EFFECTS OF GENETIC DEPLETION ON ESTIMATING RISK OF EXTINCTION OF THE ENDANGERED FLORIDA PANTHER (*Puma concolor coryi*)

An Undergraduate Research Scholars Thesis

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ANNA COLE

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ABSTRACT

Effects of Genetic Depletion on Estimating Risk of Extinction of the Endangered Florida Panther (*Puma concolor coryi)*

Anna Cole
Department of Wildlife and Fisheries Sciences
Texas A&M University

Research Advisor: Dr. Hsiao-Hsuan Wang
Department of Wildlife and Fisheries Sciences

There are over 30 species of wild cat that occupy over 90 countries of the world. Many of these species are experiencing significant population loss due to urbanization and habitat fragmentation. These forces lead to common occurrences of inbreeding and subsequent biodiversity loss. One subspecies of felid experiencing such inbreeding is the Florida panther (*Puma concolor coryi*). A subspecies of puma, the Florida panther historically resided in a large expanse of the southeast United States. Due to development and urbanization, this habitat has been reduced to two areas in southwest Florida: the Big Cypress Swamp and Everglades National Park. Due to the habitats being separated, the two remaining populations of Florida panthers are isolated and unable to interact with each other, thus limiting the amount of available genes. Physical and reproductive characteristics, such as cryptorchidism, have resulted from inbreeding. To prevent further population loss, and to increase biodiversity, 8 Texas cougars were introduced into the populations of Florida panthers in 1995. A population model was created in order to analyze the effects of genetic depletion if such conservation efforts were not implemented.

DEDICATION

I dedicate this work to my parents. I want to thank them for supporting me throughout my academic career. Whenever I felt overwhelmed with academic responsibilities due to my perfectionistic behavior, my parents never ceased to show their support. They are my rock, and I would not have made it this far without them helping me along the way.

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NOMENCLATURE

IUCN	International Union for Conservation of Nature
ENP	Everglades National Park
BCNP	Big Cypress National Preserve
FSA	Female sub-adult
FSASR	Female sub-adult survival rate
FPA	Female prime-adult
FPASR	Female prime-adult survival rate
FOA	Female older-adult
FOASR	Female older-adult survival rate
MSA	Male sub-adult
MSASR	Male sub-adult survival rate
MPA	Male prime-adult
MPASR	Male prime-adult survival rate
MOA	Male older-adult
MOASR	Male older-adult survival rate
q_{sa}	Reproductive probability of sub-adult females
q_{pa}	Reproductive probability of prime-adult females
q_{oa}	Reproductive probability of older-adult females
v_{sa}	Average number of kittens birthed by sub-adult females
v_a	Average number of kittens birthed by prime-adult females
IV	Interval Value

CHAPTER I

INTRODUCTION

Members of the wild cat family, known as Felidae, are present in over 90 countries across the world and vary in size from small housecats to large tigers (Dickman et al. 2015). Current phylogenetic evidence suggests eight lineages within Felidae, each composed of multiple genera and species (Mattern & McLennan, 2000). The number of felid species is debated, but ranges from 35-41 (Dickman et al. 2015). Most of these species are characterized as nocturnal, elusive hunters that live in various habitats to which they are best adapted (Mattern & McLennan, 2000). Due to low population sizes, the IUCN designated a ranking of at least vulnerable to slightly less than half of the global felid species (Dickman et al. 2015). Studies indicate that the conservation concerns regarding these species are primarily in response to genetic inbreeding and subsequent biodiversity loss due to urbanization and resulting habitat fragmentation (Alldredge et al. 2015). Urbanization encroaches on animal territory and can eventually break apart areas, isolating animals of the same species and creating separate populations, eventually reducing the amount of interaction and natural gene flow that provides diversity within these populations (Alldredge et al. 2015). Currently, inbreeding is evident in several wild cat species and subspecies, such as the Iberian lynx (Ganan et al. 2010). Genetic invariability in this European lynx subspecies has resulted in reproductive problems affecting sperm and semen that have consequently led to a rapid population decline, making this subspecies the most endangered wild felid in the world (Ganan et al. 2010). A similar problem of inbreeding and lack of biodiversity in the genome plagues the Florida panther, *Puma concolor coryi*, a subspecies of the *Puma concolor* orgnanism that is known as a puma, cougar, panther, or mountain lion (Mansfield & Land, 2002). This subspecies experienced a loss of habitat due to urbanization that caused a genetic bottleneck during the last century that crippled the population to practical obscurity and supposed extinction (Culver, 2008). Within the past couple of decades, efforts have been made to counteract this loss of biodiversity and help increase the population of Florida panthers (Johnson et al. 2010). The objective of this literature review and population ecology study is to examine the success of these efforts, specifically those focused on decreasing inbreeding effects, and identify the main threats of extinction to the Florida panther.

