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Genetic Estimates of the Number of Breeding Adults in Alligator Gar From the Choke Canyon Reservoir, Texas

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Genetic Estimates of the Number of Breeding Adults in Alligator Gar From the Choke
Canyon Reservoir, Texas

by

Blayne Newsome

A Thesis
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Abstract

Alligator gar were historically found across the coastal drainages of the Gulf of Mexico and up into the Mississippi River basin. However, their populations are experiencing decline in many portions of their range. Texas seems to have large populations of alligator gar, but state resource officers are seeking to better understand its biology so as to provide appropriate management recommendations to maintain the recreational fishery. In this study, I used genetic techniques to examine a cohort of 144 juvenile alligator gar collected in 2016 in the Choke Canyon Reservoir. By estimating the effective number of breeders and number of spawning adults, I sought to better understand the reproductive biology of alligator gar and the factors influencing spawning success. I estimated there to be 57 spawning adults with an estimated number of effective breeders of 51. There were no full or half sibling dyads with a probability greater than 0.9. This suggests that the 2016 cohort is the product of spawning by a large group of adults. This agrees with the hydrological conditions from that year, including a lengthy flood period in May, which appeared favorable for an above average level of spawning success. This study is the first of its kind for alligator gar and provides insight into the biology of the alligator gar and enhances our ability to manage this species both in Texas and elsewhere across its range.

Keywords: conservation, genetic techniques, hydrology, management, spawning

Acknowledgements

I would first like to thank my advisor, Dr. Brian Kreiser, for mentoring me through this project and always answering my endless questions. I would also like to thank my family and friends for their unwavering support throughout the entirety of this process. Finally, I wish to thank the Honors College and the University of Southern Mississippi for being my home for the past four years and constantly pushing me and providing me with many opportunities for intellectual growth as well as personal growth.

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Chapter I: Introduction

Alligator gar (*Atractosteus spatula*), one of seven species in the family

Lepisosteidae, is one of the largest freshwater species in North America (Buckmeier

2008). They were once found from the Ohio and Missouri Rivers down to the coastal

areas of the Gulf of Mexico (Suttkus 1963). Population declines have become an

increasing problem across the species' distribution (NatureServe 2017). This has led

states to begin efforts to manage their populations, but a lack of information has hindered

policy decisions (Binion et al. 2015). The biggest threat appears to be habitat degradation

as spawning areas have been lost or altered (Buckmeier et al. 2013). This loss has been

exacerbated by the construction of multiple dams and urbanization of former alligator gar

habitat (Mendoza et al. 2002). Therefore, each state, apart from Louisiana, has declared

regulations or limitations on harvesting alligator gar (Buckmeier et al. 2013).

Historically, management of alligator gar consisted of eradication as they were deemed to

be "trash fish" and harmful to populations of sport fish (Buckmeier et al. 2013).

However, a growing recreational fishery for trophy size alligator gar has become another

conservation threat and one that might lead to further declines of gar populations

(Buckmeier 2008).

Knowledge of alligator gar movement and habitat use are both helpful when

developing management plans for the species (Daugherty et al. 2017). The knowledge of

reproductive behavior can also be incredibly beneficial to the management plans.

Spawning in alligator gar occurs between April and June, usually in flooded backwater

areas (Sakaris et al., 2003; Buckmeier 2008; Buckmeier et al. 2013; Kluender et al.

2016). Fecundity, or the ability to produce offspring, in alligator gar can be variable due

to spawning being linked to stochastic seasonal flooding (Buckmeier 2008). During a spawning event, a single female is usually accompanied by 1-4 males as she lays a batch of eggs (Garcia de Leon et al. 2001). This polygamous mating system can also contribute to variation in reproductive success among individuals. Juveniles seem to remain in the spawning areas as they develop with sexual maturity being reached at 10-14 years. Although the young fish grow extremely fast, they still have a long lifespan of greater than 30 years (Buckmeier 2008).

