

Summer 2023

# The Effect of Genetic Relatedness on Mate Selection and Spatial Distribution in the American Alligator, *Alligator mississippiensis*

Mark Hoog

Follow this and additional works at: <https://digitalcommons.georgiasouthern.edu/etd>



Part of the [Genetics Commons](#), and the [Other Animal Sciences Commons](#)

---

## Recommended Citation

Hoog, Mark, "The Effect of Genetic Relatedness on Mate Selection and Spatial Distribution in the American Alligator, *Alligator mississippiensis*" (2023). *Electronic Theses and Dissertations*. 2619.

<https://digitalcommons.georgiasouthern.edu/etd/2619>

This thesis (open access) is brought to you for free and open access by the Jack N. Averitt College of Graduate Studies at Digital Commons@Georgia Southern. It has been accepted for inclusion in Electronic Theses and Dissertations by an authorized administrator of Digital Commons@Georgia Southern. For more information, please contact [digitalcommons@georgiasouthern.edu](mailto:digitalcommons@georgiasouthern.edu).

THE EFFECT OF GENETIC RELATEDNESS ON MATE SELECTION AND SPATIAL  
DISTRIBUTION IN THE AMERICAN ALLIGATOR, *ALLIGATOR MISSISSIPPIENSIS*

by

MARK E. HOOG

(Under the Direction of Aaron Schrey)

ABSTRACT

To date, most genetic studies on the American alligator (*Alligator mississippiensis*) have focused on population genetics and multiple paternity. There have not been any studies looking at how relatedness affects alligator behavior and movement. This study focused on three main questions: 1) what is the genetic diversity among alligators in this location? 2) can parentage be accurately defined among parents and offspring? and 3) how does individual relatedness affect their spatial distribution? We examined the relatedness of 174 unique individuals from the Okefenokee Swamp Park, in South Georgia, USA. In addition, we placed Telonics<sup>®</sup> satellite tags on 10 adult alligators and created home ranges for these animals. We extracted DNA from tissue, bone, and egg samples, screened multiple microsatellites via PCR, and characterized each individual's genotype. Mean Pairwise Relatedness (*MPR*) was used to determine the upper and lower 95% confidence limits to describe the expected random deviations of *MPR* if random mating occurred among sites. The data were categorized into six groups based on the sex and age classes of the alligators. The *MPR* was calculated among individuals with known GPS capture locations and tracking movement data to examine the effect of relatedness on spatial distribution. By including the GPS locations, it was possible to test if animals with overlapping, or disjunct home ranges differed in genetic relatedness. Observational field data and *MPR* were also used to test relatedness among all sampled alligators. Since alligators had known age classes and sex, the

*MPR* was integrated with the known physical data to assign hatchlings and juveniles to a parent pair. Overall hatchlings and juvenile males trended slightly towards inbreeding, while adult males trended slightly toward out breeding. I was able to determine parentage for three nests, one nest was consistent with multiple paternity, while the other two were consistent with having only a single sire, but all three nests were at least partially sired by the dominant male. Spatial data combined with *MPR* showed that non-related individuals are significantly more likely to have overlapping territories than related individuals. Specifically, non-related individuals had 5.3 times more hectares of overlap than related individuals.

INDEX WORDS: Alligators, Dominance, Genetics, Genetic diversity, Okefenokee Swamp, Parentage, Relatedness, Reptiles territory

THE EFFECT OF GENETIC RELATEDNESS ON MATE SELECTION AND SPATIAL  
DISTRIBUTION IN THE AMERICAN ALLIGATOR, *ALLIGATOR MISSISSIPPIENSIS*

by

MARK E. HOOG

B.S., Newberry College, 2019

A Dissertation Submitted to the Graduate Faculty of Georgia Southern University in Partial

Fulfillment of the Requirements for the Degree

MASTERS OF SCIENCE

© 2023

MARK HOOG

All Rights Reserved

THE EFFECT OF GENETIC RELATEDNESS ON MATE SELECTION AND SPATIAL  
DISTRIBUTION IN THE AMERICAN ALLIGATOR, *ALLIGATOR MISSISSIPPIENSIS*

by

MARK E. HOOG

Major Professor: Aaron Schrey  
Committee: Kimberly M. Andrews  
Alexander Collier  
Kathryn Craven

Electronic Version Approved:  
July 2023

## ACKNOWLEDGMENTS

First off, I am extremely grateful to my advisor Dr. Aaron Schrey and project PI Dr. Kimberly Andrews for taking me under their wing and guiding me through the turbulence that is research. They have been behind me every step of the way and have shared so much of their knowledge and have been so supportive and encouraging. I would like to express my deepest appreciation to the Okefenokee Swamp Park for supporting my stipend, helping to fund the project, and for making sure I have what I need when I am onsite. Many thanks go out to anyone who donated to the UGA Foundation; those donations allowed us to purchase the necessary supplies needed to keep the project and my research going. Special thanks to the Okefenokee National Wildlife Refuge for permitting us to capture alligators and conduct research in the Okefenokee Swamp. A huge thanks goes out to Chip and Joy Campbell for allowing me to use their boat and for being so enthusiastic about the research and for caring so much about the Swamp. Words cannot express my gratitude to Oscar Thompson, Bridgette Hancock, Josh Billings, Colleen Goff and Alex Crib for spending so many, buggy, muggy, late nights in the field with me helping collect data. I'd like to thank everyone who has ever helped me track or catch an alligator. I literally could not have done this without their help. A big thanks to UGA Marine Extension and Georgia Sea Grant for hosting me and allowing me access to their fleet vehicles. I would like to thank my parents for encouraging me to follow my passions and for helping me so much on my journey through life. Lastly, I'd like to give a very special thank you to my wife Megan who has not only been my biggest supporter and fan, but who has also spent a ton of time with me in the field collecting data.