CHAPTER II

METHODS

Natural history of the Florida panther

There are currently thirty unique subspecies of pumas, or *P. concolor*, across the world (Finn et al. 2013). Of these subspecies, the Florida panther (*Puma concolor coryi*) is the only one residing in the eastern United States, and is in danger of extinction (Johnson et al. 2010). First analyzed in the late 19th century, the Florida panther is an elusive and polygamous predator (Culver, 2008). Previously found throughout the southeastern United States, individuals are now restricted to the Big Cypress National Preserve and Everglades National Park in southwest Florida (Roelke et al. 1993), an area that is estimated to have a carrying capacity of 30-40 panthers (Belden et al. 1988). These populations are both located within a subtropical region rich with vegetation (Maehr et al. 2002) that experiences most of the annual rainfall in the summer months (Richardson, 2010). Today, little of the native Florida panther habitat remains (Culver, 2008). Urbanization has presented problems to the two populations of panthers, particularly the group in the Everglades (Maehr et al. 2002).

Population decline in the 20th century

Habitat loss, negative interactions with humans, disease, automobile collisions, and other causes of fatalities reduced the Florida panther population in the 20th century (Buergelt et al. 2002). Upon spreading throughout the Southeastern United States, and consequently increasing interactions with Florida panthers, humans hunted the panthers (Culver, 2008). Conflicts with humans resulted in the restriction of Florida panthers to parts of Louisiana and South Florida by the first quarter of the 20th century, which led to the federal designation of endangered in 1967

(Culver, 2008). After years of uncertain population estimates and theorized extinction, scientists calculated the population to contain 70 individuals in the late 20th century (Culver, 2008). Due to isolated habitat and small population size, concerns existed regarding inbreeding and a lack of gene flow that could continue the population decline and eventually lead to extinction (Pimm et al. 2006). Traits caused by inbreeding in the Florida panther include unique physical characteristics such as kinked caudal vertebrae and a cowlick on the back, as well as various health problems. Such problems include heart defects (Hostetler et al. 2013), reduced sperm volume (Hedrick 1995), and cryptorchidism, a testicular disorder that can cause decreased fecundity or infertility (Mansfield & Land 2002) and was present in the majority of Florida panthers in the early 1990s (Facemire et al. 1995) .Various methods were examined and utilized in order to promote gene variety. One such method was to introduce 8 female Texas cougars (*P. c. stanleyana*) into the two populations of Florida panthers in 1995 (Hostetler et al. 2013). Texas cougars were specifically chosen to interbreed with Florida panthers due to historical interaction that occasionally resulted in interbreeding (Finn et al. 2013). This introduction was completed to increase the Florida panther genome through unions of native panthers and Texas cougars, which could provide the genetic diversity necessary to prevent extinction.