Traditional techniques for determining the number of breeders in a population can be unreliable, which has led to the application of genetic techniques to estimate the number of adults who are reproducing (Jay et al. 2014). The two numbers that are important are the number of breeding individuals (N_S) and the effective number of breeders (N_e). The effective breeding number (N_b) is a measure of the effective population size (N_e) for individuals from a single breeding season. The effective population size (N_e) is the number of individuals in an ideal population that have the same possibility of random genetic drift, inbreeding, or loss of allelic diversity as the population. The N_e can be affected by reproductive success, skewed sex ratios, and population size fluctuation, and for these reasons, estimating N_e is extremely important, especially when studying endangered species (Jay et al. 2014). One analytical approach to estimating N_b and N_S is pedigree reconstruction (Jones and Wang 2010). N_b is measured as a function of the degree of relatedness among juveniles, which are characterized as either being full-siblings, half-siblings, or unrelated. N_S, the number of parents contributing to the cohort of juveniles, can be inferred from the multi-locus genotypes of the offspring (Jay et al. 2014).

For this study, 144 juvenile alligator gar from the Choke Canyon Reservoir in Texas were collected. The primary aim is to determine the degree of relatedness among the juvenile fish so that the breeding population size and effective number of breeders can be estimated. This might also allow us to make some inferences about the impact of spawning habitat quality on reproductive success since spawning areas are limited for this species due to the link to seasonal flooding. The final objective for this study is to use this information in order to further the conservation efforts and management strategies for alligator gar.

Chapter II: Methods

Young of the year alligator gar were collected at nine locations across the Choke Canyon Reservoir (Figure 1) from May-July of 2015 by Dan Daugherty and colleagues (Texas Parks & Wildlife Department). Alligator gar are thought to spawn in the Frio River and San Miguel Rivers that feed the reservoir. Sites were selected across the upper portion of the reservoir in littoral zones where young of the year alligator gar might congregate. Collections were made using several methods, including seine nets, modified fyke nets and dip nets. The total length (TL) of each individual was measured to the nearest millimeter, and a fin clip, or the entire fish in some cases, was preserved in 100% ethanol. These samples were then stored at room temperature. The size classes represented by the individuals collected was visualized with a length frequency histogram that was produced using the Fisheries Stock Assessment (FSA) package in R (Ogle 2016).

To extract the DNA from the collected samples, I used Qiagen's Purification of Total DNA from Animal Tissues protocol in the DNEasy Kit (QIAGEN Inc., Valencia, CA). DNA quality for a subset of the individuals was checked using agarose gel electrophoresis. All individuals were genotyped for nine microsatellite loci (*Asp*007, *Asp*021, *Asp*035, *Asp*054, *Asp*066, *Asp*084, *Asp*095, *Asp*159, and *Asp*341) described by Moyer et al. (2009). Conditions for the polymerase chain reaction are as follows: the 12.5 μL reactions will consist of 1X *Taq* reaction buffer (New England Biolabs), 2 mM MgCl$_2$, 200 μM dNTPs, 0.2 units of *Taq* polymerase (New England Biolabs), 0.16 μM of the M13 tailed forward primer (Schuelke 2000), 0.16 μM of the reverse primer, 0.08 μM of the M13 labeled primer (LI-COR Inc., Lincoln, NE), 50-100 ng of template DNA and water to the final volume. A GeneAmp PCR system 9700 (Applied Biosystems) was used for PCR with the cycling conditions consisting of an initial denaturing step of 94°C for 2 minutes followed by 35 cycles of 30 seconds at 94°C, 1 minute at 56°C, and 1 minute at 72°C. A final elongation step of 10 minutes at 72°C ended the cycle. Microsatellite alleles were visualized on a LI-COR 4300 DNA sequencer and allele sizes were scored through comparison to a 50-350 bp size standard (LI-COR) using the image analysis software Gene Image IR v. 3.55 (LI-COR).

Genotypic frequencies were tested for Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium using GENEPOP on the web v. 4.1 (Raymond and Rousset 1995; Rousset 2008). The alpha levels of these tests were adjusted by a sequential Bonferroni correction (Rice 1989). The level of genetic variation across loci was assessed by the number of alleles, observed heterozygosity and expected heterozygosity as calculated by GenAlEx v. 6.5 (Peakall and Smouse, 2006; 2012). The probability of identity was also

calculated using GenAlEx v. 6.5 in order to evaluate the power of the loci to perform kinship analyses. The probability of identity provides an indication of the capacity of the data to uniquely identify individuals by determining the average probability that two individuals will have an identical genotype.