All procedures involving the capturing, handling and collection of blood and tissue samples from alligators were approved by the UGA IACUC. All Animal Use Proposals and field data

collection permits are held by Dr. Kimberly Andrews UGA Marine Extension and Georgia Sea Grant [MAREX-GASG] Coastal Ecology Lab; A2018 09-010-Y3-A4; A2019 11-003-Y3-A2).



## TABLE OF CONTENTS

	Page
ACKNOWLEDGMENTS.....	2
LIST OF TABLES .....	5
LIST OF FIGURES .....	6
CHAPTER	
1 INTRODUCTION .....	7
2 METHODS .....	11
Sample Collection .....	11
Genetic Data Collection and Analyses .....	12
3 Results.....	15
Population Diversity .....	15
Parentage Assignment .....	17
Spatial Distribution .....	18
4 Discussion.....	19
5 Conclusion.....	24
REFERENCES .....	25

## LIST OF TABLES

	Page
Table 1: How Size Class was Determined.....	13
Table 2: Breakdown of Captured Alligators .....	16

## LIST OF FIGURES

	Page
Figure 1: Location of Study Site.....	7
Figure 2: Locations of Captured Alligators.....	15
Figure 3: Mean Pairwise Relatedness.....	16
Figure 4: Relatedness vs Overlap.....	18

## CHAPTER 1

### Introduction

The American Alligator (*Alligator mississippiensis*) is one of the most iconic, recognizable, and ecologically important species in the southeastern United States. They are federally listed as a threatened species due to the similar appearance of the American crocodile (*Crocodylus acutus*), also federally listed as threatened. Further, they are a vital part of the Okefenokee Swamp, which is the largest blackwater swamp in North America, covering more than 438,000 acres. Of the total area, 400,000 acres are protected and managed by United States Fish and Wildlife Service (USFWS, U.S. Fish and Wildlife Service 2023). Spanning parts of South Georgia and North Florida (Figure 1), it is home to more than 600 different species of plants and more than 400 species of animals (U.S. Fish and Wildlife Service 2023).



Figure 1. Location of Study Site. Location of the Okefenokee Swamp and the Okefenokee Swamp Park in South Georgia, USA.

The Okefenokee Swamp is one of the few natural, untouched wilderness areas that still supports this level of biodiversity. As with many natural areas it is continually threatened by the encroachment of human development and industry. With interest in industrial development adjacent to the swamp, coupled with changing environmental conditions due to climate change, understanding the American alligator in the Okefenokee Swamp is key to producing better management techniques to ensure the continued biological success of alligators and their habitat.

The American alligator is often used as an indicator species for an ecosystem. The foraging behaviors and social interactions of these apex predators reflect the accessibility of habitats and health of the ecosystem (Mazzotti et al. 2009). Fully understanding an indicator species is key to fully understanding their ecosystem. The American alligator is a long-lived species commonly reaching 50 years of age, with evidence supporting that they can live past 70 (Wilkinson 2016). Due to their longevity, it takes several years before individuals become sexually mature ranging from 10-18yrs depending on habitat, resources, and temperatures (Lance 2003; Wilkinson 2016). For alligators, much of the insights into what drives their behavior is linked to feeding and mating. Feeding is relatively simple, they are opportunistic ambush predators that will eat almost anything (Delany and Abercrombie 1986; Wolfe 1987), their mating dynamics and movement as it relates to genetics, however, is more complex.

Additionally, alligators are a wide-ranging, generalist forager whose critical role in the ecosystem influences both inter- and intra-specific interactions (Palmer and Mazzotti 2004). This helps support high levels of aquatic and terrestrial biodiversity and overall ecosystem viability

(Palmer and Mazzotti 2004). Alligators are ecosystem engineers, creating wallows that provide freshwater refugia for smaller organisms as water recedes in droughts (Palmer and Mazzotti 2004). When alligators are removed, organisms who rely on these refugia, such as amphibians, also begin to disappear whereas other cosmopolitan species, like racoons, increase (Bondavalli and Ulanowicz 1999).

To date, the majority of genetic studies on the American alligator focus on temperature dependent sex determination (TDSD, Kohno 2008), mating dynamics (Zajdel 2011), and population genetics (Ryberg 2002). One of the biggest questions in alligator genetics has been determining how TDSD works. A gene expression study in gonadal tissue during sex determination revealed the underlying genetic framework (Yatsu et al. 2016). The alligator TRPV4 ortholog (AmTRPV4) is activated at temperatures that determine sex in alligators, with AmTRPV4 channel activity affecting the gene expression patterns associated with male differentiation (Yatsu et al. 2016).

There are multiple studies on mating dynamics that document the regular occurrence of multiple paternity (Zajdel et al. 2019; Davis et al. 2001). Multiple paternity is present in many reptiles (e.g., Uller and Olsson 2008). In a study by Lance et al. (2009), mate fidelity is the only other aspect of mating dynamics that has been examined, among 10 females, seven females exhibited mate fidelity, but only three females exhibited single paternity.