Population analysis using a computer model

After conducting a thorough literature review using journal articles found through the Texas A&M online databases, a computer model was created using the STELLA® 7.0.1 program to evaluate if the introduction of the Texas cougars was successful in increasing the population of Florida panthers. The model's parameters were based on the findings and previous data collected from a study monitoring the populations of Florida panthers after the cougar introduction (Hostetler et al. 2013). The Hostetler study examined the panthers based on sex- and agestructured groups. The ages are kittens, sub-adults (FSA and MSA; females aged 1-2.5 years old and males aged 1-3.5 years old), prime adults (FPA and MPA; females aged 2.5 years or older and males 3.5 years or older), and older adults (FOA and MOA; females and males aged 10 years or older) (Hostetler et al. 2013). For the purpose of this study, the values of each state variable were estimated at 25, resulting in a beginning population size of 200. Each of these age groups had demographic parameters of survival rate, mortality rate, and natality rate. The natality rate included sub-parameters of the reproductive probability and average litter size birthed to each female age group (Hostetler et al. 2013). The survival rate was calculated based on the estimated values of Hostetler (Hostetler et al. 2013), in which the values were shown with a standard error that resulted in a baseline, minimum, and maximum value after multiplying the value by 1.96 to account for a 95% confidence interval (Hostetler et al. 2013). In order to simulate more scenarios representative of natural phenomena, additional values were calculated by finding the difference between the minimum and maximum values and multiplying it by 0.2. This resulting value was added to the minimum value until the sum equaled the maximum value, creating four interval values in between the minimum and maximum. The value of 0.2 was used to find the interval difference value because it was the optimum number for reaching the exact maximum value when applied to the minimum value. These initial values are shown below (Table 1).

Table 1- Initial parameter values calculated based on values obtained from Hostetler et al. (2013)

	Baseline	Minimum	Maximum	Difference (Max.- Min.)	Interval Difference Value (D x 0.2)
KittenSR	0.473	0.31424	0.63176	0.31752	0.063504
FSASR	0.966	0.917	1.015 (1)	0.098	0.0196
FPASR	0.906	0.84916	0.96284	0.11368	0.022736
FOASR	0.795	0.68328	0.90672	0.22344	0.044688
MSASR	0.775	0.65936	0.89064	0.23128	0.046256
MPASR	0.848	0.76568	0.93032	0.16464	0.032928
MOASR	0.682	0.5252	0.8388	0.3136	0.06272
qsa	0.252	0.06384	0.44016	0.37632	0.075264
qpa	0.301	0.0952	0.5068	0.4116	0.08232
qoa	0.025	-0.02596	0.07596	0.10192	0.020384
vsa	2.87	2.0076	3.7324	1.7248	0.34496
va	2.41	1.6652	3.1548	1.4896	0.29792

After adding the interval difference value to the minimums of each parameter, the desired interval values were calculated. The survival rates ranged from 0.31424 to 0.63176 for kittens, 0.917 to 1.015 (1) for female sub-adults, 0.84916 to 0.96284 for female prime-adults, 0.68328 to 0.90672 for female older-adults, 0.65936 to 0.89064 for male sub-adults, 0.76568 to 0.93032 for male prim-adults, and 0.5252 to 0.8388 for male older-adults. The mortality rate was found by subtracting the survival rate from 1, and the survival of each age class was found by multiplying the state variable value by the survival rate of each group. Natality affected all age groups of both sexes and was determined by multiplying the reproductive probability of sub-adult (q_{sa}), prime-adult (q_{pa}), and older-adult (q_{oa}) panthers and the average number of panthers birthed by females both sub-adult (v_{sa}) and prime-adult (v_a) panthers. The final natality value resulted after the product was multiplied by 0.5 to account for both sexes. The values of each natality component were found using the same methods as the survival rate, with minimum and maximum values and four interval values in between. The reproductive probability ranged from 0.06384 to 0.44016 for sub-adults, 0.0952 to 0.5068 for prime-adults, and -0.02596 to 0.07596 for older-adults. These parameters and state variables were arranged so the effect of each parameter on the state variables affected the next age group, as shown in the figure below (Figure 1).

