Assessment of genetic and education recovery plan objectives for the Bog Turtle (*Glyptemys muhlenbergii*)

A Dissertation Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Cassie Marie Dresser

August 2017

Copyright © 2017 by Cassie M. Dresser
All rights reserved.

DEDICATION

This dissertation is dedicated to the dearly missed Tim King (USGS) and Bern Tyron (Zoo Knoxville).

ACKNOWLEDGEMENTS

First and foremost, I would like to thank my advisor Ben Fitzpatrick for his advice, patience, support, encouragement, feedback, and for teaching me what it means to be a true collaborator. A big thank you to my adopted mentor Beth Schussler for her support and encouragement, as well as, for sharing her tips and expertise in education research which helped shape the third chapter of this dissertation. Thanks to Jim Fordyce for his advice regarding the statistical approaches used in parts of this dissertation and Joe Clark for providing a refreshing perspective pertinent to ensuring my research was applicable and accessible to conservation practitioners; to both Jim and Joe for serving on my committee. Thank you to Zoo Knoxville for their trust and support in acquiring the needed tissue samples and for introducing me to one of the most endearing and lovable creatures on the planet, the Bog Turtle: Bern Tryon, Michael Ogle, Lynn Eastin, Doug Rice, Stephen Nelson, and Brad Moxley. A huge thank you to Todd Pierson who introduced the 3RADseq method used in Chapter I and Chapter II and helped me troubleshoot in the lab. Sarah Flanagan for her assistance with some of the coding necessary for some of the analyses conducted in Chapter I. To all of the men and women who work tirelessly to protect the Bog Turtle, Tim King (USGS), Tessa Bickhart (PA), Gabrielle Graeter (NC), Thomas Floyd (GA), Bob Cherry (NPS), all other members of Project Bog Turtle, TNC, TWRA, and the private landowners. And lastly, to my support team, who have provided research, teaching, and emotional support throughout my PhD program: my fiancé Andrew Briggs, my family, Ferlin McGaskey, EEB faculty, graduate students, and undergraduate students, and my Two Rivers growth group. This dissertation was made possible thanks to all of the people and organizations that supported me along the way. Here's to the turtles!

ABSTRACT

Unprecedented declines in biodiversity are threatening the natural world as we know it. Without human intervention, two thousand species listed under the US Endangered Species Act are likely to disappear. Fortunately, these species receive federal protection and increased research effort is needed to create and satisfy the objectives outlined in the mandated Species Recovery Plan. In this dissertation, I address three conservation objectives outlined in the Recovery Plan for North America's smallest and rarest turtle, the Bog Turtle (*Glyptemys muhlenbergii*): (1) investigate the potential genetic differentiation in southern portions of the species' range, (2) investigate the genetic impacts of reintroduction, and (3) develop an effective conservation education program. Using SNP markers generated from a novel next generation sequencing technique, I determined that genetic differentiation in the south is largely a function of geographic distance, but State-designated management units may still be suitable in practice. Some populations have relatively low genetic diversity and an effective population size substantially lower than the assumed census size, suggesting that management decisions based on census size may be inappropriate. I also detected statistical patterns consistent with local adaptation, suggesting potential outbreeding depression risk associated with proposed translocations. And for a translocation program previously implemented, I observed an increase in gene diversity, but noted that the increase was less than expected assuming an equal admixture of source populations. Lastly, I explicitly compared the engagement and learning outcomes associated with teaching conservation concepts within a classroom setting. I found no evidence that verbal questioning, clicker, and worksheet active learning strategies affected student engagement and learning, but learning outcomes may differ based on content (topic and example organism used). These findings will inform the development of an education program that will aid Bog Turtle conservation, a species vulnerable to illegal poaching, and hence limited in regards to outreach opportunities. Here I demonstrate how a combination of information from the fields of genetics and education were necessary to address Bog Turtle Recovery Plan objectives, but information from a variety of additional fields will be necessary for Bog Turtle conservation and for the rescue of our other imperiled species.

PREFACE

Our tiny turtle tale begins in a small, isolated bog in Northeast Tennessee. Surrounded by lush grasses and small shrubs, I navigated through knee-deep mud in a pair of hip waders and a probing stick in hand. Dodging poison sumac, exotic reed-canary grass with razor-sharp blades, and thorny swamp rose, I poked and prodded my stick into the mud and dried tussock mounds until I heard a promising *thud* as my stick made contact with something hard and hollow. Into the mud, I submerged my arm with the hopes of pulling out one of North America's rarest and smallest turtles, the Bog Turtle (*Glyptemys muhlenbergii*).

TABLE OF CONTENTS

INTRODUCTION 1
References 8
CHAPTER I Genetic Assessment of Southern Bog Turtle (*Glyptemys muhlenbergii*) Populations: Structure, Diversity, and Adaptation 9
Abstract.....10
Introduction 10
Methods 13
Study system 13
Sampling 14
Laboratory and post-sequencing procedures.....14
Data analysis 16
Results 18
Discussion.....24
Acknowledgments 27
References 28
CHAPTER II Genomic Assessment of Tennessee's Bog Turtle (*Glyptemys muhlenbergii*) Translocation Program.....32
Abstract.....33
Introduction 34
Methods 36
Study system 36
Sampling 37
Data analysis 39
Results 41
Discussion.....44
Acknowledgments 49
References 50
Appendices 54
Appendix A1 – Zoo Knoxville Bog Turtle captive breeding records from 1986 – 2015 54

Appendix A2 – Tennessee Bog Turtle head-start program records from 2010 – 2015 54
CHAPTER III Conservation in The Classroom: How Best to Engage and Educate Students About Conservation 55
Abstract.....56
Introduction 57
Methods 60
Overview 60
Participants.....61
Experimental design.....61
Survey and assessment.....63
Data analysis 64
Results 66
Discussion.....73
Acknowledgements 76
References 78
Appendices 82
Appendix A3 – Instructor Profiles 82
Appendix A4 – Student Engagement Survey.....83
Appendix A5 – Final Assessment (Version 1).....87
CONCLUSION.....90
References 92
VITA 93

LIST OF TABLES

Table 1.1 Genetic diversity, effective population size, census size, and F_{ST} values.....22
Table 2.1 Pairwise F_{ST} values for Bog Turtle populations in translocation program 42
Table 3.1 Linear mixed model comparisons for student engagement survey.....68
Table 3.2 Fixed effects coefficient estimates for student engagement survey 69
Table 3.3 Linear mixed model comparisons for student assessment scores.....70
Table 3.4 Fixed effects coefficient estimates for student total assessment.....72

LIST OF FIGURES

Figure 1.1 Bog Turtle sampling locations in the southern portion of distribution 15
Figure 1.2 Ancestry proportions of Bog Turtle individuals.....19
Figure 1.3 Isolation by distance analysis 21
Figure 1.4 F_{ST} outlier analysis.....23
Figure 2.1 Bog Turtle sampling locations in Tennessee and North Carolina.....38
Figure 2.2 Mean gene diversity at each Bog Turtle sampling location for translocation program 43
Figure 2.3 Observed versus expected gene diversity in Bog Turtle translocation program.....45
Figure 2.4 Genetic differentiation between Bog Turtles sampled from translocation program 46
Figure 3.1 Student engagement survey responses for three active learning treatments 67
Figure 3.2 Total student assessment scores for three active learning treatments 71

INTRODUCTION

Historically, state governments functioned as the primary stewards of wildlife, and to a large extent, still do. To protect certain species, legislation was adopted to restrict import and sale within the state. Such legislation predominantly focused on protecting game species, rarely incorporated provisions to protect habitat, and was restricted to activity within the state regardless of species' ranges. With the enactment of the Endangered Species Act (ESA) in 1973, Congress legislated federal provisions to improve state efforts by extending protections across political (e.g. state) boundaries and to non-game species and their habitat (Baur and Irvin 2010). To receive federal protections, a species must first be listed as threatened (likely to become endangered in the near future) or endangered (likely to become extinct throughout all or a substantial portion of their range). Listing is overseen by the U.S. Fish and Wildlife Service (USFWS) and the National Marine Fisheries Service (NMFS), both of which are required to initiate a status review of a species following the submission of a petition to list the species. Based on the scientific data collected by the federal agencies, state governments, and local partners, the USFWS and NMFS assess whether there is enough evidence to warrant listing based on the established criteria for each protection category (threatened and endangered). Once a species is listed as endangered, "take" prohibitions are automatically applied, meaning that no person shall "harass, harm, pursue, hunt, shoot, wound, kill, trap, capture, or collect, or to attempt to engage in any such conduct" and the USFWS formulates a Species Recovery Plan to outline the objectives that need to be met in order to manage and ultimately delist the species. An additional feature of the ESA, is the designation of "critical habitat" which occurs about one year after listing to identify and protect specific areas that contain physical or biological features essential to the persistence of the species. The ESA also allows the listing of Distinct Population Segments of vertebrate species, which Congress added to give agencies flexibility to list populations in situations where other populations of the same species are healthy or data are lacking in other portions of the species' range (Waples 1998). Although the provision has been critiqued for the lack of an objective and consistent definition of Distinct Population Segment (Pennock and Dimmick 1997), a Distinct Population Segment classification is based on discreteness (is there a physical, ecological, or behavioral separation that warrant different control or management strategies), significance (is there evidence that its loss would create a significant gap in the species range or significant loss in genetic diversity), and status (when evaluated separately, does it meet the imperiled status requirement). Currently, over 2,000 species are listed as endangered or threatened under the ESA and numerous Distinct

Population Segments are included in an effort to promote the recovery and persistence of declining species in the United States and international waters.

One such species that has received protection from the ESA and the Distinct Population Segment addendum is the Bog Turtle (*Glyptemys muhlenbergii*). The Bog Turtle is a semi-aquatic turtle in the family Emydidae, a species easily identified by the prominent yellow-orange blotches on each side of their necks, and their small size (less than 115 mm in carapace length; Ernst and Barbour 1989). Females typically lay about three eggs per season, which hatch within a couple months of being laid. Female hatchlings take about 10-12 years to reach sexual maturity and males take about 6-8 years (Bury 1979; Klemens 1990). Although predators, such as raccoons, skunks, and snakes can have detrimental impacts on Bog Turtle populations, the biggest threat to Bog Turtles is habitat loss and degradation caused by humans. Humans have altered fire regimes that maintain early successional habitat, fragmented habitat through development and road construction, drained and ditched wetlands for agricultural use, and introduced exotic plant species that reduce available nesting and basking habitat (Groombridge 1982; Tryon and Herman 1990; Klemens 1993). Although bog habitat tends to be inherently patchy, these extensive anthropogenic impacts have dramatically increased the distance between suitable bog habitat patches, which is a substantial problem for a species whose average home range is only 0.05 to 2 ha and typically travel less than 20 m over the course of one week (Carter 2000). As distance between suitable habitat patches increases, mortality associated with predation, road mortality, and desiccation likely increase for individuals that attempt to disperse, reducing donor population sizes and inhibiting demographic and genetic rescue of potential recipient populations. Experts have documented unprecedented declines and numerous local extinctions throughout the species' range in both the northern region (Massachusetts, Connecticut, New York, New Jersey, Pennsylvania, Delaware, and Maryland) and the southern region (Virginia, Tennessee, North Carolina, and Georgia), which are separated by over 400 km. Between 1977 – 1997, northern populations were determined to have declined in range and number of populations by over 50% and similar declines were thought to have occurred in the south, but southern populations received less survey effort during that same time period (USFWS 2001). These declines ultimately led to the listing of the northern Distinct Population Segment of Bog Turtle as federally threatened under the Endangered Species Act in 1997, and a "Similarity of Appearance" classification for the southern populations of Bog Turtle. A Species Recovery Plan was drafted for the northern region as mandated by the U.S. Fish and Wildlife Service and although a similar plan was not drafted for the southern region, the southern region was incorporated into some of the northern recovery plan objectives.

Furthermore, some of the recovery plan objectives broadly addressed Bog Turtle conservation, aiding the southern populations as well.

This dissertation is the result of countless search hours, not unlike the experience described in the preface, for the purpose of addressing three objectives outlined in the Bog Turtle Species Recovery Plan: (1) investigate potential genetic differentiation in southern portions of the range, (2) investigate the potential genetic impacts of reintroduction, and (3) develop an effective education program.

To investigate genetic differentiation among populations, I used novel genomic techniques to estimate and model genetic properties of extant wild populations in the understudied southern region of the Bog Turtle range. I collected tissue samples from over 200 turtles across 30 sites in four states, representing the most extensive sampling in the southern region of the Bog Turtle distribution. DNA was extracted from collected samples and sequenced (RADseq) to generate 2 658 SNP loci, nearly 150 times more markers than used in any previous genetic study of this species, ultimately allowing more precise and accurate estimation of several genetic parameters. The purposes of obtaining such estimates are to provide conservation practitioners with information pertinent to establishing biologically meaningful management units, prioritizing populations for conservation, and to assess the suitability of proposed management strategies, such as translocations. Generally speaking, Bog Turtle sites within each southern state tend to be clustered together geographically, rather than distributed across the entire state, but it is important to verify that the observed geographic separation reflects genetic separation as well, in order to optimize the designation of management units. Using a Bayesian statistical program, I estimated genetically distinct groups and observed how individuals (or portions of their ancestry) were assigned to those groups. In general, the genetically distinct groups were correlated with the political boundaries of the states, which are the management units currently used. However, there were some exceptions, particularly in North Carolina where there are multiple geographic clusters of Bog Turtle populations that represent unique genetic groups. State borders themselves likely do not reflect physical barriers for Bog Turtle dispersal as the estimated genetic distances between populations were simply proportional to the geographic distances between them. Yet, given the patchy distribution of the remaining habitat, state based management units seem suitable for Bog Turtles in the southern region of their distribution. Additionally, I estimated the genetic diversity in each population and modeled the effective population size. In several instances, the effective population size was substantially less than the assumed census size, suggesting fewer or more closely related turtles are breeding, a result consistent with the relatively low genetic diversity estimates observed. Managers will have to decide whether to invest their resources in populations most likely to persist (higher genetic diversity and effective population size) or populations

most likely to be extirpated without human intervention (lower genetic diversity and effective population size); Chapter 1 (Table 1) provides some of the information necessary to apply either approach. Once populations are selected for management, the next step is to determine the strategy or strategies to implement.

One strategy that is often proposed is translocation. Translocations are often proposed to boost census sizes and minimize inbreeding depression (fitness declines associated with breeding of close relatives); however, one genetic concern associated with translocations is outbreeding depression (fitness declines associated with breeding of genetically distant individuals, such as those locally adapted to different environments). With this concern in mind, I looked for genetic signals consistent with local adaptation, in other words, I looked for SNP loci with an atypical pattern of variability compared to the rest of the genome. I did identify 20 such loci, a result consistent with divergent adaptation between local populations. However, without fitness data it is impossible to evaluate the risk of either outbreeding or inbreeding depression. Should a translocation strategy be implemented, I would recommend starting with a translocation between populations with relatively low genetic differentiation (see Chapter 1, Table 1) which would likely reflect the lowest outbreeding depression risk and to treat the translocation as an experimental effort, collecting fitness data for several generations following implementation.

To more explicitly investigate the genetic impacts of reintroduction (specific type of translocation), I used the same next generation sequencing method described in Chapter 1 on samples collected from populations involved in the Zoo Knoxville captive breeding, head start, and release program in northeast Tennessee. This program was established two years after Bog Turtles were discovered in the state of Tennessee in 1986. Several turtles were collected from a few different wild Tennessee populations (although one individual was obtained from a population in southwest North Carolina) to establish the captive breeding program. In 1991, first generation offspring of this captive population were released at 22 months of age in an experimental release site approximately 30 miles south of any known Tennessee wild sites. Although, nearly 30 years have passed since the program was initiated, first generation offspring were always released prior to maturity rather than being retained for use in the captive breeding stock. The program was later supplemented with a head start program, that incorporated offspring from additional wild populations into the release population. Offspring were obtained from wild caught females that laid their eggs in the local rearing facility and hatchlings were raised for a minimum of 9 months prior to being released. For this study, a more extensive sampling effort was done to obtain as many individuals as possible from the release site, captive population, and all wild populations that contributed individuals to the release population via either the captive breeding

program or head start program. I was able to collect a total of 124 individuals, representing all known source populations and nearly half of all extant individuals at those sites and I identified 7 030 SNPs for use in subsequent genetic analyses. The overall purpose of this chapter was to determine if the translocation program was successful, specifically whether genetic diversity was enhanced in the release population. First, I verified that the source populations were genetically distinct by estimating genetic differentiation between every pair of populations. Given the high values of genetic differentiation (F_{ST}), I then estimated the gene diversity in the release population and compared it to the gene diversity in each source population. While gene diversity was always higher in the release population, suggesting program success, when expectations were modeled based on an equal contribution from each source population to the number of released turtles, the observed diversity in the release population fell short of expectations. This shortfall could be a result of nonrandom success of founders (perhaps beyond the program's control) or an unrecorded bias in the implementation of the release program – an item that could be addressed and inform future program adjustments.

To begin addressing the last Species Recovery Plan objective, develop an effective education program, I elected to focus on conservation education in a college classroom. Endangered species education can range from informal outreach to individuals regarding specific actions to very broad campaigns to raise awareness or understanding of general conservation issues. Formal education in large college classes is one way to maximize reach per unit effort, minimize cost to conservation practitioners, and reach young adults before they before they had the opportunity to engage in land use practices that affect conservation initiatives. More than 80 % of remaining Bog Turtle habitat is privately owned, and thus is managed as that particular landowner sees fit. Academic initiations play a pivotal role in providing young adults with a foundational knowledge and appreciation of the natural world, which in most cases occurs before they become landowners themselves. Such an education would also apply to those who do not become landowners themselves, but may own or want to own pets – consider that Bog Turtles are threatened by the illegal pet trade – such an education could highlight the considerations that should be made when considering a suitable pet for their households (i.e., not Bog Turtles). An education program targeted for a college audience is also a more appropriate strategy for Bog Turtles given the concern regarding potential poachers getting access to geographic information, which eliminates wildlife tours as a viable education program and the turnover of privately owned property across the large geographic range of Bog Turtles, which reduces the feasibility of a door to door education strategy.

I compared student engagement and learning of conservation concepts for three different classroom educational strategies to determine which method students found most engaging and which

was most effective for student learning. The educational strategies I compared are commonly used active learning strategies that differ in how dialogue between students and instructors occurs (i.e., how questions are asked and answered). The three strategies I compared were (1) verbal, (2) using electronic response devices (i.e., "clickers"), and (3) in writing (i.e., worksheets). Three discrete conservation concepts (translocations, harvest quotas, reserve design) were presented to students using one of the three active learning strategies (a different strategy for each concept) in two large introductory biology courses at a large research I institution. To assess engagement, students completed a survey following the lecture that asked them to reflect on their interest in the material taught, how on-task they were during the activity, and how well they thought they understood the concept for each of the three conservation concepts. Based on the absence of statistically significant differences between strategies for these three survey items (interest, focus, and confidence) in either class, it appears that a college-based conservation education program could implement any of these strategies and have similar outcomes in regards to student engagement (although my findings may be context specific, e.g. class size, geographic location, etc.). To assess learning, students completed a formal assessment as part of their final exam for the course, comprised of combination of multiple choice questions and short answers, four questions for each conservation concept. Although, there were significant differences in student assessment scores, the highest scoring active learning strategy differed between the two classes evaluated. In fact, assessment scores were highest in each class for the same conservation topic (i.e., translocation) and the topic was frequently mentioned on the student engagement survey as justification for their level of interest, focus, and confidence in their understanding. Further study will be necessary to explore the causes of this pattern, but the educational strategy implemented may not be the only important thing to consider when designing a conservation education program for the classroom, the topic (i.e., concept and/or example) may be equally important. Fortunately, Bog Turtles have the added benefit of being a relatively charismatic species and some of the management strategies applied to this species, such as translocations are still considered controversial among conversation scientists, which consequently tends to intrigue students, so an education program that targets college students may be a viable way to educate the general public about conservation principles relevant to Bog Turtles.

This dissertation applies principles and techniques from multiple disciplines, such as population genetics from the field of biology and participant surveys from the field of education to address three key objectives in the Bog Turtle Species Recovery Plan: (1) investigate potential genetic differentiation in southern portions of the range, (2) investigate the potential genetic impacts of reintroduction, and (3) develop an effective education program. The full dissertation describes in greater detail how each

objective was addressed to reveal patterns of low genetic diversity and high differentiation among wild populations of Bog Turtles in the southern region, the successful increase in genetic diversity within a Tennessee population as a result of an implemented translocation, and that a variety of educational methods can be applied in a classroom setting with similar outcomes in regards to engagement in and learning of conservation concepts.

References

Baur DC, Irvin WMR (2010) Endangered Species Act: law, policy, and perspectives. Second edition, American Bar Association, USA

Bury RB (1979) Review of the ecology and conservation of the bog turtle, *Clemmys muhlenbergii*. United States Department of the Interior, Fish and Wildlife Service, Special Scientific Report – Wildlife 219:1-9

Carter SL, Hass CA, Mitchell JC (2000) Movements and activity of Bog Turtles (*Clemmys muhlenbergii*) in southwestern Virginia. J Herpetol 34:75-80

Ernst CH, Barbour RW (1989) Turtles of the world. Smithsonian Institution Press, Washington

Groombridge B (1982) The IUCN amphibia-reptilia red data book: Testuines, Crocodylia, Rhynchocephalia. IUCN, Gland, Switzerland

Klemens MW (1990) The herpetofauna of southwestern New England. PhD Dissertation, University of Kent, Canterbury, United Kingdom

Klemens MW (1993) The amphibians and reptiles of Connecticut and adjacent regions. State Geological and Natural History Survey of Connecticut. Bulletin 112:1-318

Pennock DS, Dimmick WW (1997) Critique of the evolutionary significant unit as a definition for "distinct population segments" under the US Endangered Species Act. Consev Biol 11:611-619

Tryon BW, Herman DW (1990) Status, conservation, and management of the bog turtle, *Clemmys muhlenbergii*, in the southeastern United States. Beaman K, Caporaso F, McKeown S, Graff M (eds) Proceedings of the first international symposium on turtles and tortoises: conservation and captive husbandry, Chapman University

United States Fish and Wildlife Service (2001) Bog turtle (*Clemmys muhlenbergii*) Northern population recovery plan. Prepared by M Klemens. Pennsylvania Field Office, Hadley, Massachusetts

Waples R (1998) Evolutionary significant units, distinct population segments, and the Engangered Species Act: reply to Pennock and Dimmick. Publications, Agencies and Staff of the US Department of Commerce, 433

CHAPTER I

GENETIC ASSESSMENT OF SOUTHERN BOG TURTLE (*GLYPTEMYS MUHLENBERGII*) POPULATIONS: STRUCTURE, DIVERSITY, AND ADAPTATION

Abstract

Genetic data are increasingly necessary to address recovery plan objectives for imperiled species. Genomic data in particular offer greater power in estimating various population parameters and inferring both past and future population dynamics relative to previous methods, such as allozyme and microsatellite markers. Here we used 2 658 SNP loci generated using a triple-digest reduced representation library preparation method from 171 individual southern Bog Turtles to address one of the recovery plan objectives for the species: investigate the potential genetic differentiation in the southern portion of the species range. We found relatively high, but variable levels of genetic differentiation among populations, which reflect the geographic distance between populations (i.e., isolation by distance). We observed low genetic diversity within populations and several instances where the census size exceeded our estimates of effective population size. Lastly, we detected 20 outlier loci consistent with signatures of local adaptation, suggesting that outbreeding depression may be a risk in some proposed translocation scenarios. Our results are pertinent to questions related to the suitability of current management units based on political boundaries relative to biological patterns, prioritization of populations for management, and relative outbreeding depression risk associated with potential translocation scenarios. This study explicitly addresses an objective from the Bog Turtle Recovery Plan (i.e., investigate the potential genetic differentiation in southern portions of the species' range). However, to advance Bog Turtle protection and recovery, explicit criteria for human-mediated intervention programs must be established. Better structures linking primary research, management interventions, and follow-up monitoring would likely benefit Bog Turtles and many other imperiled species.