There has been only one study that incorporated genetic relatedness into spatial ecology of alligators. Davis et al. (2001) failed to detect a correlation between nest proximity and genetic relatedness of the attending female. Yet, the genetic component was a small aspect of the study, which makes it difficult to draw strong conclusions. The current literature on alligator genetics leaves several questions to be answered, primarily concerning the effect of genetic relatedness on

reproductive behavior and spatial distribution. The Okefenokee Swamp is a great place to answer these questions as all age classes can be collected contemporaneously, which enables mating dynamics to be elucidated even if the specific nest is not located.

The effect that genetic relatedness plays in the life of an alligator and their daily activities has yet to be studied. The objective of this Master's thesis was to address how genetic relatedness affects mate selection and spatial distribution of alligators in the northern portion of the Okefenokee Swamp. To accomplish this, American alligators were sampled across multiple reproductive stages (adult, sub-adult, juvenile, hatchling) from the Okefenokee Swamp Park (OSP), and microsatellite loci were screened to estimate genetic diversity to identify genetic relatedness and parentage. There were three main questions: 1) what is the genetic diversity among alligators in this location? 2) can parentage be accurately defined among parents and offspring? and 3) how does individual relatedness affect their spatial distribution?

## CHAPTER 2

### Methods

#### Sample Collection

This project was conducted in collaboration with the UGA Marine Extension and Georgia Sea Grant Coastal Ecology Lab (CEL) who began collecting samples in the Okefenokee Swamp in 2017. Sampling occurred at the northern entrance of the Okefenokee Swamp, the Okefenokee Swamp Park (OSP). Alligators were captured by attaching a weighted treble hook (79.3-83.8g) to 150lb (68kg) to braided fishing line. The treble hook was cast beyond the animal and then reeled in slowly until the hook contacted the animal. Once the hook was set, the animal was reeled in until it is close enough for the animal to be snared using a cable snare attached to a 2.4m paint pole. If the animal was captured from a boat, the mouth was taped shut prior to bringing the animal aboard the vessel, otherwise the animal was restrained once on land. Rope restraints were attached to the animals' hind legs and a towel placed over the animals' eyes to keep the animal calm. Tissue samples were taken from the single row of scutes on the alligator's tail using a sterile scalpel after the area had been cleaned with alcohol and betadine. Tissue samples collected were used to obtain DNA.

In addition to the collection of samples, the CEL also monitored the movements of adult alligators within the Swamp through the use of Telonics satellite tags, a GPS logger with an online downloadable data component and built-in VHF (Model SeaTrkr-4370-4). This allowed for the estimations of home ranges that could then be compared against the results of the relatedness analyses to determine the distribution of adult alligators relative to their genealogy. Movement and home range data was available for 10 adult alligators.



### Genetic Data Collection and Analyses

DNA was extracted from a total of 174 individuals using scutes, eggs, and bone tissue following the manufacturer's protocol of the Dneasy Kit (Qiagen, Valencia CA USA). DNA concentrations were determined using a 24 NanoDrop Spectrophotometer ND-1000 (Thermo Scientific, Waltham, MA). Previously developed microsatellites loci (Glenn et.al. 1998; Subalusky 2012) were optimized for the newly collected alligator samples. Microsatellite loci were screened by PCR to determine the allelic variation across loci. PCR was conducted at a final volume of 10  $\mu$ L, containing 1 X PCR Buffer (50 mM KCl, 10mM Tris HCl pH 9.0), 2 mM  $MgCl_2$ , 200  $\mu$ M each dNTP, 0.1 unit Taq DNA polymerase, 0.9  $\mu$ M of each PCR primer (forward primers labeled with 6-Fam or HEX), and 1-20 ng template DNA. Thermal cycles were 94 °C 2 m, 94 °C 30 s, annealing 30 s (temperatures defined by Ernst et al. 2004), 70 °C 30 s, 70 °C 5 m, repeated 40 times. Resultant PCR products were analyzed via fragment analysis at the Pritzker Laboratory for Molecular Systematics and Evolution at The Field Museum (Chicago, Illinois). Resultant lane files were analyzed by the ThermoFisher Connect, Microsatellite Analysis, CE Fragment Sizing (Applied Biosystems, Waltham, MA).

Genetic diversity was characterized by estimating expected heterozygosity ( $H_e$ ), observed heterozygosity ( $H_o$ ), the system of mating inbreeding coefficient ( $F_{IS}$ ), and mean pairwise relatedness ( $MPR$ ; Queller and Goodnight, 1989) with GENALEX6 (Peakall and Smouse 2006, 2012).  $MPR$  was compared among six groups of individuals using 9999 permutations of genotypes among categories to determine the upper and lower 95% confidence limits to describe the expected random deviations of  $MPR$  if random mating occurred among sites. The data were categorized into groups based on the sex and age classes of the alligators: adult male, adult

female, sub-adult male, sub-adult female, juvenile male, juvenile female, hatchlings, egg, and unclassified (Table 1).

<b>Size Class</b>	<b>Length (cm)</b>
Adult	> 213
Sub-Adult	152-213
Juvenile	61-151
Hatchling	< 61

Table 1. Size Classes. How alligator size class was determined.

