

Evaluating the Effectiveness of Headstarting for Wood Turtle
(*Glyptemys insculpta*) Population Recovery

by

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Abstract

Headstarting is a conservation strategy that assumes raising hatchling turtles to larger body sizes increases their survivorship compared to wild non-headstarted turtles. This increased survivorship should increase population growth rate relative to wild recruitment. There are, however, few published results of long-term population recovery using headstarting. The lack of demographic assessment of population recovery has led to an overall lack of quantitative assessment of the effectiveness of headstarting as a conservation action. Headstarting needs to be efficient and effective as a poorly executed headstarting project can result in species extinction given it is often used with critically endangered species. We released 3 cohorts of headstarted Wood Turtles (*Glyptemys insculpta*) with varying degrees of headstarting to determine if headstarting increases survivorship. I showed that headstarting turtles to a larger body size confers a survival advantage, and this survival advantage should increase population growth rate relative to wild recruitment. I then quantitatively assessed the effectiveness of a 15-year Wood Turtle headstarting program by modeling population-specific demographic parameters to evaluate recovery efforts, and determine the next phase of recovery. I found some evidence of population recovery, but also identified challenges and make several management recommendations that should enhance the success of the headstarting program. Overall, I have provided support for headstarting as an effective conservation strategy, with the caveat that all headstarting projects must be paired with management plans that maintain high adult and juvenile survival.

Keywords

headstarting, conservation, recovery, species-at-risk, endangered, population modelling, population viability analyses, zoo conservation, long-term research, population demography, population dynamics, intervention ecology, turtles, VORTEX

Notice of Data Sensitivity

Turtles are the most traded, most endangered vertebrates on the planet (Dowd 2016). Wood Turtles (*Glyptemys insculpta*) are listed by the IUCN as Endangered across their global range (van Dijk and Harding 2011), and illegal collection for the pet trade is a major threat to their populations (Litzgus and Brooks 1996, this study). Individual Wood Turtles are valued at up to CAD\$1000, up to CAD\$1500 for a breeding pair, and up to CAD\$5000 per individual in overseas markets (Adkins 2015, Dowd 2016). Poachers obtain species location data in part from reading scientific manuscripts, reports, theses, and publications (Lindenmayer and Scheele 2017), thus there is justifiable reason for researchers to withhold findings. However, publishing and reporting ideas to the scientific community is of utmost importance to advance research; this data sensitivity issue clearly clashes with the foundations of science. Thus, as per recommendations by Lowe *et al.* (2017), I shall follow guidelines to publish openly, and responsibly. To ensure responsible publication, I have not included: 1) data-sensitive citations, 2) names of geographic landmarks, and 3) study site maps. Because I have not included the sensitive location information, my thesis can be freely shared.

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General Introduction

Global Conservation Crisis

Biodiversity has changed more in the past 50 years than at any other time in known human history (Millennium Ecosystem Assessment 2005). The current extinction rate for vertebrate species is up to 100x higher than the estimated background extinction rate, suggesting we have entered into a 6th mass extinction (Ceballos *et al.* 2015). From 1970–2014, vertebrate populations have declined in abundance on average by 60%, while freshwater populations have declined by 83% (WWF 2018). These declines are caused by habitat loss and degradation, overexploitation, climate change, and pollution, all of which are by-products of human life (WWF 2018). This conservation crisis will also impact human life because we depend on biodiversity and ecosystem services for many aspects of our well-being including food, water, disease management, climate regulation, spiritual fulfillment, and aesthetic enjoyment (Millennium Ecosystem Assessment 2005). A recent report by the United Nations (2017) suggested that the loss of biodiversity is an infringement on the Universal Declaration of Human Rights by violating the rights to life, health, food, water, and culture. The loss of global biodiversity also has major global financial impacts. Global ecosystem services are valued at approximately USD\$125 trillion/year (Costanza *et al.* 2014) which is almost double the global gross domestic product at USD\$73 trillion/year (World Bank 2017). The loss of biodiversity thus has vast implications for human life, and the crisis must be mitigated (Ripple *et al.* 2017).

Turtles (freshwater turtles, sea turtles, and tortoises) fulfill many important roles in maintaining healthy ecosystems. Turtles are agents of seed dispersal (Braun and Brooks 1987) and fungal dispersal (Jones *et al.* 2007), nutrient storage and cycling (Sterrett 2014), vegetation

management in sensitive ecosystems (Zavaleta *et al.* 2001), provision of seasonal food sources through their eggs (Pike *et al.* 2016), keeping water sources clean by scavenging dead animals (Schneider 1998), and acting as keystone species through habitat management (Gopher Tortoise (*Gopherus polyphemus*) burrows provide refugia for many species during fires; Russell *et al.* 1999) and predator-prey dynamics (Sea Turtle-Jellyfish dynamics help maintain healthy fisheries and promote human safety; Wilson *et al.* 2010). Given their various roles in maintaining healthy ecosystems, it is of serious concern that turtles are one of the most globally endangered taxa with approximately 61% of all species listed as globally threatened by the International Union for Conservation of Nature (IUCN; Turtle Taxonomy Working Group 2014, Lovich *et al.* 2018).

Turtle Conservation

Threats to turtles are numerous and include human exploitation for pets, food, and traditional medicine (Cheung and Dudgeon 2006), and from incidental effects of a vast human footprint including road mortality (Gibbs and Shriver 2002), climate change (Janzen 1994), habitat loss, degradation, and fragmentation (Gibbs and Shriver 2002, Bennett *et al.* 2010, Böhm *et al.* 2013), invasive species (Bolton and Brooks 2010), emerging infectious diseases (Johnson *et al.* 2008), pollution (Hopkins *et al.* 2013), and subsidized predators (Marchand and Litvaitis 2004). Given the extensive threats, a quick response is warranted. We must, however, establish well-informed conservation strategies to maximize conservation funds/effort that take into consideration the life-history characteristics of the target animal (Frazer 1992).

Turtles have evolved a suite of life-history characteristics that include long lifespans, high survivorship of sexually mature individuals and older juveniles, low survivorship of sexually immature individuals (eggs, hatchlings and younger juveniles), iteroparity, and delayed sexual

maturity (Brooks *et al.* 1991, Congdon *et al.* 1993, Keevil *et al.* 2018). Multiple mathematical models have shown that turtle populations cannot sustain chronic increases in mortality of sexually reproductive adults (Heppell *et al.* 1996, Gibbs and Shriver 2002, Enneson and Litzgus 2008, Mitrus 2008, Spencer *et al.* 2017). Turtles also may lack density-dependent responses to population declines (Brooks *et al.* 1991). A population of Snapping Turtles (*Chelydra serpentina*) that experienced a mass-mortality event from predation has shown no signs of recovery after 23 years (Keevil *et al.* 2018). Slow population growth due to lack of density-dependence results in long periods of time at small population sizes. Small populations are a conservation concern as risk of extinction (through environmental stochasticity, demographic stochasticity, and genetic deterioration) increases with a decreasing population size (Lynch *et al.* 1996). Turtles may, however, be able to buffer some of these extinctions risks, as Kuo and Janzen (2004) showed Ornate Box Turtles (*Terrapene ornata*) have relatively slow rates of genetic drift. Genetic deterioration may also be hard to detect in turtles because of their long generation times (Bennett *et al.* 2010, Kimble *et al.* 2014). Nevertheless, all these facets of turtle life history show that maintaining high adult survivorship is required for effective conservation. Because of large losses of some species, management practices have needed to focus on supplementary strategies to bolster populations (Cadi and Miquet 2004, Buhlmann and Osborn 2011, Milinkovitch *et al.* 2013) and these practices need to be informed by good science to ensure they are effective. One widely-used, but infrequently tested management practice is termed headstarting (described below).

Headstarting

In the most simplistic definition, headstarting attempts to increase survivorship of juveniles with the purpose of increasing recruitment into a population for conservation purposes (Haskell *et al.* 1996, Heppell *et al.* 1996, Eiby and Booth 2011, Burke 2015). Headstarting programs have been widely used across many taxa including fish (Kerr 2000), amphibians (Muths *et al.* 2001), lizards (Alberts 2007), snakes (King and Stanford 2006), mammals (McCleery *et al.* 2014), and birds (Pain *et al.* 2011). Various other terms are used synonymously with headstarting for chelonians such as repatriation, supplementation, stocking, augmentation, captive rearing and release, and supportive releases. I will use the term headstarting as it is the most commonly used term throughout the literature for chelonians. Turtle headstarting most often includes collecting and artificially incubating eggs, then rearing the hatchling turtles in protected ex-situ environments to a larger body size, then releasing them back into their natural habitat where their survivorship is assumed to increase compared to their wild-counterparts (Haskell *et al.* 1996, Heppell *et al.* 1996, Seigel and Dodd 2000, Vander Haegen *et al.* 2009, Eiby and Booth 2011, Bona *et al.* 2012). Headstarting to a larger body size allows turtles to bypass the life-stage of elevated mortality, which should increase recruitment into a population. The Green Sea Turtle (*Chelonia mydas*) was possibly the first chelonian to be headstarted through a large-scale conservation project started in 1959 (Huff 1989, Woody 1990). Headstarting is now widely used across the globe by many prominent conservation groups including the Turtle Survival Alliance (TSA; Burke 2015). Given that headstarting is often perceived to be the last resort in saving populations of critically endangered species (Milinkovitch *et al.* 2013, Burke 2015), a poorly executed headstarting project can result in extinction, thus gaps in knowledge must be understood and mitigated.

Gaps in Knowledge about Turtle Headstarting

Population Recovery: Short Term

There is a lack of published results on the shorter-term impacts of headstarting on population recovery. Several factors contribute to the lack of published results, including lack of rigorous post-release monitoring due to limited resources, data reported only in non-public documents, lack of publishing incentive for non-academics, waiting to publish until long-term data are available, no incentive to publish negative results, and lack of collaboration with academic institutions to aid in complex demographic modelling (Bennett *et al.* 2017). Short-term demographic modelling of population recovery can be complex for wildlife managers as early-stage headstarted populations will have a juvenile age-class bias in population structure due to releases of headstarted juveniles combined with delayed sexual maturity. Populations augmented with headstarts by definition have inflated birth-rates (headstart releases), and possibly inflated death-rates (Tuberville *et al.* 2015) given the large numbers of headstarted turtles released combined with general stochasticity of juvenile turtle survivorship (Iverson 1991). Capture probabilities differ between juveniles and adults, and within juvenile age-classes due to variations in size, survivorship (Iverson 1991, Paterson *et al.* 2012), behaviour, habitat use, and trapping/survey protocols (Congdon *et al.* 1996). As a result, juveniles are often omitted from population size estimates (Congdon *et al.* 1996, Koper and Brooks 1998, Hasler *et al.* 2015). This is not possible when evaluating a headstarting project given the likely juvenile-biased age-structure given the release of headstarted turtles. There are also likely differences in capture probabilities between sexes in headstarting projects as researchers generally invest large amounts of effort in nesting surveys to locate eggs for headstarting, which could result in an adult female capture bias compared with adult males. It has been suggested that blocking the population into

subgroups for analyses is effective if the population size is large enough (McKnight and Ligon 2017), which is likely not the case in most headstarting scenarios. Overall, the combination of juvenile age-class biased population structures combined with a small population sizes create demographic modelling challenges for wildlife managers interested in quantitatively evaluating short-term population recovery. It may, however, not be completely appropriate to thoroughly evaluate population recovery for headstarting projects on shorter time scales given the life-history characteristics of chelonians. Many shorter-term studies thus focus on individual fitness (Chapter I, Haskell *et al.* 1996, Vander Haegen *et al.* 2009, Michell and Michell 2015) as a metric of population recovery, rather than specifically modelling short-term recovery efforts (Bennett *et al.* 2017).

Population Recovery: Long Term

Broadly put, the end goal of most species' conservation projects is to have a viable, self-sustaining population in the wild that does not require human intervention. A stable population of sexually reproductive adults with high survivorship is critical for a turtle population to be self-sustaining, as shown by multiple elasticity analyses (Heppell *et al.* 1996, Mitrus 2008, Enneson and Litzgus 2008, Spencer *et al.* 2017). Turtles, however, have delayed sexual maturity, with some species not reaching adulthood for 15+ years (Congdon and Van Loben Sels 1993). Headstarting projects most often rear turtles for 1–2 years before release into the wild, resulting in a time-lag of 10–15 years (depending on age of sexual maturity) until the first cohort of headstarted turtles reaches adulthood, and does not account for the target population reaching a stable age-class distribution. Turtles have long lifespans (Congdon *et al.* 1993, University of Michigan 2016), thus achieving a stable age-class distribution could require several decades of

augmentation but given budgeting limitations, headstarting programs rarely last that long. Evidence of long-term population recovery via headstarting is rare; however, such data have increased in recent years (Milinkovitch *et al.* 2013, Shaver and Caillouet 2015, Spencer *et al.* 2017, Dreslik *et al.* 2017). Overall, these data limitations have prevented large scale, multi-population, multi-species meta-analyses, which are essential for properly analyzing the long-term impacts of headstarting on population recovery (Bennett *et al.* 2017).

Individual Fitness: Short-term

Headstarting fundamentally relies on the hypothesis that “bigger is better”, whereby body size is positively related to survivorship (Heppell *et al.* 1996, Seigel and Dodd 2000). It is assumed that headstarted turtles bypass the young life-stages of elevated mortality, which should increase recruitment into a population, thereby increasing population growth rate relative to wild recruitment. Multiple studies have observed high survivorship of headstarted individuals (Haskell *et al.* 1996, Vander Haegen 2009, Bona *et al.* 2012, Milinkovitch *et al.* 2013, Michell and Michell 2015, Tuberville *et al.* 2015); however, none of these studies compared survivorship of headstarts to that of wild non-headstarted direct-release hatchlings. Haskell *et al.* (1996) found evidence for size-related survival within a cohort of released headstarted turtles, with larger turtles having higher survivorship than smaller turtles. There is a general lack of research on the fate of wild hatchlings/juveniles (Congdon *et al.* 1993, Paterson *et al.* 2012) which makes it challenging to compare survivorship results to headstarting projects. Variance in egg, hatchling, and juvenile survivorship among sites and years (Brooks *et al.* 1991) makes cross-study comparisons difficult, thus magnifying the need for within-study comparisons to wild hatchlings. To my knowledge there are no published studies which directly compare the survival of

headstarted turtles to non-headstarted wild hatchlings within the same population during the same year.

Additionally, headstarting techniques and protocols are not standardized among programs. Husbandry protocols (i.e. diet, housing density, water flow, duration of headstarting, size at release) and field protocols (i.e. in-situ or ex-situ egg incubation, release site, timing of release, soft-release/hard-release, predator removal) vary among projects and have not been rigorously assessed (Buhlmann *et al.* 2015, Bennett *et al.* 2017). This variation in techniques and protocols makes comparisons of outcomes among headstarting projects difficult. Although standardizing headstarting techniques may seem experimentally sound, such standardization would not account for the diversity of species, goals, and budgets among programs. Nonetheless, standardizing some techniques and determining best protocols can benefit the overall practice of headstarting.

Individual Fitness: Long-Term

Turtles have delayed sexual maturity and long lifespans (Brooks *et al.* 1991, Congdon *et al.* 1993), with many species not reaching sexual maturity for 15+ years (Congdon and van Loben Sels 1993). This delayed maturity prevents wildlife managers from gathering reproductive data from headstarted turtles for many years after a project has started. The long-term effects of headstarting on reproductive physiology and behaviour, including fecundity, maternal and paternal investment, have thus yet to be evaluated. This absence of data about reproductive success of headstarted turtles has generated some deserved skepticism from many prominent turtle biologists (Woody 1990, Frazer 1992, Heppell *et al.* 1996, Seigel and Dodd 2000). It has been suggested that headstarted turtles may reproduce at a younger age, given juvenile growth rates were elevated in captivity (Hildebrand 1932, Congdon *et al.* 1993), but this hypothesis is

yet to be tested. Population manipulations for purposes of conservation can potentially have detrimental effects that could go undetected for many years after a project has started. For example, a recent study revealed that translocated non-headstarted adult male Desert Tortoises (*Gopherus agassizii*) did not sire any offspring four years post-translocation into a population (Mulder *et al.* 2017). Long-term studies investigating the possible impacts of these conservation management practices on reproductive biology are thus critical to answer concerns over the possible negative impacts.

There is a lack of published literature on the long-term effects of headstarting on individual longevity and survivorship. It has been suggested that headstarting could negatively influence behaviour, physiology, and health (Woody 1990) which could have effects on reducing long-term survival. Recently, shell disease has emerged in headstarted populations of Western Pond Turtles (*Actinemys marmorata*) which previously reported initial recovery via a headstarting program (Vander Haegen *et al.* 2009, Hallock *et al.* 2017). The possible deterioration of animal health and subsequent reduction in long-term survival resulting from the practice of headstarting has implications for long-term population viability.

History of the Wood Turtle Headstarting Project

Specific details of the study site will not be mentioned given the poaching risks threatening the species (Litzgus and Brooks 1996). This population of Wood Turtles (*Glyptemys insculpta*) is located in a watershed within the Great-Lakes-St. Lawrence forest region in Ontario, Canada. Forest cover broadly surrounding the site declined by approximately 90% due to clearing of lands for agriculture during the 19th century, but it has marginally improved with recent recovery efforts (citation removed). Within the watershed, Wood Turtles are found in 5 different

watercourses which I have identified as sub-populations, labeled as PopA, PopB, PopC, PopD, and PopE. Watercourses are small meandering creeks featuring a cobble bottom, with slow to moderate flow rates that vary seasonally. Most of the sites are on private land in an agricultural landscape which has shifted from pasture to cash crop through the course of the project. Riparian buffers between watercourses and agricultural land ranges from 2-300 m, and is highly variable among and within sites. Most watercourses have large floodplains, which aid in managing seasonal flood patterns.

The first Wood Turtle surveys were done in the 1950s (T. Lobb, pers. comm. 2014); unfortunately, these data are missing. Brief mark-recapture surveys in the late 1980s reported promising results of healthy populations. The range-wide decline of the Wood Turtle (Harding and Bloomer 1979) then prompted a larger more intensive demographic study of this population in the early 1990s. Population size in 1993 was estimated at 162 turtles for PopA, and 107 turtles for PopB. Researchers identified threats which could limit long-term population persistence including limited recruitment, lack of suitable nesting sites, relatively high adult mortality (11 dead adults over 3 years, including 8 adult females), and high levels of limb mutilation (likely from meso-predators such as Raccoons, *Procyon lotor*). Researchers also warned that the illegal collection of many turtles in a short period of time could occur given the high density of turtles present. Brief surveys (1–4 days) were conducted by several volunteers between 1994 and 1996, and low capture numbers prompted more intensive surveys in 1997. Surveys in 1997 revealed conclusively that the Wood Turtle population had decreased abruptly, likely sometime between 1994 and 1995. PopA's population size estimate declined from 162 turtles in 1993 to 26 turtles in 2001 (84% decline). PopB's population estimate declined from 107 turtles in 1993 to 58 turtles in 1998 (46% decline). The cause of the decline is unknown; however, these declines

were suspected by Brooks *et al.* (unpubl. data) to be the result of a large-scale poaching event because:

- 1) The population was thought to be well known to pet-trade enthusiasts
- 2) Two large wooden crates were found in dense forest near PopA by researchers during surveys in 1997. These crates were in good condition and had the address of a furniture manufacturing company in Detroit, USA
- 3) R.J Brooks was previously offered large amounts of money for the turtles
- 4) No carcasses were found despite intensive survey efforts, suggesting that the cause was not predation or disease

An investigation of the suspected poaching event has been on-going since the early 2000s. Regardless, without confirmation of the poached turtles, the reason for decline is still a mystery.

Given the large declines, wildlife managers wanted to know the viability of the populations long-term. Thus, a population viability analysis (PVA) was done in 2001, which predicted the extirpation of both PopA and PopB within 100 years. Researchers manipulated various demographic parameters in the PVA to determine the best plan for population recovery and showed that the population could recover in approximately 60 years if (1) survival rates of nests, hatchlings, and juveniles were held at artificially higher levels, and (2) all other demographics stayed constant (i.e. high adult survival). Manipulating these population demographics through intensive management would require extensive financial and logistical resources, but the authors of the PVA noted that any delay in implementing the headstarting program would jeopardize the likelihood of population recovery. Thus, the headstarting program began with the collection of

eggs in 2003, with the first release of headstarted turtles in 2005. Headstarting has been ongoing ever since (15 years).

MSc Research Objective

I have the rare and novel research opportunity to quantitatively examine the outcomes of a 15-year headstarting project (2003-2018) which is among the longest-running, most well-documented turtle headstarting projects globally. No data from this population have been published since population monitoring began (1988-2018, 30 years).

Chapter I

I experimentally tested if headstarting turtles to larger body sizes increases their survivorship by comparing three groups of turtles with varying degrees of headstarting: (1) 15 turtles headstarted for 2-years (2yHS), (2) 15 turtles headstarted for 1-year (1yHS), and (3) 15 turtles hatched in captivity then released (i.e. no headstarting; 0yHS). This comparison is especially informative, as no studies to date have made such a direct comparison of headstarted turtles to non-headstarted wild hatchlings. This information is critical for informing management decisions about best practices for the recovery strategy, and answering fundamental questions regarding the practice of headstarting.

Chapter II

I quantitatively assessed the effectiveness of the 15-year Wood Turtle headstarting program by modeling population specific demographic parameters to evaluate recovery efforts to date, and determine the next phase of recovery. Modeling population recovery scenarios with

comprehensive long-term data is essential for evaluating the effectiveness of headstarting projects, while continuously improving recovery efforts through adaptive management.

Thesis Rationale

Headstarting is a widely used, costly, and labour-intensive conservation strategy supported by few published results of long-term population recovery. The lack of demographic assessment of population recovery has led to an overall lack of quantitative assessment of the effectiveness of headstarting as a conservation action. Headstarting needs to be efficient and effective as a poorly executed headstarting project can result in species extinction. Thus, despite widespread use, headstarting should still be considered an experimental method of turtle conservation and it should continue to be rigorously evaluated to ensure best practices (Seigel and Dodd 2000, Buhlmann *et al.* 2015, Bennett *et al.* 2017).

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Chapter I

Is Bigger Better?
Comparing Post-Release Survivorship
Between Three Cohorts of
Wood Turtles (*Glyptemys insculpta*)
with Varying Degrees of Headstarting

Chapter I: Is Bigger Better? Comparing Post-Release Survivorship Between Three Cohorts of Wood Turtles (*Glyptemys insculpta*) with Varying Degrees of Headstarting

Abstract: Headstarting is a conservation tool that assumes raising turtles in protected ex-situ environments to a larger body size then releasing them back into the wild increases their survivorship compared to wild non-headstarted turtles. My goal was to quantitatively test this fundamental assumption by comparing three groups of turtles with varying degrees of headstarting: (1) 15 turtles headstarted for 2-years (2yHS), (2) 15 turtles headstarted for 1-year (1yHS), and (3) 15 turtles hatched incubated ex-situ then released (i.e. no headstarting; 0yHS). 2yHS and 1yHS were tracked for one year, from June 2016 until June 2017. 0yHS hatched in late July 2016, were released on 2 August 2016, and tracked for one month. All cohorts exhibited positive growth post-release. 0yHS had 73% confirmed survivorship one-month post-release and four of these turtles were confirmed to have survived their first winter. 2yHS had greater survivorship (73%) than 1yHS (40%) during the 12months post-release ($p=0.070$). My results support the fundamental assumption that bigger turtles have greater survivorship, and suggest that headstarting for 2 years increases post-release survival of turtles. Given the growing number of turtle headstarting projects globally, my study provides data that can aid in establishing best practices.