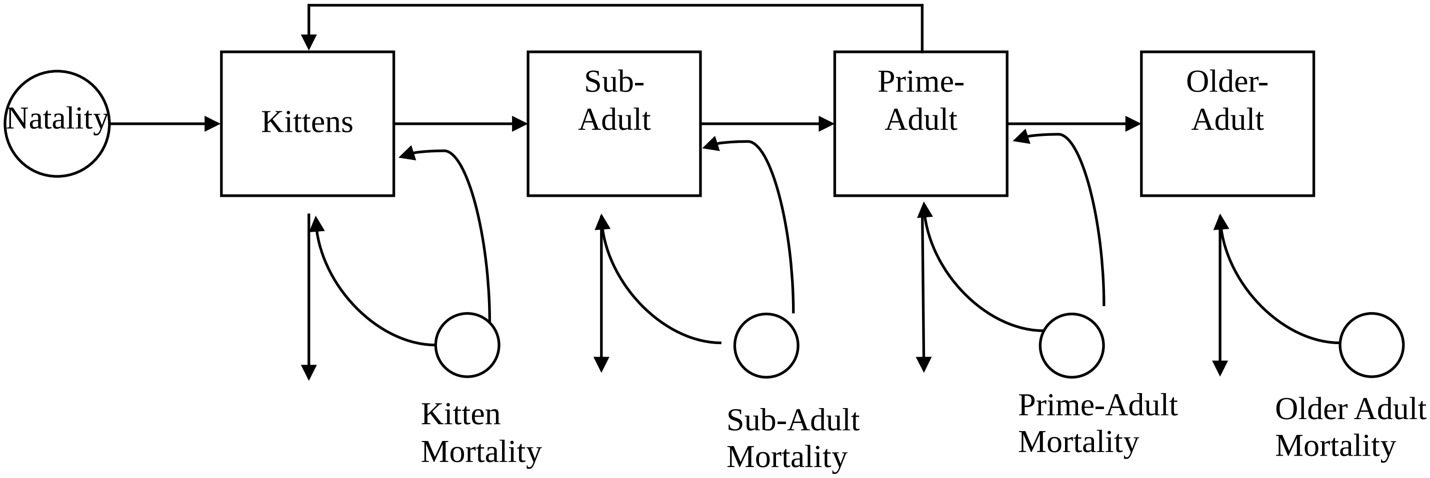

Figure 1. Conceptual model of the STELLA ® 7.0.1 population model

Prior to running the model, graphs and tables were set up to run for 25 years, the time in which the panther populations were monitored during the Hostetler study (Hostetler et al. 2013). The exact values for these parameters are shown below (Table 2).

Table 2. Complete parameter values

	Minimum	IV 1	IV 2	IV 3	IV 4	Maximum
SKitten	0.31424	0.377744	0.441248	0.504752	0.568256	0.63176
FSASR	0.917	0.9366	0.9562	0.9758	0.9954	1.015 (1)
FPASR	0.84916	0.871896	0.894632	0.917368	0.940104	0.96284
FOASR	0.68328	0.727968	0.772656	0.817344	0.862032	0.90672
MSASR	0.65936	0.705616	0.751872	0.798128	0.844384	0.89064
MPASR	0.76568	0.798608	0.831536	0.864464	0.897392	0.93032
MOASR	0.5252	0.58792	0.65064	0.71336	0.77608	0.8388
qsa	0.06384	0.139104	0.214368	0.289632	0.364896	0.44016
qpa	0.0952	0.17752	0.25984	0.34216	0.42448	0.5068
qoa	-0.02596	-0.005576	0.014808	0.035192	0.055576	0.07596
vsa	2.0076	2.35256	2.69752	3.04248	3.38744	3.7324
va	1.6652	1.96312	2.26104	2.55896	2.85688	3.1548

CHAPTER III

RESULTS

Twelve scenarios based on the model were run, resulting in twelve distinct graphs. In Scenario 1, all of the parameters were run at the baseline, minimum, and maximum values to show how varying environmental situations would affect the population. Based on these settings, the population was estimated to decrease from 200 panthers to 3 and 0.00 panthers after 25 years when the parameters were set at baseline and minimum values. When the parameters were set at the maximum values, the population increases to 1725 panthers after 25 years. These results are shown below (Figure 3).