COLONY version 2.0.6.1 (Jones and Wang 2010) was used to perform sibship analysis and parental reconstruction. This method employs a maximum likelihood analysis to estimate pedigree relationships by identifying groups of full-sibling and half-sibling families based on their multi-locus genotypes. The analysis used the full-likelihood method with high precision and a maximum run length. The mating system was set as polygamous without inbreeding for both males and females. This seems to accurately reflect what is known about the spawning behavior of alligator gar (e.g., Buckmeier et al. 2013). Since we were interested in the population from the reservoir as a whole, I performed the analysis on all individuals in order to obtain estimates of N_S (the number of adults that contributed to the juveniles of that year class) and N_b (the effective number of breeding adults).

I also examined the potential impact of environmental conditions of reproductive success by looking at the hydrographs during the spring and summer time period when spawning takes place. Buckmeier et al. (2017) reported that years with appropriate hydrologic conditions occur only periodically yet produce the largest portion of individuals that ultimately recruit into the adult population. I used United States Geological Survey (USGS) surface water data (https://waterdata.usgs.gov/nwis/rt) for the station 08206910 located on the Choke Canyon Reservoir to determine if the conditions

in 2015 met the criteria predicted to produce a strong cohort. This provides context for interpreting the N_b and N_S values calculated for the 2015 cohort.

Chapter III: Results

A total of 144 individuals were collected between late May and early July of 2015 at nine different locations across the western end of Choke Canyon Reservoir. These individuals ranged in size from 16-398 mm TL (average = 94.8; standard deviation = 81.1). Most of the collections were made soon after the main spawning period during a couple of weeks spanning late to early June, which can be seen in the large frequency of fish < 100m TL (Figure 2). Alligator gar grow quickly post hatch so it was not surprising to see some individuals reach total lengths up to 400 mm by the end of the collection period (D. Daugherty, Texas Parks & Wildlife Department, personal communication).

I genotyped 144 individuals for nine microsatellite loci (Appendix I). Of these, 11 had missing data for three or more loci and were excluded from further analysis. This included all of the individuals (n=9) from the Calliham site (#9). No loci demonstrated linkage disequilibrium after a sequential Bonferroni correction, and only one locus (*Asp*21) deviated from HWE. Since none of these loci deviated from HWE in adults collected from this locale (B. Kreiser, unpublished data), I chose to use all loci in subsequent analyses.

The power of the loci to be used successfully in sibship and parental reconstruction analyses is a function of the extent to which there is genetic diversity at these loci. Standard diversity indices are reported in Table 2. The number of alleles per

locus (N_A) ranged from 3-16 with an average of 5.778 (SE=1.352). The average

observed heterozygosity was 0.623 while the average expected heterozygosity was 0.642.

The mean probability of identity (PI) across loci was 0.195 while the mean probability of

identity of siblings (PI_{sib}) was 0.478. However, when loci are considered in combination,

both the PI and PI_{sib} approached zero as the number of loci increased (Figure 3).

The COLONY analysis of the population did not detect any full or half-sib dyads

with a probability of 0.9 or greater. The effective number of breeders (N_b) was estimated

at 51 with a 95% confidence interval of 35-76. The number of total spawning adults (N_s)

was estimated to be 57, with 25 of one parent and 32 of the other (the analysis does not

provide a way to verify the sex of the inferred parents).

Gage height at the Choke Canyon reservoir varied considerably across years

(Figure 4). Each year demonstrated a spike in height at some point during the spring-

summer period. Only 2016 showed this increase in August while in the remaining years

this occurred between May-June. The largest heights were present in 2013 and 2015 with

peaks around 25 feet (approximately 15 feet above base height). However, only 2015

possessed a large and prolonged period of above baseline water height that would

presumably be most conducive to producing favorable spawning conditions on the flood

plain.

Chapter IV: Discussion

A low degree of relatedness was seen among the 144 juveniles that were sampled across the Choke Canyon Reservoir, as none of the potential full and half sibling dyads were well supported (probability > 0.9). The values that were inferred for the effective number of breeders ($N_b = 51$; 95% CI = 35-76) and the number of spawning adults inferred ($N_s = 57$) were similar but how to interpret these values is not immediately clear. To date, there are no published studies that take a similar approach to measuring spawning success in other populations of alligator gar. However, we can interpret these values in light of what we know about the reproductive biology and spawning behavior of the fish.