Introduction

Turtles are at risk-of extinction throughout their range as over 61% of species are listed as globally threatened (IUCN; Turtle Taxonomy Working Group 2014, Lovich *et al.* 2018). Reasons for initial declines are numerous but most often include increased mortality of sexually reproductive adults and modelling has shown adults to be the most critical factor required for turtle population stability (Congdon *et al.* 1993, Enneson and Litzgus 2008, Spencer *et al.* 2017). Turtles have evolved a suite of life-history characteristics including low survivorship of eggs, hatchlings, and young juveniles that results in naturally slow population growth rate (see Keevil *et al.* 2018). Slow population growth puts small populations at risk of extinction from stochastic events (Lynch *et al.* 1995). Thus, conservation biologists have opted to look into management strategies which increase population growth rate while continuing to maintain high adult survivorship. One of these management strategies is termed headstarting. In the most simplistic

definition, headstarting attempts to increase survivorship of juveniles to subsequently increase recruitment into a population (Haskell *et al.* 1996, Heppell *et al.* 1996, Eiby and Booth 2011, Burke 2015). Turtle headstarting most often includes collecting and artificially incubating eggs, then rearing the hatchling turtles in protected ex-situ environments to a larger body size, followed by releasing the headstarted turtles back into their natural habitat where their survivorship is assumed to be greater compared to their wild-counterparts (Heppell *et al.* 1996, Haskell *et al.* 1996, Seigel and Dodd 2000, Vander Haegen *et al.* 2009, Eiby and Booth 2011, Bona *et al.* 2012, Burke 2015). Headstarting fundamentally relies on the hypothesis that “bigger is better”, whereby body size is positively related to survivorship (Heppell *et al.* 1996, Seigel and Dodd 2000). It is assumed that headstarted turtles bypass the young life-stages that have elevated mortality, which consequently should increase recruitment into a population, thereby increasing population growth rate relative to wild populations with natural recruitment. In contrast to the hypothesis, some fine-scale studies have found no evidence of larger body size conferring a survival advantage within cohorts of hatchlings (Congdon *et al.* 1999, Paterson *et al.* 2014), suggesting that further insight into this fundamental assumption is required.

Multiple studies have observed high survivorship of headstarted individuals (Haskell *et al.* 1996, Vander Haegen 2009, Bona *et al.* 2012, Michell and Michell 2015, Tuberville *et al.* 2015); however, none of these studies compared survivorship of headstarts to that of non-headstarted direct-release hatchlings. Haskell *et al.* (1996) found evidence for size-related survival within a cohort of released headstarted turtles, with larger turtles having higher survivorship than smaller turtles. There is a general lack of research on the fate of wild juveniles (specifically hatchlings; Congdon *et al.* 1993, Paterson *et al.* 2012, 2014) which makes it challenging to compare survivorship results among headstarting projects. Many headstarting projects lack control groups

of non-headstarted turtles which limits comparisons and weakens scientific validity (Bennett *et al.* 2017). Additionally, variance in egg, hatchling, and juvenile survivorship among sites and years (Brooks *et al.* 1992) makes cross-study comparisons difficult, thus emphasizing the need for within-study comparisons of the survivorship of headstarted turtles to their non-headstarted counterparts. To my knowledge, there are no published studies which have directly compared the survival of headstarted turtles to the survival of non-headstarted direct-release hatchlings within the same population, during the same year. Even with all the uncertainty, headstarting is often used as the last resort in saving populations of critically endangered species (Milinkovitch *et al.* 2013, Burke 2015). Given that a poorly executed headstarting project can result in extinction, gaps in knowledge must be filled and problems mitigated.

My main objective was to quantitatively test the hypothesis that headstarting turtles to larger body sizes increases their survivorship. I tested this by comparing post-release survivorship of three cohorts of Endangered (IUCN) Wood Turtles (*Glyptemys insculpta*) with varying degrees of headstarting: (1) 15 turtles incubated ex-situ then headstarted for 2 years (2yHS), (2) 15 turtles incubated ex-situ then headstarted for 1 year (1yHS), and (3) 15 turtles incubated ex-situ then immediately released post-hatching (i.e. no headstarting; 0yHS). This project is part of a long-term Wood Turtle mark-recapture study (1988 – present) which has used headstarting a management strategy since 2003 (Chapter II). I then used the survivorship data from this study in combination with historical and contemporary data from the long-term project to model the long-term effects of various management scenarios (i.e. headstarting turtles for 0y, 1y, or 2y). Given the novelty of this study, I also compared a variety of other post-release metrics among the three cohorts including (a) size at release, (b) spatial behaviour, and (c) somatic growth rates during captive rearing (2yHS and 1yHS) and post-release (2yHS, 1yHS, and 0yHS).

These additional comparisons provide crucial baseline data to aid in the evaluation of headstarting as a management strategy.

Methods

Study Site

Locational information will not be published as suggested by Litzgus and Brooks (1996) given that illegal collection for the pet trade is a major threat to Wood Turtle populations and poachers find species location data in part from reading scientific manuscripts, reports, and publications (Lindenmayer and Scheele 2017). This population of Wood Turtles is located in a watershed within the Great Lakes-St. Lawrence Forest Region of Ontario, Canada. Watercourses are mostly small meandering creeks dominated by cobble substrate, with slow to moderate flow rate that varies seasonally (Appendix A). Most of the sites are on private land and the overall area is characterized by agricultural lands which have shifted from pasture to cash crops through the course of the project (late 1980s). Riparian buffers between watercourses and agricultural land range from 2 m to 300 m and are highly variable between sites and within sites. Most watercourses have large naturalized floodplains, which aid in managing seasonal flood patterns.

Study Population

The population has been subject to a long-term mark-recapture study since 1988. The population size declined from 269 turtles in 1993 to 83 turtles in 1998 (69.1% decline; Brooks *et al.* unpubl. data). The cause of decline is unknown, though Brooks *et al.* (unpubl. data) suspected a large-scale poaching event. A population viability analysis (PVA) in 2001 predicted population extirpation within 100 years (Brooks *et al.* unpubl. data). The PVA, however, predicted that the

population had a chance to recover if a headstarting project began (Brooks *et al.* unpubl. data). Thus, the headstarting program began with the collection of eggs in 2003, with the first release of headstarted turtles in 2005. The headstarting project is currently in its 15th year (2003-2018).

Experimental Design

A subset of Wood Turtles (n=45; 15 randomly selected per cohort) from three separate cohorts with varying degrees of headstarting were outfitted with VHF radio-transmitters and tracked for varying amounts of time between June 2016 and June 2017 (Figure 1.1, Table 1.1). Both cohorts of headstarted turtles (2yHS, 1yHS) were outfitted with 3.6 g radio-transmitters (Model R1680, Advanced Telemetry Systems, Isanti, MN) using Waterweld (J-B Weld, Atlanta, GA) and Gorilla Super Glue (Gorilla Glue Company, Cincinnati, OH), and were radio-tracked for 12-months post-release. The radio-transmitter outfit added 2.5%, and 5.3% to the mass of 2yHS', and 1yHS', respectively. These 2 cohorts were radio-tracked weekly during the active season (April to October), and monthly during overwintering (November to March). While tracking, I collected spatial data, temperature, and habitat use weekly, while morphometric measurements (straight carapace length [SCL] and mass) were taken weekly during the first month post-release to facilitate comparisons with 0yHS, then monthly. Monthly morphometric measurements were also taken while at the protected ex-situ rearing facility (Toronto Zoo) for 2yHS and 1yHS. Turtles from the cohort of direct-release non-headstarted hatchlings (0yHS) were outfitted with 0.5 g radio-transmitters (Model R1615, Advanced Telemetry Systems, Isanti, MN) using Waterweld (J-B Weld, Atlanta, GA) and Gorilla Super Glue (Gorilla Glue Company, Cincinnati, OH), and were radio-tracked semiweekly during their 1-month tracking period. The radio-transmitter outfit added approximately 7.5% to the 0yHS' mass. While tracking, I collected

spatial data, temperature, and habitat use semiweekly; however, I only collected morphometric measurements (SCL and mass) weekly in an attempt to reduce disturbance to the hatchlings. All dead turtles found with radio-telemetry were thoroughly examined (carcass position, limb loss, and state of decomposition) in an attempt to determine cause of death.

Size at Release

I compared body size (SCL) and mass at release across all three cohorts to confirm expected differences in size given the different degrees of headstarting. My SCL and mass data were not normally-distributed ($W=0.86$, $p<0.001$; $W=0.82$, $p<0.001$ respectively). Thus, I tested for differences in SCL and mass among groups using a Kruskal-Wallis rank sum test using the *dunn.test* package in R (Dinno 2017).

Known-age wild juvenile Wood Turtle morphometric data are rare, which limits comparisons between headstarted turtles from this study and non-headstarted turtles from the literature of the same age. However, both Brooks *et al.* (unpubl. data, from this population) and Farrell and Graham (1991, population in New Jersey, USA) have morphometric data for juvenile non-headstarted Wood Turtles with ages estimated using growth lines. While growth lines are generally viewed as an inaccurate indicator of age in adult turtles (Gibbons 1976, Galbraith and Brooks 1987, Litzgus and Brooks 1998), they may be useful as a coarse estimate of age for younger turtles (Harding and Bloomer 1979, Litzgus and Brooks 1998, Stone and Babb 2005). Given the potential benefits of this comparison, I compared morphometric data between my known-age headstarted Wood Turtles to morphometric data from non-headstarted Wood Turtles whose ages were estimated using growth lines (Brooks *et al.* unpubl. data; Farrell and Graham 1991).

Somatic Growth Rates

I calculated somatic growth rates (SGR, both SCL and mass) while in captivity (2yHS and 1yHS) and for multiple time-periods post-release into the wild (2yHS, 1yHS, and 0yHS). I compared SGR in captivity to SGR post-release using a paired t-test for both 2yHS and 1yHS. I compared SGR between captivity and active season (release, 24 June 2016 → 1 Sept 2016) to assess growth post-release from captivity for 2yHS and 1yHS. I excluded a turtle (Notch=850; 2yHS) that lost its front-right limb on 10 Aug 2016 from mass growth rate analyses.

I tested all SGR data for normality using Shapiro-Wilks tests (*stats*, R Core Team, 2017). When comparing three cohorts where the data were parametric, I used a one-way analysis of variance (*stats*, R Core Team, 2017); then if applicable, I did post-hoc analyses using Tukey Honest Significant Difference test (*stats*, R Core Team, 2017). When comparing three cohorts where the data were non-parametric, I used a Kruskal-Wallis rank sum test; then if applicable, I did post-hoc analyses using a Dunn's test (*Dunn.test*; Dinno 2017). When comparing two cohorts where the data were parametric, I used a two-sample T-test (*stats*, R Core Team, 2017). When comparing two cohorts where the data were non-parametric, I used a Wilcoxon rank-sum test (*stats*, R Core Team, 2017). When comparing one cohort between two time-periods, I used a dependent T-test (paired t-test; *stats*, R Core Team, 2017). I used a Bonferroni correction for multiple comparisons and accepted significance at $p < 0.01$ when comparing mass and SCL growth rates among cohorts. Post-hoc analyses were considered to be significant at $p < 0.05$.

Survivorship

I used a combination of known-fate (from VHF radio-telemetry) and mark-recapture data to estimate survivorship. I compared differences in 1-month post-release survivorship among 2yHS, 1yHS, and 0yHS using known-fate data. I compared differences in overwinter and yearly survivorship using known-fate data for 2yHS and 1yHS, and mark-recapture data as a conservative estimate of survivorship for 0yHS. I used a Fisher's exact test (with Freeman-Halton extension) using the base *stats* package in R (R Core Team, 2017) to test for differences in survival. Where appropriate, I performed post-hoc analyses using multiple Fisher's exact tests with a Bonferroni-corrected pairwise technique. I then used the yearly survivorship values to create a Kaplan-Meier estimator (NCSS 2016) of yearly survival among cohorts.

Population Modelling

I modelled 3 management scenarios based on the survivorship of the release cohorts (2yHS, 2-Year Headstarting Program; 1yHS, 1-Year Headstarting Program; 0yHS, Hatchling Release Program). I also modelled a 4th management scenario termed "Mixed-Release Headstarting Program" in which turtles from each of the aforementioned cohorts were released (Table 1.2). I used the yearly confirmed survivorship results along with historical and contemporary population demographic data to model population recovery in VORTEX, to calculate the intrinsic rate of population increase (r) (Table 1.3, Lacy *et al.* 2005). I used Wood Turtle data from the literature to augment my model if data were not available from the long-term dataset. I then chose a model which had a high population growth while also allowing the project to maintain an experimental release design such that management scenarios (i.e., best practices for headstarting) could be continuously tested. I then performed an elasticity analysis on this chosen model using VORTEX (Lacy *et al.* 2005).

Spatial Behaviour

I assessed spatial behaviour post-release by tracking movements and calculating home-range sizes using the minimum convex polygon method (100% MCP HR) and home-range length (HRL; greatest distance between two detection locations along the watercourse). SHRs were calculated because the sinuosity of the river caused much of the area in the MCP HR calculations to be in portions of habitat where the individual turtle was never found. I tracked and recorded spatial data weekly for 2yHS and 1yHS, and semiweekly for 0yHS. I calculated 12-month post-release MCP HRs and SHRs for 2yHS and 1yHS as both cohorts were tracked for 12 months. I compared 12-month post-release MCP HR and SHRs between 2yHS and 1yHS using Kruskal-Wallis tests, as the data were not normal ($W=0.74$, $p<0.001$; $W=0.88$, $p=0.009$ respectively). I then calculated 1-month post-release MCP HR and HRL for 0yHS, as this cohort was only tracked for 1 month. To allow for comparisons among all 3 cohorts, I then calculated 1-month, post-release MCP HR and HRL for 2yHS and 1yHS. The 1-month, post-release MCP HR and HRL data were not normal ($W=0.49$, $p<0.001$; $W=0.74$, $p<0.001$). Post-hoc analyses were completed with a Dunn's test using the *dunn.test* package in R (Dinno 2017).

Results

Size at Release

Mean mass (g) at release for 0yHS, 1yHS, and 2yHS was 9.3 ($n = 15$, range = 7.5 – 10), 92.2 ($n = 15$, range = 69.6 – 114.5), and 204.4 ($n = 15$, range = 182.6 – 239), respectively. Mean SCL (mm) at release 0yHS, 1yHS, and 2yHS was 37.6 ($n = 15$, range= 35.5 – 39.4), 86.2 ($n=15$, range= 80.0 – 92.0), and 112.9 ($n=15$, 106.0 – 120.0), respectively. Mass and SCL at release

differed among cohorts ($H=39.3$, $df=2$ $p<0.001$; $H=39.2$, $df=2$ $p<0.001$; Figure 1.2). 2yHS had significantly greater mass and SCL at release than 1yHS ($p<0.001$; $p<0.001$) and 0yHS ($p<0.001$; $p<0.001$). 1yHS had significantly greater mass and SCL at release than 0yHS ($p<0.001$; $p<0.001$).

Both cohorts of headstarted turtles were larger in mass, SCL, and SPL (straight plastron length) than non-headstarted turtles of the same age (coarsely estimated using growth lines) from previous studies at this site (Brooks *et al.* unpubl. data) and a site in New Jersey, USA (Farrell and Graham 1991; Table 1.4). 2yHS were similar in size to non-headstarted turtles aged 4–6 years old. 1yHS were similar in size to non-headstarted turtles aged 2–3 years old.

Somatic Growth Rates

All cohorts exhibited positive growth in mass and SCL post-release (Figures 1.3, 1.4, 1.5, 1.6). 2yHS and 1yHS exhibited positive growth in captivity from hatch to release, and in the wild from release to 12-months post-release (Figures 1.3, 1.4, 1.5, 1.6). Both 2yHS and 1yHS had higher growth rates in captivity than 12-month post-release, though 1yHS were limited by a small sample size due to few turtles surviving 12-months post-release (Figure 1.4, Table 1.5). Two 0yHS captured 13- and 21-months post-release had carapace growth rates of 2.3 mm/month (mass = 3.2 g/month) and 1.5 mm/month (mass = 1.7 g/month). Assuming these two 0yHS grew only during the active season would result in active-season carapace growth rates of 3.3 mm/month (mass = 4.6 g/month) and 2.9 mm/month (mass = 3.4 g/month).

Survivorship

Survivorship one-month post-release did not differ among 2yHS, 1yHS, and 0yHS (12/15, 80% survival for each cohort; $p=1.0$; Figure 1.7). Overwinter survivorship was similar between 2yHS (12/13, 92.3%) and 1yHS (12/12, 100%) as both cohorts had high overwinter survivorship ($p=0.52$; Figure 1.8). Four 0yHS were confirmed to have survived the winter (4/13) which suggests a minimum of 31% hatchling overwinter survivorship (Figure 1.8). Individuals from both cohorts of headstarted turtles (1yHS and 2yHS) often overwintered near wild non-headstarted adults. Two 2yHS and two 1yHS overwintered more than 35 m away from a historic adult overwintering spot (White *et al.* unpubl. data). These four headstarted turtles were in close (< 15 m) proximity to each other and all successfully overwintered. A 0yHS used this same location and overwintered successfully in 2017.

2yHS had greater 12-month post-release survivorship (11/15, 73.3%) than 1yHS (6/15, 40%), but the difference was not significant ($p=0.070$, Figure 1.9). Two 0yHS were confirmed to have survived 12-months post-release resulting in a minimum of 13% post-release survivorship. These two 0yHS were also confirmed to have survived their second winter post-release resulting in a minimum of 13% 21-month post-release survivorship. Cause of death was most often attributed to predation for all cohorts (Table 1.6). I suspect Raccoons (*Procyon lotor*) for most mortalities of the 1yHS and 2yHS, while I suspect Eastern Chipmunks (*Tamias striatus*) for both mortalities of 0yHS. There was a suspected period of elevated mortality post-release amongst all three cohorts, and a period of elevated mortality in Spring 2016 for 1yHS (Figure 1.10).

Population Modelling

The population model predicted differences in population growth rate across the 4 management scenarios tested (Figure 1.11). Population growth rate was highest in the 2-Year Headstarting Program ($r = 0.016$, $N_{\text{predicted}} = 681$), followed by the Mixed-Release Headstarting Program ($r = 0.014$, $N_{\text{predicted}} = 665$), the 1-Year Headstarting Program ($r = 0.011$, $N_{\text{predicted}} = 601$). The population growth rate was negative for the Hatchling Release Program ($r = -0.019$, $N_{\text{predicted}} = 241$). The elasticity analysis of the Mixed-Release Headstarting Program model (Table 1.7) showed that decreasing adult survivorship to 80% caused the largest decreases in population growth rate and population size ($r = 0.002$, $N_{\text{predicted}} = 251$), while increasing juvenile survivorship to 94% caused the greatest increases in population growth rate and population size ($r = 0.039$, $N_{\text{predicted}} = 1009$).

Spatial Behaviour

One-month post-release MCP and HRL were significantly different among cohorts ($H = 30.1$, $df = 2$, $p < 0.001$, Figure 1.12; $H = 28.4$, $df = 2$, $p < 0.001$, Figure 1.12). 2yHS had significantly greater 1-month post-release MCP and HRL than 1yHS ($p = 0.002$; $p = 0.003$) and 0yHS ($p < 0.001$; $p < 0.001$). 1yHS had significantly greater 1-month post-release MCP and HRL than 0yHS ($p < 0.001$; $p < 0.001$). 2yHS had significantly greater 12-month post-release MCP and HRL than 1yHS ($p = 0.004$; $p = 0.008$; Figure 1.13). 2yHS moved further downstream from their release spot than 1yHS ($p = 0.012$; Figure 1.13). Record rainfall (160 mm in 12 h) in June 2017 led to a moderate flooding event that minimally displaced most individuals (< 100 m). One 2yHS (Notch=919), however, was displaced approximately 900 m downstream; this turtle homed back to its pre-flood location within 30 days. All cohorts generally stayed close to aquatic habitats. Only 25% of 349 observations of 2yHS, 17% of 282 observations of 1yHS, and 5% of 89

observations of 1yHS were 10 m or farther away from aquatic habitats. The farthest distance from aquatic habitats I observed was 50 m for 2yHS, 40 m for 1yHS, and 15 m for 0yHS. All cohorts used a variety of microhabitats within the riparian zone. I recaptured four 0yHS after overwintering at downstream distances of 5 m, 45 m, 148 m, and 166 m from their last tracking point.

Discussion

Size at Release and Somatic Growth Rates

As expected, body size increased with duration of headstarting. Headstarted turtles were larger than non-headstarted turtles of the same age (Table 1.4). Both 1-year and 2-year cohorts were comparable in size to non-headstarted turtles which were 2-3 years older. Quantifying the size of our headstarted individuals relative to wild juveniles is important for drawing inferences about the success of headstarting. Multiple authors report juvenile survival based on age-class, which may not be relevant for comparisons across studies of headstarted and non-headstarted turtles given the variation in body size within cohort years. It is likely more relevant to compare headstarted turtles to both non-headstarted turtles of the same age but different body sizes, and to non-headstarted turtles of the similar body size but different ages.

Growth rates of headstarted turtles post-release were generally similar to those reported for wild juvenile Wood Turtles (Farrell and Graham 1991), implying that turtles raised in captivity can successfully forage in the wild. As expected, headstarted turtles had higher growth rates in captivity than wild turtles, and had higher growth rates in captivity than after they were released, at which time growth became more variable within cohorts. Growth rates in captivity for 1yHS were roughly 2x greater, and for 2yHS were roughly 2-3x greater, than those reported for wild

Wood Turtles of the same age classes (Farrell and Graham 1991). From these comparisons, it is clear that headstarting accelerates growth thereby increasing the size of turtles for a given age, thus verifying one of the fundamental assumptions of this management technique.

Direct-release hatchling Wood Turtles grew during the late summer and early fall, before overwintering (Figure 1.3). Castellano *et al.* (2008) and Farrell and Graham (1991) reported similar shell growth rates in wild-hatched hatchling Wood Turtles. In addition, the shell growth rates of direct-release hatchlings were similar to those of 1yHS in captivity, indicating that headstarted turtles in captivity grow like wild turtles in the active season. Headstarted turtles grew larger in body mass than wild turtles, which is not surprising given the abundant nutritious food provided to captive turtles. The 12-month post-release growth data of non-headstarted hatchlings are limited ($n = 2$). However, given the lack of published hatchling growth data, these findings may be a useful addition to the growing body of literature focusing on the lost years of hatchling turtles. Future work should examine the growth rates of non-headstarted hatchlings to provide an experimental control in studies of headstarted turtles.

Survivorship

Survivorship did not differ among cohorts during the first month post-release (Figures 1.7, 1.10). I had expected direct-release (0yHS) to experience the lowest survivorship. I have two possible hypotheses for why I observed no differences in survival despite variation in body size, and these hypotheses may not be mutually exclusive. Firstly, releasing the 0yHS by the stream may have reduced the high mortality faced by wild hatchlings when they travel from their nests to aquatic habitats (Janzen *et al.* 2000). Other studies observed relatively low post-nest-emergence survivorship of hatchling Wood Turtles (11-19%; Paterson *et al.* 2012, Dragon 2014,

Wicklow as cited in Jones *et al.* 2015) compared to my relatively high observed post-release survivorship (80%). My hatchlings were artificially incubated, which could have impacted survivorship, as multiple studies across different taxa have shown that incubation conditions impact post-release survival and fitness (Brooks *et al.* 1991a, Booth *et al.* 2004, Fisher *et al.* 2014). Secondly, all headstarted turtles may experience elevated mortality post-release as they adapt to their new environment. Three 1yHS died within 31 days of release then none died until 303 days post-release, and two 2yHS died within 28 days of release then none died until 212 days post-release (Figure 1.10). Wildlife managers should look to reduce this possible period of elevated mortality post-release to maximize the conservation impact of headstarting. Some projects are starting to utilize “soft-releases” where headstarted turtles are temporarily released into protected in-situ environments to acclimatize to natural conditions before being fully released (see Ritchie 2017). This technique has yet to be evaluated, but may reduce this initial period of mortality that I observed.