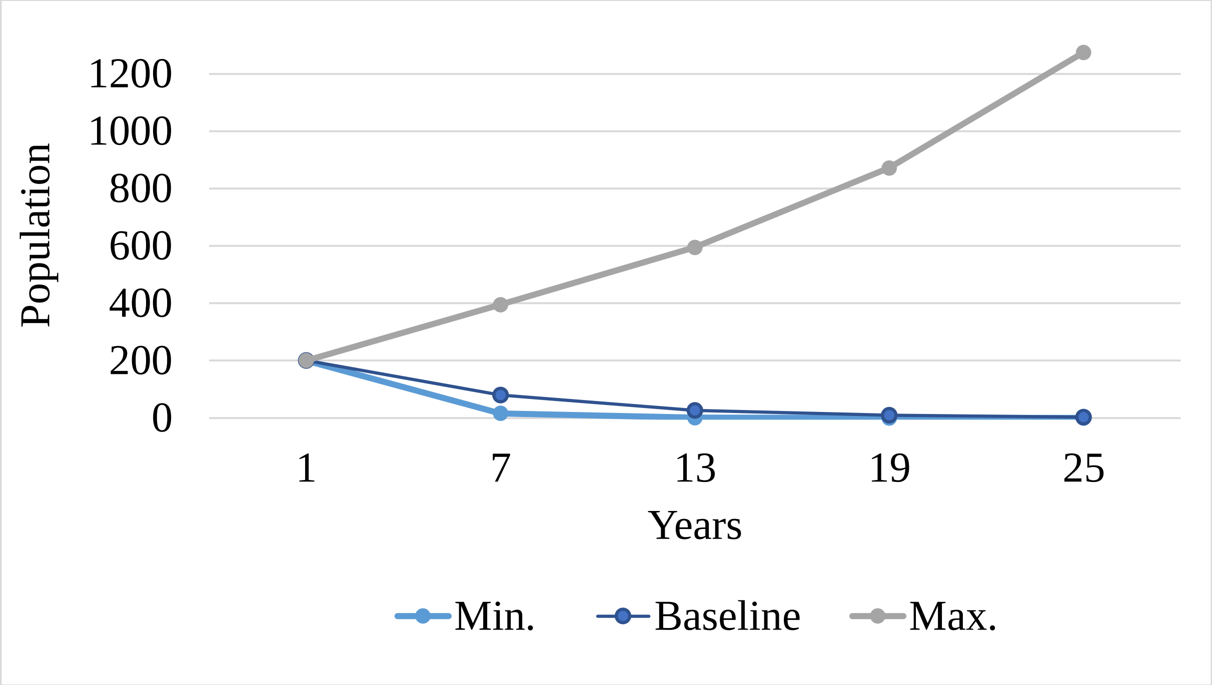

Figure 3. Estimated panther population with uniform parameters

The remaining graphs evaluated how changing each individual parameter, while keeping the other parameters at baseline values, would affect the population. The parameters representing survival rate and natality rate all consisted of six values: minimum, maximum, and four interval values. Scenario 2 represented changing the kitten survival rates, which resulted in the population decreasing from 200 to 1, 2, 3, 4, 5, and 8. The female sub-adult and prime-adult survival rates (FSASR and FPASR) were changed individually in scenarios 3 and 4, and both resulted in a population decline from 200 to 3 panthers at the end of 25 years. In scenario 5, which involved changing the female older-adult survival rate (FOASR), the different parameter values produced unique populations; while the minimum and first interval (IV 1) values each resulted in populations of 1 panther, the other three interval values (IV 2, IV 3, and IV 4) and the maximum value resulted in populations of 2, 5, 11, and 26 panthers, respectively, at the end of 25 years. The male sub-adult and prime-adult survival rates (MSASR and MPASR) were individually altered in scenarios 6 and 7, in which both of the parameters each resulted in a population decline from 200 to 3 panthers. In scenario 8, however, a varying male older-adult survival rate (MOASR) resulted in subsequent varying population values: the minimum and first three interval values (IV 1, IV 2, and IV 3) each resulted in a population of 3 panthers, the fourth interval value (IV 4) a population of 4 panthers, and the maximum value a population of 6 panthers at the end of 25 years. Scenarios 9-12 involved changing the various natality parameters while keeping the remaining parameters at baseline. While only one parameter was changed while other remained baseline during the previous scenarios, the reproductive probabilities and the average number of young birthed per female were changed together; the general natality parameter is composed of these two values. Scenario 9 specifically called for changing the reproductive probabilities for sub-adults (q_{sa}) and the average number of young birthed per sub-adult female (v_{sa}). In this scenario, the different values affected the population differently: the minimum and first interval value (IV 1) each resulted in a population of 2 panthers, and the remaining interval values (IV 2, 3, and 4) resulted in populations of 3, 4, and 5. The maximum value caused a slight population increase to 208 panthers within the first seven years before decreasing to 10 panthers at the end of 25 years. In scenario 10, the reproductive probabilities for prime-adults (q_{pa}) and the average number of young birthed per prime-adult female (v_a) were changed. The minimum value resulted in a population of 1 panther, the first and second interval values (IV 1 and IV 2) both resulted in a population of 2, and the remaining interval values (IV 3 and IV 4) and maximum value resulted in populations of 4, 7, and 14 panthers at the end of 25 years. Before decreasing to the final population of 14, the maximum value first increased to a population of 208 panthers within the first couple of years. In scenario 11, the reproductive probabilities of older-adults was changed. The minimum and first interval value resulted in a population of 1 panther, while the remaining three interval values and maximum value had populations of 2, 4, 7, and 12 panthers after 25 years. In the final scenario, all of the parameters within natality were changed at the same time. The minimum value of natality resulted in a population of 0 panthers. The first and second interval values each affected the population by causing it to decrease to a population of 1 panther. The third interval value had a final population of 7 panthers, and the maximum and fourth interval values both caused the population to increase in the first few years of the simulated scenario before decreasing. While the maximum value caused the population to slightly decrease and level off at a population of 219 panthers, the first interval value had a resulting population of 37 panthers. The population results from the natality parameters are shown below (Figure 4).