*MPR* was also calculated among individuals with known GPS capture locations, and tracking movement data, to examine the effect of relatedness on spatial distribution. By including the GPS locations, it was possible to test if animals with overlapping, or disjunct home ranges differed in genetic relatedness.

Also, for cases when a female and male alligator were observed mating, the genotypes of those individuals, and those from the resultant hatchlings, were visually inspected to ensure the genotypic data accurately reflected the known parentage. For the visual inspection, the genotype of each offspring was compared to the genotypes of both suspected parents at four microsatellite loci. If there was an allelic mismatch at more than one locus, the offspring was deemed to not belong to the parent pair.

The observational field data and *MPR* were also used to test relatedness among all sampled alligators. Since alligators had known age classes and sex, the *MPR* was integrated with the known physical data to assign hatchlings and juveniles to a parent pair. While it can be difficult to distinguish the level of relatedness between siblings and between parent and offspring

using observational field data such as, age class, size, dominance, and observed mating, it was possible to determine relationships among individuals including the genetic data. In addition to samples from live individuals, DNA also was extracted from the bones of several dead adults. These included the former dominant male in this area, who was thought to have sired many of the current adults. Including these adults, allowed relatedness to be compared over at least three generations of alligators.

## CHAPTER 3

## Results

## Population Diversity

A total of 174 unique individuals were captured at OSP (Figure 2). The breakdown for each category was; 14 adult males, 14 adult females, 5 sub-adult males, 1 sub-adult female, 20 juvenile males, 14 juvenile females, 87 hatchlings, 9 eggs, and 8 unclassified where the sex of the animal was unable to be determined (Table 2).

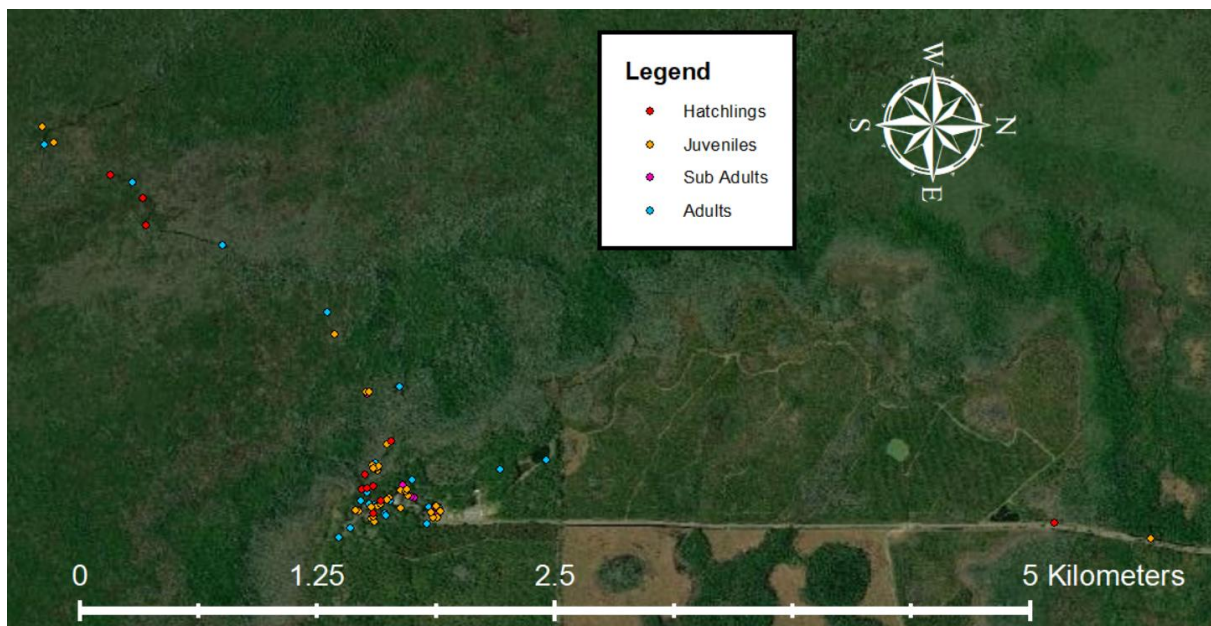


Figure 2. Locations of captured alligators. Blue = Adults, Pink = Sub-adults, Orange = Juveniles and Red = Hatchlings

Sex	Adult	Sub-Adult	Juvenile	Hatchling	Egg	Unclassified
Male	14	5	20	0	0	0
Female	14	1	14	0	0	0
Unknown	0	0	0	87	9	8

Table 2. Breakdown of Captured Alligators. Sex of American alligators screened from the Okefenokee Swamp Park with the number of individuals in each of the six reproductive categories identified.

Due to the small sample size of eggs, the eggs were combined into the hatchling category; likewise, the sub-adults were combined with the corresponding adult category in further analyses. For each category, mean pairwise-relatedness was: adult males  $MPR = -0.109$  (95% CI =  $-0.045 - 0.176$ ;  $P$ -value = 0.061), adult females  $MPR = 0.006$  (95% CI =  $-0.042 - 0.054$ ;  $P$ -value = 0.347), juvenile males  $MPR = 0.078$  (95% CI =  $0.120 - 0.037$ ;  $P$ -value = 0.028), juvenile females  $MPR = 0.021$  (95% CI =  $0.064 - -0.086$ ;  $P$ -value = 0.556), hatchlings  $MPR = 0.011$  (95% CI =  $0.939 - 0.001$ ;  $P$ -value = 0.048), unclassified  $MPR = 0.016$  (95% CI =  $0.094 - -0.126$ ;  $P$ -value = 0.498; (Figure 3).