Overwinter survivorship was high across the cohorts. The one headstarted Wood Turtle that I found dead during overwintering was likely predated as it was found near a predated wild adult female Wood Turtle. Similarly, Michell and Michell (2015) observed 100% overwinter survivorship of headstarted Wood Turtles (1 – 2 y HS time), and Szymanski (2016) observed 100% overwinter survivorship of headstarted Blanding's Turtles (2 y HS time). Overwinter survivorship of headstarted Blanding's Turtles (2 y HS time) in an artificial wetland complex ranged annually between 10-100% (Ritchie 2017), though the variation in survival may be both an artifact of the artificial wetlands at their study site, and the species nearing the northern range-limit (Ernst and Lovich 2009). Given the evidence, it seems that headstarted turtles can overwinter successfully despite not overwintering while in captivity.

I confirmed overwinter survival of four direct-release hatchlings (0yHS; 4/13, 31% survivorship); this is the first published report of overwintering survivorship of artificially incubated hatchling Wood Turtles. Additionally, I confirmed that two of these 0yHS hatchlings survived two winters post-release. Although I cannot confirm overwintering site characteristics given my limited tracking data, the 0yHS likely overwintered aquatically given their tendency throughout the tracking period to stay close to the stream. Given that Wood Turtles are likely anoxia intolerant (Ultsch 2006) this moderately high survival of artificially incubated hatchlings in harsh overwintering conditions is noteworthy. Dinkelacker *et al.* (2005) reported that hatchling Blanding's Turtles have poor survivorship in hypoxic overwintering conditions. Dissolved oxygen in winter at the site was 16.4 ppm (Riley *et al.*, unpubl. data, 2009), which is slightly higher than in the habitat of a more northern population of Wood Turtles (Greaves and Litzgus 2007). Future studies should examine the effects of dissolved oxygen on hatchling overwinter survival.

As expected, headstarted turtles had greater survivorship than direct-release hatchlings, and turtles headstarted for two years had greater survivorship than turtles headstarted for one year (Figure 1.9). Survivorship of 2yHS and 1yHS was similar until Spring 2017 when I observed an increase in mortality of 1yHS (Figure 1.10). Surveys for Wood Turtles are most productive in spring when the turtles are in relatively high densities close to watercourses and spring growth of vegetation is still incomplete (Flanagan *et al.* 2013). The predators within the system may be exploiting these same natural history attributes and opportunistically consuming a seasonally-available food source when the turtles emerge from overwintering. Early surveys at the site reported low captures of juveniles and sub-adults (Brooks *et al.*, unpubl. data). This limited recruitment was likely due to the agricultural lands subsidizing meso-predators (Gehring and

Swihart 2003) which likely reduced both nest and juvenile survivorship. While turtles have evolved to withstand low survivorship of immature life-stages, pulse recruitment is required (Spencer 2018). This possible period of elevated juvenile mortality will need to be thoroughly monitored, and mitigated.

Although the two cohorts of headstarted turtles had higher survival than the cohort of direct-release hatchlings (Figure 1.9), the survival rates were slightly lower than average annual wild juvenile survival values. Survivorship for juvenile Wood Turtles has been reported at 82% (Saumure *et al.* 2007), for Blanding's Turtles at 78% (Congdon *et al.* 1993), and for juveniles from all species of turtles at 65% based on extensive modelling (Pike *et al.* 2008). These published values are averages for the entire juvenile age-class, and thus do not provide a direct comparison to my data. My survivorship values are, however, also lower than those reported for headstarted Wood Turtles 24-months post-release (100%, 1 – 2 y HS, Michell and Michell 2015) and headstarted Blanding's Turtles 12-months post-release (72%, 1 y HS, Green 2015; 100%, 2 y HS, Szymanski 2016). Thus, my headstarted Wood Turtles have higher survivorship than their non-headstarted counterparts, but lower survivorship than those reported for headstarted turtles of the same and some closely related species.

I suspect predation is the primary cause of mortalities in my study (Table 1.6). In half of the suspected predation events (7/14), the limbs and head of the turtle were eaten, while tissue remained in the body cavity (Table 1.6). Lanszki *et al.* (2006) found that crude protein is higher in the head and limbs of European Pond Turtles (*Emys orbicularis*). This difference in protein content could explain the foraging habits of the predators in my system, though the predators may merely not be able to reach the tissue within the body cavity given the turtle's shell. Raccoons thrive in agricultural lands through crop/food availability and increased corridors for

movement (Gehring and Swihart 2003). Raccoons are a known predator of Wood Turtles, often being responsible for the mutilated limbs of adults, and mortality of all life-stages (Harding and Bloomer 1979, Farrell and Graham 1991, Brooks *et al.* 1992, Saumure 2004, Mullin *et al.* 2018). The probable overabundance of Raccoons in the agricultural lands at my site may be impairing recovery efforts. Though there are other suspect predators within the system (e.g. Red Foxes (*Vulpes vulpes*), Mink (*Neovison vison*), Domestic Cats (*Felis catus*), Striped Skunks (*Mephitis mephitis*), Virginia Opossums (*Didelphis virginiana*), Coyotes (*Canis latrans*), Bald Eagles (*Haliaeetus leucocephalus*), and Great Blue Herons (*Ardea herodias*)), which warrants further investigation. I recommend more extensive examination of the deceased turtles for specific signs to identify predators (e.g., hair samples, DNA samples) as this increased knowledge will aid in identifying and mitigating specific threats to the survival of both headstarted and non-headstarted turtles.

Population Modelling

My population models predicted similar recovery using either the 1-Year Headstarting Program, Mixed Headstarting Program, or the 2-Year Headstarting Program (Figure 1.11). Given the similarities in model outputs, I recommend implementing the Mixed Headstarting Program as it would allow the project to continue to experimentally evaluate best practices as the program releases 2yHS, 1yHS, and 0yHS concurrently. Continuing to release cohorts of non-headstarted hatchlings will allow the project to have an experimental control group with which to compare headstarted turtles. Releasing non-headstarted hatchlings will also allow various fixed-age class comparisons (e.g. survival 24-36 months post-hatch) across cohorts. These comparisons are important to contribute knowledge regarding the long-term effects of headstarting, which is

largely unknown. Mitrus *et al.* (2018) recently reported that wild non-headstarted turtles were larger in body size than headstarted turtles 7-10 years post-hatch. These findings are possibly concerning which further emphasizes that headstarting is still an experimental method of population augmentation (Buhlmann *et al.* 2015, Bennett *et al.* 2017) and needs to continuously be tested through sound experimental designs.

My elasticity analysis suggested that decreases in adult survivorship will impair recovery efforts by decreasing population growth rate (Table 1.7). These findings are consistent with previous research on turtle population demographics (Congdon *et al.* 1993, Heppell *et al.* 1996, Enneson and Litzgus 2008, Dreslik *et al.* 2017, Spencer *et al.* 2017). My model also highlighted that decreases in juvenile survival, decreases in number of females breeding annually, and decreases in egg fertilization can also reduce population growth rate and predicted population size. Even though most of the parameters tested were already known to affect turtle population dynamics, my model revealed some parameters which other models and most authors have not previously highlighted (but see Enneson and Litzgus 2008). I found that increasing juvenile survival was the most influential parameter in increasing population growth rate and size (Table 1.7). The importance of this juvenile survival parameter is possibly reflective of my juvenile-biased population structure, as 81% of the modeled population is under the age of 5 (Table 1.3). Turtle populations likely have adult-biased population structures (Brooks *et al.* 1991b, 1992), thus I caution wildlife managers about using my results before future modelling is done which examines the relationship between age-structure and recovery targets (but see Shoemaker *et al.* 2013).

Population models are hypotheses that need to be tested with field data (Bennett *et al.* 2017). Models have the potential to misinform management plans and waste valuable

conservation funds while further endangering species-at-risk if deployed without caution (Shrader-Frechette 2004, Conroy *et al.* 2006). This caveat must be recognized as we explore the population model predictions, and the impacts of the model on informing management plans. Most of my base parameters (Table 1.3) were conservative estimates. Changes in these base parameters may have large effects on population growth if they are cumulative (see elasticity analysis, Table 1.7). These parameters will need to be continually monitored with long-term data to ensure adaptive management and to continually improve recovery efforts (Buhlmann *et al.* 2015).

Spatial Behaviour

Most of the headstarted turtles established relatively small home-ranges (Figures 1.12, 1.13). The home range sizes I observed were within the ranges reported in other studies on juvenile Wood Turtles (1.6 ha by Mullin and Litzgus [unpubl. data, 2016], 2.6 ha by Jones [2009], 3.9-15.9 ha by Saumure [2004]), although small sample sizes limit meaningful comparisons. My results confirmed those of Brewster and Brewster (1991) who observed that headstarted Wood Turtles rarely moved 40 m from the watercourse; however, my study site is embedded in an agricultural landscape so there is a lack of suitable habitat beyond 40 m from the watercourse, which possibly prevented the turtles from traveling further. Headstarted Blanding's Turtles had comparable, but slightly smaller home ranges, than wild juvenile turtles (Glowacki and Kuhns 2010, Szymanski 2016). Morrison (1996) found that the movement patterns of headstarted Blanding's Turtles were highly variable until suitable habitat was found.

Although spatial behaviour was largely similar between headstarted and non-headstarted turtles, one distinction was that 2yHS moved farther downstream from their release point than

1yHS (Figure 1.13). It has been suggested that headstarting may impair swimming ability; however, headstarted Loggerhead Sea Turtles (*Caretta caretta*) showed no differences in swimming ability post-release compared to wild juveniles (Nagelkerken *et al.* 2003). If swimming ability was impaired, it is unlikely that Spinks *et al.* (2003) and Mitrus (2005) would have found no differences in recapture rates between headstarted and non-headstarted Western Pond Turtle and European Pond Turtles, respectively. Most Wood Turtles live in flood-prone river systems (Jones and Sievert 2009), thus even a slight impairment in swimming ability may have profound effects on resisting downstream currents. Interestingly, the 2yHS individual with the largest home range size (17 ha) was displaced by flooding, but was able to return to its pre-flood location, suggesting no impairment in locomotion. It is also interesting that this headstarted turtle displayed homing behaviour, a behaviour observed in wild Wood Turtles (Jones and Sievert 2009).

The larger movements I observed for 2yHS may be related to juvenile dispersal. While some studies have investigated adult and juvenile dispersal as a result of disturbances (Dodd *et al.* 2006, Jones and Sievert 2009), no study to my knowledge has looked at non-disturbance related dispersal of juvenile freshwater turtles. Avoiding agonistic encounters could be a driver of dispersal, suggesting that dispersal may be density-dependent. Agonistic behaviours have been observed between adult male Wood Turtles (Kaufmann 1992). Possible agonistic behaviours have also been observed (tail biting) between juvenile Wood Turtles at the headstarting facility though this behaviour may be related to prey misidentification. Dispersal may also be size-related, which could explain why the larger 2yHS moved farther downstream than 1yHS. Further studies are needed on wild juvenile turtle spatial behaviour, especially patterns of dispersal as they relate to population dynamics. The movements of headstarted turtles

should continue to be monitored to examine whether mortality is resulting from flooding and displacement.

Most studies, including mine, on hatchling behaviour have studied turtles for 1-2 months post-emergence from nests (Tuttle and Carroll 1997, 2005, Castellano *et al.* 2008, Jones and Sievert 2012, Paterson *et al.* 2012), largely because of limitations in tracking technology. My direct-release Wood Turtle hatchlings moved considerably less than those in the study by Castellano *et al.* (2008), perhaps because 0yHS were released at the stream (at the same locations as the headstarted cohorts), thus my hatchlings did not need to make long-distance movements from nest emergence to aquatic habitats. Additionally, 0yHS moved relatively small but variable distances between their last fall tracking location and their post-overwintering location. Mine is the first report of post-overwintering movements of hatchling Wood Turtles. As VHF technology improves (e.g. extended battery life on small VHF transmitters), so will our understanding of the spatial behaviour of hatchlings turtles.

Conclusion

I confirmed that headstarting Wood Turtles confers a survival advantage 12-months post-release in comparison to non-headstarted direct-released hatchlings. However, my survival values for headstarted turtles were lower than those reported in other studies on the same, and similar, species, possibly due to a likely overabundance of turtle predators (possibly Raccoons, though further investigation is needed) within the agricultural landscape of the study site. Interestingly, I confirmed that headstarted Wood Turtles can have high overwinter survival. My modelling predicted that headstarting could be an effective management approach to increase recruitment and thereby increase population growth rate. My elasticity analyses identified

fluctuations in adult and juvenile survivorship as important parameters which can affect population growth rates and overall recovery success. I have also confirmed that headstarted Wood Turtles can establish home ranges which are similar in size to those reported in previous studies, although the lack of published spatial data on wild juveniles limits comparisons. Future studies should follow headstarted individuals for multiple years post-release to understand site fidelity and spatial behaviour between years. In conclusion, I have provided evidence that a bigger body size confers a survival advantage, providing support for headstarting as an effective conservation strategy, with the caveat that all headstarting projects must be paired with management plans that maintain high adult and juvenile survival.

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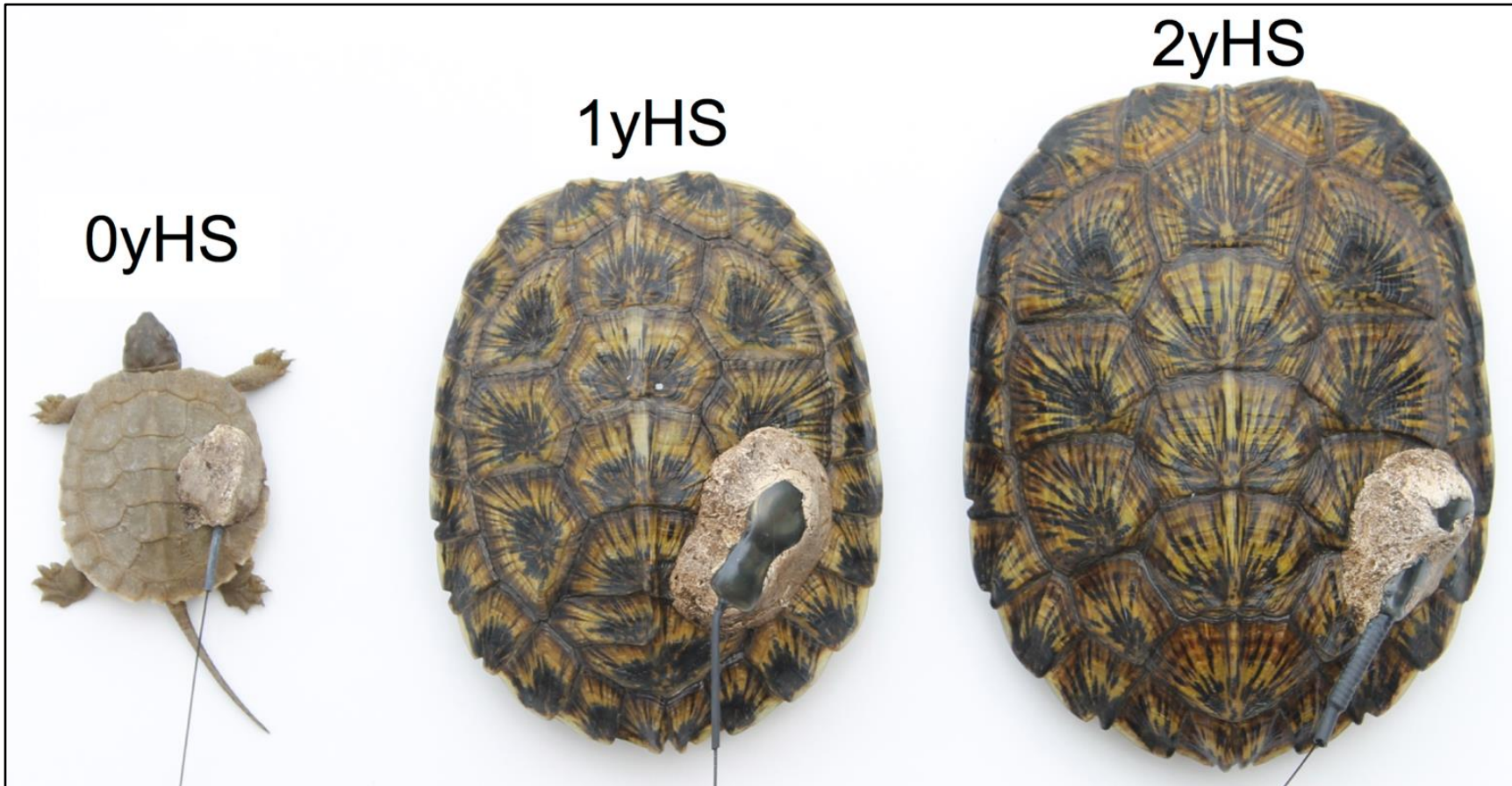


Figure 1.1. Relative sizes of each of the three experimental cohorts of Wood Turtles (*Glyptemys insculpta*) with varying degrees of headstarting shown using 1 representative individual per cohort. An individual with the closest mass (g) and SCL (mm) to the mean of the cohort was chosen as the representative for the cohort. Fifteen turtles per cohort were randomly selected and outfitted with a VHF radio-transmitter (pictured) and radio-tracked.

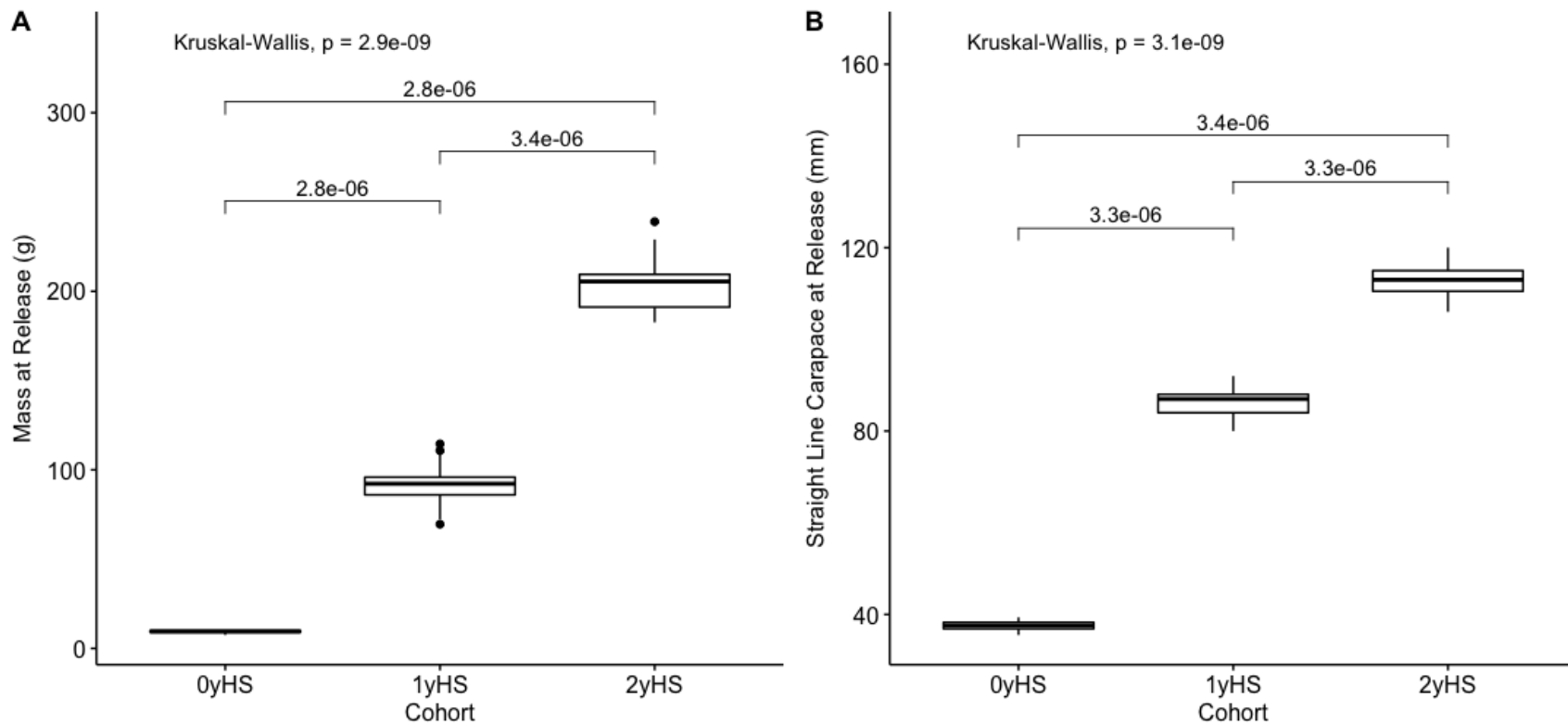


Figure 1.2. Comparisons of size at release for the experimental cohorts (2yHS, 1yHS, and 0yHS) of Wood Turtles (*Glyptemys insculpta*). Boxes display the median line between the 25th and 75th quartiles, whiskers extend to the minimum and maximum of the dataset, and black-filled circles indicate outliers. The statistical test across cohorts is overarching the boxplot with horizontal lines showing pairwise comparisons between cohorts (p-values indicated). A) Whole body mass (grams) at release. B) Straight carapace length (mm).