Introduction

Although the best way to protect an imperiled species is to prevent its decline in the first place, management intervention is often triggered long after substantial declines become apparent, if prompted at all. Some species are monitored until local, regional, or global extinction, typically in situations when monitoring programs lack pre-planned intervention programs that are implemented in a timely manner (Lindenmayer et al. 2013). For monitoring programs to be effective for species conservation, information should be gathered under the umbrella of explicit objectives linked to criteria that trigger pre-planned management interventions. For example, information on dispersal and genetic differentiation could be obtained early on in a monitoring program with the objective of designating evolutionary significant units (ESUs) providing a basis from which to allocate limited resources (i.e., time, money, and personnel) in a

biologically meaningful manner (Moritz 1994). Furthermore, these genetic data could serve multiple purposes; genetic data can be used as a minimally intrusive sampling method to quantify and compare population diversity (e.g. Tasmanian devil, Miller et al. 2011), identify populations of concern (montane aquatic mayfly, Taubmann et al. 2011), resolve population structure (Loblolly pine, Eckert et al. 2010), resolve taxonomic uncertainties (Spinks et al. 2016), detect hybridization and introgression (e.g. Atlantic salmon, Glover et al. 2013), as a forensic tool for law enforcement (e.g. bushmeat, Eaton et al. 2010), to gather basic natural history information (e.g. brown bear, Barba et al. 2010), and to inform captive breeding efforts (lesser kestrel, Alcaide et al. 2010). A critical aspect of genetic data collection and monitoring is that the information must be gathered with a clear purpose in mind, along with objective criteria to justify the implementation of a specific management strategy.

Sufficient information and clear objectives are especially important when irreversible management strategies are proposed, such as translocations. In particular, knowledge regarding historic gene flow among populations is necessary before launching a translocation to enhance gene flow (Storfer 1999). This is because gene flow can have a positive or negative effect on recipient populations. Gene flow can facilitate genetic rescue and reinforcement by maintaining or increasing genetic variation during bottleneck events (e.g. McEachern et al. 2011). Conversely, immigration can cause recipient populations to exceed carrying capacity and introduce maladaptive alleles (Garant et al. 2007). Clearly, balancing these positive and negative effects within each population is crucial for planning translocation strategies. However, determining an optimal level of gene flow is not possible without detailed information on fitness, disease transmission, and perhaps other elements of metapopulation dynamics (Hanski and Gaggiotti 2004). In the absence of such information, management programs might be best aimed at restoring or maintaining natural levels of exchange among populations. Fortunately, genetic data can provide valuable information unattainable with traditional field methods, such as identifying the presence of gene flow (in contrast to migration without breeding) and inferring historic patterns of gene flow (Schwartz et al. 2006).

In the past, geneticist have used markers such as randomly amplified polymorphic DNA (RAPDs), amplified fragment length polymorphisms (AFLPs), and microsatellites to gather genetic information; however, each marker had its limitations, some of which included inability to identify heterozygotes, assumption that DNA fragments with equal migration rates on a gel are identical, lack of repeatability, exclusion of coding regions, and high cost. The emergence of genomics (e.g. marker-based genotyping, reduced representation sequencing, and whole genome sequencing) has offered solutions to many of these limitations. For example, by increasing the number of loci, estimates such as genetic

diversity, hybridization rates, and population structure become increasingly precise. Furthermore, in the presence of individual fitness data and population growth rates acquired from long-term studies, genomics can provide previously unobtainable estimates of functional genetic variation and predictive probability for various sources of outbreeding depression (Allendorf et al. 2010).

We used genomic techniques to collect data for thousands of single nucleotide polymorphisms (SNPs) to gather information to inform three management issues: (1) evaluate whether or not management units are biologically meaningful, (2) prioritize populations for intervention programs, and (3) test for signatures of local adaptation that might need to be considered when contemplating translocation programs. Our intention was to provide conservation practitioners with genetic information necessary to make management decisions and set criteria for when intervention programs should be initiated, not to make those decisions for them as there are non-genetic inputs that should be taken into account when making such decisions (e.g. available resources, logistical constraints, and non-genetic biological concerns).

We assessed the above management issues in the context of Bog Turtle (*Glyptemys muhlenbergii*) conservation. The Bog Turtle was listed as a federally threated species in their northern range under the Endangered Species Act due to an estimated 50% decline within a 20 year period (USFWS 2001). This listing mandated a Species Recovery Plan to guide conservation and management of extant populations in New York (NY), Connecticut (CT), Massachusetts (MA), New Jersey (NJ), Pennsylvania (PA), Delaware (DE), and Maryland (MD). However, 400 km south of the southern most northern population are additional Bog Turtle populations in southern Virginia (VA), North Carolina (NC), Tennessee (TN), and Georgia (GA) that are classified as "Similar in Appearance", which prohibits the take of Bog Turtles from southern populations, but does not mandate an additional or inclusive Species Recovery Plan. Regardless, northern conservation partners did incorporate the southern populations into their plan, at least for some objectives. One such objective is Task 4, "investigate the genetic variability of the Bog Turtle throughout its range", which specifically mentions that investigation should include "southern portions of the species' range" (USFWS 2001).

To date only four genetic studies have been conducted to investigate the genetic variability of Bog Turtle populations (Amato et al. 1997, Rosenbaum et al. 2007, Pittman et al. 2011, and Shoemaker and Gibbs 2013). However, none of these studies adequately sampled the southern region; Shoemaker and Gibbs (2013) did not include any southern populations, Pittman et al. (2011) sampled only NC, Rosenbaum et al. (2007) included VA and NC, and Amato et al. 1997 included NC and GA along with populations from four northern states; however, only a total of 20 turtles were sampled. Furthermore,

Amato et al. (1997) and Rosenbaum et al. (2007) used mitochondrial markers, which represent only a single locus, and might be biased if, for example, gender-biased migration or introgression are taking place or if variation in male mating success significantly impacts effective population size. And while Pittman et al. (2011) and Shoemaker and Gibbs (2013) used nuclear markers, they used 15 - 18 microsatellite markers to represent a 3GB genome, perhaps too few markers from which to estimate population genetic parameters accurately or address questions relevant to local adaptation.

Considering the limitations of the genetic marker sets previously used for genetic assessments of Bog Turtles, the limited extent to which the southern distribution has been sampled, and the increased interest in implementing a translocation program among southern populations, we conducted an extensive genetic survey using next generation sequencing methods to more thoroughly sample the Bog Turtle genome. In addition, we sampled the geographic range more thoroughly, particularly Tennessee, which was excluded from all previous genetic assessments. From the collected data, we assessed genetic population structure to determine if current management units represented biologically meaningful patterns, designation of meaningful units promotes the preservation of adaptive genetic variance and maintenance of evolutionary potential (Fraser and Bernatchez 2001). We also estimated genetic diversity and effective population sizes of sampled populations to equip managers with information valuable for prioritizing populations for management and resource allocation. Lastly, we conducted F_{ST} outlier analysis as a preliminary test for local adaptation that might need further consideration when proposing translocations or captive breeding programs. Followed by clear intervention criteria, this genetic information was collected for the explicit purpose of addressing a recovery plan objective, which will ideally keep Bog Turtles off the list of species that were monitored to extinction.

Methods

Study system

The Bog Turtle (*Glyptemys muhlenbergii*) is a semi-aquatic turtle in the taxonomic family Emydidae; individuals are easily identified by the yellow-orange blotches on either side of their neck. Typically, females lay an average of 3 eggs per year, which hatch within 2-3 months; offspring take about 6 – 12 years to become sexually mature and have a maximum carapace length of 11.5 cm. Bog Turtles are also habitat specialists, living in spring-fed bogs, which in the southern region consist of sphagnum moss, various sedges and grasses, and shrubs. Considering these life history characteristics, Bog Turtles are

particularly vulnerable to anthropogenic activities, such as alteration of fire regimes, development, ditching and draining of wetlands, and introduction of exotic species that reduce nesting and basking habitat.

Although southern Bog Turtle populations do not have the same federal listing status as the northern Distinct Population Segment, all southern states list the Bog Turtle as an imperiled species and manage extant populations accordingly. Most conservation decisions pertaining to southern populations are managed independently by each state in conjunction with state allocated funds; with a few exceptions. The National Park Service which manages populations along the Blue Ridge Parkway in VA and NC, the USFWS which oversees the management of the species as a whole, and Project Bog Turtle (PBT), a conservation initiative of the North Carolina Herpetological Society comprised of federal, state, academic, and non-academic (e.g. zoos) representatives that meet once a year to discuss the status of Bog Turtles in the south and allocate general resources among state partners.

Sampling

We obtained tissue samples from a total of 209 Bog Turtles from 30 sites spanning all four southern states where Bog Turtles are known to occur (Figure 1.1): 13 sites in Georgia (N = 66 turtles), 4 in North Carolina (N = 53 turtles), 4 in Tennessee (N = 35 turtles), and 9 in Virginia (N = 55 turtles). Unfortunately, many of the sampled populations are estimated to have fewer than 20 individuals, and given how cryptic Bog Turtles are in their densely vegetated habitat, typically only a handful of turtles were sampled. Collaborators collected many of the samples used in this study (see Acknowledgments) during the 2014-2015 field seasons. Others were collected approximately 10 years ago for microsatellite development (King and Julian 2007). The remaining samples (all Tennessee samples and most Virginia samples) were obtained using a variety of sampling techniques, including visual surveys, probing, muddling (i.e., probing through mud and tussocks using hands), and trapping (Somers 2000; Whitlock 2002). Tissue samples were obtained from a 0.5 cm tail clip or full toenail clip and preserved in 95% ethanol and stored at – 20ºC until DNA extraction (Hughe 2010). This sampling protocol was approved by the IACUC at the University of Tennessee [2436-0316].

Laboratory and post-sequencing procedures

We extracted DNA from tissue samples using the DNeasy Blood and Tissue Kit (Qiagen Corporation, Valencia, CA). Prior to library preparation DNA quantity and quality was assessed using a

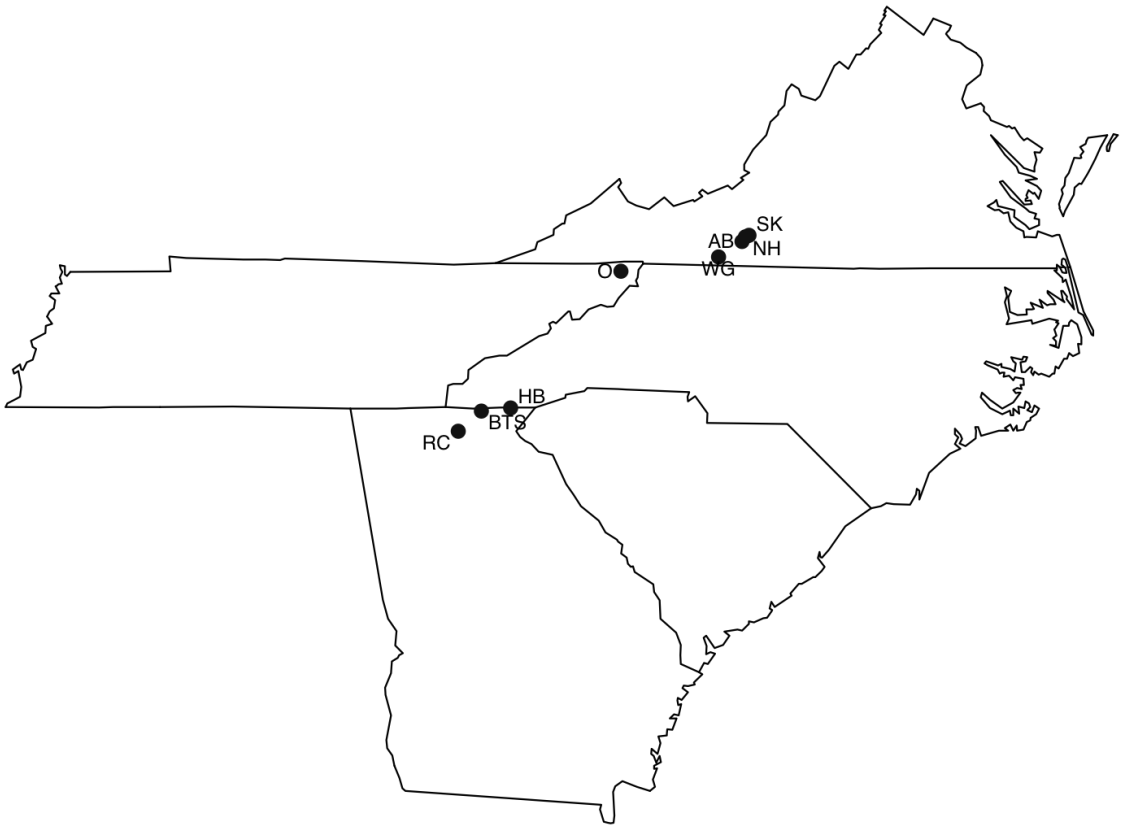


Figure 1.1 Bog Turtle sampling locations in the southern portion of their distribution. Only the sites with sufficient sampling and sequence quality (i.e., used in data analyses) are shown (11 populations), with the exception of three North Carolina sites for which geographic coordinates were not provided due to concern that the information could be intercepted by poachers. Unique site codes are shown next to their corresponding site, but the names have been omitted to protect the identity of sites.

fluorometer to quantify the amount of DNA and gel electrophoresis to confirm extracted DNA was not degraded. Samples were then digested using three enzymes (ClaI, MspI, and BamI) as part of a triple-digest restriction site associated DNA sequencing (3RADseq) library preparation protocol (T Glenn, unpublished). This procedure outperforms the more commonly used double-digest RADseq by reducing chimeras, increasing adapter ligation efficiency, and minimizing adapter dimers while simultaneously requiring less input DNA and improving sequencing efficiency through the use of variable length quadruple-index tags. The generated RADseq libraries were then pooled relative to their DNA concentration and 500bp fragments were isolated using a PippenPrep system (Sage Science Corporation, Beverly, MA) and sequenced on an Illumina NextSeq for 150-bp paired end reads for approximately 2 million reads per individual.

Prior to quality control, filtering, and assembly with the software pipeline *ipyrad* (Eaton 2015; http://ipyrad.readthedocs.io/), inner barcodes were trimmed. All *ipyrad* defaults were used, with the following exceptions: the minimum depth at which majority rule base calls are made was set to 6, the cluster threshold was set to 0.907, the maximum number of allowed mismatches between barcodes in the barcodes file and sequence reads was set to 2, the maximum number of unique alleles allowed in individual consensus reads after accounting for sequence errors was set to 2, the minimum number of samples that must have data at a given locus for it to be retained was set to 6, the maximum number of SNPs allowed per final locus was set to 20 (10 for each read in paired locus), and the maximum proportion of shared polymorphic sites in a locus was set to 0.25 (which allowed a heterozygote site to occur across a maximum of 25% of samples; i.e., to detect and remove paralogs). Subsequent filtering within the R software environment (R Development Core Team, Version 3.3.2) was necessary to confirm that all loci with more than 2 alleles were removed and all loci had a minimum minor allele frequency of 0.05.

A total of 171 turtles and 2 658 loci remained after extraction, library preparation, sequencing, and quality control and filtering (GA = 47 individuals, NC = 50 individuals, TN = 32 individuals, VA = 42 individuals). We used these remaining individuals and loci (or a subset when mentioned) for subsequent analyses.

Data analysis

To determine whether the southern Bog Turtle management units based on political boundaries (i.e., states) are biologically useful and to provide PBT with information to aid in allocation of general

resources, we assessed patterns of genetic structure using a Bayesian algorithm in *STRUCTURE* (Version 2.3.2.1; Pritchard et al. 2000). This algorithm infers the proportion of ancestry from each cluster, for an assumed number of clusters (K) from individual multilocus genotypes. The default settings were used, including an admixture model without a priori knowledge of geographic location. To determine the most likely number of clusters, we conducted a series of analyses for five independent iterations of K = 1 – 10, using a burn-in period of 10 000 repetitions and Markov Chain Monte Carlo (MCMC) of 10 000 repetitions. We examined these results using *STRUCTURE HARVESTER* (Earl and vonHoldt 2012). If state borders reflect genetic structuring of populations, ancestral proportions of individuals within the same state should be similar, where the greatest proportion of their genetic data correspond to the same genetic cluster and when K = 4 all individuals are clearly resolved by the state they reside in.

Considering the physical distribution of sampled sites, in which sites from the same state tend to be geographically clustered, we also tested for isolation by distance (i.e., proportional increase in genetic distance as geographic distance between population increases). Geographic coordinates were provided by participating state agencies, with the exception of North Carolina which feared that the information might be intercepted by poachers, thus NC was excluded from this analysis. Genetic differentiation between pairs of populations (pairwise F_{ST}) was calculated using the R package 'diveRsity' (Keenan K; R Development Core Team 2011). The significance of differentiation was assessed through the calculation of 95% confidence intervals using a bias corrected bootstrapping method with 1000 bootstraps. The estimated pairwise F_{ST} values were transformed ($\frac{F_{ST}}{1-F_{ST}}$) prior to running a Mantel test (9999 permutations) on the geographic distance and genetic distance matrices. All population level calculations in this study excluded populations with less than five sampled individuals (11 populations remained).

For use in the prioritization of populations for conservation initiatives, we calculated the genetic diversity and modeled the effective population size in sampled populations. For each population, the distribution of genetic diversity across loci and global genetic diversity (i.e., 'expected heterozygosity') was calculated using the basicStat function in the R package 'diveRsity' (Keenan K; R Development Core Team 2011). We used the linkage disequilibrium model with random mating in *NeEstimator* (Version 2; Do et al. 2014) to estimate contemporary effective population sizes (N_e) from the genetic data for each population with at least 6 sampled individuals. Parametric 95% confidence intervals were determined based on the chi-squared approximation (Waples 1989). Estimated effective population sizes were compared with estimated census sizes provided by state partners if available.

Lastly, we conducted an F_{ST} outlier analysis, to detect statistical signatures consistent with patterns of local adaptation (i.e., potentially greater outbreeding depression risk), using a Bayesian

approach implemented in *BAYESCAN* (Version 2.1; Foll and Gaggiotti 2008). BAYESCAN uses logistic regression to decompose F_{ST} coefficients into a locus-specific component (alpha) shared by all populations and a population-specific component (beta) shared by all loci. Loci potentially under selection are identified as those showing an atypical pattern of variability compared to the rest of the genome, i.e., those with a high posterior probability (q) of having a non-zero locus specific component (alpha). Positive values of alpha indicate loci potentially affected by divergent selection and negative values indicate loci potentially affected by balancing selection. Following suggestions made by Foll and Gaggiotti (2008), we used a prior odds of 10, a false discovery rate of 0.05, and chain parameters: 600 000 iterations with a thinning interval of 50 and 10 pilot runs of length 100 000 with a burn-in of 100 000. Model convergence was confirmed using Geweke's convergence diagnostic and Heidelberg Welch's convergence diagnostic and we verified non-correlated sampled parameters.

Results

We obtained a total of 296 857 917 paired-end reads, each with 150 bp for 197 individual turtles from 18 sites from four states (Virginia, North Carolina, Tennessee, and Georgia). After filtering for a minimum depth and minimum number of samples per locus of six, in *ipyrad*, we obtained 29 081 "unlinked" SNPs (only one SNP used per paired-end read). Using R, we further filtered this dataset by removing all loci with more than 50% missing data (16 297 loci), then all individuals with more than 50% missing data (12 turtles), then loci with more than 2 alleles (59 loci), and then loci with a minor allele frequency of less than 0.05 (9489 loci). Finally, we identified and removed a set of 560 putative loci that were all highly correlated with each other (within state linkage disequilibrium greater than 0.5, and predominantly at the end of the *ipyrad* output, suggesting a systematic error in designating them as distinct loci). The final dataset consisted of 2 658 markers across 171 turtles (11 populations).

The Bayesian clustering plot generated using output from *STRUCTURE* clearly showed clustering of individual turtles by their state of origin (Figure 1.2). The most likely number of clusters based on the Evanno method was K = 2, which distinguishes individuals from Georgia from other southern states based on ancestry proportions, where the cluster with the highest ancestral proportion among Georgia individuals differed from non-Georgia individuals. As we increased the number of clusters, the ancestral proportions for individuals from the same state were similar and clustered together, with the exception of individuals from one North Carolina site that formed a genetic cluster distinct from other North Carolina individuals, especially when K = 4 and 5. However, the clustering by state is likely a

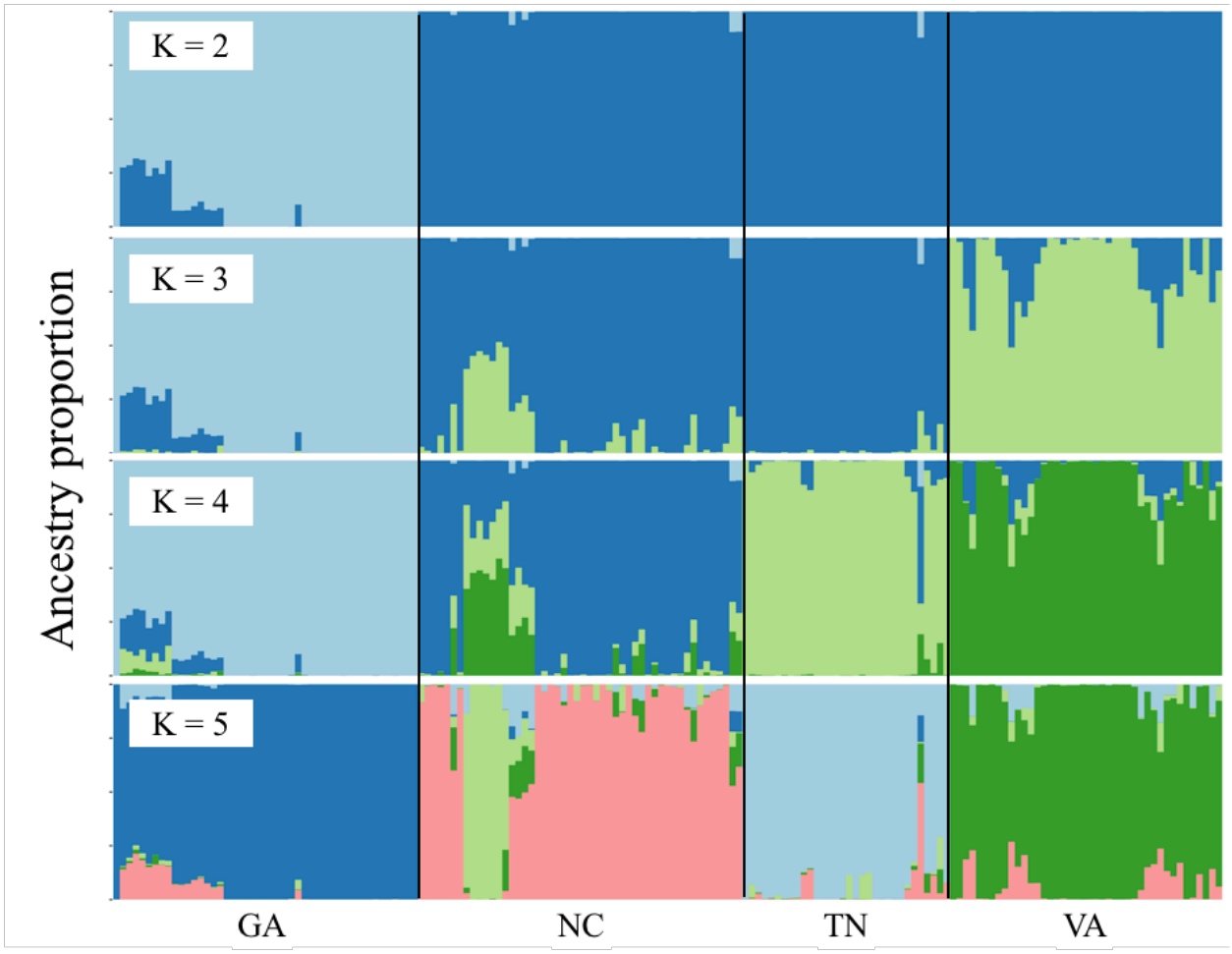


Figure 1.2 Ancestry proportions of Bog Turtle individuals, sampled in four states (GA Georgia, NC, North Carolina, TN Tennessee, and VA Virginia), to population clusters determined with the software *STRUCTURE*. The genetic data are fit to four different models, a two-cluster model, three-cluster, four-cluster, and five-cluster (K = 2 – 5). Each vertical bar represents an individual turtle; note that the ancestry proportion for some individuals is 1 for a single cluster. Each color corresponds to a distinct genetic cluster.

result of isolation by distance. Based on the pairwise comparisons between all sites with available geographic coordinates, we observed a significant positive correlation between geographic distance and the genetic distance between sites (Figure 1.3; Mantel test; $r = 0.916$, $p = 0.0002$). Thus differences in genetic distance between pairs of populations increased as expected given the geographic distance between the populations.