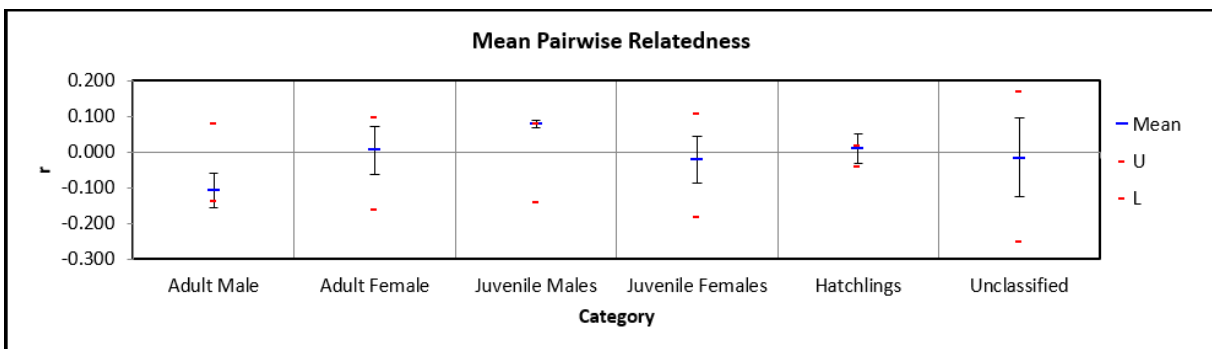


Figure 3. Mean Pairwise Relatedness. Mean Pairwise Relatedness Values for the six categories for alligators within the population at the Okefenokee Swamp Park.

### Parentage Assignment

Of the 96 hatchlings, a total of 24 could be assigned to a parent pair. These 24 hatchlings came from three nests (Nests 1, 2, and 3) from three different years (2018, 2019 and 2021). Hatchlings were assigned to five different alligators, two females, adult female 1 (AF1), adult female 2 (AF2) and three males, adult male 1 (AM1), adult male 2 (AM2) and adult male 3 (AM3). Hatchlings from Nests 1 and 3 matched the same female, AF1, and hatchlings from Nest 2 matched a different female, AF2. The mothers were observed attending the nests to which they matched hatchlings; however, despite multiple attempts, AF2 was not able to be captured, so her DNA was not available. However, it was possible to recreate AF2's genotype using the genotypes of the two males who were identified as sires of her nest. Hatchlings from Nests 1 and 3 were consistent with single paternity and were both sired by the dominant male at the time; AM1 for Nest 1 and AM2 for Nest 3. Hatchlings from Nest 2 were consistent with multiple paternity with the primary sire being the dominant male at the time, AM2, and the secondary sire being AM3. AM3 was smaller than AM2, but their genotypes were consistent with them being half-siblings ( $MPR = 0.351$ ).

In addition to assigning hatchlings and juveniles to adults, it was possible to assign current adults to parents using DNA from the bones from three adult alligators who had died, providing a unique opportunity to investigate the relatedness of the standing population of adults. Sets of bones were available from females ( $n = 2$ ), and the previous alpha dominant male from approximately 20 years ago. One female, who died in 2021, was consistent with being the mother of one adult, two sub-adults and four juveniles. Interestingly, the second female, who died in 2015, and the previous dominant male, who died in 2007, did not match any sampled offspring. Most of the current adults were negatively related to these two individuals.

### Spatial Distribution

Spatial data collected from satellite tags was available for 10 adult alligators; five males and five females. Hoog et al. (*unpublished*) created home ranges and calculated home range overlap in hectares for all 10 animals. *MPR* was separately calculated pairwise for these 10 individuals. Of the 45 pairwise *MPR* estimates, 14 had a positive *MPR* and 31 had a negative *MPR* (Figure 4).