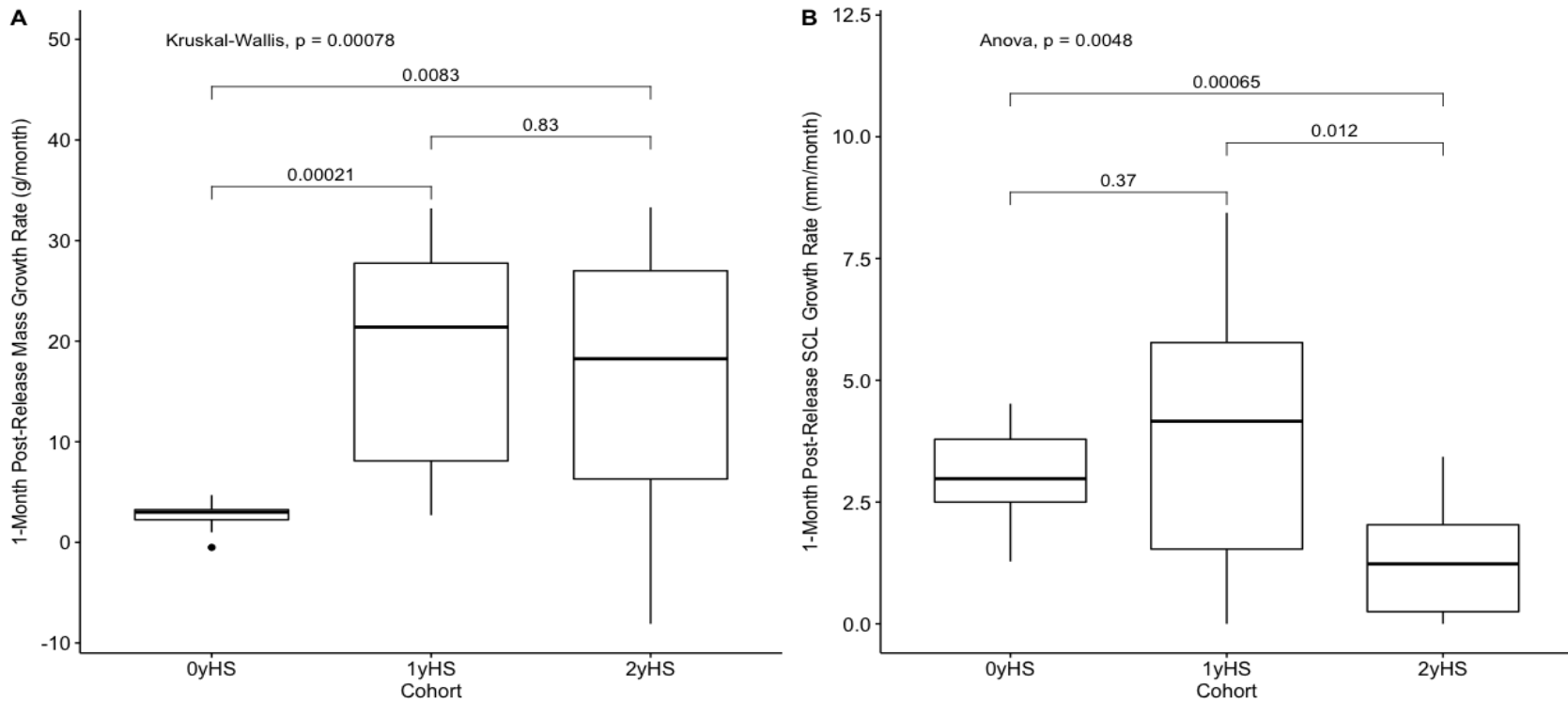


Figure 1.3. Comparison of one-month post-release growth rates for the experimental cohorts (2yHS, 1yHS, and 0yHS) of Wood Turtles (*Glyptemys insculpta*). Boxes display the median line between the 25th and 75th quartiles, whiskers extend to the minimum and maximum of the dataset, and black-filled circles indicate outliers. The statistical test across cohorts is overarching the boxplot with horizontal lines showing pairwise comparisons between cohorts (p-values indicated). A) One-month post-release mass growth rate (grams). B) One-month post-release straight carapace length (mm) growth rate.

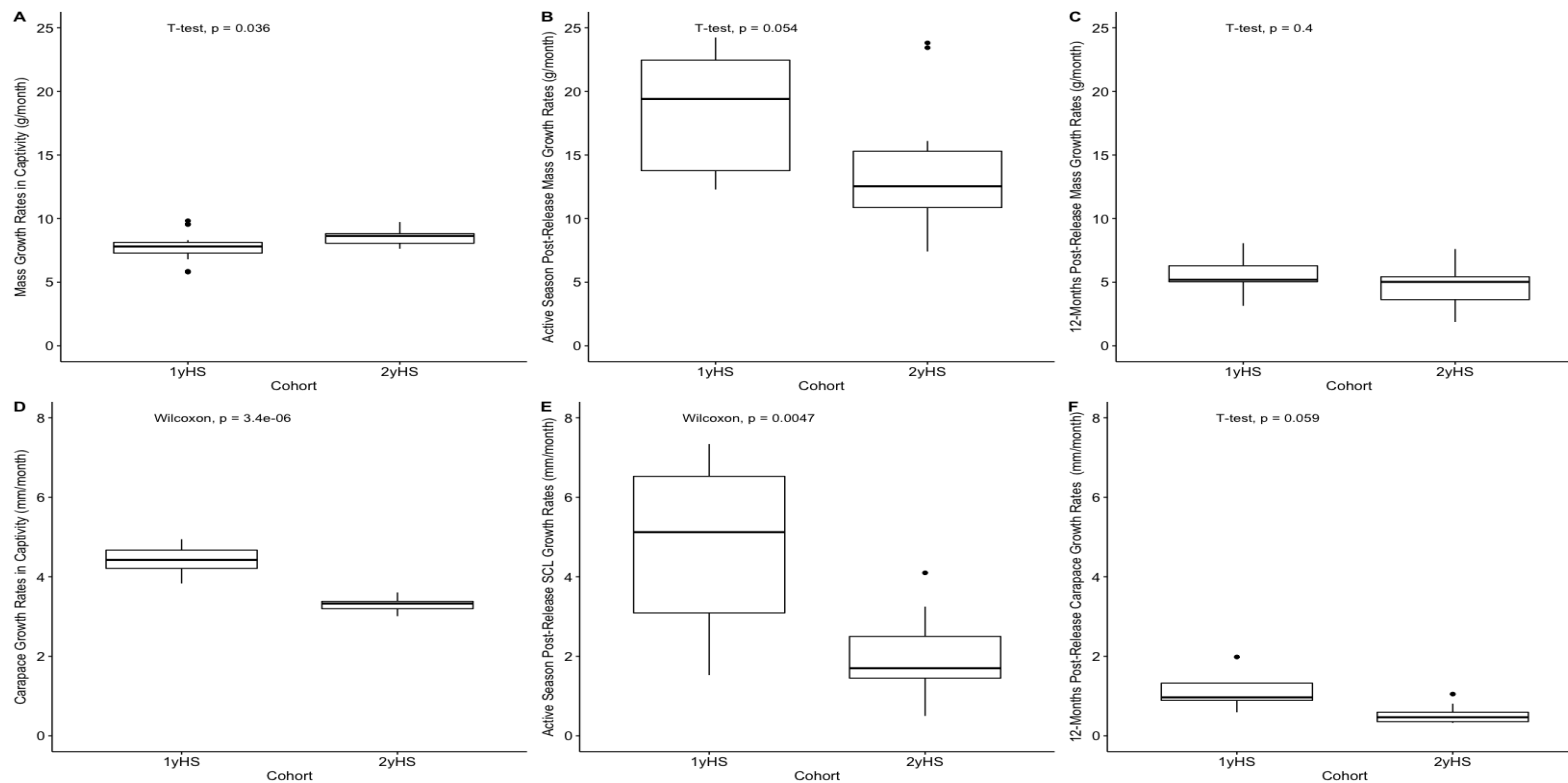


Figure 1.4. Comparisons of growth rates for the experimental cohorts 1yHS and 2yHS Wood Turtles (*Glyptemys insculpta*), over various time scales. Boxes display the median line between the 25th and 75th quartiles, the whiskers extending to the minimum and maximum of the dataset, and outliers as the black-filled circles. The statistical test between cohorts is overarching the boxplot. A) Mass growth rates in captivity. B) Mass growth rates in the active season post-release. C) Mass growth rates 12-month post-release. D) Straight carapace length growth rates in captivity. E) Straight carapace length growth rates in the active season post-release. F) Straight carapace length growth rates 12-months post release.

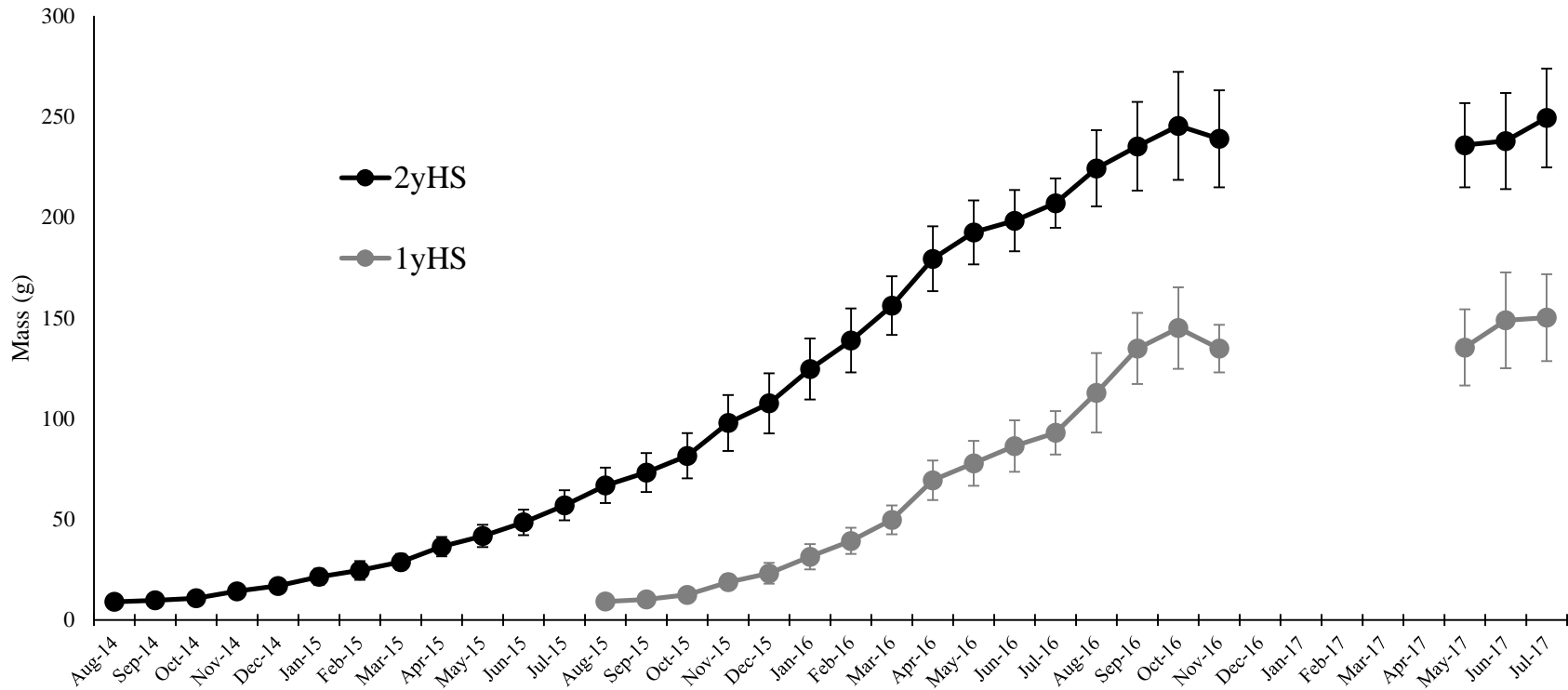


Figure 1.5. Mean monthly mass (g; +/- SD) of 2yHS and 1yHS Wood Turtles (*Glyptemys insculpta*) over time in captivity, then once released into the wild on 24 June 2016. No morphometric measurements were taken during overwintering thus explaining the gap in data between December 2016 and April 2017.

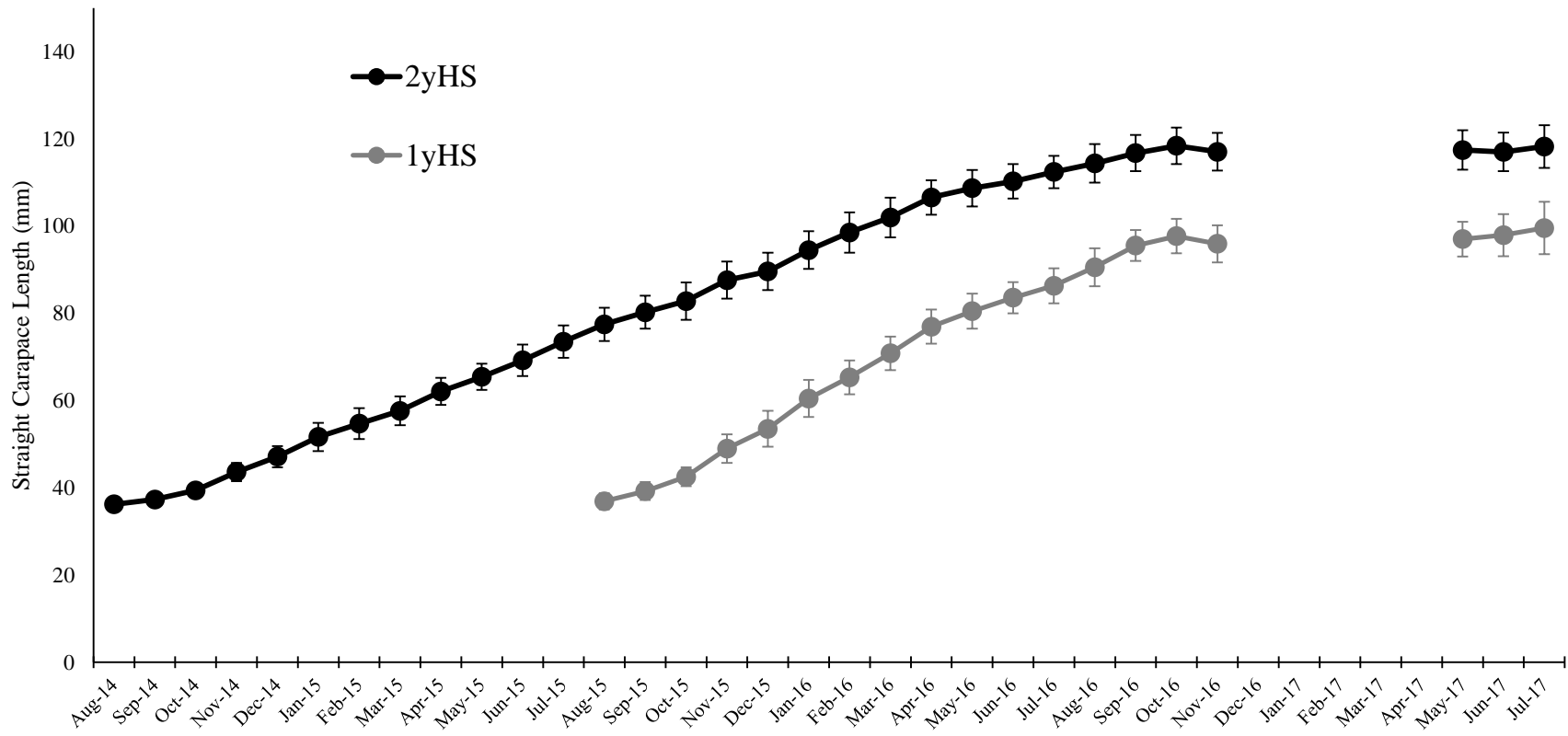


Figure 1.6. Mean monthly straight carapace length (SCL, mm; +/- SD) of 2yHS and 1yHS Wood Turtles (*Glyptemys insculpta*) over time in captivity, then once released into the wild on 24 June 2016. No morphometric measurements were taken during overwintering thus explaining the gap in data between Dec 2016 and April 2017.

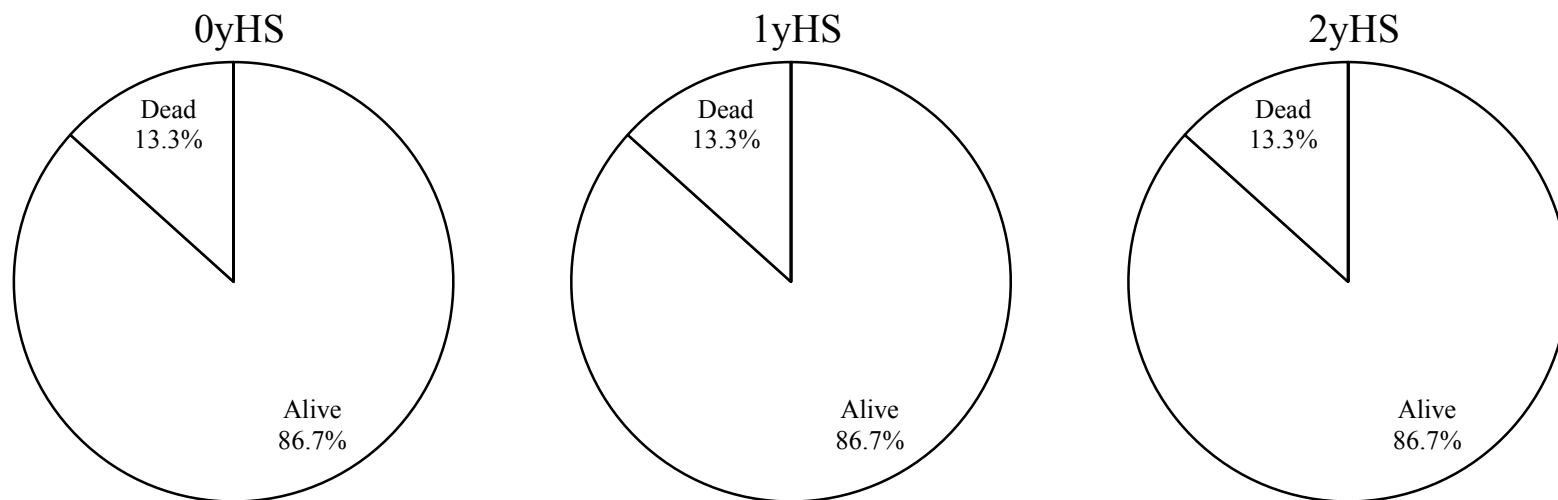


Figure 1.7. One-month post-release known-fates for the experimental cohorts (0yHS, 1yHS and 2yHS; n=15 for each cohort) of Wood Turtles (*Glyptemys insculpta*).

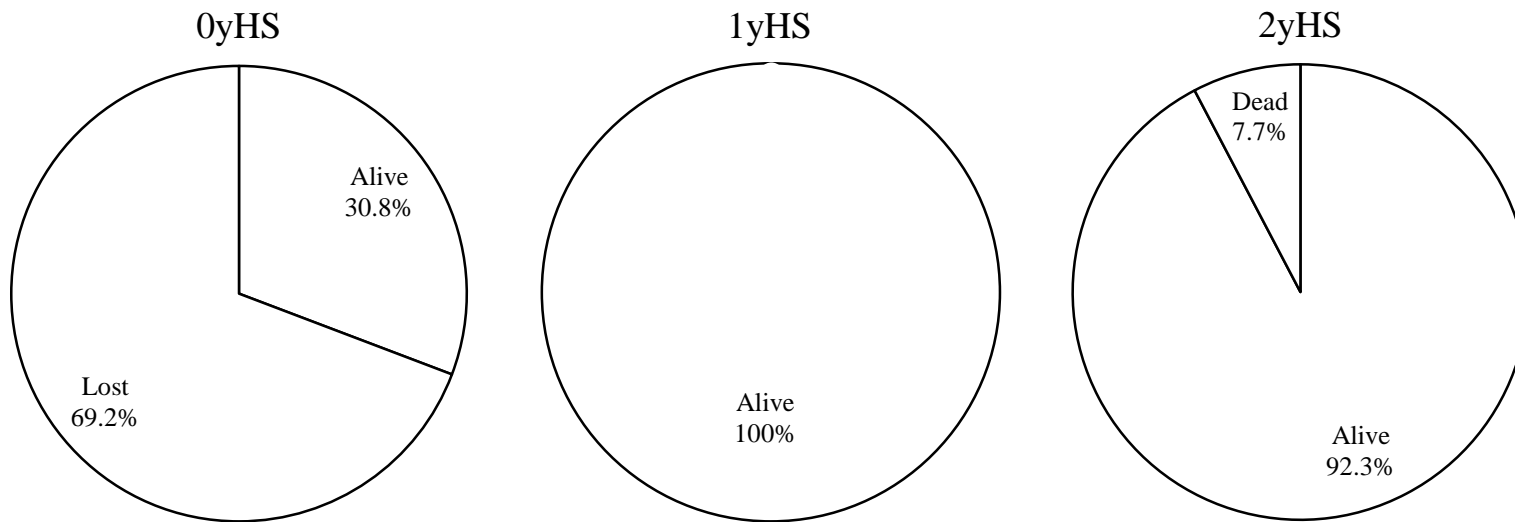


Figure 1.8. Overwintering survivorship of the experimental cohorts (0yHS, n=13; 1yHS, n=12; 2yHS, n=13) of Wood Turtles (*Glyptemys insculpta*). Known-fate overwintering survivorship of 1yHS and 2yHS and mark-recapture minimum overwintering survivorship values of 0yHS.

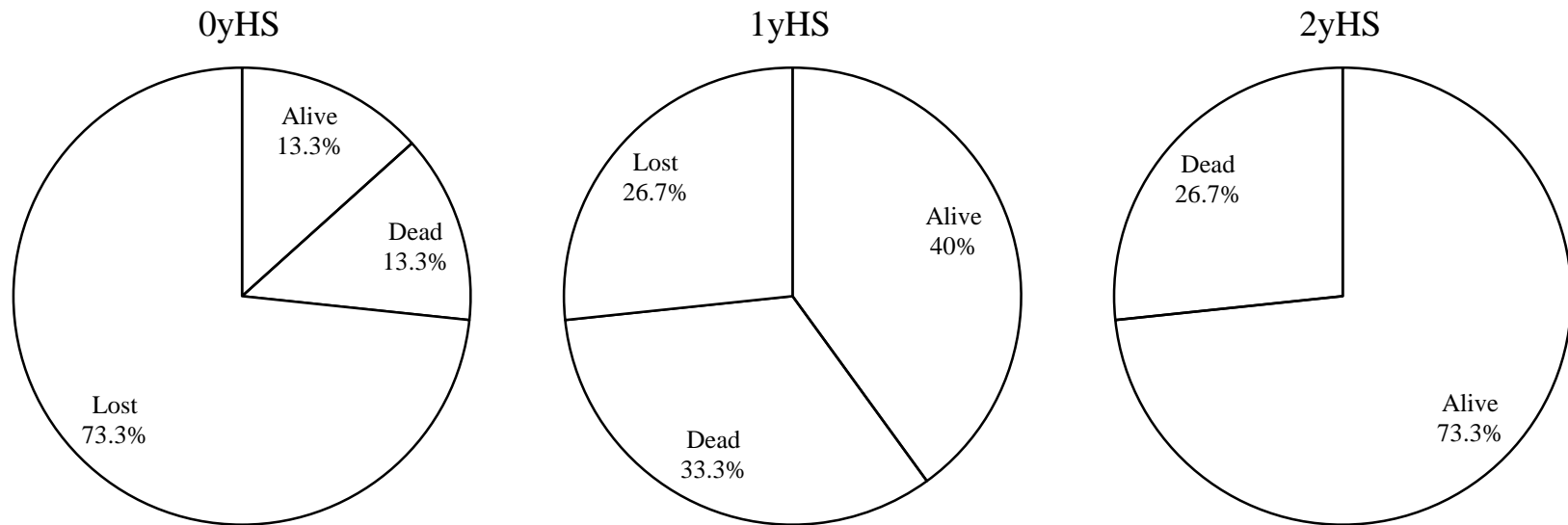


Figure 1.9. Twelve-month post-release survival of the experimental cohorts (0yHS, 1yHS and 2yHS; n=15 for each cohort) of Wood Turtles (*Glyptemys insculpta*). Twelve-month post-release known-fates of 1yHS and 2yHS and mark-recapture minimum survivorship values of 0yHS. Each cohort has a sample size of 15 turtles.