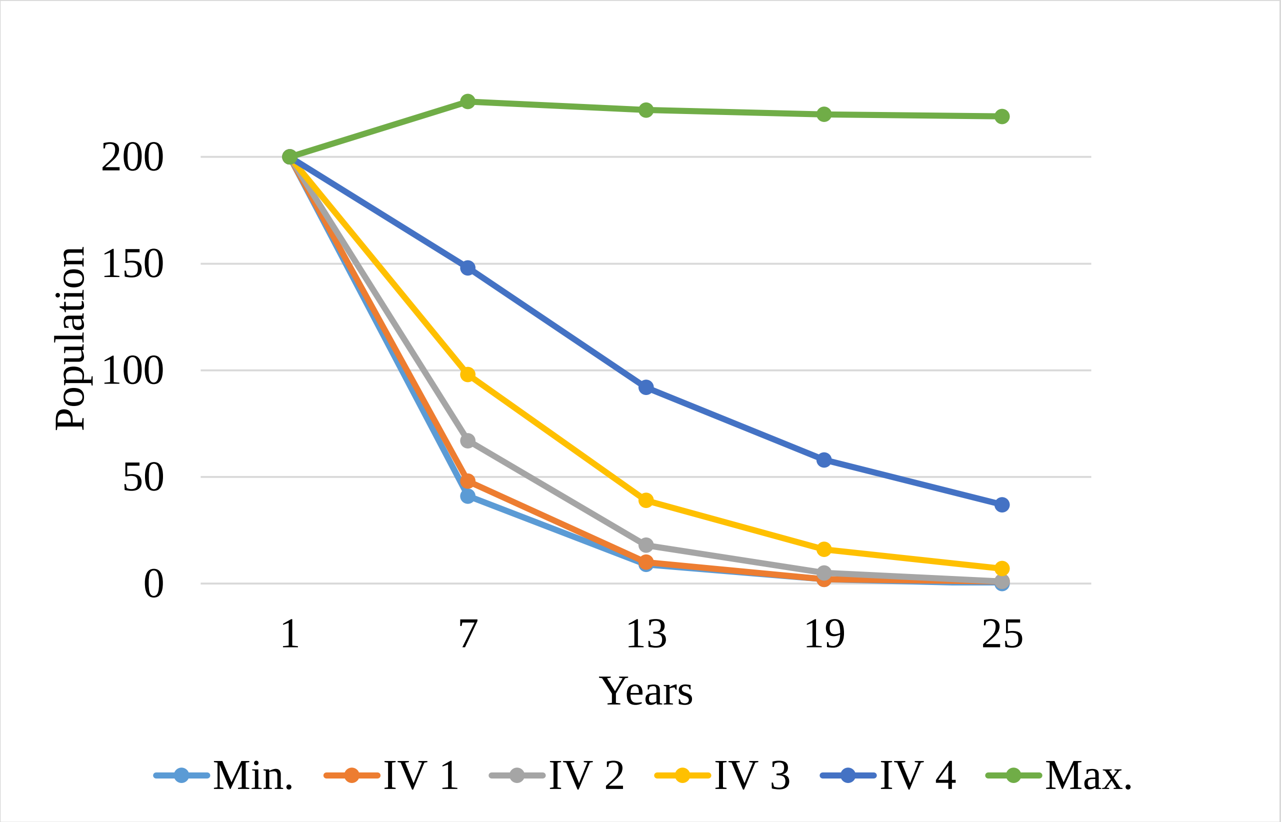

Figure 4. Population trends of the combined natality rate parameters

CHAPTER IV

DISCUSSION

Data analysis

The twelve scenarios run using the STELLA ® 7.0.1 model indicate that the introduction of the 8 female Texas cougars into the two isolated populations of Florida panthers was successful in increasing the genetic diversity and therefore the population of the Florida panthers. The simulation of scenario 1 indicated that the additional genes from the Texas cougars provided enough biodiversity to enable the panther population to increase exponentially if the survival and natality rates are at a maximum value, as opposed to the population going extinct with minimum parameter values. When parameters are set to minimum, the model mimics a situation in which natality and survival rate are low, reflecting a high occurrence of inbreeding. The continued occurrence of inbreeding effects will cause the population to go extinct within a few decades. Though most of the scenarios evaluating changing parameters individually did not show much variance in resulting population, the difference that was shown indicates that increased natality and/or survival due to gene flow will result in a smaller degree of population decrease, prolonging the existence of the subspecies. Scenarios 9-12, which involved changing the natality rates of the different age groups, showed the most varied results and the first occurrences of population increase when a single parameter is manipulated. In scenarios 9 and 10, varying values of the reproductive probabilities and average litter size differ from scenario 11; while the latter does not experience a population increase to above 200 when parameters are at maximum, the two former scenarios do show such an increase. These scenarios mimic a natural phenomenon in which inbreeding effects are at high or low occurrence, thereby