We observed low, but variable genetic diversity for each of the eleven populations (range = 0.155 – 0.219) and several instances where the effective population size was estimated to be substantially less than the assumed census size (estimated by local experts), and in some cases less than our sample size (Table 1.1). We noted two populations where the effective population size was estimated to be substantially less than the census size: Site RC in Georgia ($N_e = 3$, N = 20) and Site SK in Virginia ($N_e = 6$, N = 28). Site MG in North Carolina also had an estimated effective population size less than the census size ($N_e = 26$, N = 31), but the two values were relatively close. Although we did not have an accurate census size estimate for Site MG, we did have samples from 31 turtles which likely represents far fewer turtles than then the actual census size, so the number of turtles we sampled exceeded the effective population size we estimated. Regardless, low effective population size is unlikely a universal characteristic of Bog Turtles, but rather may warrant increased concern for particular populations that exhibit a substantial discrepancy between effective population size and census size. And although we could not estimate effective population size for all populations, several additional populations had low genetic diversity, which could correspond to low effective population sizes or biased sampling.

We found that in general, genetic differentiation was highest between populations from different states and lowest between populations from the same state. We did observe two surprising exceptions, two translocation scenarios in North Carolina, (1) between Site Z and Site SU and (2) between Site SU and MG. These pairwise comparisons had higher F_{ST} values (0.20 and 0.19, respectively) than pairwise comparisons between these NC populations and some Virginia populations (e.g. NC – Z and VA – WG = 0.08), indicating that the outbreeding depression risk associated with translocating between some NC populations is greater than translocating across state lines between NC and VA. The results of our second assessment of outbreeding depression risk, F_{ST} outlier analysis in *BAYESCAN*, were consistent with patterns of local adaptation. Populations locally adapted to different environments likely correspond to greater outbreeding depression risk, as locally adapted gene complexes would be broken up in admixed offspring, producing offspring maladapted to the present environment. Specifically, we observed 20 outlier loci, 19 of which were consistent with diversifying selection and 1 consistent with balancing selection (Figure 1.4).

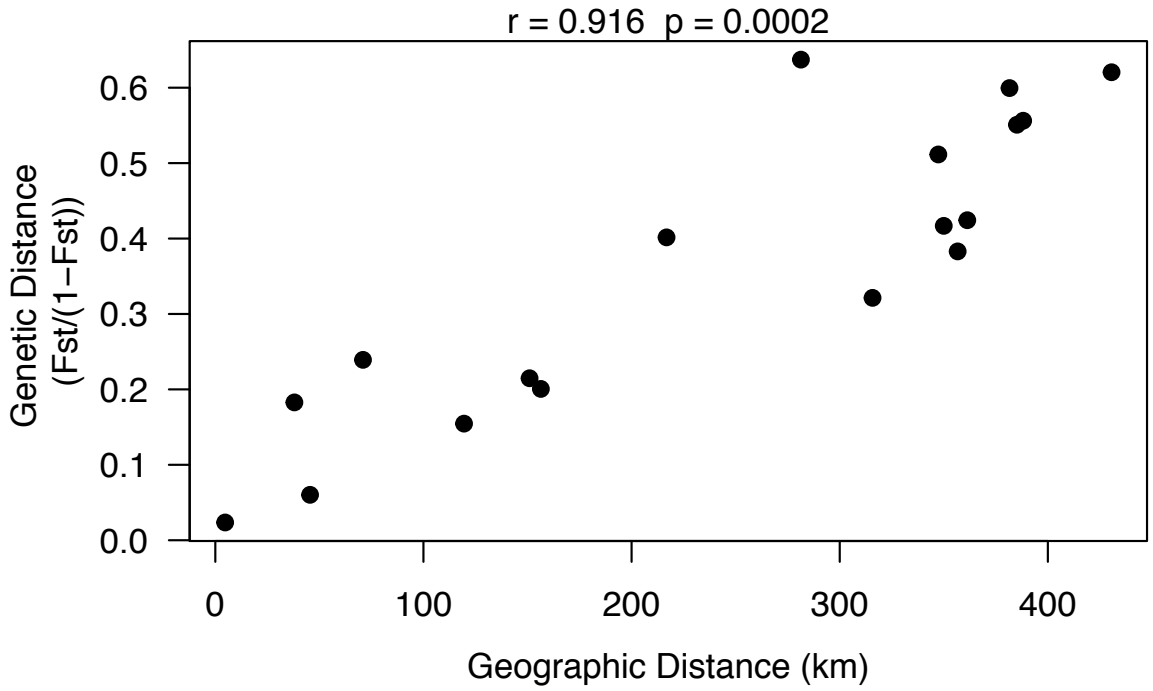


Figure 1.3 Isolation by distance analysis for Bog Turtle populations in southern Distinct Population Segment. Correlation of genetic distance (transformed pairwise F_{ST} values) and geographic distance (distance among centralized point for each site). Three North Carolina sites were excluded from this analyses as geographic coordinates were not available.

Table 1.1 Genetic diversity (GD), effective population size (N_e), assumed census size (N), and pairwise F_{ST} values for eleven Bog Turtle populations in the four United States (GA Georgia, NC North Carolina, TN Tennessee, VA Virginia).

				Pairwise F_{ST}										
Site	GD	N_e (95% HPD)	N	GA-HB	GA-BTS	GA-RC	NC-Z	NC-SU	NC-MG	TN-O	VA-SK	VA-WG	VA-NH	VA-AB
GA – HB	0.219	11 (10.7, 11.6)	9	0.00										
GA – BTS	0.195		8	0.16	0.00									
GA – RC	0.200	3 (3.0, 3.1)	20	0.19	0.15	0.00								
NC – Z	0.219			0.25	0.33	0.36	0.00							
NC – SU	0.155	18 (16.2, 21.3)		0.35	0.45	0.44	0.20	0.00						
NC – MG	0.218	26 (26.0, 26.7)	31	0.27	0.33	0.36	0.00	0.19	0.00					
TN – O	0.186		30	0.29	0.38	0.39	0.11	0.26	0.11	0.00				
VA – SK	0.201	6 (6.1, 6.2)	28	0.30	0.37	0.38	0.13	0.21	0.13	0.18	0.00			
VA – WG	0.209		20	0.24	0.34	0.36	0.08	0.21	0.10	0.13	0.06	0.00		
VA – NH	0.197		15	0.28	0.36	0.37	0.12	0.22	0.13	0.17	0.02	0.04	0.00	
VA – AB	0.194		20	0.29	0.37	0.39	0.13	0.23	0.13	0.18	0.04	0.05	0.02	0.00

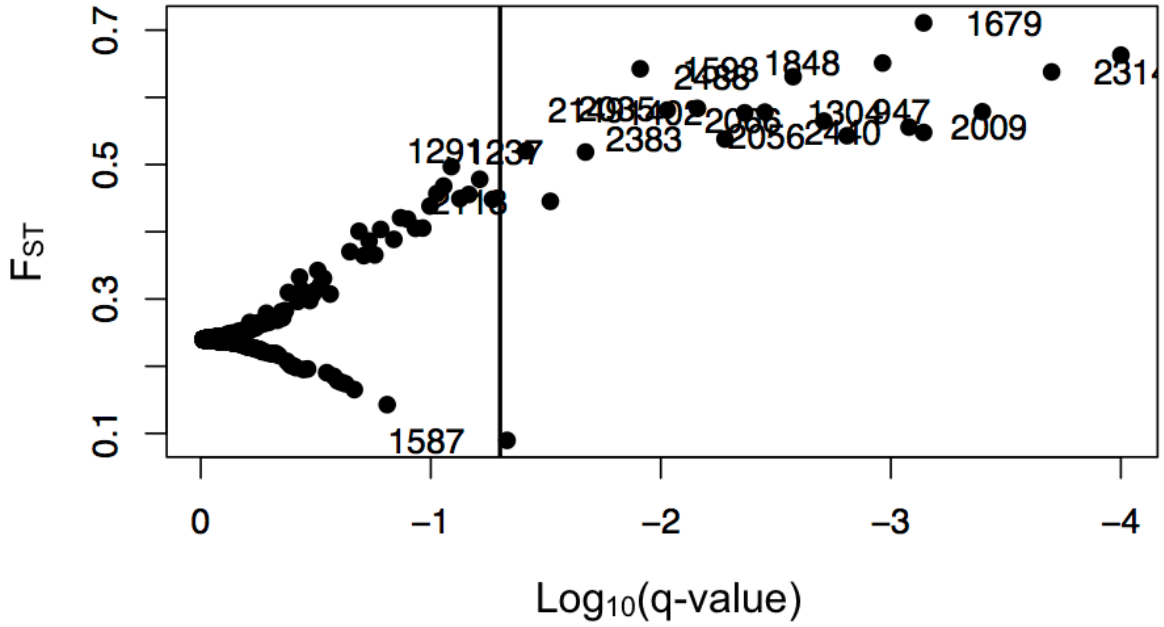


Figure 1.4 F_{ST} outlier analysis of 2 658 SNP markers in *BAYESCAN* 2.1. Pairwise F_{ST} values are plotted against the log10-transformed q-values (the minimum false discovery rate at which a locus becomes significant). Nineteen loci show greater genetic differentiation than expected under neutrality (FDR = 0.05, vertical line), consistent with diversifying selection. One locus (Number 1587) shows less genetic differentiation than expected, consistent with balancing selection.

Discussion

In 2001, the Species Recovery Plan for Bog Turtles outlined specific recovery objectives to aid in the management and protection of the federally threatened Bog Turtle. Although, the southern Distinct Population Segment was largely excluded from the mandated Recovery Plan for the northern Distinct Population Segment, one objective specifically targeted the southern portion of the species range: investigate the potential genetic differentiation in the southern portion of the species range. In this study, we used next generation sequencing to investigate this recovery plan objective and specifically addressed related questions pertinent to future management decisions: (1) are current management units biologically meaningful, (2) are there particular populations managers should be concerned about, and (3) are there signatures of local adaptation – i.e., information which would be pertinent to proposed translocation programs. We found that state-based management units are practical in that they happen to represent regional genetic groupings, differentiation among breeding populations within those groupings is substantial (likely reflecting the naturally patchy distribution of bog habitats), and that both of these patterns of differentiation can be understood as simple consequences of isolation by distance. Local populations are known to be small, but genetic estimates of effective population size were often much smaller than estimated census sizes. These results have implications for conservation prioritization and potential interventions such as translocation.

To manage and conserve species effectively, recognition of biologically meaningful units is ideal, as these units may have unique evolutionary and ecological processes that influence them and thus require different management strategies (Bernard et al. 2009). However, politically designated management units do not always reflect biologically meaningful ones. Although we found that genetic groupings of individuals corresponded to their state of origin, state borders alone did not sufficiently distinguish all distinct genetic clusters, such as Site SU in North Carolina. Furthermore, some pairwise comparisons of genetic differentiation revealed greater differentiation between populations within the same state (e.g. Site Z and Site SU in North Carolina) than between populations from different states (e.g. Site Z in North Carolina and Site WG in Virginia), but even the pairwise differentiation between sites within the same genetic cluster was substantial. Such high differentiation is likely a result of the geographic isolation of the remaining bog habitat, where genetic differentiation between populations increases in proportion to the geographic distance between sites (i.e., isolation by distance). In fact, we observed a substantially higher correlation ($r = 0.92$) between genetic and geographic distance than most other studies which considered r values between 0.62 and 0.73 to be indicative of a strong isolation by distance pattern (Kinitz et al. 2013; Ngeve et al. 2016; Grosser et al. 2017). Considering the patchy distribution of existing

habitat, where habitat tends to be clustered together within each state, the state-based management units are suitable in most cases, but some attention should also be given to individual sites.

Regardless of one's philosophy on whether we should prioritize the management of populations most likely to persist (a more secure investment) or populations likely to be extirpated without human intervention (investing most where the need is greatest), the information we provide regarding genetic diversity and effective population size (relative to the assumed census size) of sampled populations is valuable. The more secure investment would be to prioritize the populations with higher genetic diversity and populations where the effective population size meets or exceeds the assumed census size, such as Site GA – HB. In contrast, for the more uncertain investment, one would prioritize populations with low genetic diversity and effective population sizes that fall short of the assumed census size, such as Site GA – RC, NC – MG, and VA – SK. Until we know why some estimated effective population sizes were smaller than the census size, making management recommendations based on census sizes alone might be misleading. Based on our inability to estimate effective population sizes for all populations and our exclusion of seven sites from most analyses (with the exception of the population structure analysis in *STRUCTURE*) we would encourage conservation practitioners to continue taking genetic samples, especially from poorly sampled populations and collect demographic data to better estimate census sizes. These additional data would allow for the modeling of effective population sizes and to obtain more accurate and precise estimates of genetic diversity in addition to other population genetic parameters. At the very least, our estimates are likely more informative than previous estimates based on mitochondrial or microsatellite markers (Amato et al. 1997, Rosenbaum et al. 2007, Pittman et al. 2011, Shoemaker and Gibbs 2013).

Although we do not advocate for or against translocations in this manuscript, we recognize that in situations where inbreeding depression is of great concern, particularly given the small effective population sizes we observed, translocation is a strategy occasionally proposed to counter the effects of inbreeding depression. Our intention was not to evaluate the efficiency or efficacy of translocation as a management strategy for Bog Turtles (see Dresser et al. 2017 for a more direct assessment); this is because we did not have fitness data to assess whether or not inbreeding depression was occurring in the small wild populations or whether outbreeding depression was occurring in an existing translocation program at Zoo Knoxville. Such data might be feasible to acquire if substantial resources (time, money, and personnel) were dedicated to searching for nests and conducting a parentage analysis using a more targeted genomic approach (designing and using probes to target identical loci across individuals). Currently, a substantial proportion of such resources are allocated for habitat restoration and radio

telemetry studies to assess habitat use and locate hibernacula and nest sites (e.g. Lovich et al. 1992; Carter et al. 1999; Somers et al. 2007; Feaga 2010), both highly justifiable priorities particularly considering habitat loss and degradation are considered the primary threats to Bog Turtles (Copeyon 1997). We were able to provide information on the genetic differentiation between populations, where greater differentiation could be associated with greater outbreeding depression risk and we were able to detect outlier loci consistent with local adaptation, suggesting that some translocation scenarios could have increased outbreeding depression risk. Generally, lower risk was associated with hypothetical translocations between populations within the same state, with the exception of translocations between a couple North Carolina populations which were associated with higher risk relative to translocations between some North Carolina populations, across state borders, with some Virginia populations. Regardless, information regarding fitness consequences of translocating and not translocating would provide more direct evidence for or against proposed translocation scenarios. Another word of caution; we only assessed the relative genetic risks associated with each translocation scenario, non-genetic factors, such as disease (Cunningham 1996) and site fidelity (e.g. Bell et al. 2005) should be considered when discussing the suitability of translocations to meet conservation objectives. Furthermore, translocations can immediately affect non-genetic population parameters, such as census size, for example, an increase in census size reduces extinction risk by reducing demographic stochasticity.

Since the 1970s conservation has used genetic data to estimate population parameters; and over the years with the emergence of microsatellites and genomics, our power to estimate these parameters has increased greatly and has made previously inaccessible information accessible (Allendorf 2017). Here, we have demonstrated how genetic data can be useful for designation of management units, prioritization of populations for management, and for risk assessment of proposed translocation scenarios. While alternative methods are available for designating management units, such as satellite or radio telemetry to determine the extent of inter-population dispersal (e.g. Mauritzen et al. 2002), these data lack certainty in regards to effective dispersal (i.e., breeding between migrants and residents). Genetic data are also being increasingly used for conservation prioritization (e.g. Rieman and Allendorf 2001; Taylor et al. 2010; Palkovacs et al. 2013; Yumnam et al. 2014). Such data provide a wider lens in which to assess past demographic fluctuations unobtainable with recent implementation of traditional field methods (e.g. historic bottlenecks) and infer future persistence in the context of climate change (Ramey et al. 2000 and St Clair and Howe 2007, respectively).

Ideally, Species Recovery Plans, as mandated by the United States Fish and Wildlife Service for species listed under the Endangered Species Act, should provide frameworks identifying the type of data

that should be collected, as management objectives are explicitly outlined in the document. For example, we used the recovery plan objective for Bog Turtles, "investigate the potential genetic differentiation in the southern portions of the species range" to determine what type and quantity of data to collect. Most obvious, was the need to sample turtles in the southern region. Secondly, we needed to obtain the most accurate measure of genetic differentiation between populations, given certain constraints, such as the absence of a full genome and species specific probes. RADseq offered a cost and time efficient way to obtain thousands of informative genetic markers to estimate pairwise F_{ST}. In this way, we were intentional in regards to the data collected, insuring the expended resources were used to address a specific recovery objective. These data were also useful in addressing another recovery plan objective, "investigate the potential genetic impacts of reintroduction", as we were able to explore the relative risk associated with various translocation scenarios. However, as we mentioned in the introduction, such data collection and analyses need to be evaluated in the context of whether or not pre-planned management interventions should be implemented. Currently, the details of those pre-planned management interventions and associated triggers are unclear, and in some cases absent. Therefore, to optimize the useful application of our findings to Bog Turtle conservation we encourage conservation practitioners to develop explicit management intervention criteria, a recommendation that is likely applicable to the conservation and management of other species as well.

Acknowledgments

We thank Michael Ogle, Annlynn Eastin, Doug Rice, Stephen Nelson, Brad Moxley, Tessa Bickhart, and Ann Somers for their assistance collecting tissue samples from Tennessee. Additional tissue samples used in this study were generously provided by Tim King (USGS), Thomas Floyd (Georgia DNR), Bob Cherry (National Park Service – Blue Ridge Parkway), and JJ Apodaca (Warren Wilson College). We thank the private landowners and the Nature Conservancy for access to sites. A special thanks to the Project Bog Turtle organization for their support. Financial support was provided by the Association of Zoos and Aquariums (AZA) Conservation Grants Fund (CGF) [14-1245] from the Disney Worldwide Conservation Fund (DWCF), the North Carolina Herpetological Society (NCHS), the Ecology and Evolutionary Biology Department at the University of Tennessee, and Zoo Knoxville. Approved by IACUC at the University of Tennessee [2436-0316]. This chapter was co-authored by Benjamin Fitzpatrick (University of Tennessee) and Todd Pierson (University of Tennessee).

References

Alcaide M, Negro JJ, Serrano D, Antolín J, Casado S, Pomarol M (2010) Captive breeding and reintroduction of the lesser kestrel *Falco maumanni*: a genetic analysis using microsatellites. Consrv Genet 11:331-338

Allendorf FW (2017) Genetics and the conservation of natural populations: allozymes to genomes. Mol Ecol 26:420-430

Allendorf FW, Hohenlohe PA, Luikart G (2010) Genomics and the future of conservation genetics. Nat Rev 11:697-709

Amato GD, Behler JL, Tryon BW, Herman DW (1997) Molecular variation in the bog turtle, *Clemmys muhlenbergii*. In: Proceedings of the International Conference of Conservation, Restoration and Management of Tortoises and Turtles. New York Turtle and Tortoise Society, New York, pp 259-262

Barba MDE, Waits LP, Garton EO, Genovesi P, Randi E, Mustoni A, Groff C (2010) The power of genetic monitoring for studying demography, ecology, and genetics of a reintroduced brown bear population. Mol Ecol 19:3938-3951

Bell CDL, Parsons J, Austin TJ, Broderick AC, Ebanks-Petrie G, Godley BJ (2005) Some of them came home: the Cayman Turtle Farm headstarting project for the green turtle *Chelonia mydas*. Oryx 39:137-148

Bernard AM, Ferguson MM, Noakes DLG, Morrison BJ, Wilson CC (2009) How different is different? Defining management and conservation units for a problematic exploited species. Can J Fish Aquat Sci 66:1617-1630

Carter SL, Haas CA, Mitchell JC (1999) Home range and habitat selection of bog turtles in southwestern Virginia. J Wildl Manage 63:853-860

Copeyon CK (1997) Endangered and threatened wildlife and plants: Final rule to list the northern population of the bog turtle as threatened and the southern population as threatened due to similarity of appearance. Federal Register 62:59605-59623

Cunningham AA (1996) Disease risks of wildlife translocation. Conserv Biol 10:349-353

Do C, Waples RS, Peel D, Macbeth GM, Tillet BJ, Ovenden JR (2014) NeEstimator V2: re-implementation of software for the estimation of contemporary effective population size (N_e) from genetic data. Mol Ecol 14:209-214

Dresser CM, Ogle RM, Fitzpatrick BM (2017) Genome scale assessment of a species translocation program. Conserv Genet DOI: 10.1007/s10592-017-0970-6

Earl DA, vonHoldt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour 4:359-361

Eaton MJ, Meyers GL, Kolokotronis SO, Leslie MS, Martin AP, Amato G (2010) Barcoding bushmeat: molecular identification of Central African and South American harvested vertebrates. Conserv Genet 11:1389-1404

Eckert AJ, Heerwaarden J, Wergrzyn JL, Nelson CD, Ross-Ibarra J, González-Martínez SC, Neale DB (2010) Patterns of population structure and environmental associations to aridity across the range of Loblolly pine (*Pinus taeda* L., Pinaceae). Genet 185:969-982

Feaga JB (2010) Wetland hydrology and soils as components of Virginia bog turtle (*Glyptemys muhlenbergii*) habitat. Doctoral Dissertation, Virginia Tech, Blacksburg, Virginia

Foll M, Gaggiotti OE (2008) A genome scan method to identify selected loci appropriate for both dominant and codominant markers: a Bayesian perspective. Genet 180:977-993

Fraser DJ, Bernatchez L (2001) Adaptive evolutionary conservation: towards a unified concept for defining conservation units. Mol Ecol 10:2741-2752

Garant D, Forde SE, Hendry AP (2007) The multifarious effects of dispersal and gene flow on contemporary adaptation. Funct Ecol 21:434-443

Glover KA, Pertoldi C, Besnier F, Wennevik V, Kent M, Skaala Ø (2013) Atlantic salmon populations invaded by farmed escapees: quantifying genetic introgression with a Bayesian approach and SNPs. BCM Genet DOI:10.1186/1471-2156-14-74

Grosser S, Abdelkrim J, Wing J, Robertson BC, Gemmell NJ (2017) Strong isolation by distance argues for separate management of endangered blue duck (*Hymenolaimus malacorhynchos*). Conserv Genet 18:327-341

Hanski I, Gaggiotti OE (2004) Ecology, genetics, and evolution of metapopulations. Elsevier Academic Press, Burlington, Massachusetts

Hughe E (2010) Protocol for sampling and storage of painted turtle (*Chrysemys picta*) blood and tissue for genetic analysis. Genetic Sampling Protocol

King TL, Julian SE (2004) Conservation of microsatellite DNA flanking sequence across 13 Emydid genera assayed with novel bog turtle (*Glyptemys muhlenberii*) loci. Conserv Genet 5:719-725

Kinitz T, Quack M, Paulus M, Veith M, Bergek S, Strand J, Tuvikene A, Soirinsuo A, Hochkirch A (2013) Strong isolation-by-distance in the absence of genetic population structure in the eelpout (*Zoarces viviparus*, Linnaeus 1758). Ecol Indicators 27:116-122

Lindenmayer DB, Piggott MP, Wintle BA (2013) Counting the books while the library burns: why conservation monitoring programs need a plan for action. Front Ecol Environ 11:549-555

Lovich JE, Herman DW, Fahey KM (1992) Seasonal activity and movements of bog turtles (*Clemmys muhlenbergii*) in North Carolina. Copeia 1992:1107-1111

Mauritzen M, Derocher AE, Wiig O, Belikov SE, Boltunov AN, Hansen E, Garner GW (2002) Using satellite telemetry to define spatial population structure in polar bears in the Norwegian and western Russian Artic. J Appl Ecol 39:79-90