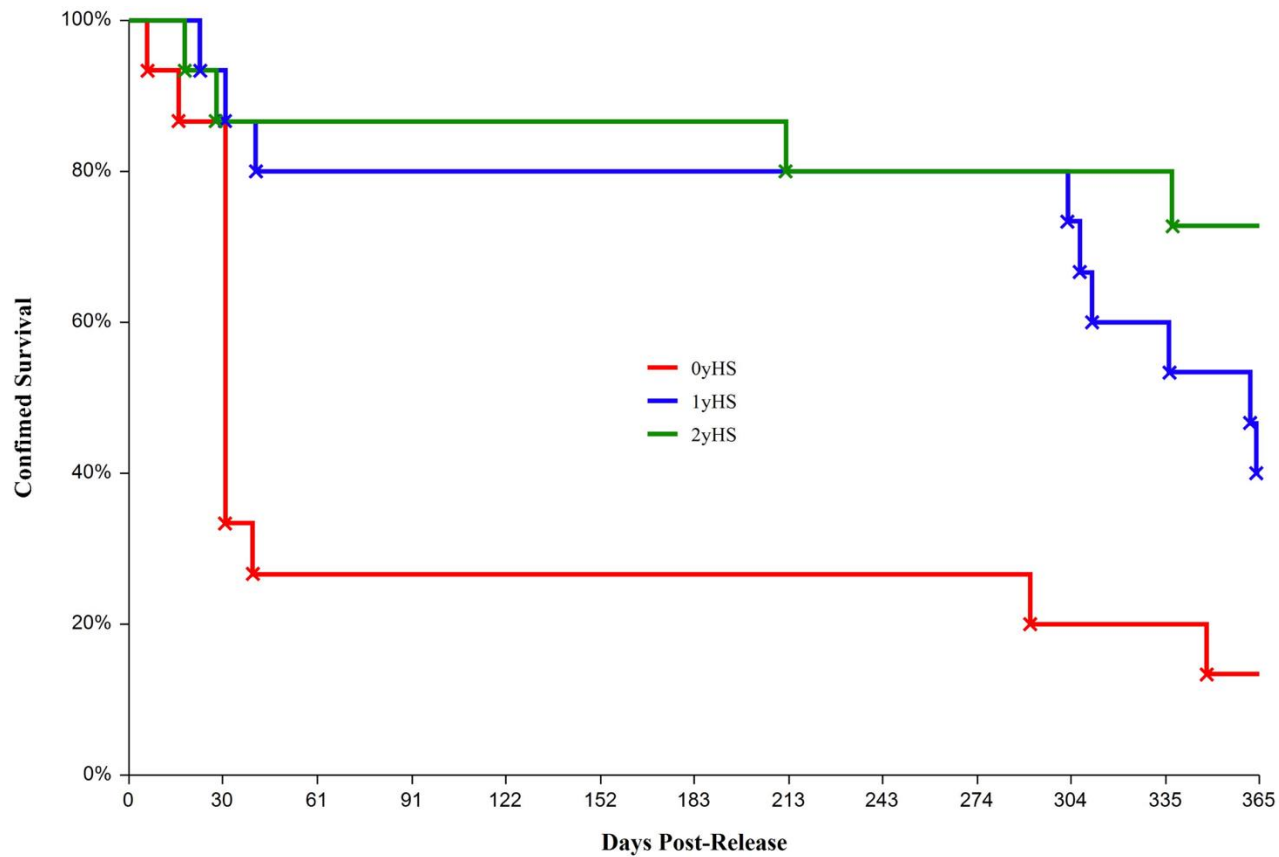


Figure 1.10. Kaplan-Meier survival analysis of the experimental cohorts (2yHS, 1yHS, 0yHS) of Wood Turtles (*Glyptemys insculpta*) over their tracking period using NCSS (2016). Dates on which turtles were lost or confirmed dead are marked with an X. I used known-fate VHF radio-telemetry data for 1yHS and 2yHS as they were tracked for 12-months (365 days) post-release. I used known-fate data for the 1-month (30 days) post-release, then used mark-recapture data as a conservative estimate for survival data for 0yHS.

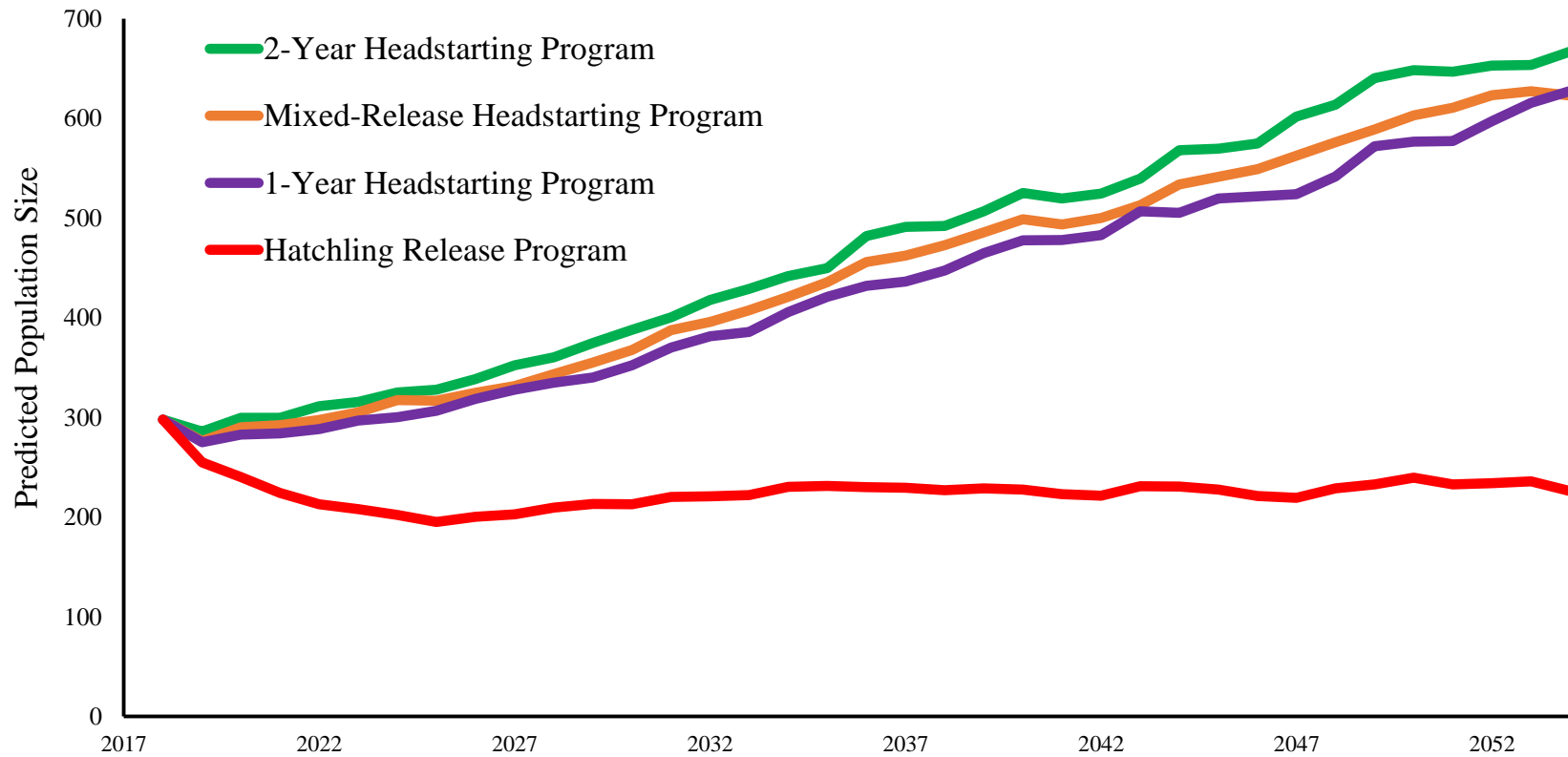


Figure 1.11. Population viability analysis model of projected population size (1000 iterations) of Wood Turtle (*Glyptemys insculpta*) population recovery using a variety of headstarting scenarios (2-Year Headstarting Program, Mixed-Release Headstarting Program, 1-Year Headstarting Program, and Hatchling Release Program) projected 36 years from present (2017), thus 50 years after the headstarting program began (2003-2053) using VORTEX. All 4 models presented are slight alterations to a base model created from population-specific life-history data where possible, and complemented by data in the literature.

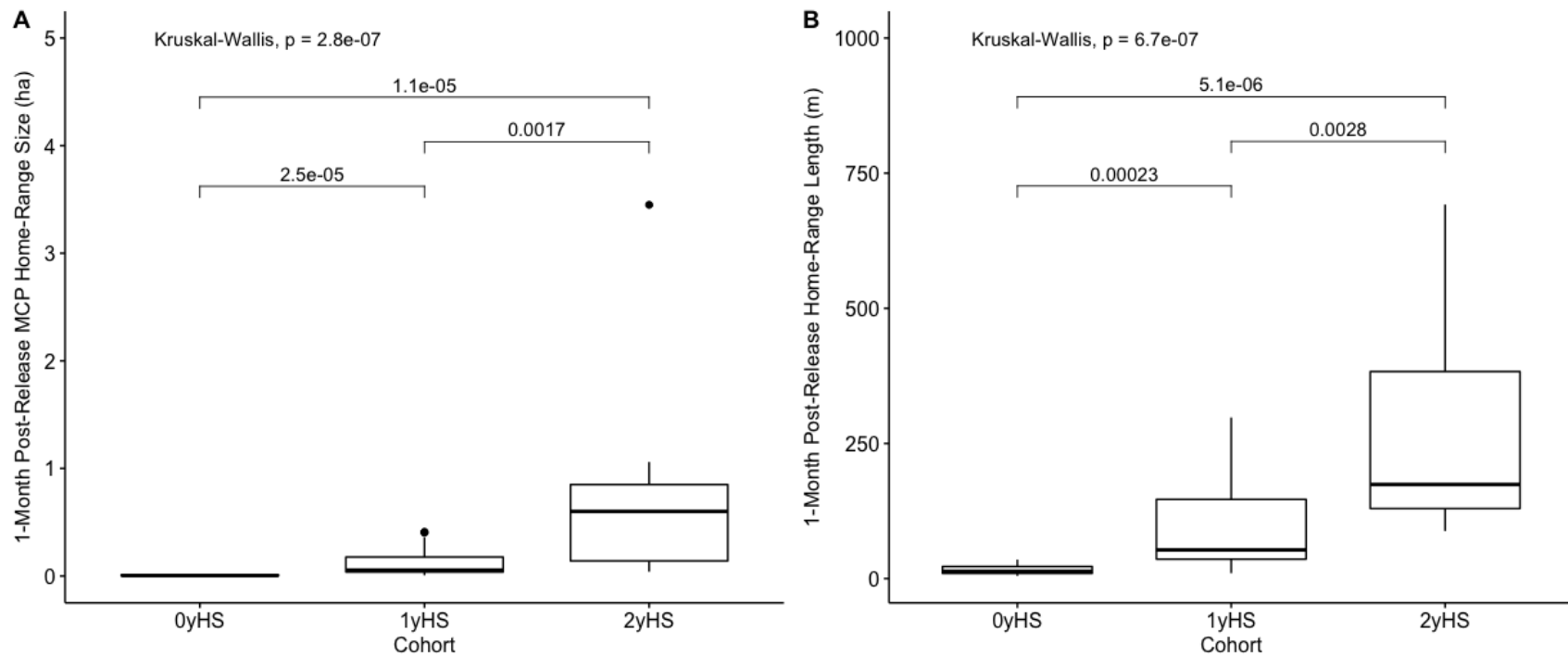


Figure 1.12. Comparisons of one-month post-release spatial behaviour metrics of the experimental cohorts (2yHS, 1yHS, and 0yHS) of Wood Turtles (*Glyptemys insculpta*). Boxes display the median line between the 25th and 75th quartiles, whiskers extend to the minimum and maximum of the dataset, and black-filled circles indicate outliers. Statistical test across cohorts is overarching the boxplot with horizontal lines showing pairwise comparisons between cohorts (p-values indicated). A) One-month post-release home-range sizes (ha) calculated using 100% minimum convex polygons (MCP). B) One-month post-release home-range length calculated as distance within the river between the furthest point downstream, and the furthest point upstream (m).

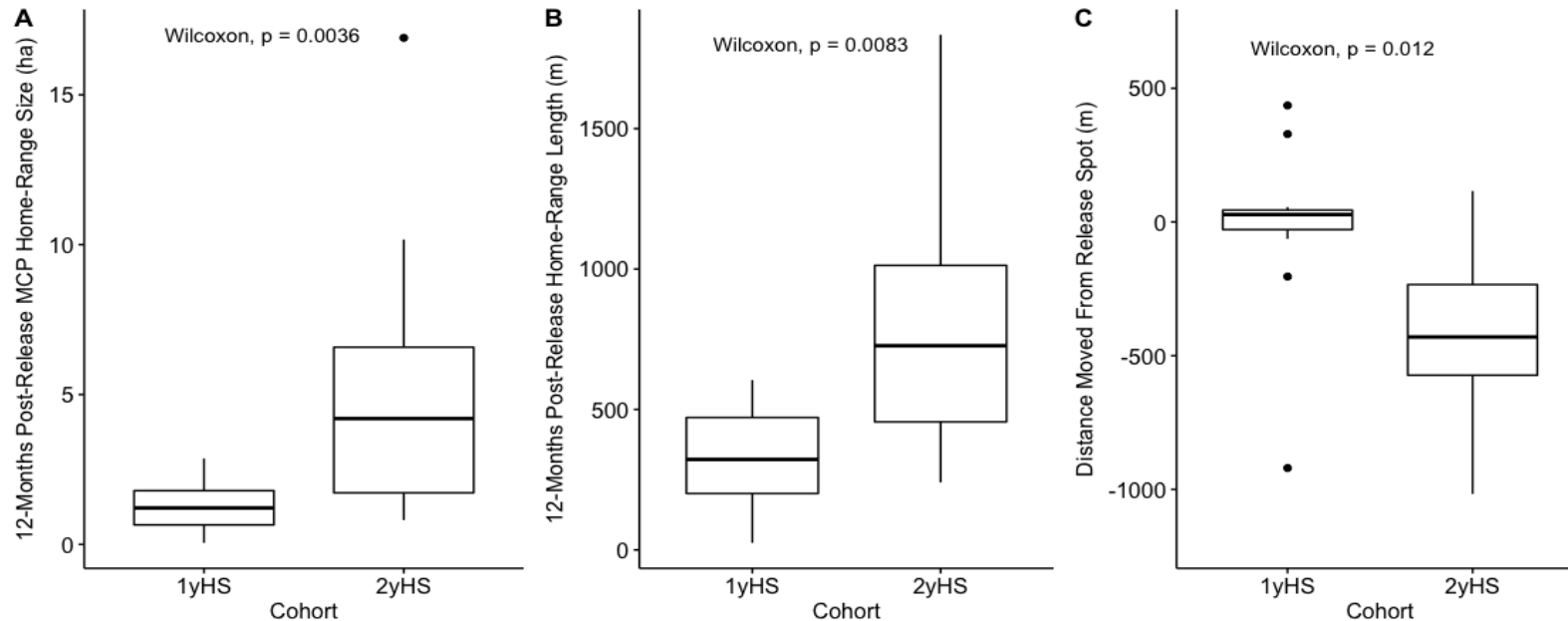


Figure 1.13. Comparisons of twelve-month post-release spatial behaviour metrics of the experimental cohorts of 2yHS and 1yHS Wood Turtles (*Glyptemys insculpta*). Boxes display the median line between the 25th and 75th quartiles, the whiskers extending to the minimum and maximum of the dataset, and outliers as the black-filled circles. The statistical test between cohorts is overarching the boxplot. A) Twelve-months post-release home-range sizes (ha) calculated using 100% minimum convex polygons (MCP). B) Twelve-months post-release home-range length calculated as distance within the river between the furthest point downstream, and the furthest point upstream (m). C) Twelve-months post-release distance (m) from their release location, positive values are coded for upstream movements while negative values are coded for downstream movements – thus all turtles with positive values moved upstream post-release, while all turtles with negative values moved downstream post-release

Table 1.1. Summary of the three experimental cohorts (2yHS, 1yHS, 0yHS) within the study. A subset (n=45; 15 randomly selected per cohort) from these Wood Turtle (*Glyptemys insculpta*) cohorts were outfitted with VHF radio-telemetry and tracked.

Cohort	Headstarting Duration (months)	Cohort Hatch Year	Release Date	Tracking Duration (months)
2-year headstarts (2yHS)	22	2014	24 June 2016	12
1-year headstarts (1yHS)	10	2015	24 June 2016	12
Direct-release hatchlings (0yHS)	0	2016	2 Aug 2016	1

Table 1.2. Headstarting management release scenarios modelled. The headstarting facility has the capacity to hold 100 individuals. I estimated approximately 100 turtles are hatched per year. Each model is a different configuration within these limitations. The 2-Year Headstarting Program was designed to release the maximum number of turtles headstarted for 2 years, while releasing the rest as hatchlings. The Mixed-Release Headstarting Program was designed to release approximately the same number of individuals in each cohort (hatchlings, turtles headstarted for 1-year, and turtles headstarted for 2-years). The 1-Year Headstarting Program was designed to release the maximum number of turtles headstarted for 1-year. The Hatchling Release Program was designed to release the maximum number of hatchling turtles, thus acting as a control for efforts of artificial incubation and release, without headstarting.

Model	Hatchlings Released	1-Year Headstarts Released	2-Year Headstarts Released
2-Year Headstarting Program (2yHS)	50	0	50
Mixed Release Program	32	34	32
1-Year Headstarting Program (1yHS)	0	100	0
Hatchling Release Program (0yHS)	100	0	0

Table 1.3. Wood Turtle (*Glyptemys insculpta*) life-history parameters for Base Model in population viability analyses using VORTEX. The Base Model represents current population demographics. Nest survivorship values are excluded given all eggs are artificially incubated. Source of parameter value is from this study, unless otherwise noted. Values were calculated based on previous studies, and are conservative estimates.

Parameter	Values	Source
Years Projected	36	50 years of headstarting (2003-2053)
Iterations per Model	1000	
Population Size	298	
Age Distribution (1-5years)	240	
Age Distribution (6-10years)	21	
Age Distribution (11-60 years)	37	
Adult Sex Ratio	1M:2F (11M:26F)	
Carrying Capacity (K)	none	Brooks <i>et al.</i> (1991a)
Breeding System	Polygynous	Galbraith (1991)
Adult Females Breeding (%)	60	
Adult Males Breeding (%)	75	Ernst and Lovich (2009)
Max Number of Clutches Per Year	1	
Clutch Size (SD)	6.63(3)	Mean number of eggs hatched per clutch
Maximum Clutch Size (eggs)	12	
Male Age at Sexual Maturity	11	
Female Age at Sexual Maturity	12	
Maximum Age of Reproduction	70	Jones (2009)
Maximum Lifespan	70	Jones (2009)
Sex Ratio (eggs)	1:1	assumed
Mortality Rates (%)		
From age 0 to 1	80 (SD=27)	Coarse estimate from the 0yHS survival data
From age 1 to 2	60 (SD=27)	1yHS minimum survivorship
From age 2 to 3	27 (SD=27)	2yHS confirmed survivorship
From age 3 to 10	18 (SD=27)	Saumure <i>et al.</i> (2007), Pike <i>et al.</i> (2008), Jones (2009)
From age 11 to 70	4 (SD=5)	

Table 1.4. Comparison of age-related Wood Turtle (*Glyptemys insculpta*) mean morphometric data between my experimental cohorts at release (0yHS, 1yHS, 2yHS), within this population from a previous study before the headstarting project began (Brooks *et al.* unpubl. data) and a non-headstarted population in New Jersey, USA (Farrell and Graham 1991). This study has known-age turtles while the reports from Brooks *et al.* (unpubl. data) and Farrell and Graham (1991) are for ages estimated using growth lines. For age, H denotes hatchling and the number denote years of age.

<i>Study</i>	<i>Age</i>	<i>n</i>	<i>Mean Mass (g)</i>	<i>Mean Straight Carapace Length (mm)</i>	<i>Mean Straight Plastron Length (mm)</i>
<i>This study</i>	H	15	10.3	37.6	30.2
	1	15	92.2	85.7	73.2
	2	15	203.7	112.4	98.0
<i>Brooks et al. unpubl. data</i>	H	11	7.5	37.8	37.3
	1	1	40.0	59.9	56.5
	2	2	50.0	68.0	61.6
	3	1	150.0	109.0	84.4
	4	9	183.3	102.8	83.8
	5	5	250.0	115.6	95.0
	6	6	276.7	125.9	99.3
<i>Farrell and Graham 1991</i>	H	10			28.2
	1	23			51.9
	2	30			69.9
	3	26			82.8
	4	15			92.4
	5	12			100.5
	6	11			102.9

Table 1.5. Multiple comparisons of somatic growth rates (SGR) between various time periods between the 2yHS and 1yHS cohorts. Greater than symbols are used to show which SGR is larger, followed by test statistic, degrees of freedom, then p-values. Significance was accepted at $p < 0.005$ to correct for multiple comparisons. Boxplots of growth rates between cohorts are in Figure 1.6.

Comparison	T	df	P
<i>Turtles Headstarted for 2 Years (2yHS)</i>			
<i>Mass</i>			
Captive Growth Rates < Active Season Growth Rates	-3.6	11	0.004
Captive Growth Rates > 12-Months Post-Release Growth Rates	6.9	9	<0.001
<i>Straight Carapace Length</i>			
Captive Growth Rates > Active Season Growth Rates	4.97	12	<0.001
Captive Growth Rates > 12-Months Post-Release Growth Rates	29.7	9	<0.001
<i>Turtles Headstarted for 1 Year (1yHS)</i>			
<i>Mass</i>			
Captive Growth Rates < Active Season Growth Rates	-7.02	9	<0.001
Captive Growth Rates > 12-Months Post-Release Growth Rates	2.6	5	0.046
<i>Straight Carapace Length</i>			
Captive Growth Rates > Active Season Growth Rates	-0.25	9	0.81
Captive Growth Rates > 12-Months Post-Release Growth Rates	10.1	4	<0.001

Table 1.6. Fates and suspected causes of death of turtles from the experimental cohorts 12-months post-release. Survival time (days) is from release (see Table 1.1) to the day I located the carcass, or the day I confirmed losing the turtle. I included lost 1yHS and 2yHS in this table, as their VHF transmitters should have allowed them to be tracked for 12-months post-release. I did not include lost 0yHS as many were likely lost due to transmitter battery failure. I noted suspected causes of death, as I cannot be confident in any observation without a visual of the predation event (i.e. a turtle could have died from disease/drowning then gotten scavenged by a predator and found on land by researchers). Table is sorted by cohort, then by survival time (days).

Cohort	Notch	Fate	Survival Time (days)	Suspected Cause of Death
0yHS	713	Dead	6	Predation; Piece of carapace found under deadfall wood
0yHS	608	Dead	16	Predation; Transmitter and piece of carapace in a small mammal burrow
1yHS	981	Dead	23	Predation; head and limbs eaten, body cavity intact
1yHS	950	Dead	24	Predation; head and limbs eaten, body cavity intact
1yHS	949	Lost	39	Predation; No turtle found, but transmitter was damaged
1yHS	962	Dead	303	Predation; Found scavenged with no tissue remaining
1yHS	1001	Lost	307	Predation; No turtle found, but transmitter was damaged
1yHS	1008	Lost	311	Unknown; lost turtle with no signal
1yHS	979	Dead	336	Predation; head and forepart of limbs eaten, body cavity intact
1yHS	971	Dead	362	Predation; head and limbs eaten, body cavity intact
1yHS	1018	Lost	363	Predation; No turtle found, but transmitter had bite marks on it
2yHS	874	Dead	17	Predation; head and limbs eaten, body cavity intact
2yHS	861	Dead	27	Predation; found missing head and forelimbs but rear legs were intact
2yHS	884	Dead	211	Predation; found missing head and limbs, found near a predated adult
2yHS	850	Dead	336	Predation; head and limbs eaten, body cavity intact

Table 1.7. Elasticity analyses to evaluate deviations in the intrinsic rate of population increase (r) of the Mixed Headstarting Program from the Base Model due to a variety of parameter changes. Parameters are sorted by causing largest decreases in population growth rate, to largest increases in population growth rate. Changes in population growth rate reflect the changes in the population growth rate between the Mixed Headstarting Program model and the Mixed Headstarting Program model + specified parameter change. Changes in predicted population size reflect the change in predicted population size between Mixed Headstarting Program model and Mixed Headstarting Program model + specified parameter change.