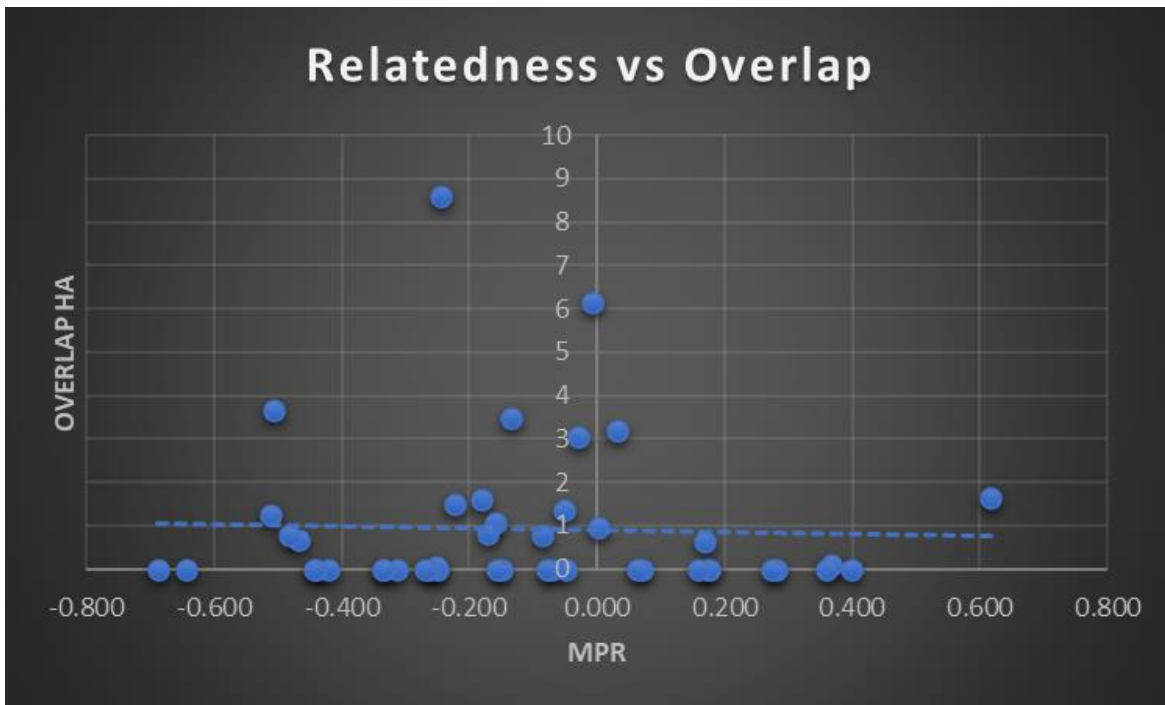


Figure 4. Relatedness vs. Overlap. *MPR* (x axis) compared to Overlap in ha (y axis)

The largest amount of overlap for individuals with a positive *MPR* was 3.20 ha and the largest amount of overlap for individuals with a negative *MPR* was 8.59 ha. The total overlap for individuals with an *MPR* over 0.3 was 1.72 ha and the total overlap for individuals with an *MPR* of less than 0.3 was 6.37 ha. The total overlap for individuals with a positive *MPR* was 6.54 ha and the total amount of overlap for individuals with a negative *MPR* was 34.85 ha.

## CHAPTER 4

### Discussion

The Okefenokee Swamp Park is a unique study site as it has been open to the public for 75 years and offers guests the chance to experience the swamp via tours. This meant that we had access to historical alligator data that proved critical when looking at relatedness over multiple generations. However, because it has been open to the public for 75 years, that also means that the ecosystem had previously been heavily modified. Channels have been carved through the usually dense bald cypress (*Taxodium distichum*) and shrub forests creating artificial pathways through which alligators can travel. These deeper areas of water have also become central hubs in which alligators live and congregate.

Aside from habitat modification, the alligator population itself has also been impacted by human activity. Specifically, there was a legacy of feeding and removing wild alligators. These actions no longer occur at OSP, but the effects can still be seen in the alligator population. The feeding has left a few of the older adults in the population curious of humans rather than wary. This resulted in alligators being removed as a consequence of becoming too friendly with people on account of a positive association and attraction to humans after imprinting from feeding. We have attempted to combat this through behavioral conditioning in which we attempt to re-associate a fear of humans via mark-recapture, which has demonstrated positive results as an alternative management technique in order to prevent removal from the population (Andrews et al. 2018).

The legacy of removal, specifically the removal of reproductive adults and hatchlings, resulted in a temporary reduction in reproduction and recruitment, which can be seen in the low number of sub-adults (six) captured over the course of the study. The animals removed were



placed into captivity to ensure the public was able to see both large and baby alligators on their visit. While these animals were not killed literally, from the standpoint of population genetics, they were non-contributors and thereby, functionally eliminated from the population. We were able to collect samples from these individuals in captivity, so it did not affect our ability to look at relatedness over multiple generations. Currently, Okefenokee does not allow feeding or the removal or harvest of any alligator in the Okefenokee Swamp unless there is an immediate threat to human safety.

To date, there has not been a genetics study on alligators that incorporates this many individuals, of this many age classes, over this many generations. With these data, it was possible to characterize the genetic diversity of the OSP population. As it pertains to genetic diversity, inbreeding does not refer to consanguineous mating but rather that the individuals within the subpopulation were more related to each other than one would expect by random chance. It is important to understand if the population is trending towards inbreeding or outbreeding as either extreme can affect, reproductive fitness, disease resistance, genetic diversity and overall fitness (Ralls 2013). The first finding of note was that hatchlings tended towards inbreeding, which is to be expected as the 96 hatchlings in the study came from four nests. This means there were four sets of brothers and sisters screened, not a random sampling of hatchlings across several nests. Thus, the trend toward inbreeding reflects their actual relatedness, rather than a systemic population-level phenomenon of consanguineous mating. Second, juvenile males also trended toward inbreeding. This could be explained by the dominant male chasing out other males that may compete for mates but allowing related juveniles to stay in the immediate area, thus increasing their chance of survival and continuing his lineage. Also of note, the 95% confidence intervals for adult males and adult females did not overlap

indicating a significant difference in the genetic diversity between the two groups. The adult females had a  $MPR = 0.06$ , very slight inbreeding, but the adult males had a  $MPR = -0.109$ , stronger outbreeding. A trend towards outbreeding is consistent with the behavior of males, which have been documented to have larger home ranges and travel longer distances in search of mates (Goodwin and Marion 1979).