McEachern MB, Van Vuren DH, Floyd CH, May B, Eadie JM (2011) Bottlenecks and rescue effects in a fluctuating population of golden-mantled ground squirrels (*Spermophilus lateralis*). Conserv Genet 12:285-296

Miller W, Hayes VH, Ratan A, et al. (2011) Genetic diversity and population structure of the endangered marsupial *Sarcophilus harrisii* (Tasmanian devil). PNAS 108:12348-12353

Moritz C (1994) Defining 'Evolutionary Significant Units' for conservation. TREE 9:373-375

Ngeve M, Van der Stocken T, Menemenlis D, Triest L (2016) Contrasting effects of historical sea level rise and contemporary ocean currents on regional gene flow of *Rhizophora racemosa* in Eastern Atlantic Mangroves. PLoS one DOI:10.1371/journal.pone.0150950

Palkovacs EP, Hasselman DJ, Argo EE, Gephard SR, Limburg KE, Post DM, Schultz TF, Willis TV (2013) Combining genetic and demographic information to prioritize conservation efforts for anadromous alewife and blueback herring. Evol Appl 7:212-226

Pittman SE, King TL, Faurby S, Dorcas ME (2011) Demographic and genetic status of an isolated population of bog turtles (*Glyptemys muhlenbergii*): implications for managing small populations of long-lived animals. Conserv Genet DOI:10.1007/s10592-011-0257-2

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genet 155:945-959

Ramey R, Luikart G, Singer FJ (2000) Genetic bottlenecks resulting from restoration efforts: the case of bighorn sheep in Badlands National Park. Restor Ecol 8:85-90

Rieman BE, Allendorf FW (2001) Effective population size and genetic conservation criteria for bull trout. North Am J Fish Mana 21:756-764

Rosenbaum PA, Robertson JM, Zamudio KR (2007) Unexpectedly low genetic divergences among populations of the threatened bog turtle (*Glyptemys muhlenbergii*). Conserv Genet 8:331-342

Schwartz MK, Luikart G, Waples RS (2006) Genetic monitoring as a promising tool for conservation and management. TREE DOI:10.1016/j.tree.2006.08.009

Shoemaker KT, Gibbs JP (2013) Genetic connectivity among populations of the threatened bog turtle (*Glyptemys muhlenbergii*) and the need for a regional approach to turtle conservation. Copeia 2013:324:331

Somers AB (2000) A population of Bog Turtles in the Piedmont of North Carolina: habitat preferences, capture method efficacy, conservation initiatives, and site enhancement. In: Report submitted to Natural Resources Conservation Service Wetlands Institute

Somers AB, Mansfield-Jones J, Braswell J (2007) In stream, streamside, and under stream bank movements of a bog turtle, *Glyptemys muhlenbergii*. Chelonian Conserv Biol 6:286-288

Spinks PQ, Thomson RC, McCartney-Melstad E, Shaffer HB (2016) Phylogeny and temporal diversification of the New World pond turtles (Emydidae). Mol Phylogenet Evol 103:85-97

St Clair JB, Howe GT (2007) Genetic maladaptation of coastal Douglas-fur seedlings to future climates. Global Change Biol 13:1441-1454

Storfer A (1999) Gene flow and endangered species translocations: a topic revisited. Biol Conserv 87:173-180

Taubmann J, Thissinger K, Feldheim KA, Laube I, Graf W, Haase P, Johannesen J, Pauls SU (2011) Modelling range shifts and assessing genetic diversity distribution of the montane aquatic mayfly *Ameletus inopinatus* in Europe under climate change scenarios. Conserv Genet 12:503-515

Taylor EB, Tamkee P, Keeley ER, Parkinson EA (2010) Conservation prioritization in widespread species: the use of genetic and morphological data to assess population distinctiveness in rainbow trout (*Oncorhynchus mykiss*) from British Columbia, Canada. Evol Appl 4:100-115

United States Fish and Wildlife Service (2001) Bog turtle (*Clemmys muhlenbergii*) Northern population recovery plan. Prepared by M Klemens. Pennsylvania Field Office, Hadley, Massachusetts

Waples RS (1989) A generalized approach for estimating effective population size from temporal changes in allele frequency. Genet 121:379-391

Whitlock AL (2002) Ecology and status of the Bog Turtle (*Clemmys muhlenbergii*) in New England. Dissertation, University of Massachusetts Amherst

Yumnam B, Jhala YY, Qureshi Q, Maldonado JE, Gopal R, Saini S, Srinivas Y, Fleischer RC (2014) Prioritizing tiger conservation through landscape genetics and habitat linkages. PLoS One DOI: 10.5061/dryad.c7v41

CHAPTER II

GENOMIC ASSESSMENT OF TENNESSEE'S BOG TURTLE (*GLYPTEMYS MUHLENBERGII*) TRANSLOCATION PROGRAM

A version of this chapter was originally published by Cassie M. Dresser, R. Michael Ogle, and Benjamin M. Fitzpatrick:

Dresser CM, Ogle RM, Fitzpatrick BM (2017) Genome scale assessment of a species translocation program. Conserv Genet DOI 10.1007/s10592-017-0970-6

This copyrighted material is owned by or exclusively licensed to the author(s) and is reproduced here with their permission.

The research in this article was conducted by CM Dresser, with help from BM Fitzpatrick and RM Ogle. Dresser wrote the manuscript, both Dresser and Fitzpatrick ran analyses, and all co-authors assisted with revisions.

Abstract

Despite increased use of species translocations, controversy remains regarding the efficacy and efficiency of the strategy in obtaining conservation goals. Much of this controversy results from vague program objectives, unclear definitions of success, and lack of follow-up monitoring. We used the translocation program initiated by Zoo Knoxville for the federally threatened Bog Turtle (*Glyptemys muhlenbergii*) as a case study to demonstrate how genomic assessments not only assess the success of program objectives, but also allow managers to quickly obtain baseline data from which program objectives and explicit definitions of 'success' can be determined. Here we used 7 030 SNP markers derived from RADseq data to confirm the premise that different source populations are genetically differentiated. Then we tested whether the release population has enhanced genetic diversity, as expected from a deliberate admixture. Although the release population had greater diversity than any source population, variation was lower than expected from modeling admixture with equal source contribution. Our results support the premise that genetic diversity can be maximized by including representatives from as many natural populations as possible. But failure to achieve the expected level of diversity could result from nonrandom success of founders from different sources or unrecorded bias in the implementation of the release program. Many existing and future translocation programs would benefit from genetic assessment similar to that conducted here with Bog Turtles.

Introduction

Habitat destruction, degradation, and fragmentation are shrinking population sizes and altering historic patterns of gene flow in numerous species (Andrén 1994; Bender et al. 1998; Cushman 2006; Swift and Hannon 2009; Quesnelle et al. 2013). Without human intervention, many of these populations would face an early extinction. Such interventions are often outlined in species action plans. One intervention in particular has become increasingly common since the 1970s: human-mediated migration (i.e., translocation – including introduction, relocation, reintroduction, and supplementation). Despite increased use of species translocations, less than 50% have been formally assessed and only a small fraction of those have been deemed successful (Fischer and Lindenmayer 2000; Tarszisz et al. 2014). Lack of assessment (i.e., poor follow-up monitoring) has likely contributed to uncertainty regarding the efficacy and efficiency of translocations: numerous studies advocate for translocations (Marsh and Trenham 2001; Strum 2005; Parker 2008; Decesare et al. 2011; Estrada 2014; Watson and Watson 2015), but many others advocate against them (Dodd and Seigel 1991; Struhsaker and Siex 1998; Ricciardi and Simberloff 2009; Godefroid et al. 2011; Oro et al. 2011). And those studies that do not explicitly advocate for or against translocations emphasize that extreme caution should be taken when considering translocations as a conservation strategy (Cope and Waller 1995; Menges 2008; Schwartz et al. 2012). One factor likely contributing to the lack of consensus regarding translocations is that program objectives are often unclear or nonexistent, resulting in vague criteria for success (Weeks et al. 2011; Ewen et al. 2014).

Genomic assessments offer an objective means to monitor translocation programs and establish specific criteria to characterize success, yet such assessments are rarely incorporated in program evaluations (Frankham et al. 2014). This is particularly surprising considering the growing realization that genetic factors often impact populations prior to their extinction (Spielman et al. 2004). Historically, and to a lesser extent presently, probability of persistence was inferred by estimating census population sizes using mark-recapture techniques (Seber 1982), management units were based solely on political boundaries, translocations were implemented based on expert opinion, and taxonomic uncertainties were resolved using morphology. However, the emergence of new genomic techniques now allows thousands of markers to be examined with relative ease, making previously unattainable information accessible and previously accessible information more reliable and objective (Allendorf et al. 2010). Such advances are changing the way populations are monitored, how they are managed, and how uncertainty is addressed.

Here we illustrate how genetic assessment can be used to evaluate a translocation program using Tennessee's Bog Turtle (*Glyptemys muhlenbergii*) captive breeding and release program. This program

presents itself as a useful case study because several aspects of the program mirror those of many extant translocation programs, namely the lack of clear objectives when the program was first initiated, subjective success criteria, and missing breeding and release records for a species notoriously difficult to monitor using conventional survey methods.

The Tennessee Bog Turtle captive breeding and release program was initiated nearly 30 years ago by Zoo Knoxville to aid in the conservation of this federally threated species. The goal was to successfully breed Bog Turtles in captivity and release the offspring in the wild. Bog Turtles from North Carolina and Tennessee were successfully bred in captivity and over 100 turtles were released to a single, experimental release site between 1991 and 2015 with an 84% annual survival rate. Was this program successful? Well, that depends on what the program objectives were. If the only objectives were to successfully breed Bog Turtles in captivity and that the released offspring survived, then the program successfully met those objectives. However, if the objective was to create a self-sustaining population, then the success of the program has not yet been determined. Specifically, if the objective was to maximize the long-term sustainability of the introduced population by maximizing genetic variation, then records documenting the release and survival of an equal number of offspring (particularly females) from each source population (assuming each source was a genetically distinct population) would suggest this objective was met. However, some breeding and release records were missing or undocumented and all but two founding captive individuals were predated by raccoons, which prohibited retroactive genetic analysis to assign missing parentage records (see Appendix A1 and A2 for details).

Another motivating factor, one of primary importance to agencies and personnel managing Bog Turtle populations for conducting a genetic assessment on the Tennessee captive breeding and release program is the fact that the Species Recovery Plan (USFWS 2001) mandates the "investigation of the genetic impacts of reintroduction". Many captive breeding programs, including the Bog Turtle program are intentionally managed to avoid breeding close relatives (i.e., inbreeding avoidance) to minimize loss of genetic variation due to inbreeding. A formal genetic assessment would indicate whether the implemented breeding strategy achieved said goal or if adjustments are necessary in the future. Such evaluation is timely as conservation options are dwindling as Bog Turtle populations continue to plummet and managing partners are expressing interest in implementing similar translocation programs in other parts of the Bog Turtle range. Since most interested partners will face implementation challenges associated with limited time, money, and personnel, our approach also demonstrates how managers could determine whether or not female turtles from *all* wild populations are necessary for a sustainable head-

start program based on the degree of genetic differentiation among populations (i.e., whether or not the inclusion of the focal population maximizes the genetic variation in the release population).

We use reduced representation next-generation sequencing (RADseq) to evaluate whether the Bog Turtle translocation program implemented by Zoo Knoxville successfully maintained genetic variation within the release population relative to neighboring wild populations and source populations. First, we tested the working assumption that different source populations are genetically differentiated, and therefore of equal importance in establishing a reservoir of genetic variation. Then we compared the estimated genetic variation in the release site to the expected level based on an assumed even admixture of source populations. Taken together, these genetic measures not only satisfy the requirements mandated in the Species Recovery Plan, but also inform current and future management programs for the federally threatened Bog Turtle. Furthermore, our genetic assessment highlights how careful record keeping can complement and even inform quantitative standards of success in regards to program objectives, bringing us one step closer to resolving the uncertainty surrounding translocation as a viable conservation strategy.

Methods

Study system

North America's smallest semi-aquatic turtle, the Bog Turtle (*Glyptemys muhlenbergii*) continues to require conservation action as populations are declining throughout their range, from northern Georgia to upstate New York and Massachusetts due to habitat destruction (Gibbons et al. 2000), road mortality (Mitchell 1994), and illegal collection for the pet trade (Tesauro 2001). Long-term programs are particularly valuable considering the life history of the species; Bog Turtles typically reach reproductive age at 6-12 years old, with males maturing before females and each female only lays an average of three eggs per year. A handful of Bog Turtle head-start programs have been launched, but the Tennessee program is the largest and longest running. The program has a blended approach, combining captive breeding and head-starting: (1) captive breeding and release, where initial breeding pairs were obtained from wild populations in the southern United States and offspring were released into a wild experimental population and (2) head-starting, where local, wild females are tracked using radio-telemetry, brought into a local laying facility to deposit their eggs and immediately returned while the eggs and eventually hatchlings are raised in a secure environment for approximately nine months before release into the wild

experimental population (although early on in the program, hatchlings were raised for 22 months before release).

The initial captive population included one individual from western North Carolina (Site F; Figure 2.1) and a few individuals from northeast Tennessee. To date, only first generation (F1) offspring from the captive breeding program have been translocated to the release site. Females for the head-start program were obtained from three sites in northeast Tennessee (Site L, O, and Q; Figure 2.1). A fourth wild site exists in Tennessee (Site B; Figure 2.1), but permission to access the property has been revoked for an unknown period of time and thus is no longer part of the program. Like most Bog Turtle sites, the experimental release site (Release Site; Figure 2.1) is also on private land (only Site O is entirely on public land), at a pristine high-elevation bog approximately 48 km south of the nearest known wild site in Tennessee. The wild sites in Tennessee are relatively close to each other geographically, particularly Site L and Site O for which previous radio-telemetry data has shown turtles occasionally moving across the 2 km agricultural field between the two wetland sites (A. Eastin, personal communication).

Sampling

We collected tissue samples from a total of 124 individual turtles, representing all known Tennessee populations and nearly half of all extant individuals at accessible sites. All Tennessee turtles were hand-captured using visual and tactile methods (Whitlock 2002) and augmented with trapping as needed (Somers 2000; Whitlock 2002) between April and October of 2014 and 2015. For each new capture a tissue sample was collected (< 0.5 cm distal portion of the tail) and was immediately placed in 95% ethanol and stored at -20°C until DNA extraction (Hughe 2010). We were able to confirm that each sample represented a different individual because all turtles had or were given a unique notching pattern on their marginal scutes (method modified from Cagle 1939).

Of the estimated number of extant turtles from each site, 63% of turtles from the Release Site were sampled (N = 57), 71% of Site L (N = 5), 30% of Site O (N = 9), 57% of Site Q (N =12). Two turtles from Site F were donated by the North Carolina Wildlife Resources Commission and J. Apodaca (Warren-Wilson College), all extant captive individuals (N = 5) were donated by Zoo Knoxville, four turtles from Site B were donated by Tim King (USGS), and the remaining samples came from juveniles at the head-start facility (N = 30). This is the most extensive and complete genetic sampling ever obtained for the Bog Turtles in Tennessee, an impressive feat considering the cryptic nature of this species and the logistical constraints on the efficiency of capture methods because of habitat characteristics at some sites. Considering that only one captive breeding pair survived the raccoon predation at the outdoor exhibit at

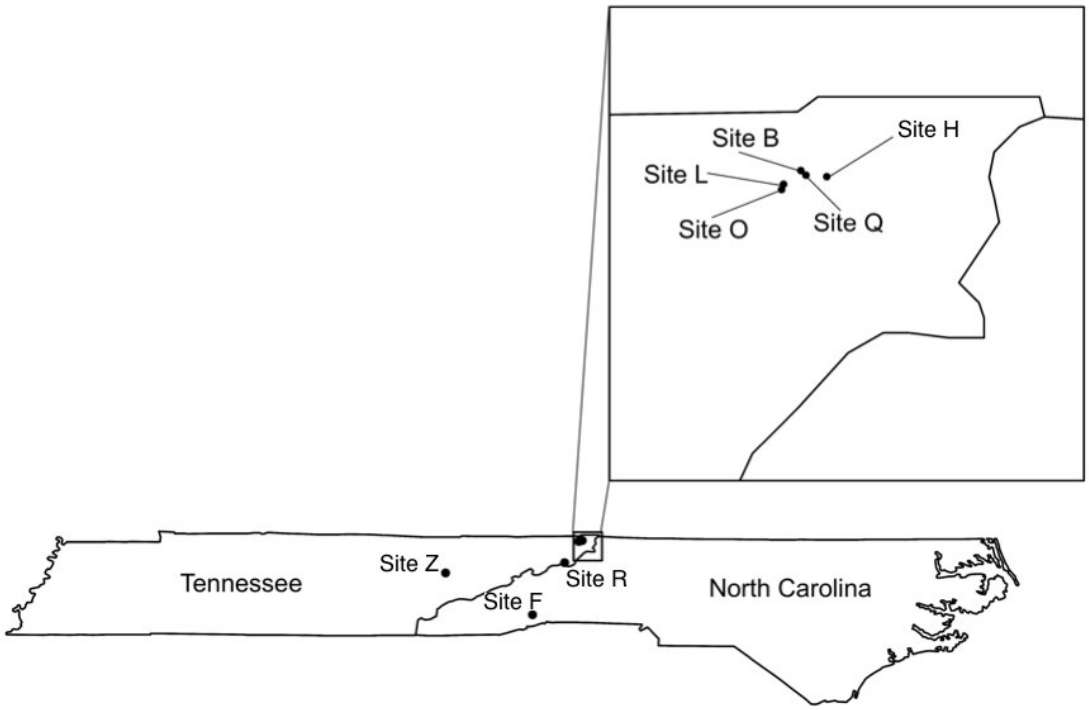


Figure 2.1 Bog turtle sampling locations in Tennessee and North Carolina. Site F represents the source population for one individual used in the original captive breeding program at Zoo Knoxville (Site Z), for which offspring were introduced to the release site (Site R) located south of the wild Tennessee Bog Turtle populations (Site O, L, B, and Q). The location of the head-start facility where offspring of wild females were raised for nine months prior to release is shown adjacent to the wild sites within the map inset

Zoo Knoxville, we supplemented the sampling of the extant captive population with samples previously collected from several turtles at the North Carolina source (Site F) and the fourth Tennessee site (Site B).

Laboratory and post-sequencing procedures

We isolated DNA from tissue using the DNeasy Blood and Tissue Kit (Qiagen Corporation, Valencia, CA), quantified extracted products using a fluorometer, and visualized DNA using gel electrophoresis to confirm that the DNA was not degraded. We digested DNA products using three enzymes (ClaI, MspI, and BamI) rather than the two enzymes typically used in double-digest restriction site associated DNA sequencing (ddRAD; Peterson et al. 2012) to reduce chimeras, increases the efficiency of adapter ligation, and minimize the occurrence of adapter dimers (3RADseq; T Glenn, unpublished). The 3RADseq protocol requires less input DNA and adapters and uses variable length quadruple-index tags to improve sequencing efficiency and allow pooling of more samples. We successfully generated 3RAD libraries for 113 individuals. Individual RADseq libraries were pooled relative to their DNA concentrations prior to size selection of 500 bp fragments using a PippenPrep system (Sage Science Corporation, Beverly, MA) and sequenced on an Illumina NextSeq for 75-bp paired end reads for approximately 2 million reads per individual.

After the inner barcode and cut site were trimmed, quality control and filtering of the genotypes for each sample were performed with the software pipeline, *pyRAD* (Eaton 2014). The default parameter values were used, with the following exceptions: the minimum depth of coverage was set to 10, the clustering threshold was 0.907 (allowing no more than 6 bp mismatches), the minimum number of samples required per locus was 2, and the maximum number of shared polymorphic sites allowed per locus was 3 bp. After library preparation, sequencing, and quality control of Illumina reads, data from 7 030 markers and 95 individual turtles remained (2 turtles from Site F, 2 from Site Z, 22 from Site H, 3 from Site B, 4 from Site Q, 6 from Site O, 4 from Site L, and 52 from Site R).

Data analysis

To assess genetic differentiation among source populations, we estimated F_{ST} for each pair of samples to represent the between-population fraction of genetic variation (Allendorf and Luikart 2009). Specifically, we calculated Weir and Cockerham's F_{ST} for comparative purposes; an unbiased test statistic with respect to sample size (Weir and Cockerham 1984). We used parametric randomization to evaluate statistical significance while avoiding potential bias arising from missing data (i.e., when resampling individuals with varying amounts of missing data, the resampled distributions are not comparable because

they vary in the number of usable loci). For each pair of populations, we estimated population allele frequencies and pooled allele frequencies for the subset of markers with shared data for the particular pair of populations. Then, for 10 000 replicates we generated two simple random samples of two alleles per locus from the pooled allele frequencies to get a distribution of F_{ST} under the null hypothesis of no population differentiation. We also generated one random sample from each population to get a distribution of F_{ST} under the alternative hypothesis of differentiated populations. Put simply, we used this procedure to test the hypothesis of differentiation based on the probability of identity of alleles rather than observed genotypes, by literally taking an allele at random and asking if it was different from a second allele, randomly drawn from either the same site or different site. Gene diversity is the probability that two randomly sampled alleles are different; often misleadingly called heterozygosity because it corresponds to the *expected* proportion of heterozygous genotypes under Hardy-Weinberg assumptions (Nei 1987, Gillespie 2004). Hardy-Weinberg assumptions are explicitly not met in the experimental release population because it still includes first generation transplants (Wahlund 1928). Therefore, we restricted our analyses to those based on allele frequencies, and made no assumptions regarding genotype frequencies. Two alleles per locus per population is the minimal sample of alleles suitable for estimating F_{ST}, resulting in maximal sampling variance and minimal risk of Type I error. We estimated p-values as the proportion of replicates in which F_{ST} under the null hypothesis was greater than or equal to F_{ST} under the alternative hypothesis of distinct populations.

To compare the genetic diversity within the release population vs. within each natural population, we estimated gene diversity for each population. We estimated bootstrap 95% confidence intervals by resampling from the full set of 7 030 loci with replacement to generate 10 000 bootstrap samples.

To specifically test whether the gene diversity in the release population (Site R) was consistent with an equal contribution from each source population, we used parametric bootstrapping to estimate the expected distribution of gene diversity in an equal mixture. The unweighted mean allele frequencies were calculated for each locus across source populations to estimate the expected allele frequencies in a source pool with equal representation from each source population. Then, 10 000 replicate random samples of two alleles per locus were generated from the pooled allele frequencies to get a distribution of sample gene diversities under the equal mixture model. We compared this distribution to the parametric bootstrap distribution of sample gene diversities generated by drawing samples from the observed allele frequencies within the release population.

As a follow-up to the previous analysis that compared observed versus expected gene diversity in the released population (Site R), we assessed expectations of gene diversity loss associated with genetic

drift assuming a constant population of 176 individuals (which was the number of individuals released in Site R) comprised of an equal number of founders from each source population. Using standard theory for the loss of gene diversity owing to drift (Gillespie 2004), we calculated the number of generations it would take for the expected gene diversity to match the observed gene diversity. We used 10 000 stochastic simulations to assess the variability of gene diversity loss owing to drift.

Finally, to evaluate whether the release population was more genetically similar to some source populations than others, we used the pairwise F_{ST} parametric bootstrap routine to estimate bootstrap distributions of F_{ST} and Jost's D (Jost 2008) between the release site and each source.

Results

We obtained a total of 163 139 257 paired-end reads, each 150 bp across 113 individual turtles in four wild Tennessee populations, one North Carolina wild population, the captive population at Zoo Knoxville and the head-start population at the local rearing site. After filtering for a minimum coverage depth of 10 and a minimum number of samples per locus of two, 7 030 total markers were found across 95 turtles, for which nine loci had three alleles.

Partitioning of genetic diversity as estimated using pairwise F_{ST} values ranged from 0.411 (Head Start, H – Release Site, R) to 0.717 (Site L - Site O) (Table 2.1). The null distributions of F_{ST} values produced from parametric randomizations indicated that all pairwise F_{ST} values were significantly higher than would be expected if the sites were parts of a single panmictic population (Table 2.1). The observed genetic differentiation among wild source populations did not correspond to expectations given the geographic proximity of populations. For example, Site L was geographically closest to Site O (within 2 km of each other), but the pairwise F_{ST} value between these two sites was higher than pairwise F_{ST} values between either site and the other, more geographically distant wild sites (Site Q and Site B).