A recent population estimate for the Choke Canyon alligator gar was 5437 (95% CI 3215-9195; Binion et al. 2015). The length-frequency histogram suggests that this population has a low rate of exploitation and is capable of supporting trophy fishery. Furthermore, sex ratios appeared to be skewed towards males (1:4 female to male). The reasoning for this skew was not apparent, and the authors made sure to emphasize that there could be multiple explanations, including sex specific differences in survival and habitat use, or that it is an artifact of sampling bias in the methods. For our purposes, if the adult population size is about 5400, then the population should be comprised of roughly 1350 females and 4050 males. If most of these adults spawn in a given year, that would give us a number well in excess of our estimate of the number of spawning adults which was 57. This value is closer to 1% of the population. One explanation for the apparent disconnect between the two numbers is the large size of the population, as we may just have not sampled enough juveniles to achieve an accurate estimate of the

number of spawning adults. However, knowledge gaps about the spawning behavior of alligator gar mean that we also have a hard time discounting other explanations. For example, we are still unaware if all of the alligator gar in a population spawn synchronously or if individuals vary temporally in their spawning activity. It is not unlikely that some adults (particularly females) may spawn at various times over the summer. Since our samples only cover a small portion of the summer spawning period, we could be detecting only a subset of the total spawning population in the Choke Canyon Reservoir.

Alligator gar practice a periodic life history strategy (Ferrara 2001; as defined by Winemiller and Rose 1992). This means that they mature late and have high fecundity with a low population turnover. They use a bet hedging strategy where they spread out reproduction over multiple years and among a large number of potential offspring in case of reproductive failure in a given spawning season. Buckmeier et al. (2017) found that annual recruitment is highly variable in the systems that they studied (Trinity and Choke). In the Choke Canyon Reservoir, they saw above expected levels of recruitment in about 30% of the years studied. The two best recruitment years (1997 and 2002) made up about half of the population. They determined that successful years corresponded to suitable hydrologic conditions—large, long duration flood pulses in June and July where the flood plain was covered for greater than 20 days.

In the years since the Buckmeier et al. (2017) study, those hydrological conditions do not seem to have been fully met in the Choke Canyon Reservoir (Figure 4). The exception to this is 2015, the year these samples were collected. There was a large flood pulse in May, which was when our samples were collected. Although this was not during

the June-July time frame proposed by Buckmeier et al. (2017), the May flood may still be sufficient to produce a large spawning event. This may explain the fact that related juveniles were not detected in our collections. They represent a sample of a large cohort of individuals spawned by a large group of adults. These adults seem to be contributing equally to the reproductive success as the N_b and N_S were similar. If there was a lot of variance in reproductive success among the adults (some parents contributed more offspring than others), we would expect to see a smaller N_b value relative to the number of inferred spawners. While the hydrologic conditions and genetic data seem to indicate that there was above average spawning success in 2015, it remains to be seen if this ultimately leads to the recruitment of a strong cohort of adults such as the ones spawned in 1997 and 2002.

Kinship analysis has been used increasingly to learn more about how recruitment is influenced by environmental factors and the spawning behavior of a species. This study represents the first time these techniques have been applied to alligator gar. These results do provide some insight into the alligator gar population in the Choke Canyon Reservoir. Although there may be some ambiguity in the connection between the genetic results and what is known about the biology of the fish, these gaps point to areas where further work is necessary. Ultimately, a better understanding of the biology of the alligator gar will enhance our ability to manage this species both in Texas and elsewhere across its range.

