cortisol levels between males and females, or between the female age classes. Although no difference was seen between female age classes, significant differences in cortisol levels were measured between male age classes. Among male fishers, juveniles had higher cortisol levels than subadults and adults which may reflect the biology and lifestyle of these animals. In the late summer, juveniles disperse and attempt to find and establish their own home range. During this time, they suffer significantly higher mortality than other male age classes (Sweitzer et al. 2015). Adult males are territorial, and may pose a threat to juvenile males seeking to establish a home range as has been seen in other mammal species (Le Boeuf 1974, McLean 1983, Pusey and Packer 1987). In contrast, subadults have established home ranges where they are more familiar with their surroundings, yet are too young to mate, so they do not spend the spring leaving their home ranges in pursuit of adult females. Adult males had intermediate cortisol levels, possibly because of their familiarity with their established home ranges and their large body size, while also spending the spring leaving their home ranges in search of adult females for breeding. Gabriel et al. (2015) found that depredation of fishers is highest in the spring when male fishers leave their home ranges in search of a mate. Thus, adult males may experience more stress than subadult males because of their breeding activity. Furthermore, Bonnet et al. (1999) documented a pattern of high juvenile (due to

dispersal) and adult (during breeding season) mortality with low subadult mortality in snakes which is consistent with our male age class findings.

A significant difference was found between cortisol levels for fishers sampled in the KR study area and the SP study area, with the KR population having higher cortisol levels. This could be due to observed differences in the amount of tree mortality documented in the SP and KR study areas (see Chapter 2). In the SP study area, home ranges (95% kernel) for males had an average of 10.45 dead trees per acre and female ranges had an average of 7.5 dead trees per acre. In contrast, in the KR study area, male ranges had an average of 19.71 dead trees per acre, and female ranges had an average of 26.04 dead trees per acre. The large difference in tree mortality between the two study areas is most likely the key variable influencing the significantly different levels of cortisol in these two study populations. We suggest further research to determine why tree mortality is so much lower in the SP study area. Topographic and microclimatic differences between these 2 study sites is likely influencing different rates of beetle infection and subsequent tree mortality.

Chronic stress has also been shown to increase an individual's vulnerability to different diseases (Schneiderman et al. 2005, Glaser and Kiecolt-Glaser 2005, Blaustein et al. 2012), therefore possibly influencing survival. We found that female fishers with low cortisol levels had higher annual survival rates than females with medium and high cortisol levels (see Fig. 3.5). Although females with high cortisol levels had a slightly higher survival rate than females with medium cortisol levels, these two groups did not have significantly different survival rates. Decreased survival in female fishers could greatly influence the future of this small, isolated population.

With the loss of trees and decreased cone production, small mammal populations will likely decline during the following years (Hansson 1979). Numerous studies have shown that prey declines can lead to changes in reproduction and reduce body condition in carnivores (Ward and Krebs 1985, O'Donoghue et al. 1997). If the small mammal prey base declines, this isolated fisher population could be adversely impacted. Furthermore, if the amount of late-successional forest continues to decrease with increased tree fall (van Mantgem et al. 2009), these animals will have less cover to avoid predation, and fewer trees to use for shelter and dens. Climate change is continuing to increase the average temperatures of this region as well (Stewart et al. 2004). With all of these factors at play, it is increasingly important to monitor this isolated fisher population moving forward. If cortisol levels continue to increase, we may begin to see greater impacts of physiological stress on the fitness and health of individuals in this population.

During this study, cortisol levels increased over the 3 years of sampling. Because of this increase, we believe our fitness results may become more drastic in the near future if tree mortality continues to increase and decreases the amount of late-successional forest that fishers prefer (Thompson et al. 2011). We concluded that the drought response as mediated through tree mortality is creating a landscape of stress within this isolated fisher population. With anticipated increases in drought severity with climate change, this landscape of stress will likely continue to persist and expand. Measures of cortisol concentrations in hair samples from captured fishers appeared to be a viable technique for measuring physiological stress and could prove useful for other species of concern. By understanding how climate change is influencing this sensitive species, managers may be able to take action in preserving the late-successional forest that fishers inhabit. This study is the first to show the link between tree mortality and physiological stress in fishers, and furthermore, the link between increased cortisol and decreased female survival.

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