In total, 24 hatchlings could be assigned to a parent pair, these hatchlings came from three nests, two were consistent with a single sire and the third was consistent with multiple paternity. This data is consistent with previous studies where multiple paternity was found in some but not all nests (Lance et. al. 2009; Zajdel et al. 2009). The current findings also support previous studies that hypothesized dominance plays a role in mate selection (Zajdel et al. 2009). All three nests were at least partially sired by the dominant male. Dominance has been found to play a significant role in reproduction in another predatory organism the elephant seal (*Mirounga angustirostris*) where male reproductive success is directly correlated to dominance rank (Haley et. al. 1994). However, due to the limited sample size of nests and the low number of hatchlings where parentage could be assigned, further work is needed to show the precise effect dominance has on paternity.

One of the most interesting discoveries in this effort was that the previous dominant male, who died in 2007 and who was believed to have sired many nests in his time, was not consistent with being the father, or grandfather, of any sampled alligator. Given the longevity of alligators (50-70yrs, Wilkinson 2016), we are confident that the previous dominant male's offspring would still be alive as many of them would have reached sexual maturity and would be reproducing themselves. One explanation is that there are usually multiple large alligators and, if they are not tagged, it can be difficult to distinguish between them. However, the sheer size of

this alligator, over 4m long, make it unlikely he was misidentified. Alligators typically do not get that big, and the likelihood of multiple alligators of that size cohabiting the same area is relatively low. The succedent dominant male however had many individuals who were strongly related to him. This suggests a hypothesis to explain these findings is that when a dominant male dies or vacates his territory and a new male takes over, the new dominant male attempts to rid the area of any alligators not related to him. Male alligators are territorial and larger males are able to hold and defend territories and have been known to chase off other males (Garrick and Lang. 1977; Joanen and McNease. 1971). If the dominant male were to only chase off sexually mature alligators, this would create a pattern of what I am referring to as “male-biased dispersal,” an occurrence seen in some other large vertebrate predators, such as African lions (*Panthera leo*) where juvenile males are forced out of their natal pack at onset of sexual maturity (Pusey and Packer. 1987). My results on genetic diversity support this hypothesis, as the adult males trend toward outbreeding and the juvenile males are significantly more related to each other than expected by chance. If a dominant male remains in one area for an extended period of time, it is theoretically possible for him to have cleared the area all males related to the previous dominant male. This would leave only male animals related to the current alpha male (and possibly some alternate sires that were offspring of the alpha dominant male). This would skew the population towards inbreeding. This is further supported by finding the one animal that was related to the previous alpha male was a small female.

Integrating spatial data with *MPR* estimates found a very clear pattern that animals who are less related are more likely to have overlapping territories. This marks the first study to pair an *MPR* comparison with home range and home range overlap in crocodylians. The data was compared in two ways, one was the total overlap of animals with a positive *MPR* vs the total

overlap of animals with a negative *MPR*. The second was comparing the total overlap of animals above an *MPR* of 0.3 vs total overlap of animals with an *MPR* less than -0.3. For both comparisons, the amount of spatial overlap for non-related individuals was significantly higher than that of related individuals. For positive vs negative *MPR*, the amount of overlap for negatively related individuals was 5.3 times higher than that of positively related individuals. The fixed *MPR* level showed negatively related individuals had 3.7 times more overlap than related individuals. This trend of establishing territories that overlap more with unrelated individuals would be evolutionarily beneficial as mating with unrelated individuals would increase the fitness of their offspring by avoiding inbreeding depression.

Multiple paternity and mating in alligators has been heavily studied and the results gathered from this study further support previous findings. Davis et al. (2001) found that the attending female had a genotype consistent with the clutch she was guarding, which is expected given the high level of maternal care in alligators. The attending female captured in our study was also consistent with this finding. The occurrence of multiple paternity in the present study was 33% which is consistent with the findings of Davis et al. (2001), but considerably lower than the 51% average reported by Lance et al. (2009). Multiple paternity seems to be highly variable among sites which may have to do with the variability of habitat across the alligator's range.

While we are confident in the data collected, we acknowledge that four loci are fewer than most microsatellite studies and, from an abundance of caution, suggest limited inferences should be drawn from the current results. Adding additional loci would increase confidence in the results, and investigating alligators from multiple locations would support more broad inferences to be drawn. The next step for this research is to expand to other locations in the Okefenokee Swamp and to add questions about population genetics and epigenetics.

## CHAPTER 5

### Conclusion

In conclusion, alligators in the OSP are consistent with a hypothesis that new dominant males drive out nonrelated males, leaving only males related to the dominant male. The dominant male also seems to have a significant impact on reproduction as all three nests were at least partially sired by the dominant male. Of special note, a previous dominant male, from the early 2000s, was only strongly related to one female in the current population. As there were many individuals related to the succedent dominant male, this suggests that when a new dominant male is introduced, he attempts to rid his territory of any individuals related to the previous dominant male. In addition to the genetic data, spatial data compared to relatedness shows that individuals who are unrelated were more likely to have overlapping territories than related individuals. The present study supported previous findings of multiple paternity, but also finds several novel properties centering on the dominant male.