Figures

Figure 1. Map of the Choke Canyon Reservoir with Labeled Site Locations. The base map is from Google Maps (https://www.google.com/maps) and shows the general location of Choke Canyon Reservoir in Texas. The inset map (generated by https://mapmaker.nationalgeographic.org/) indicates the location of sites within the reservoir.
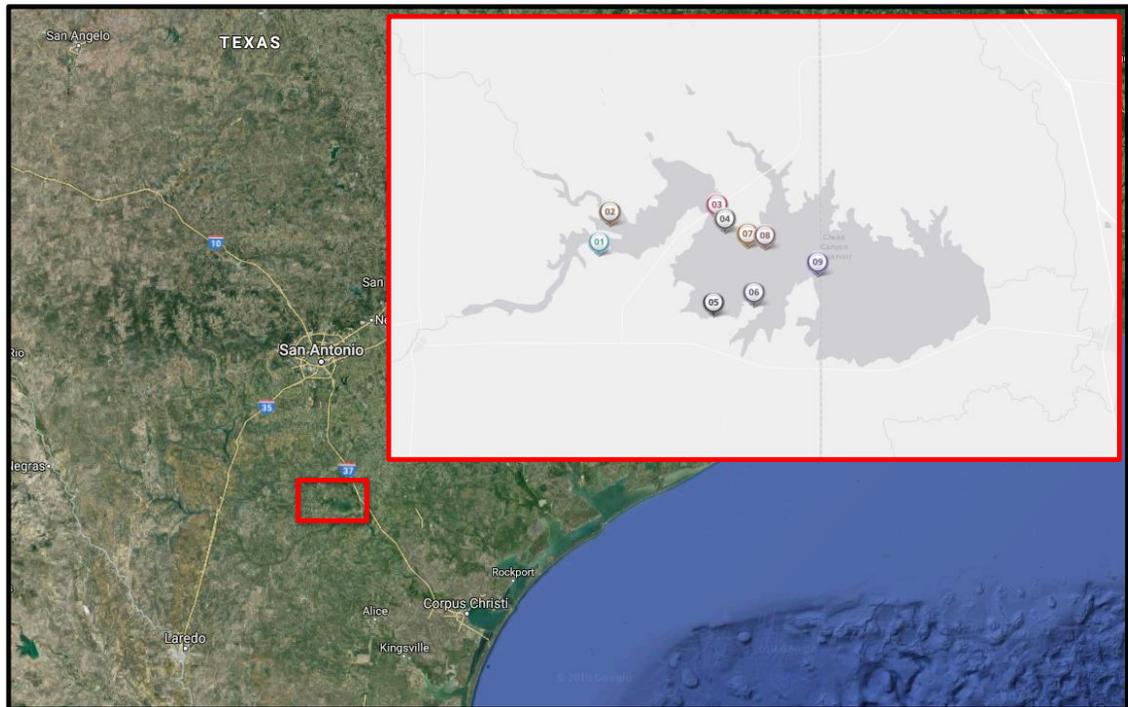
Figure 2. Length frequency histogram (total length in millimeters) of the alligator gar samples collected from Choke Canyon Reservoir in 2015.
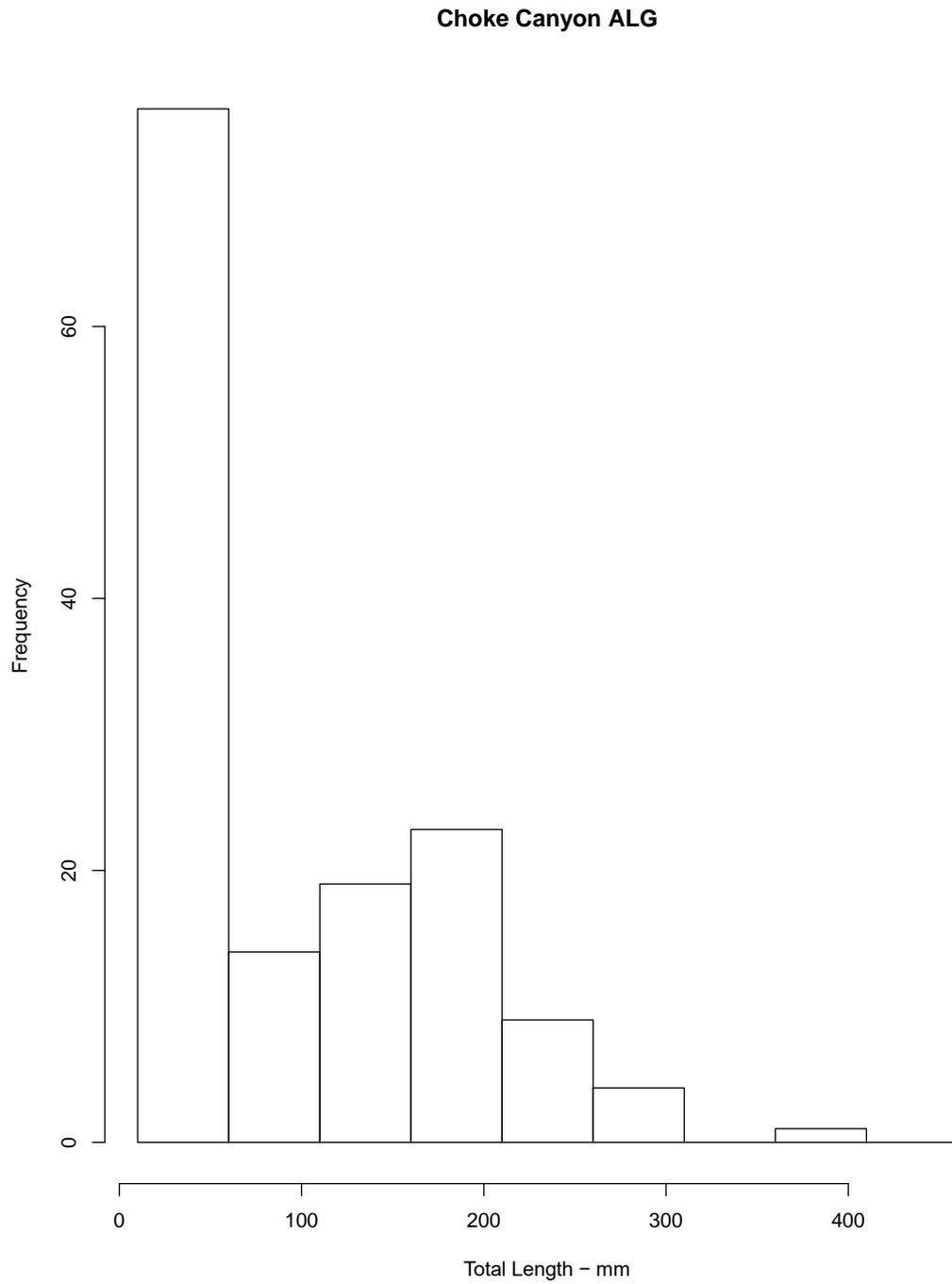