limiting or not impacting the total population. Scenario 11 is different from 9 and 10 because older adult females are not shown to reproduce often regardless of inbreeding due to natural decreased fertility chances that occur as panthers age. The scenario that shows the most revealing results regarding the effect of decreasing the inbreeding is scenario 12. In this scenario, which mimics how the degree of inbreeding affects natality rates of all female panthers, low natality rates resulting from a high occurrence of inbreeding would lead to certain extinction, while high natality rates characteristic of a lack of inbreeding would result in an increasing and/or stable population of panthers. Specifically, the resulting population range of 0 to 1725 panther after 25 years indicates that, assuming genetic viability remains the only factor affecting individuals, high reproductive probabilities and the high averages of young birthed per female will lead to an exponential population increase. Though the current population of Florida panthers numbers less than 200, possibly less than 100, it has grown from the approximate population of 70 in 1999 (Schwab & Zandbergen 2011). An increase of an estimated 30-130 individuals over the course of two decades is not much in comparison to population increases, however, it is a significant increase in a population of only 100-200 animals. To confirm the implications of the model results, studies analyzed in the literature review show that the first progeny of the female Texas cougars and the male Florida panthers had decreased occurrences of kinked tails, cowlicks, and cryptorchidism, though further research is required to validate these results (Hedrick & Fredrickson 2010). If the reductions of the inbreeding traits are to this estimated degree, these effects will further diminish with time if the population continues increasing.