Gene diversity measures within wild source populations ranged from 0.08 (Site L) to 0.14 (Site Q) (Figure 2.2). The captive population at Zoo Knoxville (Site Z) and the head-start population at the local rearing site (Site H) had higher gene diversity values relative to the wild source populations (Figure 2.2). Pertinent to our overall research question, regarding whether the release program successfully increased gene diversity in the experimental release population, we did observe a substantially higher gene diversity value in the released population relative to its source populations (Figure 2.2). However, the observed population level gene diversity in the released population falls significantly short of the expected gene diversity based on the admixture model where all source populations contributed equally to

Table 2.1 Pairwise F_{ST} values for Bog Turtle populations (see Figure 2.1 for site locations). Pairwise F_{ST} values are shown above the diagonal and p-values calculated using parametric randomizations from 1000 replicates are shown below the diagonal

	Site F	Site R	Site O	Site Q	Site L	Site H	Site B	Site Z
F	-	0.482	0.561	0.524	0.630	0.508	0.507	0.478
R	<0.0001	-	0.465	0.433	0.501	0.411	0.454	0.419
O	<0.0001	<0.0001	-	0.584	0.717	0.477	0.554	0.535
Q	<0.0001	<0.0001	<0.0001	-	0.687	0.463	0.523	0.412
L	0.0007	<0.0001	<0.0001	<0.0001	-	0.570	0.635	0.641
H	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	-	0.488	0.441
B	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	-	0.477
Z	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	-

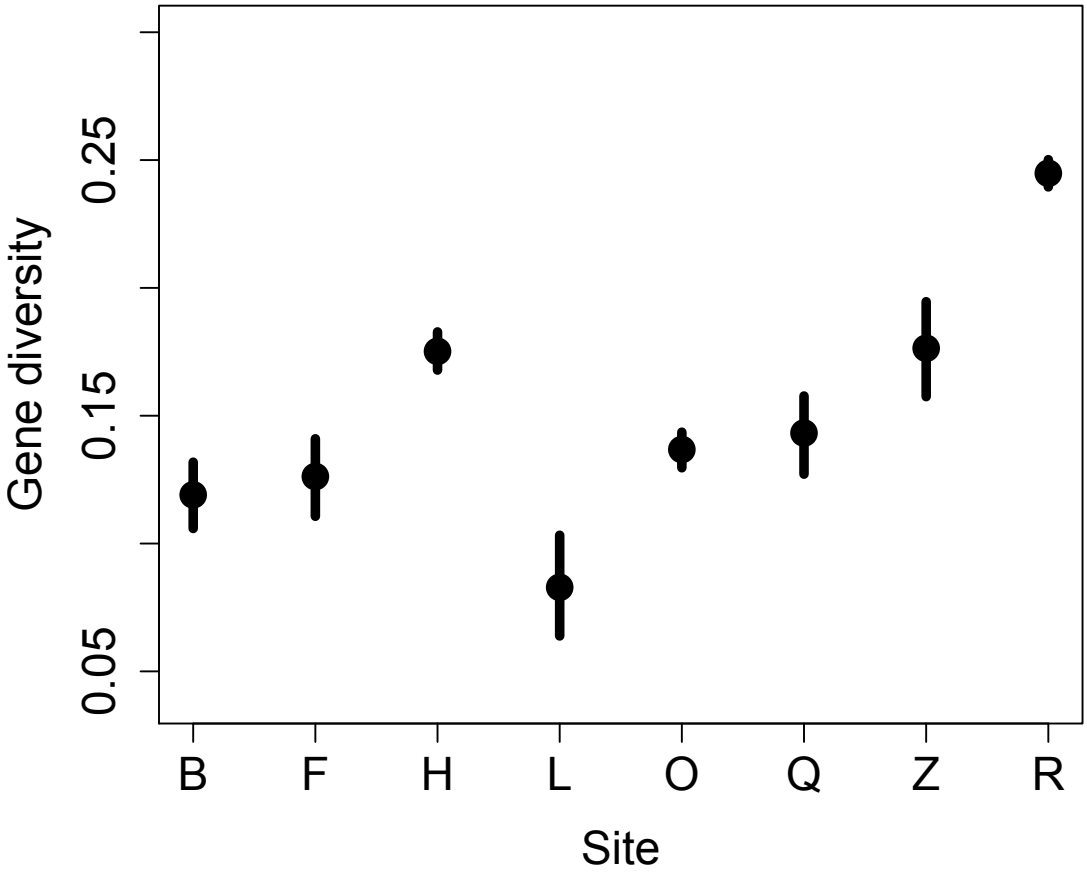


Figure 2.2 Mean gene diversity at each Bog Turtle sampling location (see Figure 2.1) across 7030 SNP markers. Ninety-five percent bootstrap confidence intervals are shown around each mean

the released population (Figure 2.3). In fact, it would take, on average, about 47 generations (perhaps 400-500 years) for genetic drift to reduce the expected gene diversity of 0.280 to the observed gene diversity of 0.245 (see File 2 cited in published manuscript).

Measures of genetic differentiation were also inconsistent with equal contribution of source populations (Figure 2.4). The release population had the greatest genetic similarity to populations Q and B and the Zoo population (Z), and the greatest differentiation from populations O, L, and F (Figure 2.4). The head-start population was surprisingly dissimilar to the release population according to Jost's *D*, while F_{ST} between them was relatively low (likely reflecting the relatively high within-population variation seen in both samples, see Figure 2.2).

Discussion

Nearly thirty years ago, Zoo Knoxville started a captive breeding and release program for the federally endangered Bog Turtle (*Glyptemys muhlenbergii*) as a preemptive conservation measure to combat the observed decline of populations throughout their range. Within ten years, the IUCN listed the Bog Turtle as endangered, and the US government listed populations within the northern region as Threatened under the ESA. As a result, a species recovery plan was drafted (USFWS 2001), which included an objective to genetically assess the impacts of reintroductions (i.e., 'translocations'). Although the particular long-term objectives of the Tennessee program were either vague or unwritten when the program was initiated nearly 30 years ago, implementation of the program has generally been guided by the idea that the experimental release population should be composed of individuals from many distinct natural populations to maximize genetic diversity (presumably maximizing adaptive capacity and minimizing risks associated with inbreeding depression). Here we confirmed the inferred premise that the source populations represent distinct gene pools, and showed that the experimental released population has greater genetic diversity than any of the possible source populations. However, the level of genetic diversity falls short of the predicted diversity of a truly equal mixture from all sources. Understanding the causes of this shortfall could help guide future management decisions.

Experts believed the Bog Turtle was in decline long before their addition to the IUCN Red List in 1996 (Ernst and Barbour 1972; Behler 1974; Bury 1979; Chase et al. 1989). With each passing year, the IUCN Red List grows; in fact since 2000 the number of listed threatened species has more than doubled (IUCNredlist.org). Many of these additions result from delayed assessments of less well-known groups or the eventual filling in of the knowledge gaps from insufficient preliminary assessments (e.g. marine

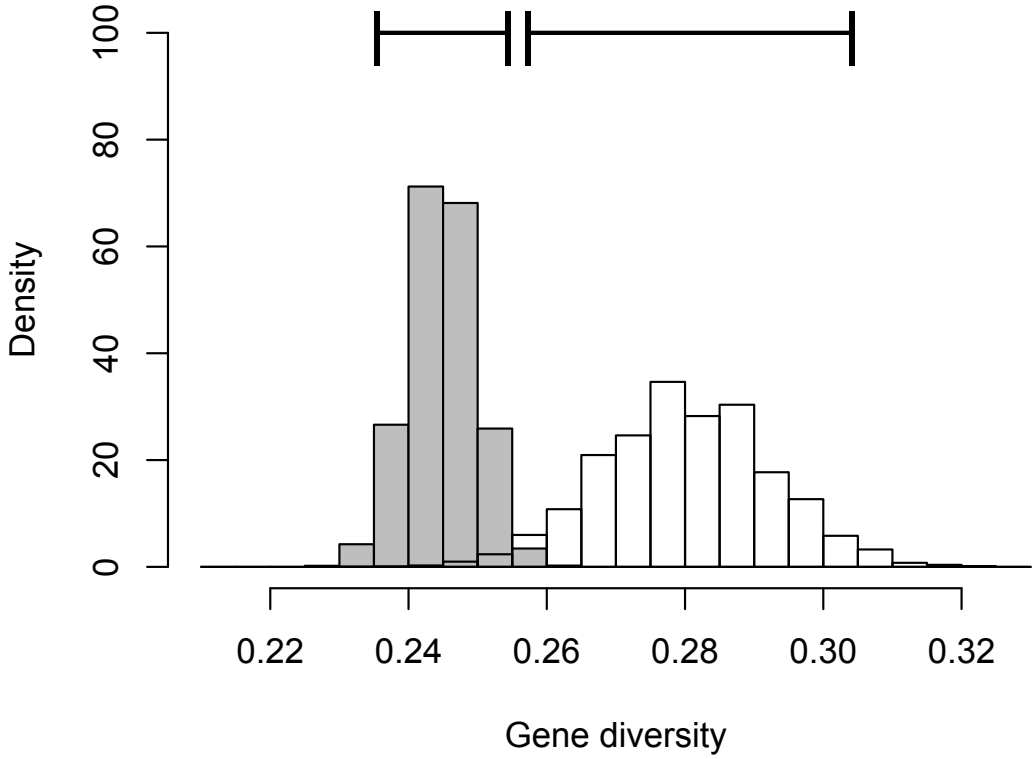


Figure 2.3 Observed (gray) versus expected (white) gene diversity for the experimental Bog Turtle release population (Site R) based on 1000 bootstrap replicates. Expected gene diversity was calculated assuming an equal contribution from the captive breeding population (Z) and each potential wild source population (Sites F, O, L, B, and Q in Figure 2.1)

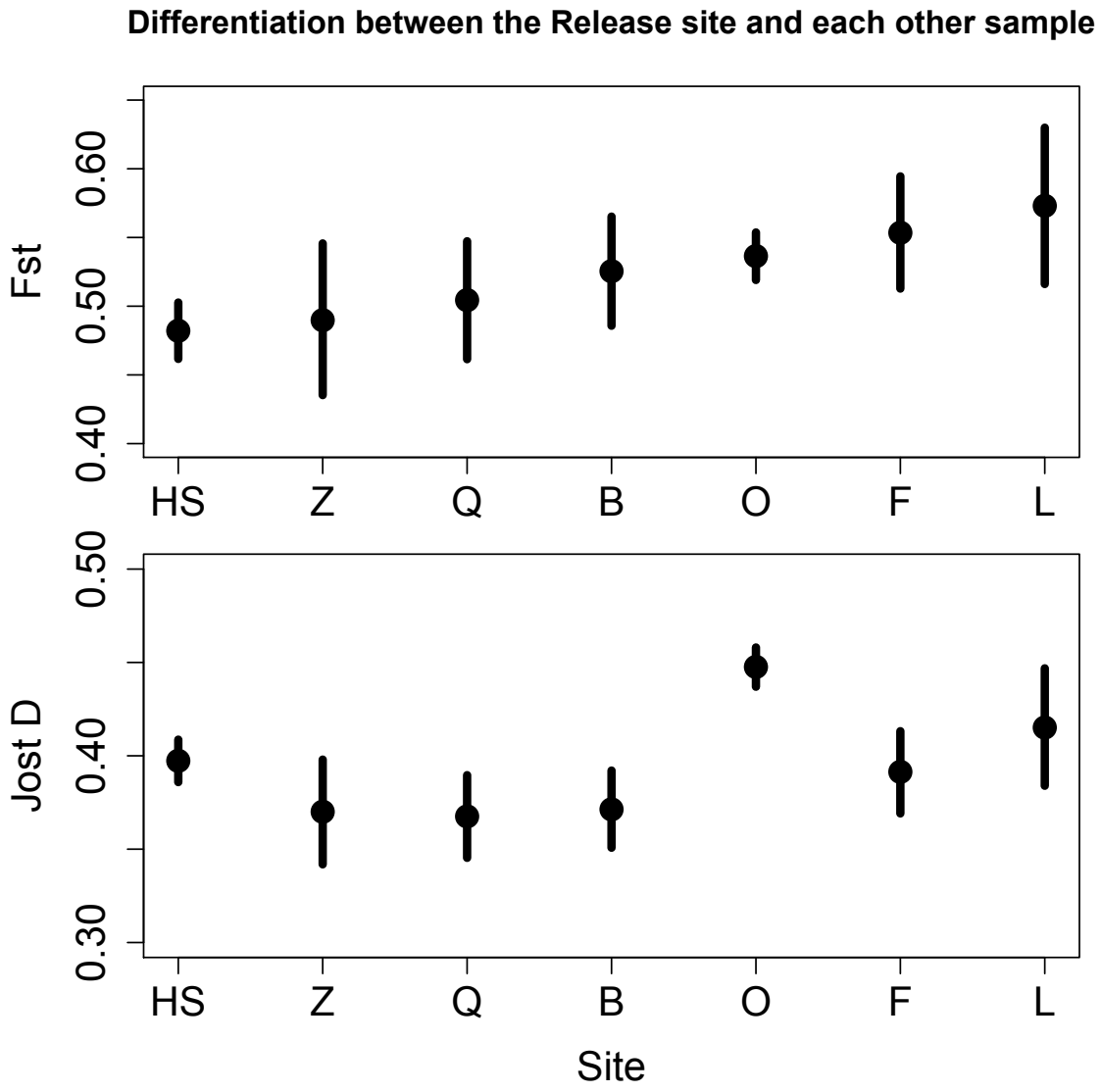


Figure 2.4 Genetic differentiation between Bog Turtles sampled from the release site and other samples with 95% parametric bootstrap confidence intervals

turtles; Seminoff and Shanker 2008). Although the IUCN is often regarded as the world's primary authority on the conservation status of species (Mrosovsky 1997), within the United States federal protections are not established until they are listed under the Endangered Species Act. For Bog Turtles, ESA listing came the following year (although only for the northern populations), but for most IUCN-listed species ESA listing has never followed (Harris et al. 2011). Unfortunately, delayed prioritization and protections can leave managers with fewer conservation options with potentially greater uncertainty regarding their outcomes. In situations such as these, translocations may be proposed to artificially restore historic gene flow or introduce a population to a more suitable habitat (e.g. freshwater mussel; Cosgrove and Hastie 2001). However, to increase the likelihood of success, translocations should be considered long before they become a last resort (Griffith et al. 1989). Clearly, endangered species conservation programs could benefit from a greater understanding of the efficacy and efficiency of controversial translocation strategies.

Genetic, or more recently genomic assessments offer a way to address the current deficiency in our ability to evaluate translocation as a possible strategy by providing a relatively quick method for collecting baseline data from which to formulate quantitative program objectives and conduct follow-up monitoring. Specifically, we used RADseq to characterize the genetic variation present in potential source populations (i.e., starting gene diversity) to serve as a baseline to compare to the translocated population, where greater gene diversity would provide an objective measure of 'success'.

Pairwise F_{ST} values indicated that all wild source populations are genetically distinct and thus worthy of inclusion in the program. This is immediately pertinent considering several of the populations are continually threatened by human-caused habitat degradation. Thus the offspring currently existing in the release population may soon be the only genetic representatives left from some natural populations. With continued climate change on the horizon and the unpredictable impacts of anthropogenic activities, the variants of a gene that will be beneficial in the future will be hard to predict, thus preserving genetic variation may be the most conservative strategy (Bonin et al. 2007) – in which case the Bog Turtles of Tennessee are in a better position than they were in the recent past.

Careful and complete bookkeeping of any translocation program would also reduce ambiguity in assessments of success. In our case, the absence of complete breeding and release records inhibit our ability to distinguish between two possible explanations for the lower than expected gene diversity in the translocated population; (1) unrecorded bias in the implementation of the release program and (2) nonrandom success of released individuals (including the dominance of one or a few breeding males). If more individuals were released from one source relative to another then our assumption of equal

contribution from each source would yield unrealistic expected gene diversity; however, if these data were available it would be an easy assumption to update by using weighted means for each source population. If our assumption of equal mixture was incorrect, our simulations indicate that genetic variation could be increased by 25% if an equal number of offspring from each genetically distinct source population were successfully released. On the other hand, if our assumption of equal contribution was correct, then the lower than expected gene diversity is likely a result of unequal survival or reproductive success between individuals of different source populations, rather than inbreeding depression or genetic drift. Many captive populations are specifically managed to prevent inbreeding, as is true for the captive breeding program at Zoo Knoxville. Offspring of captive turtles were released rather than added to the captive breeding stock and over 170 turtles were released from five different wild source populations (either via the captive breeding program or head-start program) over the past three decades, minimizing breeding between close relatives. Furthermore, only a maximum of three generations of turtles are present at the release site compared to the 47 generations necessary for genetic drift to produce the observed gene diversity (assuming an equal contribution from each source population). In fact, the 99% interquantile of our simulated data under genetic drift did not overlap the 99% interquantile range of the bootstrap distribution based on the real data until 22 generations. Thus, there has not been enough time for drift to explain the discrepancy between the expected and observed gene diversity in the release population. However, a disparity in reproductive success is possible as Bog Turtles are hypothesized to have a polygamous mating system. Such a mating system was observed in their close relative, the Wood Turtle (*Glyptemys insculpta*) where high-ranking males were found to father more offspring (Galbraith 1991) and half of all clutches had multiple sires (Pearse and Avise 2001). An extremely skewed effective sex ratio could lower the effective population size enough to achieve the observed level of gene diversity in one or two generations (Gillespie 2004) and a milder skew could be a contributing factor. Such a diagnosis would be valuable to those implementing the program and would help guide managers in making appropriate adjustments to improve the program.

Hundreds of translocation programs have been implemented (Fischer and Lindenmayer 2000), providing a great sample size for which to implement genomic assessments to evaluate program success, at least in terms of genetically relevant objectives. Of course, other assessments will be valuable to determine other dimensions of success, such as habitat assessments (e.g. bighorn sheep; Zeigenfuss et al. 2000) and health screenings to monitor the spread of disease (Griffith et al. 1993). Broad implementation of genetic assessments would help evaluate the success of previously implemented translocations, providing managers with more concrete direction regarding whether translocation is a viable strategy in

general. Moreover, genetic assessments could provide managers with a baseline from which specific program objectives could be developed and hypothetical outcomes of alternative strategies could be modeled. Conservation decisions are often challenged with uncertainty and urgency, but *post hoc* genetic analyses of existing translocation programs and *a priori* assessments of any population that may be considered in future translocation programs could remove some of the uncertainty surrounding the idea of translocations as a viable conservation strategy.

Acknowledgments

This translocation project was the idea of Bern W. Tryon (late Director of Animal Collections/Herpetology) at Zoo Knoxville. We thank the private landowners and the Nature Conservancy for living accommodations and access to sites, our volunteers and/or field technicians, Annlynn Eastin, Doug Rice, Stephen Nelson, Brad Moxley, Tessa Bickhart, and Ann Somers for their assistance collecting tissue samples. A special thanks to Todd Pierson for his invaluable assistance in the lab and the Project Bog Turtle organization for their support. Financial support was provided by the Association of Zoos and Aquariums (AZA) Conservation Grants Fund (CGF) [14-1245] from the Disney Worldwide Conservation Fund (DWCF), the North Carolina Herpetological Society (NCHS), the Ecology and Evolutionary Biology Department at the University of Tennessee, and Zoo Knoxville. Approved by IACUC at the University of Tennessee [2436-0316].

References

Allendorf FW, Hohenlohe PA, Luikart G (2010) Genomics and the future of conservation genetics. Nature Reviews 11:697-709

Allendorf FW, Luikart GH (2009) Conservation and the genetics of populations. Wiley-Blackwell

Andrén H (1994) Effects of habitat fragmentation on birds and mammals in landscapes with different proportions of suitable habitat: a review. Oikos 71:355-366

Behler JL (1974) Efforts to save the bog turtle. Herpetol Rev 5:44

Bender DJ, Contretas TA, Fahrig L (1998) Habitat loss and population decline: a meta-analysis of the patch size effect. Ecology 79:517-533

Bonin A, Nicole F, Pompanon F, Miaud C, Taberlet P (2007) Population adaptive index: a new method to help measure intraspecific genetic diversity and prioritize populations for conservation. Conserv Biol 21:697-708

Bury RB (1979) Review of the ecology and conservation of the bog turtle, *Clemmys muhlenbergii*. In: US Fish Wildl Serv Spec Sci Rep – Wildl. Washington DC, No 219, p 9

Cagle FR (1939) A system of making turtles for future identification. Copeia 1939:170-173

Chase JD, Dixon KR, Gates JE, Jacobs D, Taylor GJ (1989) Habitat characteristics, population size, and home range of the Bog Turtle, *Clemmys muhlenbergii*, in Maryland. J Herpetol 23:356-362

Cope WG, Waller DL (1995) Evaluation of freshwater mussel relocation as a conservation and management strategy. Regulated Rivers: Research and Management 11:147-155

Cosgrove PJ, Hastie LC (2001) Conservation of threatened freshwater pearl mussel populations: river management, mussel translocation and conflict resolution. Biol Conserv 99:183-190

Cushman SA (2006) Effects of habitat loss and fragmentation on amphibians: a review and prospectus. Biol Conserv 128:231-240

Decesare NJ, Whittington J, Hebblewhite M, Robinson H, Bradley M, Neufeld L, Musiani M (2011) The role of translocation in recovery of woodland caribou populations. Conserv Biol 25:365-373

Dodd CK, Seigel RA (1991) Relocation, repatriation, and translocation of amphibians and reptiles: are they conservation strategies that work? Herpetologica 47:336-350

Eaton DAR (2014) PyRAD: assembly of *de novo* RADseq loci for phylogenetic analyses. Bioinformatics DOI:10.1093/bioinformatics/btu121

Ernst CH, Barbour RW (1972) Turtles of the United States. University of Kentucky Press, Lexington

Estrada J (2014) Finding correlations among successful reintroduction programs: an analysis and review of current and past mistakes. Thesis, University of Michigan

Ewen JG, Soorae PS, Canessa S (2014) Reintroduction objectives, decisions and outcomes: global perspectives from the herpetofauna. Animal Conserv 17:74-81

Fischer J, Lindenmayer DB (2000) An assessment of the published results of animal relocations. Biol Conserv 96:1-11

Frankham R, Bradshaw CJA, Brook BW (2014) Genetics in conservation management: Revised recommendations for the 50/500 rules, Red list criteria and population viability analysis. Biol Conserv 170:56-63

Galbraith DA (1991) Studies of mating systems in wood turtles (*Clemmys insculpta*) and snapping turtles (*Chelydra serpentina*) using DNA fingerprinting. Dissertation, Queen's University, Ontario

Gibbons JW, Scott DE, Ryan TJ, Buhlmann KA, Tuberville TD, Metts BS, Greene JL, Mills T, Leiden Y, Poppy S, Winne CT (2000) The global decline of reptiles, déjà vu amphibians. BioScience 50:653-666

Gillespie JH (2004) Population genetics: A concise guide, 2nd edn. Johns Hopkins University Press, Boston

Godefroid S, Piazza C, et al (2011) How successful are plant species reintroductions. Biol Conserv 144:672-682

Griffith B, Scott JM, Carpenter JW, Reed C (1989) Translocation as a species conservation tool: status and strategy. Science 245:477-479

Griffith B, Scott JM, Carpenter JW, Reed C (1993) Animal translocations and potential disease transmission. J Zoo Wildlife Med 24:231-236

Harris JBC, Reid JL, Scheffers BR, Wanger TC, Sodhi NS, Fordham DA, Brook BW (2011) Conserving imperiled species: a comparison of the IUCN Red List and US Endangered Species Act. Conserv Letters 00:1-9

Hughe E (2010) Genetic Sampling Protocol: protocol for sampling and storage of painted turtle (*Chrysemys picta*) blood and tissue for genetic analysis. CRD Species at Risk Information and Collabative. http://www.speciesatrisk.hat.bc.ca

Jost L (2008) G_{ST} and its relatives do not measure differentiation. Mol Ecol 17:4015-4026