Choke Canyon ALG

Figure 3. The average probability of identity (PI) and probability of identity of siblings (PI$_{sib}$) as a function of increasing numbers of loci for juvenile alligator gar in Choke Canyon Reservoir.
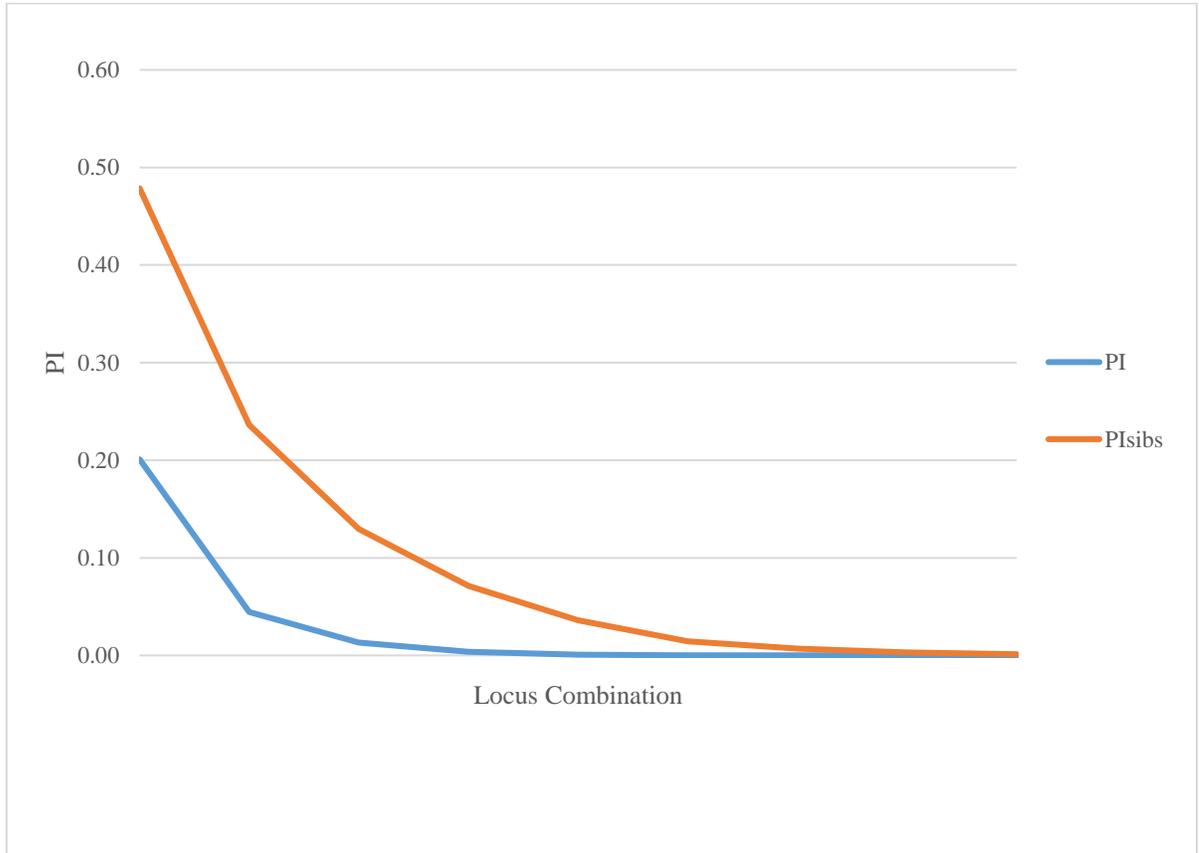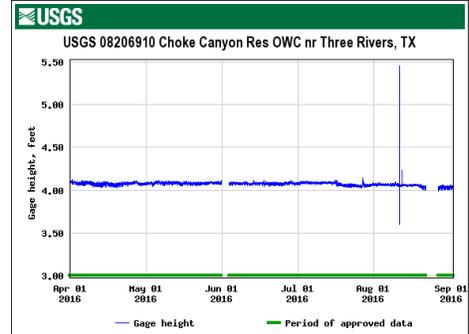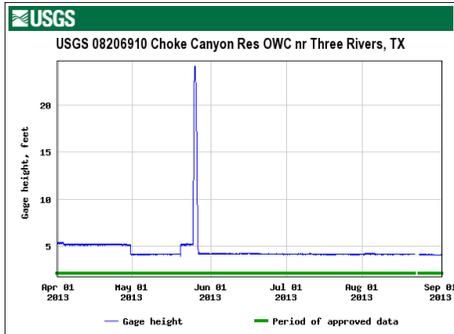
Figure 4. Gage height between April 1-September 1 of 2012-2016 at the United States Geological Survey station 08206910 located on the Choke Canyon Reservoir.