Continued research of the presence of inbreeding in Florida panthers is required to prevent further biodiversity loss from occurring.

Continuing problem of habitat loss

Drainage of the Everglades

Though current genetic evidence indicates a low occurrence of the negative inbreeding effects, Florida panthers face a continued threat of extinction due to habitat fragmentation from urbanization (Kautz et al. 2006).In the past century, Florida panther habitat has shrunk to a small portion of Southwest Florida (Murrow et al. 2013). In the late 19th century, action was taken to drain the Everglades of water so that the land could be converted to be used for farmland and development, however the impact of drainage didn’t escalate until the mid-20th century (Sklar et al. 2005). During this time, plans were developed to create the current water-management systems, including canals, which exist in Florida (Sklar et al. 2005). The drainage of the Everglades involved diverting runoff water from Lake Okeechobee, the main water source to the Everglades, east to the Atlantic Ocean and West to the Gulf of Mexico (Sklar et al. 2005). Since drainage began, around 400,000 km² of the Everglades has been converted into farmland and communities (Sklar et al. 2005), with only 5,650 km² remaining in Everglades National Park (Richardson, 2010). Plans to conserve and expand the Everglades have been developed in recent years. The most recent of these is the Comprehensive Everglades Restoration Plan, or CERP, which details plans to reallocate water to the Everglades by decreasing current water paths and reinstating historic flows feeding to ENP (Richardson, 2010). Created in 2000, CERP has yet to make headway in restoring the Everglades due to rising costs and other conflicts, though research and action is being taken to begin projects (Guinto & Reed, 2008).

Habitat fragmentation from interstates and highways

In addition to drainage of the Everglades, the area has also been fragmented by increasing roads including highways and interstate; the main interstate crossing through most of Florida, I-75, travels through BCNP and connects to ENP via highways (Gunto & Reed, 2008). Due to the high traffic on these roads occurring in close proximity to panther habitat, many Florida panther fatalities occur from collisions with automobiles (Schwab & Zandbergen 2011). In response to the increase in panther mortalities from car accidents due to home ranges lying adjacent to I-75, over 20 underpasses have been constructed to aid animals in traveling between areas without risk (Schwab & Zandbergen 2011). However, studies of the success of such underpasses show that panthers do not cross the underpasses of high-trafficked roads as much as those of smaller, less traveled roads (Schwab & Zandbergen 2011). Statistics show that the development of Florida and the subsequent road traffic is not going to yield, but exponentially increase. When drainage of the Everglades began, population of south Florida was less than 80,000 (Clarke, 2003). At the start of the 21st century, the population of Florida was just over 15 million, and is projected to nearly double in the next decade (Guinto & Reed, 2008).

Need for further research

In order to increase the population of Florida panthers to a sustainable level when the human population surrounding the habitat continues to rise, research needs to be done regarding how the sub-species can be conserved without disrupting current developments. Specific research topics could analyze future plans for develop as well as historic and current panther behavior to evaluate if negative anthropogenic behaviors are present. It is vital to know exactly how panthers are being affected in order to create the most effective conservation plan. To ensure the survival of the Florida panther, further research regarding habitat and human-panther relations need to be conducted in conjunction with increasing and maintaining the genetic diversity of the sub-species.

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