Marsh DM, Trenham PC (2001) Metapopulation dynamics and amphibian conservation. Conserv Biol 15:40-49

Menges ES (2008) Restoration demography and genetics of plants: when is a translocation successful? Aust J Bot 56:187-196

Mitchell JC (1994) The reptiles of Virginia. Smithsonian Institution Press, Washington DC

Mrosovsky N (1997) IUCN's credibility critically endangered. Nature 389:436

Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York

Oro D, Martínez-Abraín, Villuendas E, Sarzo B, Mínguez E, Carda J, Genovart M (2011) Lessons from a failed translocation program with a seabird species: determinants of success and conservation value. Biol Conserv 144:851-858

Parker KA (2008) Translocations: providing outcomes for wildlife, resource managers, scientists, and the human community. Restor Ecol 16:204-209

Pearse DE, Avise JC (2001) Turtle mating systems: behavior, sperm storage, and genetic paternity. J Hered 92:206-211

Peterson BK, Weber JN, Kay EH, Fisher HS, Hoekstra HE (2012) Double digest RADseq: an inexpensive method for *de novo* SNP discovery and genotyping in model and non-model species. PLOSone 7:e37153

Quesnelle PE, Fahrig L, Lindsay KE (2013) Effects of habitat loss, habitat configuration and matrix composition on declining wetland species. Biol Conserv 160:200-208

Ricciardi A, Simberloff D (2009) Assisted colonization is not a viable conservation strategy. Trends Ecol Evol 24:248-253

Schwartz MW, Hellmann JJ, et al (2012) Managed relocation: integrating the scientific, regulatory, and ethical challenges. BioScience 62:732-743

Seber GAF (1982) The estimation of animal abundance and related parameters. Blackburn Press, New Jersey

Seminoff JA, Shanker K (2008) Marine turtles and IUCN Red Listing: A review of the process, pitfalls, and novel assessment approaches. J Exp Mar Biol Ecol 356:52-68

Somers AB (2000) A population of Bog Turtles in the Piedmont of North Carolina: habitat preferences, capture method efficacy, conservation initiatives, and site enhancement. In: Report submitted to Natural Resources Conservation Service Wetlands Institute

Spielman D, Brook BW, Frankham R (2004) Most species are not driven to extinction before genetic factors impact them. PNAS 101:15261-15264

Struhsaker TT, Siex KS (1998) Translocation and introduction of the Zanzibar red colobus monkey: success and failure with an endangered island endemic. Oryx 32:277-284

Strum SC (2005) Measuring success in primate translocation: a baboon case study. Am J Primatol 65:117-140

Swift TL, Hannon SJ (2009) Critical thresholds associated with habitat loss: a review of the concepts, evidence, and applications. Biol Reviews 85:35-53

Tarszisz E, Dickman CR, Munn AJ (2014) Physiology in conservation translocations. Conserv Physiol 2:1-19

Tesauro J (2001) Restoring wetland habitats with cows and other livestock. Conservation 2:26-31

United States Fish and Wildlife Service (2001) Bog turtle (*Clemmys muhlenbergii*) Northern population recovery plan. Prepared by M Klemens. Pennsylvania Field Office, Hadley, Massachusetts

Wahlund S (1928). Zusammensetzung von Population und Korrelationserscheinung vom Standpunkt der Vererbungslehre aus betrachtet. *Hereditas* 11:65–106

Watson DM, Watson MJ (2015) Wildlife restoration: mainstream translocations to keep common species common. Biol Conserv 191:830-838

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution 38:1358-1370

Whitlock AL (2002) Ecology and status of the Bog Turtle (*Clemmys muhlenbergii*) in New England. Dissertation, University of Massachusetts Amherst

Weeks AR, Sgro CM, Young AG, Frankham R, Mitchell NJ, Miller KA, Byrne M, Coates DJ, Eldridge MDB, Sunnucks P, Breed MF, James EA, Hoffmann AA (2011) Assessing the benefits and risks of translocations in changing environments: a genetic perspective. Evol Appl 4:709-725

Zeigenfuss LC, Singer FJ, Gudorf MA (2000) Test of a modified habitat suitability model for bighorn sheep. Restor Ecol 8:38-46

Appendices

Appendix A1 – Zoo Knoxville Bog Turtle captive breeding records from 1986 – 2015

Female ID	Source population	Clutches laid	Number of laid eggs	Number of fertile eggs	Number of hatched eggs
1	Site O	19	70	65	60
2	Site O	8	25	24	23
3	Site Q	6	19	17	15
4	Site F	19	68	49	41
5	Site Q	9	41	23	21
Unknown	-	8	18	18	18
TOTAL		69	241	196	178
Died prior to release					28
Stolen prior to release					4
Released					146

Appendix A2 – Tennessee Bog Turtle head-start program records from 2010 – 2015

Source population	Year	Number of clutches
Site O	2010	5
	2011	4
	2012	4
	2013	4
	2014	3
	2015	1
Site Q	2010	2
	2011	3
	2012	2
	2013	2
	2015	4
Site L	2013	1
	2014	3
	2015	2

*Records do not reflect the number of clutches or eggs (average of 3 per clutch) that were actually released, as some eggs were infertile and some hatchlings died before release

**Records prior to 2010 were lost as a result of a hard drive crash

CHAPTER III

CONSERVATION IN THE CLASSROOM: HOW BEST TO ENGAGE AND EDUCATE STUDENTS ABOUT CONSERVATION

Abstract

The success of conservation management relies on the support of the local community, in regards to both political and financial support. However, such support assumes that citizens share values (i.e., beliefs or attitudes) consistent with conservation and that these values translate into practice (e.g. recycling, voting in favor of conservation initiatives, etc.); this is why development of an education program is often an objective in species recovery plans. To optimize such an education program, a few considerations should be made. First, conservation goals for one species are often similar to the goals for others, so it may be possible to develop an education program mutually beneficial to multiple species. This is especially valuable considering the limited time, personnel, and money available to individual conservation programs. Such an approach would be beneficial to species with poaching risk, such as the Bog Turtle (*Glyptemys muhlenbergii*), as some outreach programs may require the use of sensitive information (e.g. geographic location) that could jeopardize the protection of species. With the previous considerations in mind, we conducted a study to determine the most effective teaching method for conveying conservation concepts within an undergraduate classroom setting that could ultimately benefit Bog Turtle conservation efforts. Specifically, we compared student engagement and learning among three commonly used active learning approaches, verbal questioning, clickers, and worksheets, where each technique was paired with a different conservation topic. Teaching methods were randomized among the topics independently for two large introductory biology courses at a large research I university. We found no significant difference among treatments in student self-reported confidence in understanding and interest in the material, but did observe significant differences in student reported focus in one class. Additionally, we found treatment level differences in student assessment scores on the final exam. However, the highest scoring active learning treatment differed between the two classes evaluated. Intriguingly, assessment scores were highest in each class for the same conservation topic and the topic was frequently mentioned in student responses to the open-ended survey questions as justification for their interest and focus. Based on our findings, the Bog Turtle conservation education program could probably implement any of the three active learning strategies in the classroom and observe similar outcomes in regards to engagement and learning. However, the results suggest that some content (concepts and/or examples) might have superior potential to achieve broader educational goals. Next steps should include a systematic study of the impact of content on engagement and learning, and how engagement and learning during a student's academic career translates into their conservation biology values and practices in future life stages.

Introduction

The fate of imperiled species, ecosystem services, and wildlife habitat depends on our ability to educate others – our ability to give citizens the tools with which to evaluate impacts and policy (Jacobson et al. 2015). The relative importance of education has been recognized not only by conservation practitioners, but also by many public funding agencies. The National Science Foundation (NSF), for example, provides financial assistance for scientific inquiry within and beyond the field of conservation by instituting an expectation of Broader Impacts, often met through the education of audiences intimately tied to academia (Nadkarni and Stasch 2013). This may be in part because of the impact education can have on human behavior and attitudes about science. For example, an increase in recycling was observed following a public outreach program in East Harlem, New York (Margai 1997) and a change in public attitudes regarding wolf (*Canis lupus*) reintroduction was perceived following educational efforts outlined in the US Wolf Recovery Plan (Troxwell et al. 2009). Over the years, a variety of educational outreach strategies have been implemented by a plethora of organizations that vary in scope. For example, zoos play a role, not only in the maintenance of viable captive populations, but also in the preservation of natural habitats through public education (Kleiman et al. 1986). International programs have been mobilized to help promote awareness across national borders (e.g. Dhar et al. 2002). And some programs have even used visual and performing arts to elicit an emotional connection between people and nature (Jacobson et al. 2006).

Each educational method is implemented with a target audience in mind, such as local landowners, the interested public, and the general public. Although educating landowners likely to have vulnerable species on their property is the most direct method, such a strategy requires more extensive resources in the form of time, money, and personnel to deliver the message to each individual landowner. And although stronger relationships are created via this method, absentee landowners may also reside outside of the jurisdiction of these programs. Absentee landowners are more likely to live in urban areas and less likely to be engaged in active management practices (Petrzelka et al. 2013). This is particularly concerning considering that landowners most interested in allowing conservation easements (i.e., a voluntary legal agreement between a government agency and a landowner that ensures land use does not compromise conservation values) are active land users (Brenner et al. 2013). One mechanism to improve buy-in from landowners is to gather information about landowner attitudes, behaviors and concerns toward proposed management strategies. This strategy can help conservation practitioners better introduce their management strategies by framing them in such a way that landowner concerns and goals are addressed, although those concerns and goals may be quite variable between landowners (Morton et al.

2010). One reason that educational programs designed to target individual landowners can be challenging and resource intensive is that property can change hands frequently and suddenly, which in the absence of a conservation easement leaves the habitat and extant species unprotected despite previously implemented educational outreach. It may also take several educational outreach visits before progress is made toward the reduction of detrimental land use practices.

Another educational approach, which generally requires fewer resources relative to its reach (i.e., number of people informed) includes educational booths at festivals and farmer's markets, wildlife tours, and naturalist programs. Each of these approaches informs a greater number of people per implementation of the educational program than the landowner education strategy. Personal encounters with wildlife can contribute to pro-environmental attitudes and even elicit longer term intentions to engage in conservation actions that benefit the species encountered (Zeppel and Muloin 2008). Some approaches even reduce future resource requirements (such as the number of conservation practitioners needed to inform the public) by implementing a teach-the-teacher framework, where participants are prepared and motivated to share learned skills and information with others in both a formal and informal setting (e.g. Florida Master Naturalist Program; Main 2004). Furthermore, these approaches can have the added benefit of funding future conservation efforts by gathering revenue during public events (e.g. Hvenegaard 2011). However, the participants in these programs often already have a vested interest in or curiosity about nature and conservation. Thus, a large proportion of the voting public is excluded by this educational outreach method.

There is another audience conservation educational programs could target, that includes both interested and uninterested persons, orders of magnitude more people reached, and requires fewer resources – students in a college or university setting. Academic institutions play a pivotal role in an individual's discovery of nature and natural processes by providing students with a foundational knowledge about human interactions with the environment (Bjorkland and Pringle 2001). Ideally, the benefit of a personal encounter described in the educational approach previously mentioned can be incorporated into the academic experience by integrating students in fieldwork so they can experience the practical value of science, i.e., how scientific findings can be applied to conservation management and how conservation management can direct scientific inquiry (Brewer 2002). Unfortunately, such an experience is not suitable in all contexts, such as for species threatened by poaching like the Bog Turtle (*Glyptemys muhlenbergii*).

The Bog Turtle was listed as a federally threatened species under the Endangered Species Act in 1997 following unprecedented declines in the number and range of populations in the northeast United

States. Primary threats to Bog Turtles include alteration of fire regimes that previously maintained early successional habitat, human development which reduced the available habitat and dispersal corridors, draining and ditching of wetlands for agricultural purposes, and introduction of exotic species that reduced the available basking and nesting habitat (USFWS 2001). Another prominent concern for the persistence of Bog Turtles is poaching for the illegal pet trade. This threat restricts the particular education programs that can be implemented, such as personal encounters via tours or field experiences, for fear that public knowledge of population locations would make them more vulnerable to poachers.

Considering the poaching concerns for Bog Turtles and interest in maximizing the reach of implemented education programs with minimal resources, we opted to assess how best to convey conservation concepts to the general public in a classroom setting. Our ability to engage, entice, and educate students about conservation in general would not only benefit Bog Turtles, but other imperiled species as well by providing the foundational knowledge necessary to understand, evaluate, and hopefully support conservation initiatives. We also incorporated other species and systems into our experimental design in an effort to further minimize resource costs associated with development of educational programs for additional species. By taking a general approach rather than a species-specific approach to conservation educational program design, we can avoid the multiple, labor-intensive, door to door visits to landowners about each individual species by instilling conservation values during their academic experience. The key is to determine how best to convey conservation knowledge and values in a classroom, specifically how best to engage students and facilitate learning.

Techniques that require undergraduate students to spend time engaged in answering questions, problem solving, and reflecting on received feedback, such as group problem-solving, worksheets, tutorials, clickers, and peer instruction (i.e., active learning) have been shown to increase student performance and understanding of course content (Freeman et al. 2007; Freeman 2014). While extensive research exists supporting the use of active learning over traditional lecturing methods for student learning (Springer et al. 1999; Lake 2001; Armbruster et al. 2009; Freeman et al. 2011; Haak et al. 2011), few studies have directly compared engagement and learning outcomes of individual active learning approaches. Here we compared three commonly used active learning approaches; verbal, clicker, and worksheet delivery (i.e., question and response activities) to determine which, if any, were more engaging to students and had greater learning outcomes, under the presumption that greater engagement and learning reflects an increased likelihood of upholding conservation values.

While we evaluated verbal, clicker, and worksheet activities in part because they are some of the most commonly used active learning strategies in STEM, we also elected to compare these approaches

based on the benefits revealed in previous studies. Greater learning gains were observed for students given the opportunity to vocalize their understanding via verbal questioning (Obenland 2013), but even students that remained silent reported attempting to think of an answer even if they didn't raise their hand (Obenland 2012). However, some students perform better on assessments that hold students accountable for a response, such as those using clicker response devices (Barr 2014), although studies on the influence of clicker use on academic performance have yielded mixed results (Good 2013). Lastly, given the fact that handwritten responses improve student performance on exams (Mueller 2014) we might assume that completion of an in-class worksheet would improve student learning more than other active learning approaches. But no studies have explicitly compared these three active learning approaches for engagement and learning, particularly in the context of conveying conservation concepts.

To fill this knowledge gap and gather information to better inform Bog Turtle managers on how educational programs could best be implemented, we randomly assigned three different conservation concepts (i.e., different learning objectives) to the three active learning approaches (verbal, clicker, and worksheet) in two large introductory biology courses at a large, Research I institution. We focused specifically on (1) which active learning strategy was most engaging and (2) which was most effective for learning, as these outcomes likely correspond to the presence of a knowledge base consistent with conservation values.

Methods

Overview

We compared student engagement and learning outcomes for three active learning approaches for three distinct conservation topics. All three teaching approaches were implemented sequentially during a single class period (50 minutes) in each of two large, introductory biology classes at the participating university in the fall semester of 2016. Each class was taught by a different instructor, but all active learning approaches compared in this study were implemented by a single, experienced guest lecturer. A single class period was divided into three distinct time periods, one for each treatment (i.e., combination of topic and active learning approach). The content covered in the two classes (Class A and B) was identical, as were the questions asked of the students; however, the delivery method of those questions and how students were asked to respond differed between the three treatments within the class period – (1) verbal question and answer (i.e., verbal), (2) written question and remote response (i.e., clicker), and

(3) written question and written response (i.e., worksheet). Furthermore, to disassociate the teaching approach from the content, the pairing of conservation topic and active learning approach was different in each class. Unfortunately, with three topics and three active learning methods, we could not test all possible combinations with only two classes. At the end of the class period, students completed an online survey to assess their interest, focus, and confidence in the material taught for each of the three conservation topics. Approximately one week later, the students completed a summative assessment to evaluate their understanding of the learning objectives covered for each of the three conservation topics. Student engagement and assessment scores for each treatment (i.e., conservation topic and associated active learning approach) were compared using a linear mixed modeling approach with random effects.

Participants

All students enrolled in two of the Introductory Organismal and Ecological Biology lecture courses at a large, southern public research university participating in our study in the Fall of 2016 were eligible to participate. This course is typically the first of two lecture courses students take as the introductory majors' sequence at the university. Approximately 225 students were enrolled in each lecture course and each course was taught by a different instructor (see Appendix A3 - Instructor Profiles), but the major course content and textbooks were identical between the two courses. The majority of participants were first year college students between the ages of 18-20 and majoring in either biology or a pre-professional field. All students met twice a week for 50 minutes in a large lecture hall with their respective instructor; additionally, small groups of approximately 25 students met once a week for a 50 minute discussion session led by a graduate teaching assistant.

Students were given the opportunity to opt out of our study (i.e., have their data removed from the study) prior to completing the post class survey; regardless, all students were expected to attend the class taught by the guest lecturer and take the final assessment to satisfy course requirements. All procedures used in this study were approved by the Institutional Review Board for Human Subjects.

Experimental design

As mentioned in the Overview, each instructor agreed to allow a guest lecturer to teach a single class period at the end of the semester (1-2 classes prior to the final exam). To minimize overlap in covered content between the guest lecture and previous lectures, the topic (conservation biology) was

agreed upon at the beginning of the semester. Specifically, instructors were asked to avoid readings or lecture examples that addressed the following learning objectives that would be covered by the guest lecturer: (1) discuss the positive and negative genetic consequences of translocations (conservation at the population level), (2) explain the different quota regimes and their limitations in regards to the conservation of harvested species (conservation at the species level), and (3) compare and contrast different IUCN reserve designs, taking into account the interests of various stakeholders (conservation at the ecosystem level). These learning objectives were selected for four reasons, (1) minimal overlap among the three conservation topics, (2) minimal overlap with planned course content, (3) to complement the expertise of the guest lecturer, and (4) difficult to understand using simple intuition, yet appropriately challenging for an introductory course, thus requiring class attendance to master the content. Furthermore, each learning objective was illustrated using a novel example not previously presented to the students; genetic concerns associated with a Bog Turtle translocation program (Learning Objective 1; LO 1), the use of harvest quotas to manage game fish (LO 2), and the role of competing agendas of various stakeholders in reserve design in the Serengeti (LO 3).

One week prior to the guest lecture on conservation biology, students were given a no-credit homework assignment by their respective instructor to read six short Science Daily articles (e.g. Great Barrier Reef marine reserves combat coral disease) to serve as a primer to the topic of conservation rather than an in-depth overview of the content to be covered in lecture. Students were also asked to complete an online homework assignment through Survey Monkey that asked them six questions regarding their personal opinions about some hot topics in conservation (e.g. who is the most responsible for conserving nature: rank the following: federal government, state government, non-profit organizations, industry / developers, scientists, citizens).

The appearance of a guest lecturer was not announced prior to the conservation biology lecture. The guest lecturer was introduced by the regular lecturer at the beginning of the class period. After the introduction, the guest lecturer introduced the basic structure of the class period (i.e., three sections on three distinct topics), and explained that they would use their electronic devices during one of the sections to respond to questions (otherwise electronic devises were to be stowed away), and emphasized that all of the content covered would be on their final exam, so they should pay special attention to the learning objectives posted at the beginning and end of each section in the PowerPoint, which would be posted after class. The active learning approach (verbal, clicker, or worksheet) was assigned to the particular conservation topic (i.e., learning objective) for each class *a priori*, but was not announced to students at any point. For Class A, LO 1 was clicker, LO 2 was verbal, and LO 3 was written, but for Class B, LO 1

was written, LO 2 was clicker, and LO 3 was verbal. The questions students responded to for each learning objective were identical regardless of the active learning approach used, including "clickers", because Poll Everywhere was used rather than traditional proprietary clicker software or devices. Poll Everywhere allowed us to collect student answers to both multiple choice and open-ended questions (one of our instructors did not require their students to buy clickers).

Best practices for active learning were followed throughout each section, including encouraging students to discuss the questions in pairs or groups before answering and providing explanations for correct and incorrect answers after each question. A minimum of one external observer was present in each class to record the amount of time spent on predefined classroom events such as: lecture, explanation, student questions, verbal questions, clicker questions, and worksheets. This observation and data collection was done using a protocol previously developed (Auerbach and Schussler 2016). The delivery of each section for each class was also audio-recorded.

Survey and assessment

At the end of class, students were asked by the guest lecturer to complete an anonymous survey (22 items) on their electronic devices before leaving (see Appendix A4 – Engagement Survey). The survey asked students to identify the active learning approach used for each section of content from a list of approaches, their level of interest, ability to stay on-task, and level of confidence in their understanding of the material presented in each section (each of three topics). Students were also asked about the use of active learning approaches by their regular instructors, but these data were not used in this study. Individual Instructor Profiles were created from a self-reported practices survey all instructors were asked to complete (see Appendix A3 – Instructor Profiles). Students were randomly assigned one of two response formats, either a continuous sliding scale bar or a Likert scale (i.e., a single student responded to all survey questions using one of the two formats), which was an experimental design relevant to a separate study. For the purposes of this study, we used only continuous sliding scale bar responses in our analyses, as these data provide greater resolution to detect any differences between active learning approaches because they could be treated as continuous variables for parametric statistical analyses. There was also a second hierarchical level for survey version which inverted the wording of sets of survey questions. For example, question 2 of version 1 was "I was interested in the topic discussed in Section 1 [strongly disagree to strongly agree]", while question 2 of version 2 was "I was not interested in the topic discussed in Section 1 [strongly disagree to strongly agree]". For the purposes of this study, we converted

all responses to reflect the version that used positive wording (i.e., "I was interested"). The survey also contained several open-ended questions that allowed students to expand on their continuous-scale bar selections and explain their preference for a particular active learning strategy (should they have a preference). None of the authors viewed any of the engagement survey data until after the semester was over and grades had been turned in and responses were never viewed by the regular instructors.

At the end of the semester (approximately one week following the guest lecture on conservation biology), students took their final exam for the course, which contained twelve questions on conservation biology (see Appendix A5 – Final Assessment). This portion was at the end of the exam for both classes and consisted of 12 questions based on the learning objectives, four for each conservation topic (i.e., learning objective). Of the four questions for each section, two were multiple choice and two were short answer questions (each worth 1 point). Within each course, students were randomly given one of two versions of the exam. The versions were identical except the multiple-choice questions were converted to short answer questions on the second version and the short answer questions were converted to multiple choice questions. We presented each question in both formats to avoid confounding question format with experimental treatment. In addition to the graded questions, the students were asked three survey questions on their final exam: (1) did you attend the Conservation Biology lecture given by Instructor X, (2) if so, was your attendance helpful in answering these questions, and (3) how much time did you spend studying this material (excluding class time). Students were awarded up to 2 bonus points for answering these additional survey questions, but these points were not included in statistical analyses. The conservation biology portion of the final exam was graded by the guest lecturer.

Data analysis

The engagement survey data were extracted from the web-based survey environment for each course separately and analyzed both together and separately to determine whether active learning strategy and topic influenced student interest in the topic, focus during the activity, and confidence in the material covered. Our analyses included only students that correctly identified the active learning method used and responded to the continuous scale bar questions. We processed these quantitative data using a linear mixed modeling approach in the program R (R Development Core Team 2008). An arcsine square root transformation was used to transform each response variable (interest, focus, and confidence) prior to model fitting and comparison. The fixed effects in our combined class models were active learning strategy (AL; verbal, clicker, and worksheet) and topic (Topic; genetic consequences of translocation

using a turtle example – LO1, harvest quotas as a management tool using a fish example – LO2, and reserve design using the Serengeti as an example – LO3). We used Akaike Information Criteria (AIC) to compare the null model – intercept only, no fixed effects – to the following three alternative models: (1) full model - both fixed effects and an interaction coefficient (AL + Topic), (2) only the fixed effect AL, and (3) only the fixed effect Topic. When classes were analyzed separately, the full model could not be assessed due to the experimental design because AL and Topic were confounded within class. All models included random effects; in the combined class models, student was nested within class and in the single class models, student was treated as a single random effect. The model with the lowest AIC value and $\Delta AIC > 2$ was interpreted as the best model given our dataset. We used the Anova function in R to obtain parameter estimates, standard error, and test statistics for each fixed effect in cases where an alternative model rather than the null model was identified as the best model.