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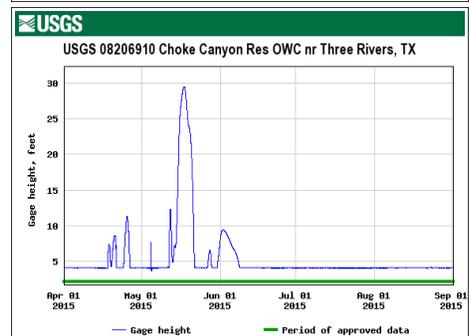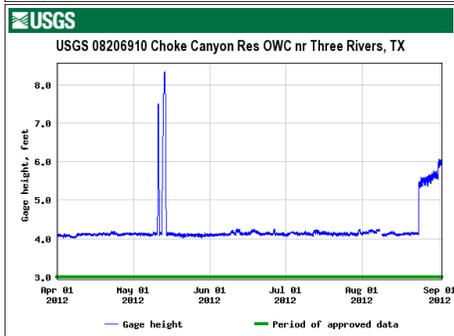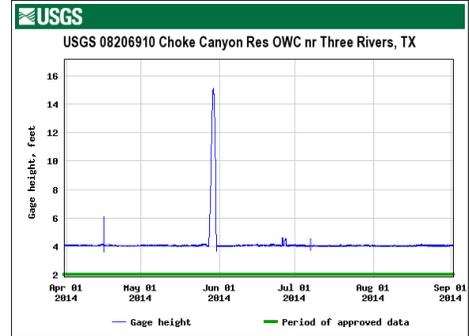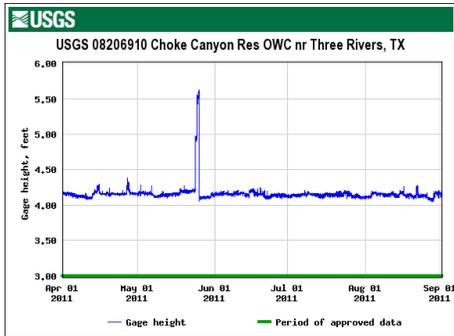
Tables

Table 1. Collection site locations (latitude and longitude), site name, and sample sizes of alligator gar collected from Choke Canyon Reservoir in 2015. Site numbers correspond to those in Figure 1.