Parameter Change	Change in Population Growth Rate	Change in Predicted Population Size
Adult Mortality Rates Increase to 20%	-0.012	-414
Adult Mortality Rates Increase to 10%	-0.012	-283
Juvenile Mortality Rates Increase to 30%	-0.005	-393
40% if Adult Females Breed Annually	-0.005	-144
Fertilized Eggs/Clutch Decreases 25%	-0.005	-131
Juvenile Mortality Rates Increase to 24%	-0.004	-268
Lifespan Increases to 80 Years	-0.002	-72
Age at Sexual Maturity Increases by 1	-0.002	-59
Age at Sexual Maturity Increases by 2	-0.002	-48
Lifespan Drops to 60 Years	-0.001	-33
Lifespan Drops to 50 Years	-0.001	-18
Fertilized Eggs/Clutch Increases 25%	-0.001	+3
Age at Sexual Maturity Decreases by 1	0	+21
80% of Adult Females Breed	0	+25
BASE MODEL	N/A	N/A
Lifespan Increases to 90	0	-37
Stable Age Distribution	0.001	+17
Age at Sexual Maturity Decreases by 2	0.001	+41
Adult Mortality Rates Decrease to 2%	0.004	+117
Juvenile Mortality Rates Decrease to 12%	0.011	+1032
Juvenile Mortality Rates Decrease to 6%	0.025	+344

Chapter II

Mixed Results of Population Recovery for an Endangered Turtle after a 15-year Headstarting Program

Chapter II: Mixed Results of Population Recovery for an Endangered Turtle after a 15-year Headstarting Program

Abstract: Two populations (PopA and PopB) of endangered Wood Turtles (*Glyptemys insculpta*) were studied extensively beginning in 1988. The populations sharply declined by approximately 70% in the mid 1990s. A population viability analysis (PVA) predicted the extirpation of both populations if no intervention was undertaken, and so a headstarting project was initiated in 2003 and the first cohort was released in 2005. My objective was to quantitatively assess the effectiveness of a 15-year headstarting program by modeling population specific demographic parameters to evaluate recovery efforts to date, and determine the next phase of recovery. To date, 123 and 330 headstarted turtles have been released at PopA and PopB, respectively. PopA has continued to decline despite headstarting efforts and now has a population size of approximately 18 turtles (11 adults, 7 juveniles). PopB has partially recovered and now has a population size of approximately 117 turtles (31 adults, 86 juveniles). PopA and PopB had relatively high adult annual apparent survivorship (89%, 93%) but low 1-year post-release annual apparent survivorship of headstarted turtles (36%, 52%). A PVA predicted that PopA will continue to slowly decline whereas PopB will slowly recover. However, a secondary PVA also predicted that both populations may recover if a predator-reduction strategy which increases juvenile and adult survivorship is incorporated alongside the headstarting program. Six headstarted turtles have reproduced suggesting both populations may become self-sustaining. Mycotic shell disease, ranavirus, and *Glyptemys herpesvirus-2* have recently been discovered in the populations. Overall, headstarting alone is not enough to save both populations from local extinction as these populations face multi-faceted problems for which management is challenging. I thus make 7 recommendations to enhance the success of this project. Modeling population recovery scenarios with comprehensive long-term data is essential for evaluating the effectiveness of headstarting projects, while continuously improving recovery efforts through adaptive management.

Introduction

Large declines in biodiversity are now almost routinely reported within the literature with the World Wildlife Foundation (2018) reporting that vertebrate populations have declined by 60% since the 1970s, while freshwater populations more specifically have declined by 83%. This global decline in biodiversity has caused some to suggest that we are entering into a 6th mass extinction (Ceballos *et al.* 2015). Given the importance of biodiversity to human life (United Nations 2017, Ripple *et al.* 2017), the recovery and conservation of the natural world should be a top priority. Recovery strategies vary in scope and scale, and there is much debate about the most efficient use of conservation funds (Martin *et al.* 2018). Recovery strategies featuring

intervention ecology in which humans actively manage ecological systems are growing in popularity (Hobbs *et al.* 2011). A review by Fischer and Lindenmayer (2000), however, reported that only 47% of 180 published animal relocation studies (includes reintroductions, supplementation, and translocations) were considered “successful”. Using limited conservation funds on ineffective management strategies is not a sustainable solution for protecting global biodiversity. Conservation programs must be well-informed by evidence-based methods to ensure best management practices are established (IUCN/SSC 2013, Bennett *et al.* 2017).

Turtles are of great conservation concern as approximately 61% of all species are listed as globally threatened with extinction (Turtle Taxonomy Working Group 2014, Lovich *et al.* 2018). These declines are substantial as turtles not only have important ecological roles, but also have central roles in human culture. Turtle recovery strategies vary widely (Bennett *et al.* 2017), and increasingly integrate aspects of intervention ecology. One highly controversial conservation strategy that is widely used in the recovery of turtle populations is called headstarting. Turtle headstarting most often includes collecting and artificially incubating eggs, then rearing the hatchling turtles in captivity to a larger body size, followed by releasing them back into their natural habitat where their survivorship is assumed to increase compared to their wild-counterparts (Heppell *et al.* 1996, Haskell *et al.* 1996, Seigel and Dodd 2000, Vander Haegen *et al.* 2009, Eiby and Booth 2011, Bona *et al.* 2012).

Headstarting is often perceived to be the last resort in saving populations of critically endangered species (Milinkovitch *et al.* 2013, Burke 2015). A poorly executed headstarting project could further endanger imperiled species while also wasting limited conservation funds. Effective conservation programs must consider the life history of the species (Frazer 1992). Turtles have evolved a suite of life-history characteristics that includes long lifespans, high

survivorship of sexually mature individuals, generally low but stochastic survivorship of sexually immature individuals (eggs, hatchlings and juveniles), iteroparity, and delayed sexual maturity (Brooks *et al.* 1991, Congdon *et al.* 1993, Keevil *et al.* 2018, Spencer 2018). Multiple mathematical models have shown that because of this life history, turtle populations cannot sustain chronic increases in mortality rates of sexually reproductive adults (Heppell *et al.* 1996, Gibbs and Shriver 2002, Enneson and Litzgus 2008, Mitrus 2008, Spencer *et al.* 2017, Keevil *et al.* 2018). Maintaining high adult survivorship should hence be the primary focus for turtle conservation projects. However, even with high adult survivorship, turtles are ill-adapted to recover from catastrophic declines as turtles likely lack density-dependent responses to population declines (Brooks *et al.* 1991, Keevil *et al.* 2018). A prime example is that of a population of Snapping Turtles (*Chelydra serpentina*) which experienced a mass-mortality event from predation and has shown no signs of recovery after 23 years (Keevil *et al.* 2018). Small populations are a conservation concern as risk of extinction (through environmental stochasticity, demographic stochasticity, and genetic deterioration) increases with a decreasing population size (Lynch *et al.* 1995). Turtles may, however, be able to buffer some of these extinctions risks, as Kuo and Janzen (2004) reported that Ornate Box Turtles (*Terrapene ornata*) have relatively slow rates of genetic drift, though genetic deterioration may also be hard to detect in turtles because of their long generation times (Bennett *et al.* 2010, Kimble *et al.* 2014). Contrasting to these perceived threats from small population sizes, Shoemaker *et al.* (2013) predicted through demographic modelling that small populations (<50 individuals) of Bog Turtles (*Glyptemys muhlenbergii*) likely would persist for multiple generations in a stable environment. Regardless, larger population sizes are generally more desirable given increased stability when populations are faced with naturally occurring stochasticity. Hence, increasing population size by increasing

population growth rate with headstarting is an attractive tool for wildlife managers. Headstarting is, however, considered an experimental conservation strategy given uncertainties regarding its effectiveness (Seigel and Dodd 2000, Buhlmann *et al.* 2015, Bennett *et al.* 2017).

Headstarting programs fundamentally rely on the assumption that headstarting increases juvenile survivorship, which consequently should increase recruitment into a population, thereby increasing population growth rate relative to wild recruitment. Several short-term studies have provided support that headstarted turtles have relatively high post-release survivorship (Haskell *et al.* 1996, Vander Haegen *et al.* 2009, Bona *et al.* 2012, Michell and Michell 2015, Tuberville *et al.* 2015); however, these studies are missing essential control groups of non-headstarted juveniles of similar age classes (but see Chapter I). Evidence of long-term population recovery via headstarting was quite rare until quite recently (Milinkovitch *et al.* 2013, Shaver and Caillouet 2015, Spencer *et al.* 2017, Dreslik *et al.* 2017). There are also a few longer-term studies that have determined that populations have not recovered despite headstarting efforts. In one such study, headstarting was used but threats to adult survivorship were not mitigated (Frazer 1992), thus the turtle population continued to decline despite intensive headstarting efforts (Smeenk 2010). This highlights the importance of using headstarting only as a supplementary strategy in stable environments. There are also examples of turtle populations continuing to decline despite intensive headstarting efforts due to unforeseen novel problems such as increased predation rates (Dreslik *et al.* 2017). It is possible that the increased predation rates reported by Dreslik *et al.* (2017) were an effect of captive rearing; hence the necessity of wild controls in studies on headstarted turtles (Bennett *et al.* 2017). There is also concern that headstarting may impair behaviour, physiology, and health (Woody 1990, Frazer 1992) which could affect short, and long-term survival. For example, a Western Pond Turtle (*Actinemys*

marmorata) headstarting project which previously reported on short-term recovery (Vander Haegen *et al.* 2009) is now reporting on novel disease outbreaks which are limiting long-term recovery goals (Hallock *et al.* 2017).

Turtles have delayed sexual maturity and long lifespans (Brooks *et al.* 1991, Congdon *et al.* 1993) which means detecting a stable population of sexually reproductive headstarted adult turtles could require several decades of research. These life-history attributes may prevent wildlife managers from gathering data to evaluate the long-term effects of headstarting on individual health, which has generated criticisms of headstarting (Woody 1990, Frazer 1992, Heppell *et al.* 1996, Seigel and Dodd 2000). A recent study revealed that translocated non-headstarted adult male Desert Tortoises (*Gopherus agassizii*) did not sire any offspring four years post-translocation (Mulder *et al.* 2017), suggesting that population manipulations for conservation, such as translocations and/or headstarting, can potentially have detrimental effects that could go undetected for many years after a project has started. Long-term studies investigating the possible impacts of these management practices on reproductive biology are thus critical to answer concerns over the possible negative impacts of headstarting.

This chapter presents the results of a 30-year Wood Turtle (*Glyptemys insculpta*, Endangered – IUCN (van Dijk and Harding 2011) mark-recapture study which has featured a headstarting component for the past 15 years. Two populations (PopA and PopB) of Wood Turtles have been studied since 1988. A suspected poaching event caused PopA's population size to decline from 162 turtles in 1993 to 26 turtles in 1998 (84% decline) and PopB's population size to decline from 107 turtles in 1993 to 52 turtles in 1998 (51% decline, Brooks *et al.* unpubl. data). A population viability analysis (PVA) conducted in 2001 predicted combined population extirpation within 100 years (Figure 2.1). The PVA also predicted that a headstarting

program would result the best chance recovery (Brooks *et al.* unpubl., Figure 2.1, Table 2.1). Thus, the headstarting program began with the collection of eggs in 2003, with the first release of headstarted turtles to their maternal streams in 2005. The headstarting project is currently in its 15th year (2003-2018). Here I use historical and contemporary data to report on population demography. I then use the population-specific demographic data alongside population modeling tools to quantitatively assess the effectiveness of headstarting as a recovery strategy for this population to date. From this, I make recommendations to enhance the success of this project

Methods

Study Site

Detailed information on the location of the study site will not be published as recommended by Litzgus and Brooks (1996) given that illegal collection for the pet trade is a major threat to Wood Turtle populations and poachers get species' location data in part from reading scientific manuscripts, reports, and publications (Lindenmayer and Scheele 2017). The two populations (PopA and PopB) of Wood Turtles are located approximately 6 km apart within a watershed in Ontario, Canada. Migration between populations is limited to a few individuals across the 30 years of our study. Watercourses are mostly small meandering creeks featuring a cobble bottom, with slow to moderate flow rate which is highly variable through seasonal changes (Appendix A). Riparian buffers between watercourses and agricultural land range from 2 to 300 m throughout the sites and are highly variable between sites and within sites. Most watercourses have large naturalized floodplains, which partially aid in managing the seasonal flood patterns. Most of the sites are on private land and the overall area is characterized by prime

agricultural lands which have shifted from pasture to cash crop through agricultural intensification over time.

Mark-Recapture Study

A mark-recapture study on the two (PopA and PopB) populations of Wood Turtles has been ongoing since 1988. Turtles were located using a combination of techniques including spring emergence, nesting, canine, and VHF radio-telemetry surveys. Survey effort has varied throughout the project, as is common in long-term studies. Turtles were marked using three methods for identification throughout the study: an “X” carved into the plastron (batch mark; used only from 1988-1989), triangular notches in the marginal scutes (Cagle 1939), and Passive Integrated Transponder (PIT) tags. Standard morphological measurements (max carapace length (MaxCL), midline carapace length (MidCL), max plastron length (MaxPL), mid plastron length (MidPL), carapace width (CW), carapace height (CH), mass), spatial data, general habitat data, behaviour, overall health, and basic thermal data were collected. The sex of adult turtles captured was determined using secondary sexual characteristics as described by Harding and Bloomer (1979). Dead turtles were thoroughly examined to determine possible cause of death.

Headstarting

Nesting and VHF radio-telemetry surveys were conducted from 2003 to the present, to locate gravid/nesting female Wood Turtles. Turtles were identified and processed post-oviposition to associate maternity with eggs collected. Nests were excavated and clutches of eggs were collected, then transferred to vermiculite and artificially incubated ex-situ. Hatchlings were initially stored on moist paper towel until they absorbed their yolk sacs. After absorbing yolk

sacs, hatchlings were processed, including morphological measurements and marking using marginal scute notches (hatchlings were too small for PIT tags). Most hatchling turtles were then sent to a captive rearing facility to be headstarted; though a small proportion of hatchlings were direct-released into the wild without headstarting. Most turtles were headstarted for 2 years although, headstarting time has varied between 1–4 years over the 15-year study. Turtles were headstarted at the Ontario Turtle Conservation Centre (formerly the Kawartha Turtle Trauma Centre) in 2003–2009, then at the Toronto Zoo in 2010-present. Morphological measurements (MidCL and mass) of the headstarted turtles were taken each month to monitor their growth while in the captive rearing facility. Headstarted turtles received PIT tags before release using methods similar to Buhlmann and Tuberville (1998). Timing of release has varied throughout the project, though most turtles were released in June or July. Exact release locations have also varied, though most turtles were released back into their maternal streams.

Data Analyses

Population Demography of Wild Turtles

I used RMark (Laake 2013) in R (R Core Team 2017) to create binary capture histories, then I used a Jolly-Seber (Jolly 1965, Seber 1965) model through POPAN (Schwarz and Arnason 1996) in Program MARK (White and Burnham 1999) to estimate adult population size and apparent adult survival across PopA and PopB in 1991–2017. Apparent survival is different than true survival as turtles that have emigrated from the populations are impossible to distinguish from turtles that have died. Spatial Cormack-Jolly-Seber (Cormack 1964, Jolly 1965, Seber 1965) models can estimate true survivorship (Schaub and Royle 2014) which presents future research opportunities using this dataset. I then remodelled the same parameters and

models for both PopA and PopB in 1997-2017 to reduce the variability from the large population decline in 1993-1997. I then calculated annual sex ratios for both populations in 1997–2017 by blocking PopA and PopB by sex while only including captures between emergence and 15 May in attempt to maintain homogeneity in capture probability between sexes (McKnight and Ligon 2017). I fitted 3 models per scenario where probability of entry (p_{ent}) was held constant while apparent survival (ϕ) and capture probability (p) were either constant (.) or time-dependent (t). I chose models based on QAIC_c (quasi-Akaike's information criterion, Akaike 1974) then QDeviance. I also scrutinized all models to ensure they were biologically valid and reflected the known declines in population size (time-dependent apparent survival) and known yearly variation in survey effort (time-dependent variation in capture probability).

Population Demography of Headstarted Turtles

Many demographic analyses omit juvenile turtles given the difficulties in meeting assumptions of population estimators, partially due to stochastic survivorship and consequently catchability (Congdon and Gibbons 1996, Koper and Brooks 1998, Hasler *et al.* 2015). This is not possible for evaluating a headstarted turtle population which likely has a juvenile-biased age structure given the delayed sexual maturity of the taxa and large number of headstarted turtles released. However, our populations are intensively surveyed each year and most individuals are captured every year, which provides an extensive mark-recapture dataset similar to census data. I used individual turtle last capture data within this extensive dataset to infer conservative estimates of both population size and apparent survival for headstarted turtles post-release, to prevent violating assumptions of heterogeneity in capture probabilities in traditional mark-recapture models. My method assumes that a headstarted turtle has survived every year from

release to their year of last capture. For example, a turtle with the binary capture history (1=captured, 0=not captured) of 10111101111000 had their binary capture history converted to 11111111111000 thus their capture histories can be created by knowing release year, and year of last capture. I did not include release year or within 1-year post-release captures for last capture data when estimating population size in order to increase stability of the population size estimates given the large amounts of headstarted turtles released. I did, however, include all turtles released when calculating post-release apparent survivorship. This method only crudely estimates apparent survival values as it does not consider capture probabilities which is an important parameter to compensate for imperfect detection of individuals. This model, when complemented with known-fate radio-telemetry data (See Chapter I), provides an extensive understanding of post-release survival of headstarted turtles.

Reproductive Biology of Headstarted Turtles

I used the extensive mark-capture dataset to report on size and age at maturity of headstarted Wood Turtles. I confirmed reproductive status in males based on observed mating attempts and in females through gravidity. I compared these values to known values from the literature and from previous studies within this population by Brooks *et al.* (unpubl. data).

Individual Turtle Health

All turtles had brief health assessments completed upon each capture. All turtles with what researchers determined to be serious health complications were brought into captivity for further assessment. Turtles with significant health issues were sent to either Dr. Sue Carstairs at the Ontario Turtle Conservation Centre (Peterborough, Canada) or veterinarians at the Toronto Zoo

Wildlife Health Centre (Toronto, Canada). Dr. Sue Carstairs sent biological samples to the British Columbia Ministry of Agriculture Animal Health Centre (Abbotsford, Canada) for further analyses. Veterinarians at the Toronto Zoo sent biological samples to Dr. Daniel Woodburn at the University of Illinois (Champaign, USA) for further analyses. Dead turtles were sent to the Canadian Wildlife Health Cooperative (University of Guelph, Guelph, Canada) for necropsies if the carcasses were in relatively good condition and had not been extensively scavenged.

Population Modelling

I compiled population-specific demographic parameter estimates, including historical data from Brooks *et al.* (unpubl. data), to perform population viability analyses in VORTEX (Lacy *et al.* 2005) to calculate r (intrinsic rate of population increase) for both PopA and PopB. I used data available in the peer-reviewed literature to augment my model if data were not available from the long-term dataset. I modeled and compared 4 management scenarios which included (1) continue incubating eggs and headstarting hatchlings, (2) incubate eggs and direct-release hatchlings without headstarting, (3) protecting nests and incubating eggs in-situ then releasing hatchlings, and (4) no management thus not protecting nests. I performed an elasticity analysis on two models in VORTEX (Lacy *et al.* 2005): (1) which included a continuation of headstarting and (4) in which no management was undertaken. I chose model (1) because the headstarting project likely will continue, thus further evaluating parameters to inform best management practices. I also chose model (4) to evaluate parameter sensitivity of the population if no management was done. I then recommended a management strategy based on my elasticity analyses and re-ran all 4 models with this recommended management strategy using theoretical parameter changes to establish clear goals for the headstarting program.

Results

Population Demographics: PopA

A total of 123 headstarted turtles were released into PopA between 2005 and 2017 (Appendix B). The population has not recovered to pre-decline levels (Figure 2.2, Table 2.2). I estimated the population size in 2017 to be approximately 18 individuals comprising 7 wild adults, 4 headstarted adults, and 7 headstarted juveniles. Only 2 of the 7 remaining wild adults are male. One of the adult males is missing both front limbs and may be unable to copulate successfully. The other adult male was first captured in Spring 2018. This male is currently being tracked with VHF radio-telemetry. The population has seen some evidence of recruitment of released headstarted turtles to adulthood as the population currently has 4 headstarted adults (1 male, 3 females). I estimated the sex ratio to be 1:3.96 (M:F) in 2014–2017 which is much higher than the sex ratio I estimated for 1991-1993 of 1:1.96 (M:F). Approximately 47% of females nested annually in 2014–2017. An adult headstarted female turtle (Notch=550, Age=15, MaxCL=184 mm) was not gravid in 2016, 2017, or 2018 despite being of age and size of sexual maturity. Only 1 unmarked adult has been captured in 2010-2018, suggesting that most of the adult population is marked.

I estimated adult survivorship in PopA to be 88.8% in 1997–2017 (95% CI: 84.3-92.1%, Table 2.2). Adult male survivorship (92.5%, 95% CI: 87.3-95.7%, Table 2.2) was greater than adult female survivorship (89.0%, 95% CI: 84.7-92.2%, Table 2.2) in 1997-2017. I estimated 1-year post-release apparent survival of headstarted turtles to be 35.8% (Figure 2.3, Appendix B). Post-release apparent survival then gradually increases with years post-release from 61.9% 2-years post release to 100% 7-years post release, though sample sizes are small in later years

(Figure 2.4, Appendix B). Apparent survival of headstarted turtles after 7-years post-release was not stable (Figure 2.3, Appendix B). Four subadult/adult-sized turtles were confirmed dead in 2016-2018 which included 1 wild adult female (Notch=250, MaxCL=178 mm), 1 wild adult male (Notch=756, MaxCL=194 mm), 1 headstarted subadult male (Notch=388, Age=7, MaxCL=165 mm), and 1 headstarted adult female (Notch=503, Age=11, MaxCL=167 mm). These mortalities may be underrepresented in my reported survival estimates given the large number of mortalities in a short period of time, as adult survival was otherwise relatively stable (Appendix D). A total of 34 turtles were confirmed dead in 2004–2017, and of these it is suspected that 16 were predated, 3 died in agricultural equipment-related collisions, 1 was run over by a gravel truck in an aggregate pit, 1 was run over by a personal lawn tractor, and 13 died from unknown causes (Appendix D). I suspect Raccoons (*Procyon lotor*) to be the main predator as their tracks were consistently found near many of the dead turtles found at both PopA and PopB (Figure 2.4). Raccoon hairs were also found in 2017 on a dead adult female at PopB (Mullin *et al.* 2018). Twelve non-headstarted hatchlings (eggs incubated ex-situ) were released in 2009; none have been recaptured. Thirty (15 per year) non-headstarted hatchlings (eggs incubated ex-situ) outfitted with VHF transmitters were released in 2016 and 2017 in PopA and PopB, and the 1-year post-release apparent survivorship for both release years was 13% (Chapter I). There is limited evidence of natural recruitment within this population, partly due to all eggs found being collected for the headstarting program.

Population Demographics: PopB

A total of 330 headstarted turtles were released into the population between 2005 and 2017 (Appendix C). The population has recovered to above pre-decline levels (Figure 2.2, Table 2.2).