We used thematic analysis to identify categories that would represent the majority of student responses to each of the open-ended survey questions. Initial categories for each question were independently derived by two different researchers, who each read all student responses and created their own categories. These two researchers then met to compare and reconcile category names and definitions. Once categories were identified, each went back to the data to sort responses into each category and then met again to discuss categorization. These discussions altered some of the categories. For example, student responses regarding their interest in the material presented was initially categorized into a positive and a negative component (e.g. liked the organism versus didn't like the organism). These two components were compacted into a single category for data presentation as they both reflected the driver of their interest (e.g. whether or not they liked the organism). If a student response expressed more than one idea, the response could be placed into more than one category. Initial agreement of categorizing between researchers ranged from 77 – 87%, and discrepancies were resolved in the final meeting. Final categories were identical between each lecture class.

Student learning outcomes were compared among the three treatments implemented in this study by using assessment scores. The total assessment score for each section (i.e., active learning approach and conservation topic) was calculated for each student (out of 4 points per section), as well as the multiple-choice score for each section for each student (out of 2 points) and the short answer score for each section for each student (out of 2 points). We used the same linear mixed modeling approach previously described for the quantitative engagement survey data on student assessment score data (response variables; total score, multiple choice score, and short answer score).

Results

All of the students who attended the guest lecture on conservation and stayed through the entire class period submitted an engagement survey. Recall that only students who completed the continuous sliding scale bar version of the survey were included in this study; a total of 128 students from Class A and 114 students from Class B. After removing the responses from students that failed to respond to any of the survey questions, 54 students remained in Class A and 45 students remained in Class B. Most students reported being confident in their understanding of the conservation topics covered, being on task during class, and being interested in the material covered, more so in Class A than Class B (Figure 3.1). However, neither the active learning strategy used, nor the topic significantly influenced student interest or confidence in the material covered (Table 3.1). In regards to student focus, we did identify instances where a model that included one or more fixed effect (i.e. active learning approach and topic) best fit our data, this was not true for both classes (Table 3.1). In cases where an alternative model was the best fit, the active learning strategy used was more influential than Topic (Table 3.1). When the two classes were analyzed together, students reported being more focused during the worksheet activity than during verbal questioning and were the least focused during clicker questioning (Figure 3.1; Table 3.2), but when analyzed separately, Class B reported being the most focused during the worksheet activity and the least focused during verbal questioning (note that the null model was the best model for Class A).

A total of 190 students from Class A and 213 students from Class B completed the final assessment at the end of the semester. After removing the students that were absent the day of the guest lecture, 161 students remained in Class A and 171 students remained in Class B. In all model comparisons, when classes were analyzed together and separately, an alternative model fit the student assessment score data better than the null model (Table 3.3), suggesting that the active learning strategy implemented and the topic covered significantly influenced student assessment scores (total scores, multiple choice scores, and short answer scores). In Class A, student assessment scores were significantly higher for the learning objective taught using clickers as the active learning strategy than either verbal or worksheet methods, while in Class B, scores were significantly higher for the worksheet treatment than either the verbal or clicker active learning strategies (Figure 3.2; Table 3.4). The same patterns were observed when we looked specifically at scores on multiple choice questions and short answer scores (Table 3.3; Table 3.4).

Student open-ended responses (123 students from Class A and 108 from Class B) revealed that the learning objective regarding the genetic consequences of translocations (LO 1), introduced using a local turtle translocation as an example, always received the highest total exam score regardless of the

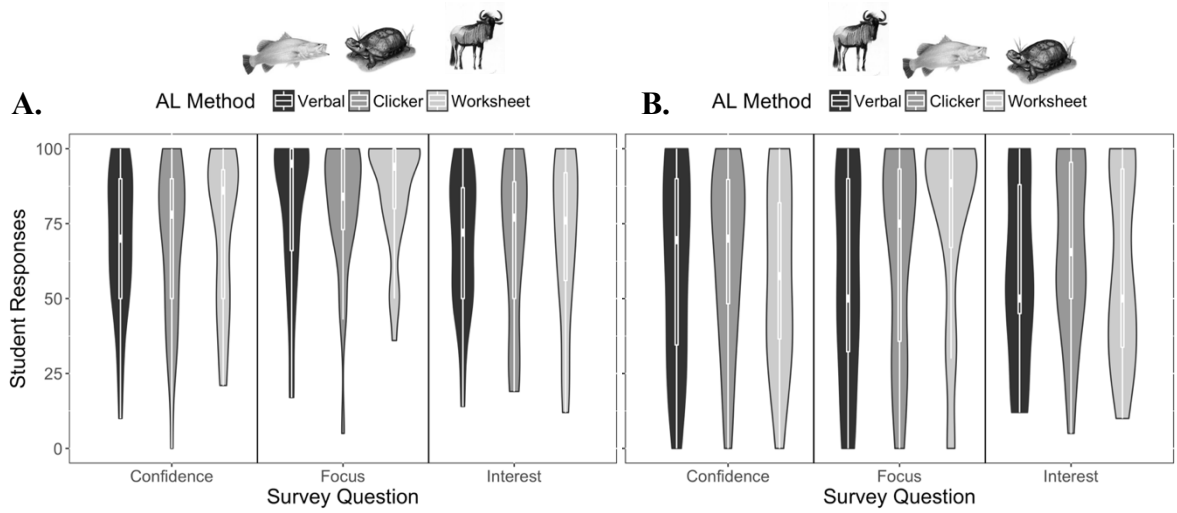


Figure 3.1 Student engagement survey responses for three active learning (AL) treatments. Survey questions pertained to students' confidence in their understanding (Confidence), focus during class (Focus), and interest in the material taught (Interest). A single class session was partitioned into three sections and randomly assigned one of three AL methods; questioning and responses were given and received verbally (Verbal, dark gray), using clickers (Clicker, medium gray), or via a worksheet (Worksheet, light gray). Experimental treatments were implemented by a guest lecturer in two large introductory biology classes, each with a unique regular instructor (A = Class A; B = Class B). The AL method applied to each of the three sections differed between the two lecture classes. Each animal symbol represents the example used to introduce one of three learning objectives (see text). These data only include students that correctly identified the AL method implemented for each section (Class A = 54 students; Class B = 45 students). Data are displayed using a boxplot overlaid on a violin plot. The violin plot shows the density distribution of student responses between 0 and 100, where values closer to 100 correspond to a strong level of agreement with the survey statement (e.g. "I was interested in the topic discussed in Section 1"). The boxplot shows the first and third quartiles with whiskers that extend to the highest and lowest values (data beyond the end of the whiskers are outliers) and the median displayed as a thick white bar.

Table 3.1 Linear mixed model comparisons for student engagement survey responses to questions pertaining to interest, focus, and confidence in presented material for three active learning (AL) methods and three topics for two classes analyzed together and separately (Class A and B). Each response variable represents the level (0 – 100%) to which individual students agreed with the survey statement (see Appendix A4). The fixed effects for each model were AL method (i.e., verbal, clicker, and worksheet) and Topic (i.e., genetic consequences of translocation using turtle example, use of harvest quotas to manage game species using fish example, and role of stakeholders in reserve design using the Serengeti as an example). The random effects for the combined classes analyses were Class (i.e., A and B) and Student, with Student nested within Class. For individual classes analyses, the only random effect was Student and the model AL + Topic was not compared as AL and Topic were confounded within class. The p-value is shown when an alternative model was more strongly supported than the null model (ΔAIC of alternative model < 2) for both the fixed effects, AL and Topic and the response variable is highlighted in bold font.

Classes Combined	AIC	ΔAIC	p	Classes Separately	AIC	ΔAIC	p
INTEREST				Class A			
Null	128.7	0.0		INTEREST			
Topic	129.6	0.9		Null	57.6	0.0	
AL	132.3	3.6		AL/Topic	60.2	2.6	
AL + Topic	133.4	4.7		FOCUS			
FOCUS				AL/Topic	82.7	0.0	
AL + Topic	198.6	0.0		Null	84.5	1.8	
AL	199.3	0.7	0.004	CONFIDENCE			
Null	206.3	7.7		Null	5.7	0.0	
Topic	210.1	11.5	0.090	AL/Topic	8.1	2.4	
CONFIDENCE							
Null	16.4	0.0		Class B			
Topic	19.5	3.1		INTEREST			
AL	19.7	3.3		Null	70.8	0.0	
AL + Topic	22.8	6.4		AL/Topic	73.0	2.2	
				FOCUS			
				AL/Topic	112.1	0.0	0.009
				Null	117.3	5.2	
				CONFIDENCE			
				Null	10.7	0.0	
				AL/Topic	14.7	4.0	

Table 3.2 Fixed effects coefficient estimates for student engagement survey items for instances where an alternative model was more strongly supported than the null model (i.e., no fixed effects) in Table 3.1. The parameter estimates (Est), standard error (SE) and test statistic (t) are shown for each treatment variable. Estimates are relative to the verbal active learning treatment (AL) and LO1 (Topic). For the fixed effect, Topic, LO1 was the genetic consequences of translocation using turtle example, LO2 was the use of harvest quotas to manage game species using fish example, and LO3 was the role of stakeholders in reserve design using the Serengeti as an example.

Fixed Effect	Treatment Variable	Est	SE	t
Combined Classes - FOCUS				
AL	Clicker	-0.025	0.041	-0.612
	Worksheet	0.127	0.041	3.093
Topic	LO2	0.045	0.041	1.096
	LO3	-0.045	0.041	-1.080
Class A - FOCUS				
AL	Clicker	-0.070	0.045	-1.558
	Worksheet	0.038	0.045	0.837
Topic	LO2	0.070	0.045	1.558
	LO3	0.108	0.045	2.395
Class B - FOCUS				
AL	Clicker	0.064	0.056	1.143
	Worksheet	0.171	0.056	3.050
Topic	LO2	-0.107	0.056	-1.907
	LO3	-0.171	0.056	-3.050

Table 3.3 Linear mixed model comparisons for student assessment scores for three active learning (AL) methods for two classes analyzed together and separately (Class A and B). Each response variable represents the number of points an individual student received for the entire assessment (TOTAL), multiple choice questions only (MC), and short answer questions only (SA). The fixed effects for each model were AL method (i.e., verbal, clicker, and worksheet) and Topic (i.e., genetic consequences of translocation using turtle example, use of harvest quotas to manage game species using fish example, and role of stakeholders in reserve design using the Serengeti as an example). The random effects for the combined classes analyses were Class (i.e., A and B) and Student, with Student nested within Class. For individual classes analyses, the only random effect was Student and the model AL + Topic was not compared as AL and Topic are confounded within Class. The p-value is shown when an alternative model was more strongly supported than the null model (ΔAIC of alternative model < 2) for both the fixed effects, AL and Topic, and the response variable is highlighted in bold font.

Classes Combined	AIC	ΔAIC	p	Classes Separately	AIC	ΔAIC	p
TOTAL				Class A			
AL + Topic	2719.4	0.0		**TOTAL**			
Topic	2721.4	2.0	< 0.001	AL/Topic	1324.8	0.0	< 0.001
AL	2822.5	103	0.050	Null	1364.3	39	
Null	2836.5	117		**MC**			
MC				AL/Topic	968.5	0.0	< 0.001
Topic	1951.6	0.0	< 0.001	Null	1009.9	41	
AL + Topic	1953.1	1.5		**SA**			
AL	2047.7	96		AL/Topic	917.9	0.0	0.009
Null	2072.1	120		Null	923.3	5.4	
SA							
AL + Topic	1918.3	0.0		Class B			
Topic	1919.1	0.8	< 0.001	**TOTAL**			
Null	1932.6	14.3		AL/Topic	1396.1	0.0	< 0.001
AL	1936.3	18.0	0.090	Null	1473.0	76	
				MC			
				AL/Topic	987.3	0.0	< 0.001
				Null	1064.7	77	
				SA			
				AL/Topic	999.6	0.0	0.002
				Null	1008.4	8.8	

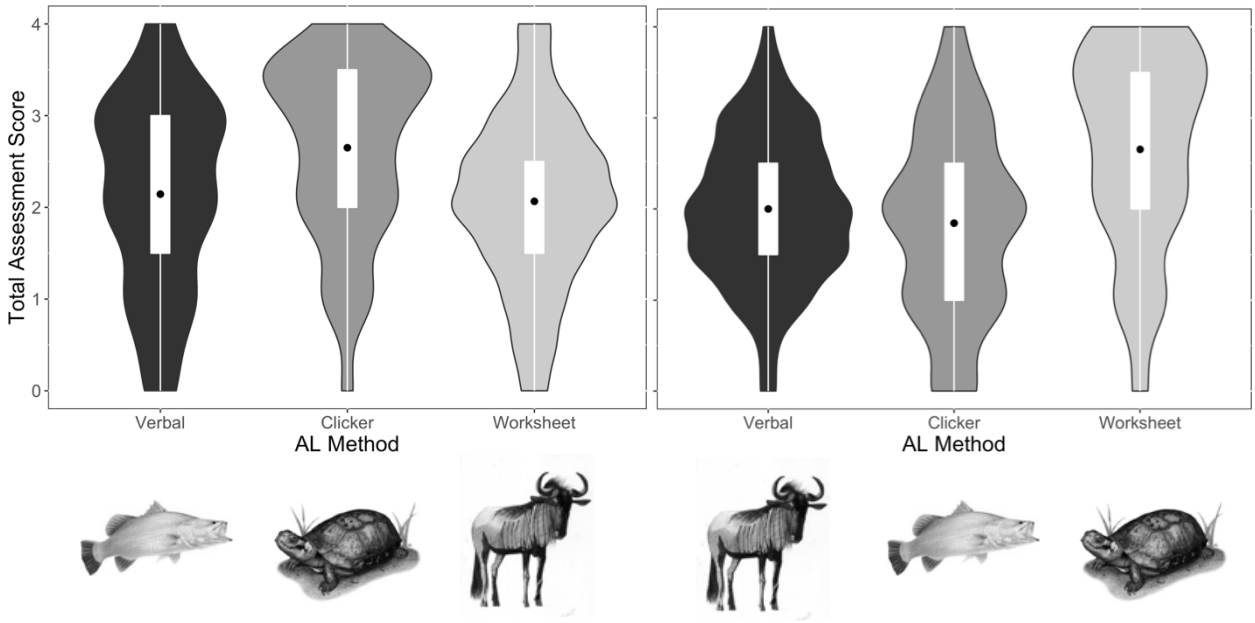


Figure 3.2 Total student assessment scores for three active learning (AL) treatments. Student assessment scores were calculated as the total number of points out of 4 students received on two multiple choice and two short answer questions for topic discussed in a previous class. One of three AL methods: questioning and responses given and received verbally (Verbal, dark gray), using clickers (Clicker, medium gray), or using a worksheet (Worksheet, light gray) were randomly assigned to each topic (i.e., section). Experimental treatments were implemented by a guest lecturer in two large introductory biology classes, each with a unique regular instructor (A = Class A; B = Class B). The AL method applied to each of the three sections differed between two lecture classes. Each animal symbol represents the example used to introduce one of three learning objectives (see text). Two versions of the exam were given to each lecture class: both versions were identical except multiple choice questions on version 1 were short answer questions on version 2 and vice versa. These data only include students that attended the lecture. Data is displayed using a boxplot overlaid on a violin plot. The violin plot shows the density distribution of student scores between 0 and 4, where a value of 4 indicates that students' received full credit on all questions pertaining to that topic (each question was worth 1 point). The boxplot shows the first and third quartiles with whiskers that extend to the highest and lowest values and the mean is displayed as a black point. Significant differences are indicated in Table 3.3.

Table 3.4 Fixed effects coefficient estimates for student TOTAL assessment scores for instances that an alternative model was more strongly supported than the null model (i.e., no fixed effects) in Table 3.3. The parameter estimates (Est), standard error (SE) and test statistic (t) are shown for each treatment variable. Estimates are relative to the verbal active learning treatment (AL) and LO1 (Topic). For the fixed effect, Topic, LO1 was the genetic consequences of translocation using turtle example, LO2 was the use of harvest quotas to manage game species using fish example, and LO3 was the role of stakeholders in reserve design using the Serengeti as an example.

Fixed Effect	Treatment Variable	Est	SE	t
Combined Classes - TOTAL				
AL	Clicker	-0.179	0.075	-2.390
	Worksheet	-0.055	0.075	-0.737
Topic	LO2	-0.685	0.075	-9.155
	LO3	-0.708	0.075	-9.462
Class A - TOTAL				
AL	Clicker	0.506	0.093	5.428
	Worksheet	-0.078	0.093	-0.838
Topic	LO2	-0.506	0.093	-5.43
	LO3	-0.584	0.093	-6.27
Class B - TOTAL				
AL	Clicker	-0.156	0.090	-1.731
	Worksheet	0.653	0.090	7.252
Topic	LO2	-0.809	0.090	-8.980
	LO3	-0.653	0.090	-7.250

active learning strategy implemented. When students were asked to explain why they found particular sections of the lecture interesting or uninteresting, 65% of students from Class A and 52% from Class B mentioned liking the example organism or that they found the topic relevant. An additional 14% from Class A and 11% from Class B mentioned liking that the material covered was new as another reason for their interest. Interest in organism and topic was mentioned again by 22% and 10% (Class A and Class B, respectively) when asked why they were more or less on-task during particular sections. Teaching method was only mentioned as a reason for being on or off-task (22% in Class A and 20% in Class B) and to explain their confidence or lack thereof in their understanding of the learning objectives (30% in Class A and 19% in Class B).

Discussion

Active learning in the classroom is a powerful tool to improve student performance and comprehension of scientific concepts (Carini et al. 2006; Freeman et al. 2014), including concepts relevant to conservation of imperiled species. But few studies have empirically compared effectiveness among active learning strategies, most have compared active learning to traditional lecturing (e.g. DeNeve and Heppner 1997). Here we compared student engagement and learning following the implementation of three active learning strategies (verbal questioning, clickers, and worksheets) to teach three conservation topics (genetic consequences of species translocation, the use of harvest quotas to manage game species, and the role of stakeholders in reserve design). We found no significant differences in student survey responses regarding their confidence, focus, or interest based on the active learning method used (and conservation topic covered), with the exception of two significant differences, focus in Class B and focus when classes were analyzed together. Although the active learning strategy implemented appears to be more important than the topic in regards to student focus, the particular strategy that corresponds to the greatest focus differed between classes. And although we found significant differences in assessment scores, the active learning treatment with the highest assessment scores differed between our two classes, but aligned with the same conservation topic (i.e., same learning objective). Together these results suggest that for the three active learning methods compared, active learning approaches are equally effective in regards to student engagement and become conflated with other factors such as conservation topic (maybe example organism) when it comes to student learning. Thus, as long as a topic of interest is chosen, any of the active learning strategies could probably be used

in an academic educational program to convey conservation concepts pertinent to Bog Turtles and have similar engagement and learning outcomes.

Although further study is needed to test the hypothesis that conservation topic was the driver of the class level differences we observed in assessment scores, some characteristics of the topics used in this study have been found to influence attitudes and how new information is processed. Arguably, a major component that makes a topic engaging to students is the organism used to introduce a learning objective. For example, results from a standardized survey issued to over 400 students from primary and secondary academic institutions indicated that butterflies, birds, and most mammals were more highly appreciated than other insects, amphibians, and species that were unfamiliar (Schlegel and Rupf 2010). Furthermore, Schlegel and Rupf (2010) found that these attitudes were confounded by the institution they attended, which could reflect differential exposure to nature due to socioeconomic, cultural, or regional factors. Outside of the classroom, stereotypical, charismatic species dominate publically available media to maximize public interest and financial contributions. Popular US conservation and nature magazines tend to put mammals and birds on their covers, rather than invertebrates, fishes, amphibians, or plants to draw the eye and interest of viewers in hopes that they are more likely to purchase the magazine (Clucas et al. 2008). And it works; the use of charismatic species elicits an increase in 'willingness to pay' attitudes for the purchase of habitat required for conservation of the flagship species (Kontoleon and Swanson 2003). Flagship species have also been used to stimulate tourism and visitor interest in zoo exhibits (Veríssimo et al. 2009; Moss and Esson 2010, respectively). Given the success that the use of charismatic species has had on the private and public sectors of conservation, and the fact that turtles are usually considered more charismatic than the other species and systems we used in this study, example organism may explain why students had a better understanding of the learning objectives taught using the turtle example. The inclusion of mathematical equations within the presentation of the learning objective on fish quota regimes may have also contributed to lower exam scores for that learning objective, considering many undergraduates admit to having anxiety or fear of math (Arnett and Van Horn 2009). Another potential contributor for the lower scores on the reserve design learning objective was the lack of a single correct answer for the class example. The contradictory interests of stakeholders may have been challenging to students, as such a paradox may have required greater cognitive effort and discussion among group members presenting different views (Lewis and Dehler 2000). Although we made every effort to minimize overlap between the conservation learning objectives and previously covered course content, the learning objective on the genetic consequences of translocations was the most likely to overlap with previously covered content such as population genetics. Since students usually construct

knowledge not by taking it in through dissemination, but rather by linking new information to previously acquired information (Cross 1998), the knowledge from this learning objective may have been easier for them to recall on the final assessment.

The experimental design of our study allowed us to minimize some confounding factors; however, there may still be some confounding factors and limitations, such as topic, which we previously mentioned. First, we did not explore all active learning strategies currently in use, such as concept maps, free write, panel discussions, role playing, and simulations – to name a few (Zayapragassarazan and Kumar 2012), rather we focused on the three most commonly used in STEM disciplines. Considering the multidisciplinary nature of conservation, conservation educational programs would benefit from similar research with other active learning approaches in other disciplines, such as sociology and political science. Second, we designed our own survey, but alternative surveys are available to measure engagement, such as the National Survey of Student Engagement (e.g. Junco et al. 2010), the Student Course Engagement Questionnaire (Handelsman et al. 2010), and ASPECT (Wiggins et al. 2017). Regardless of the survey used, self-reported student surveys often contain some degree of error that may compromise the validity (extent to which the survey question actually measures what is intended) and reliability (consistency in how the same individuals respond to the same question) of the data (Takalkar et al. 1993). Although this would not affect our pairwise comparisons, the final assessment scores may be generally high due to students completing the engagement survey and should not be assumed to reflect the potential outcome of a similar educational program that lacks such a survey component. Observed learning outcomes could simply be a result of taking the survey, as in some contexts, students who engage in reason justification (as we requested students to do to explain why they were or were not interested, focused, and confident) scored higher on assessments (Lin and Lehman 1999). Additionally, subconscious bias (e.g. differences in clarity of explanation and enthusiasm) may have been introduced into the study as a result of the guest lecturer's personal research interests which were closely tied to the learning objective with the consistently higher assessment score. Other biases may have been introduced as a result of familiarity with the implemented active learning strategy; the instructor for Class A frequently used clickers, while the instructor for Class B frequently used worksheets; and student assessment scores were highest for the conservation topic taught using clickers in Class A and worksheets in Class B. Although we did replicate the entire experiment twice (i.e., in two different classes) to avoid making broad generalizations based on a single class, a better statistical approach would be to replicate the experiment in ten or more independent classes, treating each class, rather than each student as an independent data point. However, such sampling is unlikely to be logistically feasible as ten or more

independent classes of introductory level organismal biology are rarely if ever taught concurrently in the same semester at the same institution by different instructors with the same general syllabus. Lastly, given the nature of active learning approaches, which if implemented as recommended based on best teaching practices, require student-student interaction and discussion. This inherently means that each student is not an independent data point, but may be influenced by another student or students. However, this characteristic was true for all active learning strategies implemented and considering the fact that students could not discuss their answers during the final exam, in-class interaction likely had a minimal impact on observed learning outcomes on the assessment that took place over a week later, but none the less should be considered given the statistical framework used.