Site #	Site name	N	Latitude	Longitude
1	Frio	27	28.50361	-98.46514
2	San Miguel	4	28.51352	-98.42454
3	Hwy 99	4	28.52159	-98.38913
4	Upper Reservoir	49	28.51109	-98.38814
5	Mason Point	13	28.48156	-98.38960
6	Mason/Calliham	9	28.48248	-98.37029
7	Reservoir	13	28.48763	-98.38984
8	Lower Reservoir	4	28.49806	-98.37820
9	Calliham	9	28.49534	-98.34145

Table 2. Summary statistics of genetic diversity for the 2015 collection of alligator gar juveniles from Choke Canyon Reservoir. Sample size (N), number of alleles (N_A), observed heterozygosity (H_o) and expected heterozygosity (H_e) is provided for each locus as well as the average probability of identity for unrelated individuals and sibs (PI and PI_{sib}). The mean and standard error (SE) for these values across all loci are also provided.

Locus	N	N_A	H_o	H_e	PI	PI_{sib}
Asp007	131	4	0.550	0.643	0.201	0.479
Asp341	131	3	0.527	0.624	0.221	0.494
Asp021	128	3	0.617	0.551	0.299	0.549
Asp035	132	4	0.508	0.546	0.287	0.549
Asp054	130	7	0.608	0.590	0.199	0.505
Asp066	129	5	0.822	0.743	0.110	0.406
Asp095	132	4	0.614	0.634	0.205	0.485
Asp159	131	6	0.664	0.704	0.145	0.434
Asp084	132	16	0.697	0.746	0.086	0.399
Mean	130.667	5.778	0.623	0.642	0.195	0.478
SE	0.471	1.352	0.032	0.025	0.024	0.018

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Appendices

Appendix I. Allele frequencies and sample size for each of the nine loci genotyped in the

2015 collection of alligator gar juveniles from Choke Canyon Reservoir.

Locus	Allele/n	Population
Asp007	N	131
	165	0.015
	171	0.397
	177	0.408
	180	0.179
Asp341	N	131
	227	0.424
	233	0.164
	236	0.412
Asp021	N	128
	201	0.520
	204	0.418
	207	0.063
Asp035	N	132
	251	0.004
	257	0.580
	260	0.083
	263	0.333
Asp054	N	130
	172	0.608
	181	0.150
	217	0.004

	223	0.100
	226	0.077
	229	0.019
	232	0.042
Asp066	**N**	129
	254	0.252
	257	0.349
	260	0.105
	263	0.054
	266	0.240
Asp095	**N**	132
	207	0.004
	211	0.292
	213	0.223
	217	0.481
Asp159	**N**	131
	260	0.267
	263	0.057
	266	0.008
	269	0.321
	272	0.344
	275	0.004
Asp084	**N**	132
	199	0.455
	211	0.098
	215	0.057
	219	0.011

	223	0.152
	227	0.008
	231	0.027
	241	0.011
	245	0.004
	249	0.080
	253	0.011
	261	0.004
	269	0.004
	281	0.004
	285	0.064
	289	0.011

Appendix II. Letter of IACUC Approval.

THE UNIVERSITY OF
SOUTHERN MISSISSIPPI.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

118 College Drive #5116 | Hattiesburg, MS 39406-0001
Phone: 601.266.6791 | Fax: 601.266.4377 | iacuc@usm.edu | www.usm.edu/iacuc

NOTICE OF COMMITTEE ACTION

The proposal noted below was reviewed and approved by The University of Southern Mississippi Institutional Animal Care and Use Committee (IACUC) in accordance with regulations by the United States Department of Agriculture and the Public Health Service Office of Laboratory Animal Welfare. The project expiration date is noted below. If for some reason the project is not completed by the end of the approval period, your protocol must be reactivated (a new protocol must be submitted and approved) before further work involving the use of animals can be done.

Any significant changes should be brought to the attention of the committee at the earliest possible time. If you should have any questions, please contact me.

PROTOCOL NUMBER: 17101202 (Renewal of 11092206)

PROJECT TITLE: Population Genetics & Systematics of Freshwater Fishes and Herps

PROPOSED PROJECT DATES: 10/2017 – 09/2020

PROJECT TYPE: New

PRINCIPAL INVESTIGATOR(S): Brian Kreiser

DEPARTMENT: Biological Sciences

FUNDING AGENCY/SPONSOR: N/A

IACUC COMMITTEE ACTION: Full Committee Approval

PROTOCOL EXPIRATON DATE: September 30, 2020

_____   10/18/2017
Jake Schaefer, PhD   _____
IACUC Chair   Date