## REFERENCES

- Andrews KM, Skupien GM, and Bauer RG. 2018. Alternative management tools for American alligators. *In*: S. Henke and C. Eversole (eds.), pp. 329-347. *American Alligators: Habitats, Behaviors, and Threats*. Nova Publishing, Hauppauge, NY, USA.
- Bondavalli C. and Ulanowicz RE. 1999. Unexpected Effects of Predators Upon Their Prey: The Case of the American Alligator. *Ecosystems* 2: 49–63
- Davis LM, Glenn TC, Elsey RM, Dessauer HC. and Sawyer RH. 2001. Multiple paternity and mating patterns in the American alligator, *Alligator mississippiensis*. *Molecular Ecology*. 10: 1011-1024.
- Delany MF. and Abercrombie CL 1986. American alligator food habits in northcentral Florida. *The Journal of Wildlife Management* 50: 348-353.
- Ernst JA, Branch LC, Clark AM, and Hokit DG. 2004. Polymorphic microsatellite markers for the Florida scrub lizard (*Sceloporus woodi*). *Molecular Ecology Notes* 4: 364-365.
- Garrick LD. and Lang JW. 1977. Social signals and behaviors of adult alligators and crocodiles. *American Zoologist* 17: 225-239.
- Glenn TC, Dessauer HC. and Braun MJ. 1998. Characterization of microsatellite DNA loci in American alligators. *Copeia* 1998: 591-601.
- Goodwin TM. and Marion WR. 1979. Seasonal activity ranges and habitat preferences of adult alligators in a north-central Florida lake. *Journal of Herpetology* 13: 157-163.
- Haley MP, Deutsch CJ, and Le Boeuf BJ. 1994. Size, dominance and copulatory success in male northern elephant seals, *Mirounga angustirostris*. *Animal Behaviour* 48: 1249-1260.

- Joanen T. and McNease L. 1971. Propagation of the American alligator in captivity. In Proceedings of the Annual Conference, Southeastern Association of Game and Fish Commissioners 25: 106-116.
- Kohno S, Bermudez DS, Katsu Y, Iguchi T. and Guillette, Jr LJ. 2008. Gene expression patterns in juvenile American alligators (*Alligator mississippiensis*) exposed to environmental contaminants. *Aquatic Toxicology* 88: 95-101.
- Lance VA. 2003. Alligator physiology and life history: the importance of temperature. *Experimental Gerontology* 38: 801-805.
- Lance SL, Tuberville TD, Dueck L, Holz-Schietinger C, Trosclair III PL, Elsey RM. and Glenn TC. 2009. Multiyear multiple paternity and mate fidelity in the American alligator, *Alligator mississippiensis*. *Molecular Ecology* 18: 4508-4520.
- Mazzotti FJ, Best RG, Brandt LA, Cherkiss MS, Jeffery BM. and Rice KG. 2009. Alligators and crocodiles as indicators for restoration of Everglades ecosystems. *Ecological Indicators* 9: 137-149.
- USFWS (US Fish and Wildlife Service). 2023. *Okefenokee National Wildlife Refuge*. FWS.gov. (2023, January 13). Retrieved January 17, 2023, from <https://www.fws.gov/refuge/okefenokee>.
- Palmer ML and Mazzotti FJ. 2004 Structure of Everglades alligator holes. *Wetlands* 24: 115-122.
- Peakall R and Smouse PE. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*. 6: 288-295.
- Peakall R and Smouse PE. 2012. GenALEX 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics* 28: 2537-2539.

- Pusey, AE and Packer C. 1987. The evolution of sex-biased dispersal in lions. *Behaviour* 101: 275-310.
- Queller DC and Goodnight KF. 1989. Estimating relatedness using genetic markers. *Evolution* 43: 258-275.
- Ralls K, Frankham R, and Ballou JD. 2013. Inbreeding and outbreeding. *In*: Levin SA. (ed.), pp. 245-252. *Encyclopedia of Biodiversity*, 2nd edition, Volume 4, Academic Press, Waltham, MA, USA.
- Ryberg WA, Fitzgerald LA, Honeycutt RL. and Cathey JC. 2002. Genetic relationships of American alligator populations distributed across different ecological and geographic scales. *Journal of Experimental Zoology* 294: 325-333.
- Subalusky AL, Garrick RC, Schable NA, Osborne J. and Glenn TC. 2012. Development and characterization of tetranucleotide microsatellite loci for the American alligator (*Alligator mississippiensis*). *Conservation Genetics Resources* 4: 567-570.
- Uller T and Olsson M. 2008. Multiple paternity in reptiles: patterns and processes. *Molecular Ecology* 17: 2566–2580.
- Wilkinson PM, Rainwater TR, Woodward AR, Leone EH, and Carter C. 2016. Determinate growth and reproductive lifespan in the American alligator (*Alligator mississippiensis*): evidence from long-term recaptures. *Copeia*, 104: 843-852.
- Wolfe JL, Bradshaw DK, and Chabreck RH. 1987. Alligator feeding habits: new data and a review. *Gulf of Mexico Science* 9: 1.
- Yatsu R, Miyagawa S, Kohno S, Parrott BB, Yamaguchi K, Ogino Y, Miyakawa H, Lowers HR, Shigenobu S, Guillette JL, and Iguchi T. 2016. RNA-seq analysis of the gonadal



transcriptome during *Alligator mississippiensis* temperature-dependent sex determination and differentiation. *BMC Genomics* 17: 1-13.

Zajdel J, Lance SL, Rainwater TR, Wilkinson PM, Hale MD, and Parrott BB. 2019. Mating dynamics and multiple paternity in a long-lived vertebrate. *Evolution and Ecology* 9: 10109–10121.