Our findings can help inform conservation practitioners interested in developing education programs for their specific species, particularly for species where poaching concern is a risk, like the Bog Turtle. Traditional outreach programs, such as personal encounter opportunities for the general public or targeted outreach to all potential landowners may put Bog Turtles in unnecessary risk by providing poachers easy access to Bog Turtle sites or may simply not be feasible given resource constraints relative to the number of potential landowners. Furthermore, landowners that have Bog Turtles on their property will often also have other vulnerable species, such as the green pitcher plant (*Sarracenia oreophila*), which could result in an overwhelming number of conservation officers knocking on a single landowner's door. Hence, in such situations we advocate for educational programs implemented in the classroom, where orders of magnitude more individuals can be reached for relatively little investment of resources, geographic anonymity of populations can be maintained, and such efforts would be an altruistic approach to conservation – serving multiple species rather than just a single species. Furthermore, reaching younger generations can help instill values consistent with conservation before destructive land use behaviors are employed. Although our study did not explicitly test how learned information is retained or applied to behaviors relevant to conservation, previous work has suggested that opinion-forming early in life is critical to the appreciation of wildlife later in life (Eagles and Muffitt 1990) and using an active learning approach in the classroom can help students better retain new knowledge long-term (Price 2004).

Acknowledgements

First and foremost, thank you to the faculty members that sacrificed one of their last class days of the semester and a portion of their final exam, which allowed us to implement a stronger experimental design. Thank you to all of the students that willingly participated in this study. We also appreciate the

time and effort of our classroom observers that verified consistency between the guest lectures in the two classes and observed the regular instructors to provide us with individual instructor profiles for each class. Financial support was provided by the National Science Foundation [DUE 1245215]. Approved by IRB at the University of Tennessee [14-08940-XP]. This chapter was co-authored by Elisabeth Schussler (University of Tennessee) and Jennifer Brigati (Maryville College).

References

Armbruster P, Patel M, Johnson E, Weiss M (2009) Active learning and student-centered pedagogy improve student attitudes and performance in introductory biology. CBE Life Sci Educ 8:203-213

Arnett A, Van Horn D (2009) Connecting mathematics and science: a learning community that helps math-phobic students. J College Sci Teach 30-34

Auerbach AJ, Schussler EE (2016) Instructor use of group active learning in an introductory biology sequence. J Coll Sci Teach 45:67-74

Barr M (2014) Encouraging college student active engagement in learning: the influence of response methods. Innov High Educ 39:307-319

Bjorkland R, Pringle CM (2001) Educating our communities and ourselves about conservation of aquatic resources through environmental outreach. BioSci 51:279-282

Brenner JC, Lavallato S, Cherry M, Hileman E (2013) Land use determines interest in conservation easements among private landowners. Land Use Policy 35:24-32

Brewer C (2002) Outreach and partnership programs for conservation education where endangered species conservation and research occur. Conserv Biol 16:4-6

Carini RM, Kuh GD, Klein SP (2006) Student engagement and student learning: testing the linkages. Res High Educ 47:1-32

Clucas B, McHugh K, Caro T (2008) Flagship species on covers of US conservation and nature magazines. Biodivers Conserv 17:1517

Cross KP (1998) Opening windows on learning: the cross papers number 2. League for Innovation in the Community College and Educational Testing Service, Mission Viejo, California

DeNeve KM, Heppner MJ (1997) Role play simulations: the assessment of an active learning technique and comparisons with traditional lectures. Innovative Higher Education 21:231-246

Dhar U, Rawal RS, Airi S, Bhatt ID, Samant SS (2002) Promoting outreach through conservation education programmes – Case study from Indian Himalayan Region. Curr Sci 82:808-815

Eagles PFJ, Muffitt S (1990) An analysis of children's attitudes toward animals. J Environ Educ 21:41-44

Freeman S, Eddy SL, McDonough M, Smith MK, Wenderoth MP, Okoroafor N, Jordt H (2014) Active learning increases student performance in science, engineering, and mathematics. PNAS 111:8410-8415

Freeman S, Haak D, Wenderoth MP (2011) Increased course structure improves performance in introductory biology. CBE Life Sci Educ 10:175-186

Freeman S, O'Connor E, Parks JW, Cunningham M, Hurley D, Haak D, Dirks C, Wenderoth MP (2007) Prescribed active learning increases performance in introductory biology. CBE Life Sci Educ 6:132-139

Good KC (2013) Audience response systems in higher education courses: a critical review of the literature. IJITDL 10:19-34

Haak D, HilleRislambers J, Pitre E, Freeman S (2011). Increased structure and active learning reduce the achievement gap in introductory biology. Science 332:1213-1216

Handelsman MM, Briggs WL, Sullivan N, Towler A (2010) A measure of college student course engagement. J Educ Res 98:184-192

Hvenegaard G (2011) Potential conservation benefits of wildlife festivals. Event Manage 15:373-386

Jacobson SK, McDuff M, Monroe MC (2006) Promoting conservation through the arts: outreach for hearts and minds. Conserv Biol 21:7-10

Jacobson SK, McDuff M, Monroe MC (2015) Conservation education and outreach techniques. Oxford University Press, Oxford, United Kingdom

Junco R, Heiberger G, Loken E (2010) The effect of Twitter on college student engagement and grades. J Comput Assist Lear 27:119-132

Kleiman DG, Beck BB, Dietz JM, Dietz LA, Ballou JD, Coimbra-Filho AF (1986) Conservation program for the Golden Lion Tamarin: captive research and management, ecological studies, educational strategies, and reintroduction. In Benirschke K (eds) Primates. Proceedings in Life Sciences. Springer, New York

Kontoleon A, Swanson T (2003) The willingness to pay for property rights for the Giant Panda: can a charismatic species be an instrument for nature conservation? Land Econ 79:483-499

Lake D (2001) Student performance and perceptions of a lecture-based course compared with the same course utilizing group discussion. Physical Therapy 81:896-902

Lewis MW, Dehler GE (2000) Learning through paradox: a pedagogical strategy for exploring contradictions and complexity. J Manage Educ 24:708-725

Lin X, Lehman JD (1999) Supporting learning of variable control in a computer-based biology environment: effects of prompting college students to reflect on their own learning. J Res Sci Teach 36:837-858

Margai FL (1997) Analyzing changes in waste reduction behavior in a low-income urban community following a public outreach program. Environ Behav 29:769-792

Main MB (2004) Mobilizing grass-roots conservation education: the Florida Master Naturalist Program. Conserv Biol 18:11-17

Morton LW, Regen E, Engle DM, Miller JR, Harr RN (2010) Perceptions of landowners concerning conservation, grazing, fire, and eastern redcedar management in tallgrass prairie. Rangeland Ecol Manage 63:645-654

Moss A, Esson M (2010) Visitor interest in zoo animals and the implications for collection planning and zoo education programmes. Zoo Biol 29:715-731

Mueller PA, Oppenheimer DM (2014) The pen is mightier than the keyboard: advantages of longhand over laptop note taking. Psychol Sci 25:1159-1168

Nadkarni NM, Stasch A (2013) How broad are our broader impacts? An analysis of the National Science Foundation's Ecosystem Studies Program and the Broader Impacts requirement. Front Ecol Environ 11:13-19

Obenland CA, Munson AH, Hutchinson JS (2012) Silent students' participation in a large active learning science classroom. J Coll Sci Teach 42:90-98

Obenland CA, Munson AH, Hutchinson JS (2013) Silent and vocal students in a large active learning chemistry classroom: comparison of performance and motivational factors. Chem Educ Res Pract 14:73-80

Petrzelka P, Ma Z, Malin S (2013) The elephant in the room: absentee landowner issues in conservation and land management. Land Use Policy 30:157-166

Prince M (2004) Does active learning work? A review of the research. J Eng Educ 93:223-231

Schlegel J, Rupf R (2010) Attitudes towards potential animal flagship species in nature conservation: a survey among students of different educational institutions. J Nat Conserv 18:278-290

Springer L, Stanne M, Donovan SS (1999) Effects of small-group learning on undergraduates in science, mathematics, engineering, and technology: a meta-anlaysis. Rev Educ Res 69:21-51

Takalkar P, Waugh G, Micceri T (1993) A search for truth in student responses to selected survey items. Presented at AIR Forum, Chicago

Troxwell PS, Berg KA, Jaycox H, Strauss AL, Struhsacker P, Callahan P (2009) Education and outreach efforts in support of wolf conservation in the Great Lakes Region. In Wydeven AP et al. (eds) Recovery of gray wolves in the Great Lakes Region of the United States, Springer, New York

United States Fish and Wildlife Service (2001) Bog turtle (*Clemmys muhlenbergii*) Northern population recovery plan. Prepared by M Klemens. Pennsylvania Field Office, Hadley, Massachusetts

Veríssimo D, Fraser I, Groombridge J, Bristol R, MacMillan DC (2009) Birds as tourism flagship species: a case study of tropical islands. Anim Conserv 12:549-558

Wiggins BL, Eddy SL, Wener-Fligner LS, Grunspan DE, Timbrook JP, Freisem K, Crowe AJ (2017) ASPECT: a survey to assess student perspective of engagement in an active-learning classroom. CBE Life Sci Educ DOI: 10.1187/cbd.16-08-0244

Zayapragassarazan Z, Kumar S (2012) Active learning methods. NTTC Bulletin 19:3-5

Zeppel H, Muloin S (2008) Conservation and education benefits of interpretation on marine wildlife tours. TME 5:215-227

Appendices

Appendix A3 – Instructor Profiles

Instructor profiles for the regular instructors for each of the two large introductory courses used in this study.

Description	Class A	Class B
General		
Percent of total grade based on in-class activities	21 – 30 %	0 – 10 %
How often each active learning strategy was used		
Verbal questions	Every class	Often
Clickers	Every class	Never
Worksheets	Rarely	Occasionally
Verbal questions		
Number per class	9 – 10	3 – 5
Percentage cold call	< 10 %	None
Clickers		
Number per class	6 – 10+	NA
Graded as…	Accuracy and participation	NA
Points per question	0.5 – 1	NA
Worksheets		
Graded as…	Participation	Participation
Points per worksheet	10	5

Appendix A4 – Student Engagement Survey

STUDENT OPINION SURVEY

Biology Education Research Study

THE UNIVERSITY of TENNESSEE KNOXVILLE

STUDENT OPINION SURVEY

You are invited to participate in a research study evaluating the effectiveness of various teaching strategies. Please read the study information below and indicate whether or not you wish your responses to be included in the study.

PARTICIPANTS' INVOLVEMENT IN THE STUDY: Participants will indicate how strongly they agree or disagree with 23 statements and XX open-ended questions about their experiences in the class. The statements and questions will ask for participant opinions regarding the three sections of today's class as well as opinions about the course overall. Participants can choose to answer all, some, or none of the questions. If all questions are answered the survey should take approximately 5-10 minutes.

RISKS: Students may worry that their instructors may see their responses or that their responses would impact their course grade. Such risk will be minimized by having students complete the survey during a class period led by a guest lecturer and with the instructor of record absent. Furthermore, the instructor will not be able to view any individual responses at any point in time and will only have access to compiled response data after final grades have been submitted to the University registrar.

CONFIDENTIALITY: All data will be kept confidential and stored on a secure password-protected computer in a locked University office. Data analyses will be conducted using automatically assigned ID numbers that replace participant names and summary results (not individual responses) will be reported when publishing findings.

COMPENSATION: There is no compensation for the participants.

CONTACT: If you have questions at any time about the study, procedures, or how the results will be reported, you may contact the corresponding researcher: Cassie Dresser, Ecology and Evolutionary Biology, 537 Hesler, cdresser@vols.utk.edu, 865-974-6188. If you have questions about your rights as a participant, contact the Office of Research Compliance Officer at the University of Tennessee, 865-974-7697.

PARTICIPATION: Your participation in this study is voluntary and should you decline to participate or withdraw you will not be penalized in any way. If you decide to participate, but wish to withdraw from the study prior to completing the survey, you may do so by simply exiting out of the Internet browser window without clicking 'submit'. Due to the nature of the computer generated ID assignment, you cannot withdraw from the study after submitting the survey because your name is no longer tied to the responses. Checking the boxes below and submitting your survey responses constitutes your consent to participate. The survey will launch automatically following your consent submission. If you do not wish to participate simply close your Internet browser now.

You may receive one or more of the following question formats: closed scale, sliding scale, or open ended. For the closed scale format, choose one of the six options that best indicates your level of agreement with the provided statement. For the sliding scale questions, please scroll the sliding scale to indicate where your opinion falls on the labeled scale below the scroll bar. If the statement or question is not applicable select the "NA" option. For the open-ended questions, please type in your response.

☑ I am at least 18 years old, I have read the above information, understand the potential risks of participation, and agree to participate in this study.

Continue

STUDENT OPINION SURVEY

Select your regular Instructor: Guffy

IMPORTANT: The term 'clicker' used in this survey includes any audience response technology, including but not limited to Turning Point Response clickers, Poll Everywhere, Text Message Polling, and Learning Catalytics

The following questions pertain only to today's class, which was taught by a guest lecturer. Today's class was broken up into 3 sections, each focusing on a different topic within the field of conservation biology.

1. Identify the teaching method used for each section (verbal questioning by the instructor, reflective journaling, group worksheet, roleplaying, or other)

Section 1: -----
Section 2: -----
Section 3: -----

2. I was not interested in the topic discussed in Section 1.

Strongly disagree — Strongly Agree

3. I was not interested in the topic discussed in Section 2.

Strongly disagree — Strongly Agree

4. I was not interested in the topic discussed in Section 3.

Strongly disagree — Strongly Agree

5. For today's class, explain why you found particular topics interesting or uninteresting.

6. I was on-task during Section 1.

Strongly disagree — Strongly Agree

7. I was on-task during Section 2.

Strongly disagree — Strongly Agree

8. I was on-task during Section 3.

Strongly disagree — Strongly Agree

9. Explain why you were more on task or less on task during particular sections of today's class.

10. I am not confident in my understanding of the material presented in Section 1.

Strongly disagree — Strongly Agree

11. I am not confident in my understanding of the material presented in Section 2.

Strongly disagree — Strongly Agree

12. I am not confident in my understanding of the material presented in Section 3.

Strongly disagree — Strongly Agree

13. I don't think the type of teaching strategy used today contributed to how much I learned about a particular topic (section).

Strongly disagree — Strongly Agree

14. Explain your answer to the previous question. (Why did you learn more in one section versus another?)

14. Explain your answer to the previous question. (Why did you learn more in one section versus another?)

The following questions pertain to the entire course, taught by your regular instructor.

15. Estimate how often each teaching strategy has been used during this course (average number of times per class)

Verbal questioning: 0

Clickers: 0

Group Worksheets: 0

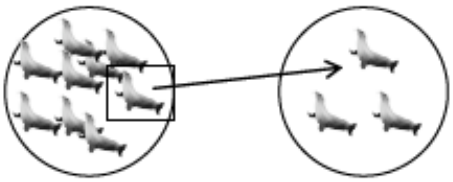
Other Strategies: 0

22. Which teaching strategy (verbal questioning, clickers, or worksheets) do you prefer? Why?

Submit

Appendix A5 – Final Assessment (Version 1)

The Hawaiian monk seal is a highly endangered species, particularly within the Hawaiian Island. Less than 2,000 individuals are spread out in separate populations throughout the Islands. These populations face numerous threats including entanglement in gillnets, intentional killing by humans, and food limitation.



Johnston Island (outside Hawaii) — Laysan Island (in Hawaii)

** NOTE: The latitudinal distance between Johnston Island and Laysan Island is equivalent to the distance between Knoxville and Miami.

1. Scientists have recommended a variety of approaches to aid declining populations of Hawaiian monk seals. Name the conservation strategy depicted in the diagram above.

2. During the last three decades this strategy has been implemented for its potential genetic benefit to the population at Laysan Island. What genetic benefit did we discuss in lecture that this conservation strategy could provide? (Circle answer)
   A. Increased population size
   B. Increased number of heterozygotes
   C. Reduced inbreeding depression
   D. Reduced frequency of outbreeding depression

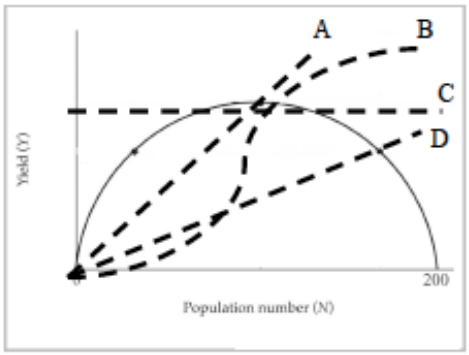
3. Managers were delighted after the first breeding season following implementation of this strategy because numerous healthy pups were born; however, after five years, most of their offspring had died. Name and describe the genetic explanation for this fitness decline.

4. Fortunately, the scenario described in the previous question did not occur and the conservation strategy has been deemed a success for the Hawaiian monk seal. How common is such success (when this conservation strategy is implemented for other threatened and endangered species)? (Circle answer)
   A. Success is common
   B. Success is uncommon
   C. Success is largely uncertain

Prior to settlement, Michigan had an abundant deer herd that was naturally limited by wolves and other predators. In the absence of these predators, hunting is now necessary to manage deer populations, but has far exceeded sustainable harvest recommendations, resulting in a rapid population decline.

5. Use the Michigan deer scenario to briefly describe the theory of the tragedy of the commons.

6. At what population size is the maximum sustainable yield for the Michigan population of deer shown in the graph below? (Circle answer)
   A. 0 (there is none)
   B. 55
   C. 100
   D. 200



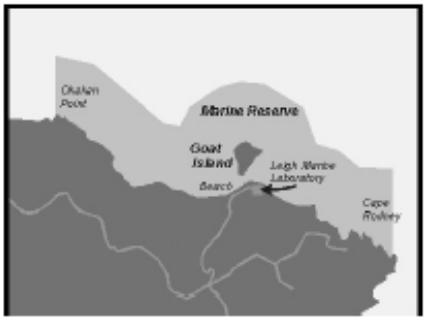
7. Most of the dotted lines on the graph above represent **sustainable** harvest quotas for this Michigan deer population.

   Name the quota regime (type) associated with each dotted line (if one or more lines is not a quota regime we talked about, write "NA").

   A =
   B =
   C =
   D =

8. A constant harvest MSY quota has the potential to be perfect, but what might make it difficult to achieve? (circle answer)
   A. Carrying capacity is dynamic and hard to estimate
   B. Offspring die early on
   C. As population size increases, yield decreases
   D. Harvest rate is proportional to population size
   E. None of the above

One of the world's largest marine protected areas has been proposed in an area off the coast of Atlantis. Some stakeholders are proposing the area be open for scientific projects to study climate change impacts on ocean biodiversity, but be strictly closed off



to fishers.

The governor of Atlantis made the following statement regarding the proposed reserve, "The health of our natural resources is crucial to the quality of our lives. Islanders know the limit of the natural environment, so they make the best stewards of such an important reserve."

However, the proposed fencing off of the marine area for protection and research emerged as a controversial issue. Fishers were opposed to these conservation efforts claiming that marine-protected areas stifled local innovations in sustainable fishing. Representatives from Atlantis's fishing industry said that local fishers were making huge strides in developing sustainable fishing technologies, using hooks and lines instead of nets, which produce wasteful bycatch. Their exclusion from the reserve would slow down such innovations whilst forcing Atlantis to import more fish from abroad, they argued.

7 IUCN Categories: (Ia) strict nature reserve, (Ib) wilderness reserve, (II) national park, (III) natural monument, (IV) habitat / species management area, (V) protected landscape / seascape, and (VI) managed resource protected area

9. Generally speaking, the seven IUCN protected area management categories listed above classify protected areas according to what?

10. Which of the following may be stakeholders in the proposed reserve design? (Circle answer)
    A. Scientists
    B. Atlantis government
    C. International shipping industry
    D. Fisherman
    E. All of the above
    F. Only B and D

11. Name one key difference between a strict nature reserve and a national park.

12. Should the proposed reserve design be implemented, what are two potential drawbacks of this large reserve? (Circle ALL correct answers)
    A. Low buffering capacity
    B. May lack local community support
    C. May be difficult to enforce restrictions
    D. May not be able to absorb impacts of climate change
    E. Proportion of habitat has already been substantially weakened

Bonus Questions (Optional; 2 pts.)

Did you attend the Conservation Biology lecture given by Cassie Dresser?

YES NO UNSURE

If so, was your attendance helpful in answering these questions?

YES NO IDK

How much time did you spend studying this material (excluding class time)?

_____
00:00 (hrs:mins)

CONCLUSION

The research presented in this dissertation mirrors the multidisciplinary nature of conservation. Just as there is no single field of study that prepares one to be a conservation biologist, conservation does not focus on input from any single area of expertise. Conservation relies on expertise from biology, ecology, genetics, chemistry, statistics, sociology, policy, economics, philosophy, education, and many more disciplines to inform species management, reserve design, ecological economics, restoration ecology, conservation marketing, conservation journalism, and environmental ethics just to name a few (Groom et al. 2006). Here I have shown you how genetics and education can inform the species management of the federally threatened Bog Turtle (*Glyptemys muhlenbergii*).

In Chapter I, I showed how recent advances in genetics have allowed us to obtain more accurate estimates of population parameters, such as genetic diversity and allowed us to begin exploring previously unreachable areas of interest, such as the potential of local adaptation. Exploration of genetic population structure in the south can help ensure the most biologically meaningful management units are designated. Estimates of genetic diversity and effective populations sizes can help us avoid ill-advised prioritization of populations, such would be the case for using Bog Turtle population census sizes as the basis for decision making. Preliminary checks for signatures of local adaptation can also aid managers in evaluating the relative risks of different translocation scenarios.

In Chapter II, I used a Bog Turtle translocation program to demonstrate how genomic techniques can be used to assess the efficiency and efficacy of translocations and aid in the formulation of clear and objective criteria of success. First, I confirmed the assumption that contributing source populations were genetically distinct using pairwise F_{ST}. Then I estimated the gene diversity of the source populations and compared those diversities to the gene diversity of the release population to determine if the program successfully increased gene diversity. But, I took it a step farther by determining whether this increase in gene diversity was a high as we would expect from the deliberate and equal admixture of sources.

In Chapter III, I considered the resource limitations and poaching concerns of Bog Turtle conservation practitioners, as well as, the overlapping conservation values between Bog Turtle conservation initiatives and initiatives for other imperiled species, by pursuing an educational program best suited for these qualities – general conservation education in an academic setting. Through a meticulously designed experiment, I was able to determine that a variety of approaches (i.e., verbal questioning, clickers, and worksheets) could be used to convey conservation concepts with similar

outcomes in regards to student engagement and learning, but that topic and organism example should be given careful consideration.

My findings directly address three key objectives in the Bog Turtle Species Recovery Plan, but the general process of genetic inquiry and comparison of educational methods could easily be applied to other species of conservation concern. As new genomic techniques continue to emerge, our genetic population estimates will continue to improve and more avenues of questioning will become accessible (Allendorf et al. 2010). Furthermore, long-term studies on how knowledge of conservation concepts introduced in an academic setting is retained and applied in later life stages, will be valuable for informing conservation practitioners on how to more effectively instill conservation values into the hearts and minds of the general public. Not only will I pursue questions in both of these disciplines (genetics and education) in the future, but I will continue to expand and explore the many other disciplines that inform the field of conservation biology.

References

Allendorf FW, Hohenlohe PA, Luikart G (2010) Genomics and the future of conservation genetics. Nature Reviews 11:697-709

Groom MJ, Meffe GK, Carroll CR (2006) Principles of conservation biology: third edition. Sinauer Associates, Sunderland, Massachusetts, pp 20

VITA

Cassie Marie Dresser was born on May 4th, 1988 to Kim and Lee Dresser. Her passion for biology and education came early on in life while she was in high school at South Seneca Central School (Ovid, NY). She earned a Bachelor's degree in biology with a minor in mathematics at Hartwick College (Oneonta, NY), where she was also a dual-sport NCAA Division III athlete (volleyball and basketball). Upon graduation, she immediately began a Master's degree in conservation biology at Central Michigan University (Mount Pleasant, MI) where she completed a dissertation on the behavioral and genetic aspects of rusty crayfish invasion, as well as, the impact of legislation on the rate of spread of rusty crayfish. After a summer working for the Michigan Department of Natural Resources at Pontiac Lake Recreation Area as a Park Explorer Guide she enrolled in the doctoral program in the Ecology and Evolutionary Biology Department at the University of Tennessee in 2013. She received her doctorate in 2017 for her multidisciplinary research on bog turtle conservation and education, knowledge she will share with the students at Lyman Briggs College, Michigan State (East Lansing, MI) and with her soon to be husband, Andrew S. Briggs, a fish biologist with the U.S. Fish and Wildlife Service (Waterford, MI).