The population demography includes a strong juvenile bias (Figure 2.2). I estimated the population size in 2017 to be approximately 117 individuals which includes 22 wild adults, 4 wild juveniles, 9 headstarted adults, and 82 headstarted juveniles (Figure 2.2). I estimated the sex ratio (M:F) of adults in 2014–2017 to be 1:2.35. The sex ratio of headstarted adults is also female-biased at 1:2. Approximately 64% of females nested annually between 2014 and 2017. Only 1 unmarked wild adult (Male, Notch=380, growth rings >20) has been encountered in 2010–2018, suggesting most of the adult population is marked.

I estimated PopB adult survivorship to be 92.8% in 1997–2017 (95% CI: 89.5-95.2%, Table 2.2). Adult female survivorship (93.0%, 95% CI: 89.1-95.6%, Table 2.2) was higher than adult male survivorship (91.64%, 95% CI: 86.9-94.8%, Table 2.2) in 1997–2017. I estimated 1-year post-release apparent survival of headstarted turtles to be 52.4% (Figure 2.3, Appendix C). Post-release apparent survival of headstarted turtles then gradually increases with years post-release from 60.63% 2-years post release to 100% 6-years post release, though sample sizes are small in later years (Figure 2.3, Appendix C). Apparent survival of headstarted turtles after 6-years post-release from captivity was relatively stable (Figure 2.3, Appendix C). Seven adult turtles were confirmed dead in 2016–2018 which included 1 wild adult male (Notch=370, MaxCL=203 mm), 3 wild adult females (Notch=60, MaxCL=178 mm; Notch=94, MaxCL=182 mm; Notch=150, MaxCL=194 mm), and 3 headstarted adult females (Notch=434, Age=14, MaxCL=171 mm; Notch=446, Age=11, MaxCL=167 mm; Notch=478, Age=11, MaxCL=166 mm). These mortalities may be underrepresented in my reported survival estimates given the large number of mortalities in a short period of time, as adult survival was otherwise relatively stable. A total of 71 turtles were confirmed dead in 2005–2017. Researchers suspected 45 were predated, 3 drowned, 2 were crushed by vehicles, and 21 died from unknown causes (Appendix

D). Included in this total is one headstarted turtle who was found dead 40 km downstream from the release spot 11-months post-release. Thirty-one non-headstarted hatchlings (eggs incubated ex-situ) were released in 2009; one of these released hatchlings was last captured in 2016 (Notch=42, Age=7, MaxCL=164 mm, Mass=530 g). This turtle has not been recaptured in 2017 or 2018 despite intensive survey efforts. No other hatchlings released in 2009 have been recaptured.

Reproductive Biology of Headstarted Turtles

Female headstarted turtles matured at a minimum of 10 years and 166 mm carapace length, whereas headstarted males matured at a minimum 10 years and 172 mm carapace length (Table 2.3). Research in the early 1990s found that wild non-headstarted females matured at 10 years (growth line estimated age) and 158 mm carapace length and wild males matured at 12 years (growth line estimated age) and 173 mm carapace length. Headstarted females matured at larger sizes than wild females but similar ages, whereas headstarted males matured younger but at a similar size than wild males, though low samples sizes of confirmed reproduction in headstarted females (n=4) and headstarted males (n=2) limit extensive comparisons. A headstarted female turtle from PopB (Notch=522, Age=15, MaxCL=163 mm) was observed mating on three occasions between 2015 and 2018 but she has never reproduced. An adult female headstarted turtle from PopA (Notch=550, Age=15, MaxCL=184 mm) is well above the size/age at sexual maturity which I determined for this population, but she has never reproduced.

Population Modelling

Life-history parameters were compiled from the demographic analyses (Table 2.4). My population models predicted that both populations would (1) grow with continued headstarting efforts, (2) decline if hatchlings are direct-released post-incubation, (3) decline if nests are incubated in-situ and protected, (4) decline if no management is implemented (Figure 2.5). My elasticity analysis predicted that decreasing adult and/or juvenile survivorship would cause the largest decreases in projected population size, whereas increasing adult and/or juvenile survival caused the greatest increases in population growth rate (Table 2.5). Based on my elasticity analysis, I recommended a management strategy that incorporates predator reduction and a split-release scenario (equal number of releases between populations). I then re-ran all 4 models incorporating this recommended management strategy, along with anticipated changes in parameters to help inform management decisions (Figure 2.6, Table 2.6). Primary anticipated changes in demographic parameters from the management strategy included increased juvenile and adult survivorship (Table 2.6) assumed from the reduction in predators within the system given that predators were suspected of many deaths observed within this population (Figure 2.4, Appendix D).

My recommended management strategy model predicted population recovery in PopA for (1) continued incubation of eggs and headstarting ($r=0.075$), (2) incubating eggs and direct-releasing of hatchlings ($r=0.049$), (3) protecting nests ($r=0.018$) and (4) no management ($r=0.005$, Figure 2.6). My recommended management strategy predicted population recovery in PopB for (1) continued incubation of eggs and headstarting ($r=0.027$) and (2) incubating eggs and releasing hatchlings ($r=0.005$), but predicted population declines with (3) protecting nests ($r=-0.025$) and (4) no turtle management ($r=-0.044$; Figure 2.6). Low population growth of PopB in comparison to PopA is likely due to headstarted turtles being removed from PopB to

supplement PopA due to the split-release scenario. To confirm this, I again changed the management strategy and removed the supplementation aspect and remodelled PopB. Without the removal of eggs for supplementation of PopA, I predicted population recovery for PopB using all non-headstarting management strategies including (2) incubating eggs and direct-releasing of hatchlings ($r=0.048$), (3) protecting nests ($r=0.023$), and (4) no management ($r=0.006$; Figure 2.6).

Disease Ecology

Most headstarted turtles from the 2015-2018 release cohorts have mycotic shell disease (Figure 2.7). The disease worsened as turtles emerged from overwintering. Two turtles with extensive infections were sampled and analyzed by Dr. Daniel Woodburn (University of Illinois) in January 2018. One turtle had an infection which was consistent with *Nannizziopsiaceae* spp. and the other turtle had an infection consistent with *Pureocillium lilacinum*. Lab diagnostics (through cultures, histopathology, and computerized tomography scans) reported that there is only superficial growth of fungal agents in the outer keratinized layer of the carapace, which does not extend into deeper tissues. There was also no dermal inflammation or evidence of a deeper fungal infection or systemic infection. Supplementary studies are ongoing to further our understanding of these infections. One headstarted juvenile was found dead during its first winter post-release in 2018 and sent for a necropsy in February 2018. Final diagnostics revealed that the turtle was emaciated, had pulmonary mineralization, hepatic necrosis, and preliminarily tested positive for ranavirus, though future study is needed to reconfirm this diagnosis and the extent of infection within the population. One adult female headstarted turtle (Notch=477, Age=11, MaxCL=166 mm) was found in spring 2018 with most clinical symptoms of ranavirus infections

in turtles. Preliminary diagnostic testing revealed that this turtle did not have ranavirus but instead had *Glyptemys herpesvirus*.

Discussion

Population Demography and Modelling

Both PopA and PopB have benefited from headstarting, although their long-term viability requires continuation of management intervention. PopA has continued to decline despite headstarting efforts (Figure 2.2), and is projected to continue to decline if not actively managed (Figure 2.5). Recovery of PopA has been limited by several factors including low number of headstarted turtles released, low survival of headstarted turtles post-release, low adult survival, and low number of wild females nesting annually. In contrast, PopB has partially recovered (Figure 2.2) likely due to the greater number of headstarted turtles released, their higher survival post-release, and the slightly higher adult survival and number of nesting females.

High adult survival is the most important demographic parameter for turtle population persistence, as it helps offset naturally stochastic survivorship of eggs and juveniles (Iverson 1991, Congdon *et al.* 1993, Heppell *et al.* 1996, Enneson and Litzgus 2008, Spencer *et al.* 2017). Schneider *et al.* (2018) reported 97% adult survival in a growing population of Wood Turtles. My reported adult survival values for PopA (89%) and PopB (93%) are lower, suggesting that the recovery of our study populations will require an increase in adult survival. Additionally, my estimates of overall adult survivorship may be overestimates because stable survivorship across most years may mask infrequent events of increased adult mortality such as that observed in 2016-2018. Given the established importance of adult survivorship to population growth and

persistence, and the current relatively low adult survivorship in my populations, increasing adult survivorship should be a primary goal for this Wood Turtle recovery program.

Other researchers have reported high post-release survival of headstarted turtles (Haskell *et al.* 1996, Vander Haegen 2009, Bona *et al.* 2012, Michell and Michell 2015, Tuberville *et al.* 2015). Post-release survival of headstarted turtles in my study was much lower, especially at 1-year post-release (Figure 2.2, 2.3, and see Chapter I). Thirty headstarted turtles were tracked with VHF radio-telemetry in 2016-2017 (Chapter I). Known-fate survival values were 40% for turtles headstarted for 1 year, and 73% for turtles headstarted for 2 years (Chapter I). The greatest threat to juvenile survivorship at our sites appears to be abundant predators, primarily Raccoons. Researchers suspected 58% of the 105 confirmed turtle mortalities resulted from predation, and this is likely a conservative estimate as the causes of 40% of the 105 mortalities are unknown. Dreslik *et al.* (2017) also suspected Raccoon predation as a major factor negatively impacting the success of an Alligator Snapping Turtle (*Macrochelys temminickii*) headstarting program. Both PopA and PopB are located within prime agricultural lands which provide food subsidies (i.e. corn and soy) for a variety of species, including Raccoons. Agricultural fields fragment habitats while increasing edge habitat and providing travel corridors (Gehring and Swihart 2003). There are also regular culls of coyotes within the study sites (R.C. White, pers. comm), which likely reduces predation pressures on meso-predators. These landscape level habitat alterations change community composition and impact predator-prey dynamics (Oro *et al.* 2013) which may be impairing recovery efforts. Additionally, heavy equipment is another cause of mortality to both juvenile and adult Wood Turtles within agricultural lands (Saumure *et al.* 2007).

In summary, both adult and juvenile survivorship are lower than what is required for long-term population viability. I predicted with my elasticity analyses that increasing adult and

juvenile survivorship will increase population growth rate (Table 2.5) which is a similar finding to other elasticity analyses (Enneson and Litzgus 2008, Gasbarrini 2016). Accordingly, I predicted both populations could recover if a predator-reduction management strategy was implemented which increased both adult and juvenile survivorship. My predator-reduction model is, however, limited as I used hypothetically estimated parameter changes of increased adult and juvenile survivorship (based on data from Congdon *et al.* 1993, Schneider *et al.* 2018, and Brooks *et al.* unpubl. data) and not population-specific parameters. However, other studies do support the effectiveness of predator removal including both Engeman *et al.* (2005) and Urbanek *et al.* (2016) reporting that a predator removal management strategy increased nest survivorship of Sea Turtles and Blanding's Turtles (*Emydoidea blandingii*), respectively. Pramuk *et al.* (2013) also predicted that recruitment of wild Western Pond Turtles could be increased by removing invasive predatory American Bullfrogs (*Lithobates catesbeianus*). Additionally, through personal communication, I know that predator removal is being used by other Wood Turtle biologists, although none of the results are published. An extensive meta-analysis on multiple species of birds reported that predator-removal was an effective conservation strategy, however, there are both practical and ethical limitations (Smith *et al.* 2010). Further research on the effectiveness of predator-removal for increasing juvenile and adult Wood Turtle survivorship is needed, and should be undertaken as a collaborative effort with other researchers who are employing this technique.

Sex ratios were female-biased in both PopA and PopB. Reported sex ratios vary among studies of Wood Turtles but are predominantly reported as either female-biased (Brooks *et al.* 1992, Schneider *et al.* 2018) or equal (Harding and Bloomer 1979, Walde *et al.* 2003). Lovich *et al.* (1990) suggest that reports of female-biased Wood Turtle populations may be an artifact of

sampling effort; however, given the extensive survey effort and lack of new captures at our sites, this is likely not the case for our populations. Possibly undetected sex-biased mortality from predators may have devastating impacts on recovery projects. Future research should investigate the potential causes of female-biased sex ratios to understand threats and further develop mitigation and management strategies for the study populations.

A potentially overlooked threat (but see Jones and Sievert 2009) to headstarted juveniles may be seasonal flooding events (Appendix A) or dam failures that wash turtles downstream. The primary river which connects PopA and PopB has a series of dams/spillways upstream which affect natural flow regimes. Jones and Sievert (2009) reported that seasonal floods impact Wood Turtles, specifically through displacement and direct mortality. Keevil *et al.* (2018) also reported that approximately 8 adult Snapping Turtles died following the failure of a dam. Flooding may be more detrimental to smaller, weaker, naïve juveniles and hence could negatively impact recruitment. Wood Turtles can, however, migrate back to their home ranges following flooding events (Jones and Sievert 2009, Chapter I). Jones (2009) proposed that intense seasonal flooding from snow melt in the North may limit population distribution by frequently displacing individuals during the dormant season, and through direct mortalities of adult turtles. Future research should investigate seasonal flooding as a potential threat to juvenile survivorship.

I predicted through my elasticity analyses that the number of females breeding annually impacts population growth (Table 2.5). Studies that assume 100% adult females breeding (e.g., Spencer *et al.* 2017) likely overestimate this parameter which will consequently reduce the precision of population viability analyses and may misinform management plans. Number of adult females breeding annually (female reproductive frequency) is a difficult metric to estimate;

however, it is an important parameter for population modelling (Gibbons 1982, Moll and Iverson 2008, Keevil *et al.* 2018, this study). The number of adult females breeding annually in PopA (47%) and PopB (64%) is higher than the 33% previously reported for this population (PopA and PopB combined in 1991-1993), though researchers had trouble locating nesting females (Brooks *et al.* unpubl. data). The values reported for this population are lower than values previously reported for Wood Turtles by Jones (2009, 71%) and Walde *et al.* (2007, 83%). Encounter rates between females and males may be rare given the small population size and female-biased sex-ratio. Hence female Wood Turtles in both of our study populations may be reproducing primarily using stored sperm (Gist and Jones 1989). A large female headstart (Notch=550, Age=15, MaxCL=184 mm) in PopA has yet to reproduce despite being of reproductive size (minimum MaxCL=166 mm, Table 2.3) and age (minimum age=10). Notch 550 may not have encountered and mated with a male, which is possible given the low number of adult males in PopA. The fertility of eggs using stored sperm decreases over time due to sperm depletion (Gist and Jones 1987). If the study populations continue to decline, then encounter rates between adult males and females could become more infrequent, which may result in decreases for both egg survivorship and number of adult females breeding annually.

Finally, differences in population growth rates between PopA and PopB are likely related to differences in the number of eggs collected, and consequently the number of headstarted turtles released into each site. The number of headstarted turtles released is clearly going to affect population growth rate (Gasbarrini 2016, Dreslik *et al.* 2017, this study). The collection of eggs for headstarting at PopB has been facilitated by artificial nesting sites (similar to Buhlmann and Osborn 2011). Similar nesting sites were built in PopA, but females seem to prefer nesting in other locations. Nonetheless, the artificial nesting sites in PopB may be important to the future of

the project because they encourage turtles not to nest in the cropland, which may present an ecological sink (Mui *et al.* 2016).

Reproductive Biology of Headstarted Turtles

A subset of the headstarted turtles have reached sexual maturity and reproduced, which is important information supporting a trajectory towards a self-sustaining population. A lack of observed reproduction by headstarted turtles is an aspect of headstarting that has been heavily criticized (e.g., Woody 1990, Frazer 1992, Heppell *et al.* 1996, Seigel and Dodd 2000). However, I cannot confirm that headstarting has no impacts on reproductive development because one headstarted female who is of reproductive size has not yet produced eggs (see above, Notch=550). Further analyses are needed to examine maternal investment (egg size, clutch size, body size relationships) in headstarted female turtles. It has also been suggested that increasing juvenile growth rates decreases age at maturity (Hildebrand 1932, Congdon and Van Loben Sels 1993). Thus, headstarted turtles should mature at younger ages than their wild counterparts. Our dataset on the age at maturity of headstarted turtles is limited, but provides some support for the acceleration of maturity. The outcomes of population manipulations in long-lived species can go undetected for many years after a management project has started. Long-term studies investigating the possible impacts of headstarting on reproductive biology and physiology are thus critical, as we do not want our mitigation measures to incur negative consequences.

Disease Ecology

Several unexpected cases of disease in the Wood Turtle headstarting program have been observed, including mycotic shell disease, ranavirus, and herpesvirus. Mycotic shell disease with likely fungal causative agents has been reported in freshwater turtles by Hallock *et al.* (2017; Western Pond Turtle, *Actinemys marmorata*), in tortoises by Nardoni *et al.* (2011; Hermann's Tortoise, *Testudo hermanni*) and Rose *et al.* (2001; Texas Tortoise, *Gopherus berlandieri*), and in Sea Turtles by Cabanes *et al.* (1997; Loggerhead Sea Turtle, *Caretta caretta*). A fungal infection on the shell can be highly infectious, chronic, and debilitating and can lead to secondary infections which can kill the infected individual, and individuals who recover retain pitted scutes (Wallach 1975). Hatt (2010) reported poor husbandry is most often the cause of fungal infections in chelonians, which is relevant as all reported cases above are of chelonians which either had direct or indirect ties to captive-rearing. Hallock *et al.* (2017) reports a similar situation to our study with prevalence of the disease in a population comprised of both headstarted and non-headstarted turtles. Hallock *et al.* (2017) reported infection rates ranged between 29–49% in each of the 6 study populations of headstarted Western Pond Turtles. The disease was primarily observed in headstarted turtles, although three wild non-headstarted turtles showed clinical symptoms (Hallock *et al.* 2017). No clinical symptoms have been detected on wild non-headstarted turtles in our study population of headstarted Wood Turtles, although further monitoring is needed.

The detection of ranavirus in our population is concerning. This is the first confirmed case in Ontario, along with one Snapping Turtle (Canadian Wildlife Health Cooperative 2018). Ranavirus is reported to have caused mass mortalities in Eastern Box Turtles (*Terrapene carolina*; Kimble *et al.* 2017) and is thought to be potentially lethal to other turtles (Johnson *et al.* 2008, Allender *et al.* 2013). Further in-depth studies should aim to confirm the presence and

prevalence of ranavirus within the study population and establish management guidelines to prevent unintentional spread via mark-recapture study.

One turtle in our population has tested positive for *Glyptemys herpesvirus 2* (GlyHV-2), a novel alphaherpesvirus (Ossiboff *et al.* 2015a). The only other study on this herpesvirus was by Ossiboff *et al.* (2015a) who confirmed the virus in 5/9 (55%) Wood Turtles tested. GlyHV-2 was not observed in other related species (Bog Turtles, *Glyptemys muhlenbergii* and Spotted Turtles, *Clemmys guttata*), suggesting the disease can only be transmitted within host species (Ossiboff *et al.* 2015a). Ossiboff *et al.* (2015a) suggested that GlyHV-2 is part of the natural disease ecology (host-pathogen evolution) of Wood Turtles, and that this virus is likely not a significant threat to healthy individuals or populations. Nonetheless, given the lack of substantial evidence regarding the impact of GlyHV-2 on populations, the precautionary principle should be applied as there are other herpesviruses which have resulted in mortalities in other turtle species, including but not limited to, Emydidae herpesvirus 1 (EmyHV-1) in Northern Map Turtles (*Graptemys geographica*; Ossiboff *et al.* (2015b)), Testudinid herpesvirus 3 (TeHV-3) in many species of tortoises (Oraggi 2012), and Chelonid fibropapilloma-associated turtle herpesviruses (CFPHV) in all 7 sea turtle species (Alfaro-Nunez *et al.* 2014). Inadvertently transferring novel diseases to wild populations through conservation management strategies can have devastating effects (Smith 2015). The Wood Turtle infected with Gly-HV2 is currently being quarantined to prevent the potential spread of this disease. The turtles with mycotic shell disease have not been quarantined given the abundance of turtles infected along with the lack of evidence of severe physiological impairment from lab diagnostics. Turtles with mycotic shell disease may be quarantined if researchers notice deteriorating health. The negative effects of infectious diseases may be exacerbated when working with endangered species due to small population sizes and

reduced genetic diversity (Flanagan 2000). Thus, diseases within population of endangered species need to be well understood and the effects mitigated.

Conclusion

After 15 years of headstarting, our two populations of Wood Turtles have shown some evidence of recovery, though long-term survival of the metapopulation will require additional intervention, including continued headstarting, predator reduction, and disease management. PopA will require greater intervention than PopB. PopB has higher adult survival, higher post-release survival of headstarted turtles, more eggs collected and hence more headstarted turtles released, and a higher proportion of females breeding annually, all of which has led to greater recovery in comparison to PopA. In comparison to other headstarting projects, both PopA and PopB have seen limited evidence of recruitment, although some of the earliest headstarted turtles released are now reaching sexual maturity, indicating that a pending shift in population demographics may eventually lead to self-sustainability. Importantly, headstarting alone is not enough to save both PopA and PopB from local extinction; these populations face multi-faceted complex problems for which management is challenging.

Management Recommendations

- 1) Only continue headstarting if additional management is undertaken (e.g., predator reduction).
- 2) Commence an annual predator-reduction management strategy to increase juvenile and adult survivorship. Both non-lethal and lethal wildlife control methods should be considered. Collaborate with other Wood Turtle biologists that use predator removal to ensure that we

are all using data-driven management strategies and not unnecessarily killing animals. Publish these results regardless of anticipated public response.

- 3) Continue investigating the presence of diseases and their effects on the population through collaboration with a disease ecologist. Share data to ensure best husbandry practices are established.
- 4) Supplement PopA with headstarted turtles from PopB to improve recovery efforts once a predator management strategy has been established in both PopA and PopB.
- 5) Investigate alternative uses for the land as cash cropping has direct, and indirect negative impacts for Wood Turtles. Alternative uses for the land could include pasture farming.
- 6) Implement a dispersal study to understand the effects of seasonal flooding on post-release survival and spatial ecology of headstarted turtles.
- 7) Study nest survivorship for Snapping Turtles and Midland Painted Turtles as a potential proxy for natural nest survivorship as all three species (including Wood Turtles) have similar nesting seasons and similar nest predators. This parameter may also be used to evaluate the effectiveness of predator-management strategies given that some baseline data is available.

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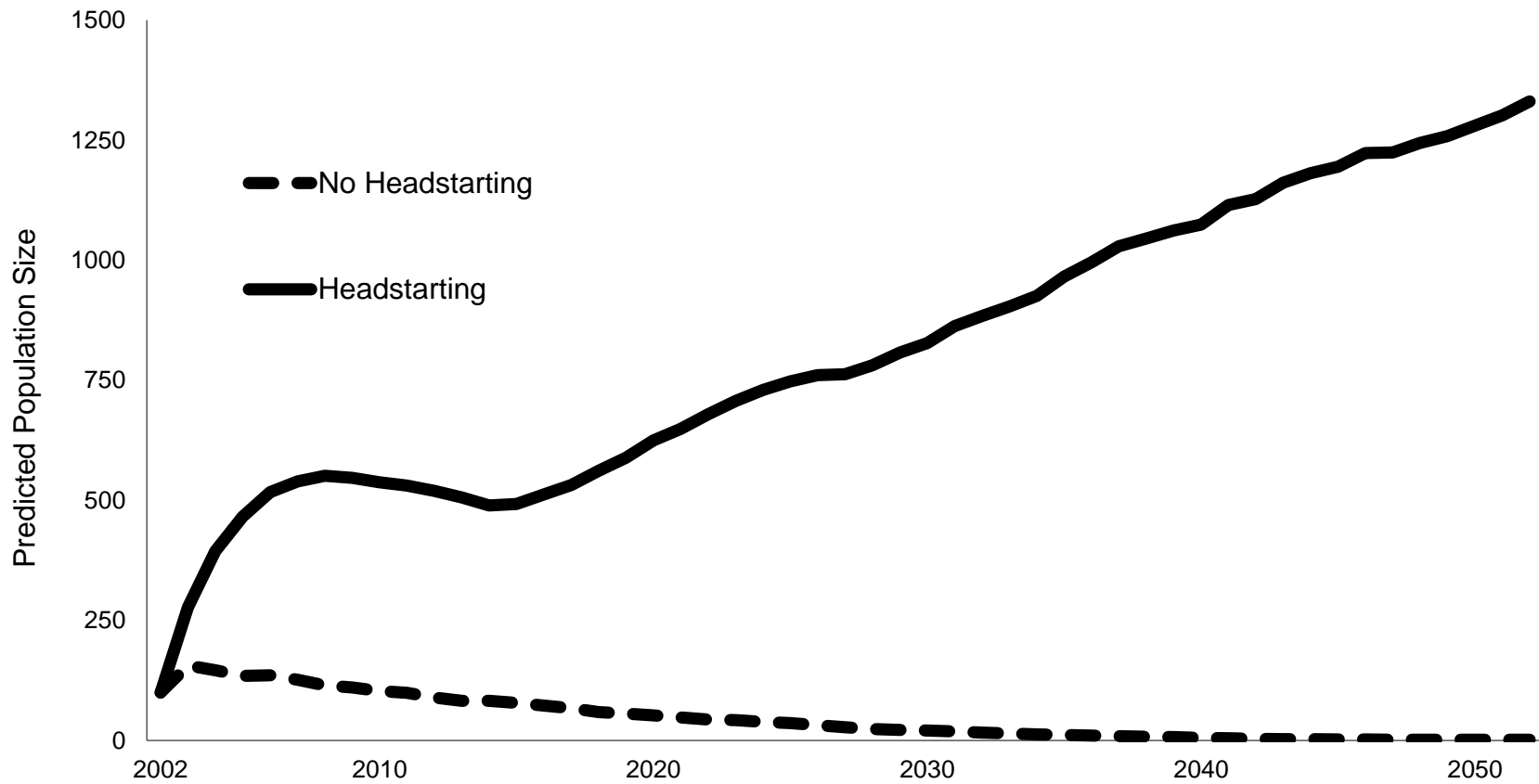


Figure 2.1. Brooks *et al.* (unpubl. data) population viability analysis of Wood Turtle population size projected 50 years from 2002 created with data for PopA and PopB combined. The “No Headstarting” model used population-specific demographic parameters from 2002. The “Headstarting” model was a modification of the “No Headstarting” model that included changing juvenile survival rate and age at maturity to predicted values under a headstarting program (Table 2.1).

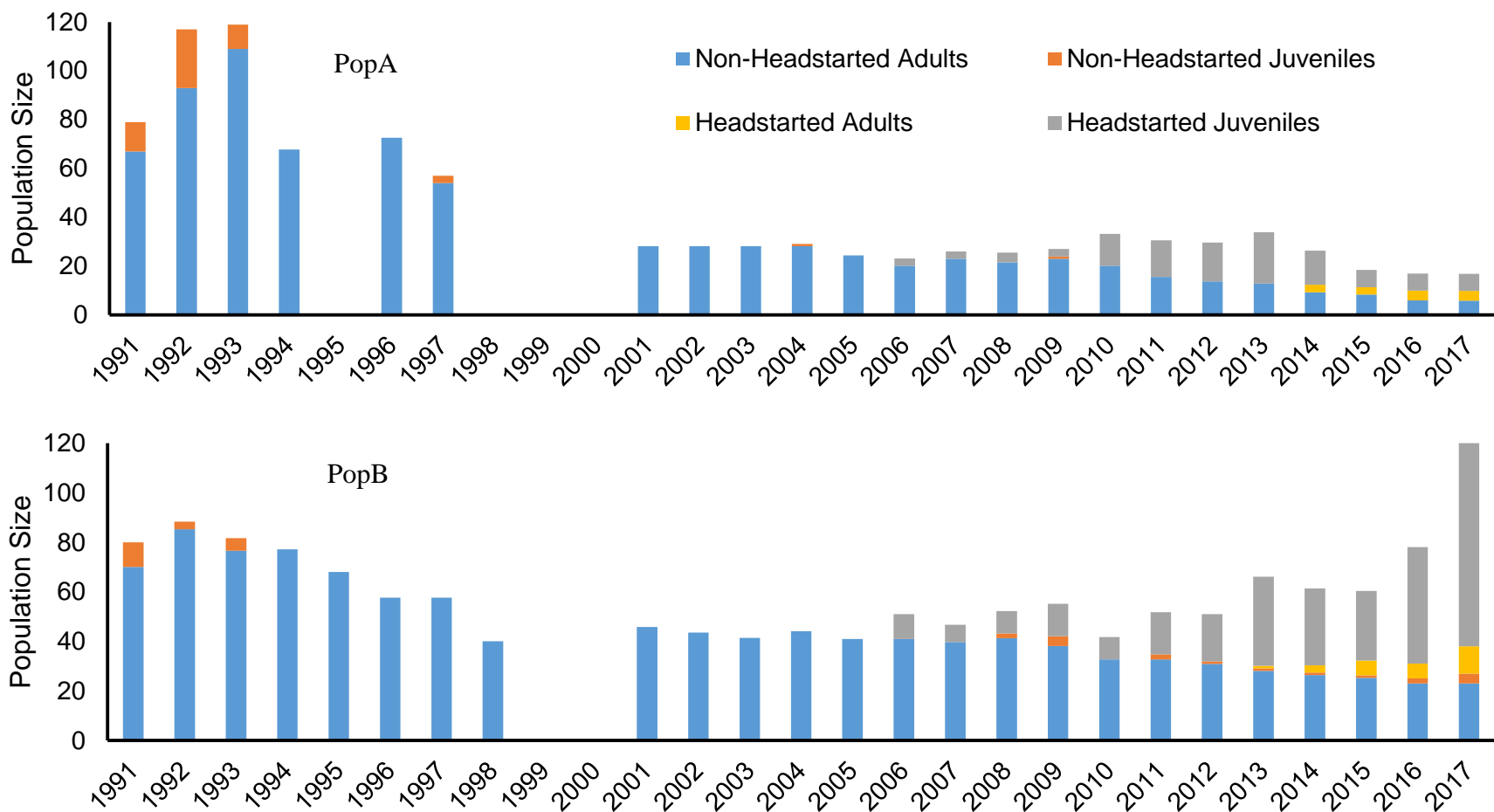


Figure 2.2. Population size and demographics of PopA (top) and PopB (bottom) between 1991 and 2017. See *Methods* for details regarding the population size estimates. The suspected poaching and associated decline occurred between 1994-1997. The headstarting program began with the collection of eggs in 2003, with the first release of headstarted turtles in 2005. Headstarted turtles were not included in the population until 1-year post-release.

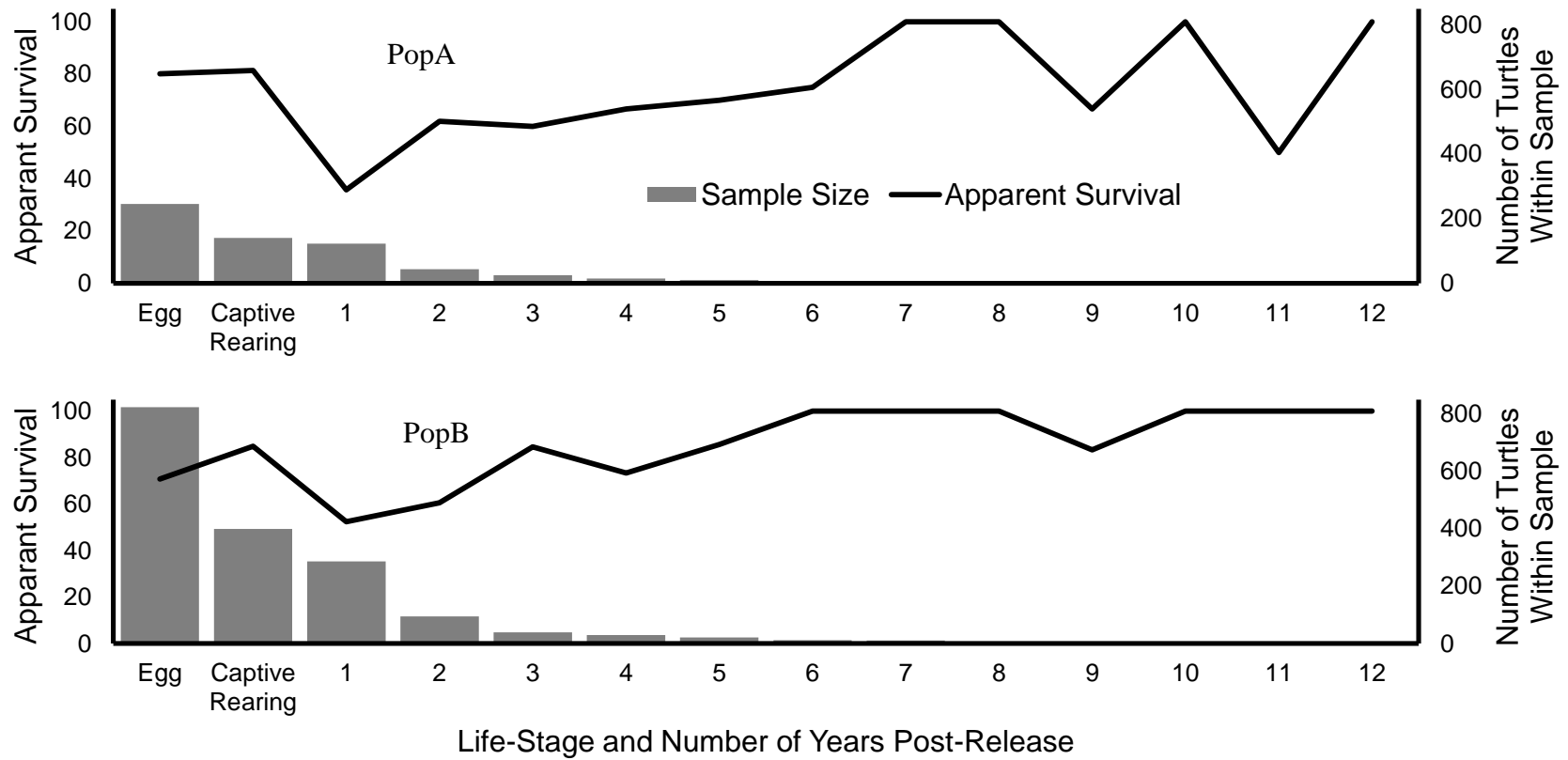


Figure 2.3. Apparent survival (line graph, left y-axis) and sample size (bar graph, right y-axis) of eggs incubated ex situ, of hatchlings in captive rearing facility during headstarting, and of headstarted juveniles post-release at PopA (top) and PopB (bottom) for 2003-2017. Procedure for estimating apparent survival of headstarted turtles is available in *Methods*.



Figure 2.4. Dead headstarted juvenile Wood Turtle (*Glyptemys insculpta*) found in spring 2017 with Raccoon (*Procyon lotor*) tracks in close proximity. This juvenile Wood Turtle was found missing its head, and the distal parts of the hind and fore limbs. Many dead Wood Turtles were found with similar injuries (see Appendix D).

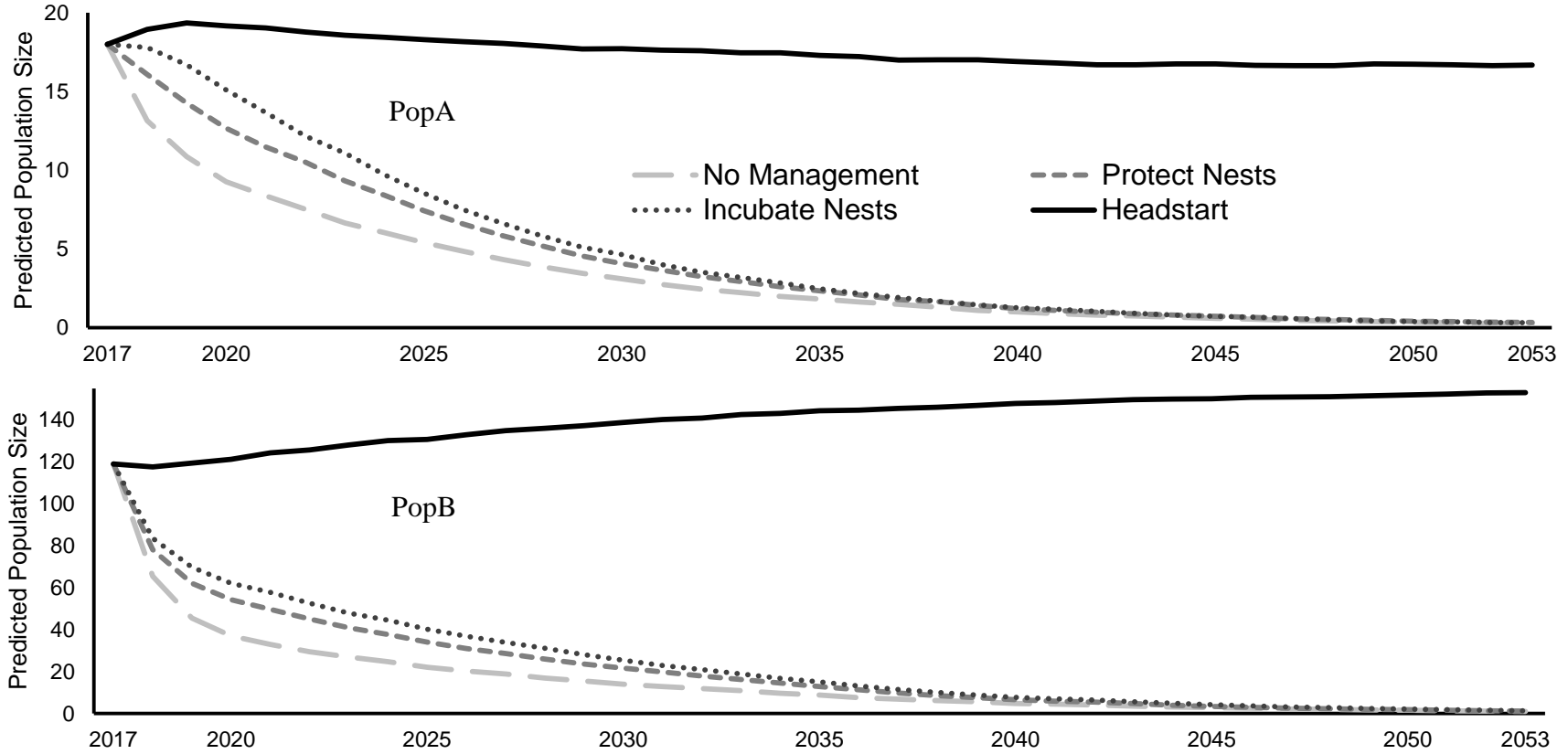


Figure 2.5. Population viability analysis models (VORTEX) of projected population sizes (1000 iterations) of Wood Turtles using a variety of management scenarios (No Management, Protect Nests, Incubate Nests, and Headstart; see *Methods – Population Modelling*) projected 36 years from 2017, thus 50 years after the headstarting program began (2003-2053). All 4 models presented are slight alterations to a base model created from population-specific life-history data where possible, and complemented by data from the literature. PopA (top) and PopB (bottom). Note the differences in the Y-axes.

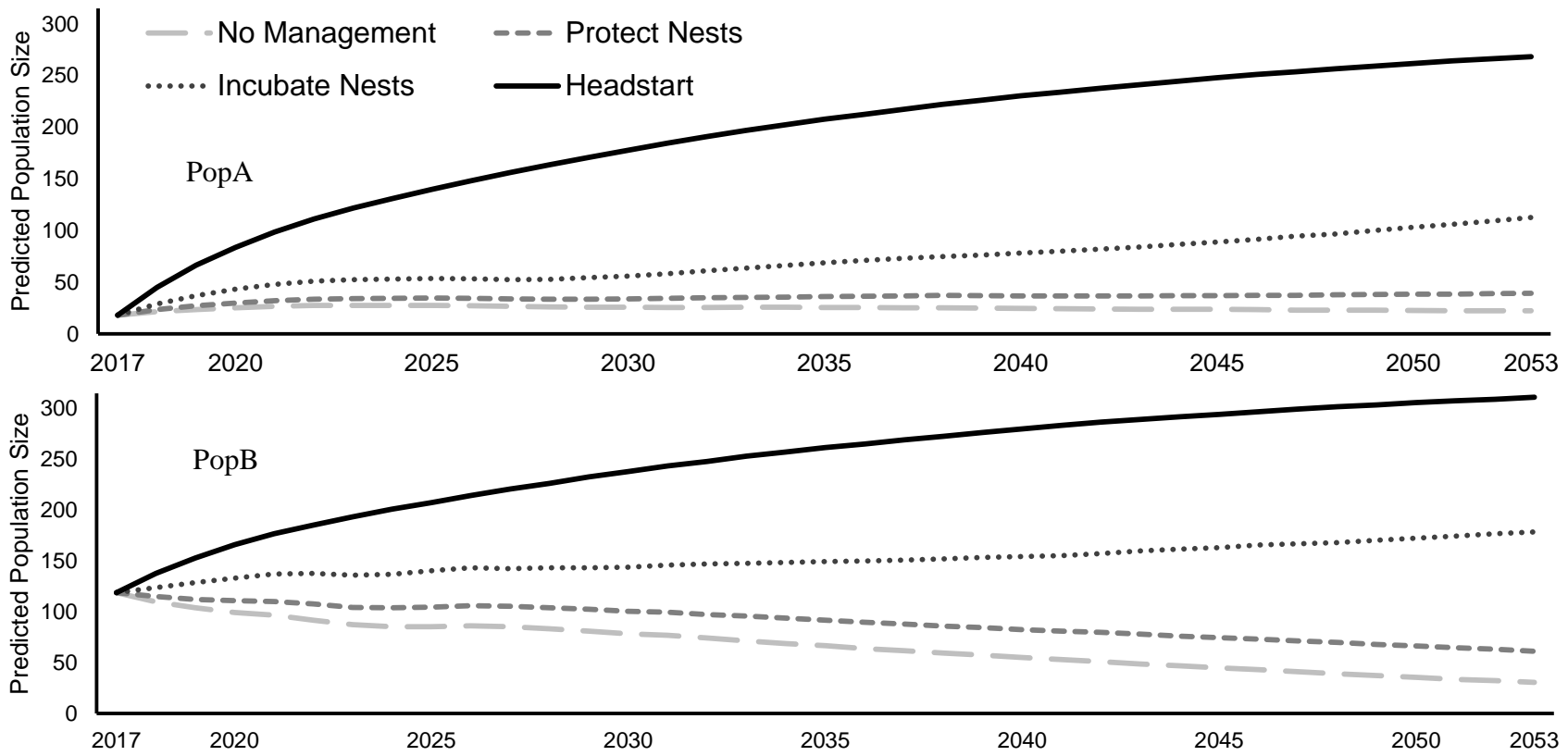


Figure 2.6. Population viability analysis models (VORTEX) of projected population sizes (1000 iterations) of Wood Turtles using a variety of management scenarios which all incorporate a predator reduction strategy (No Management, Protect Nests, Incubate Nests, and Headstart; see *Methods – Population Modelling*) projected 36 years from 2017, thus 50 years after the headstarting program began (2003-2053). All 4 models presented are slight alterations to a base model created from a combination of population-specific life-history data along with predicted parameter changes influenced by implementing a predator management strategy, complemented by data from the literature. PopA (top) and PopB (bottom). Note the similarities in Y-axes.

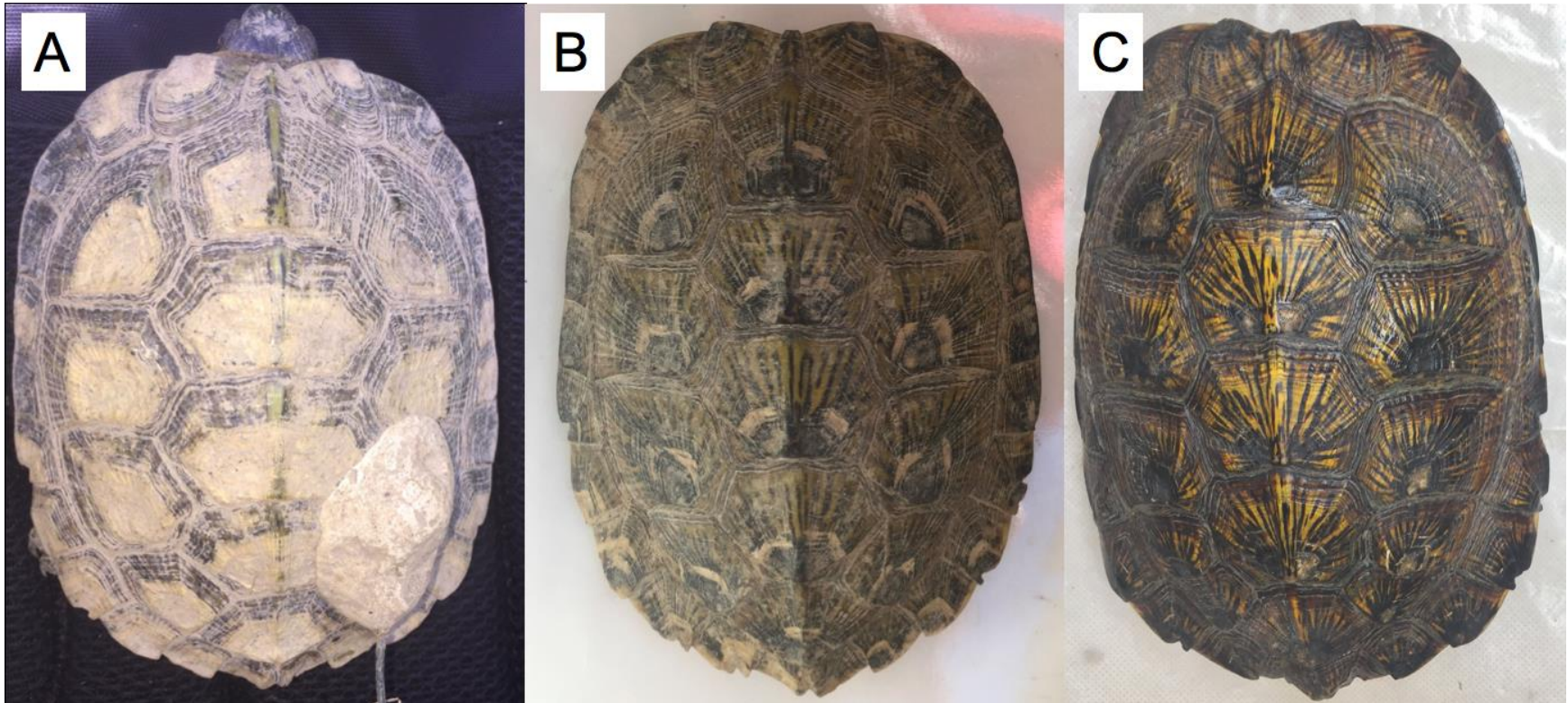


Figure 2.7. Three juvenile headstarted Wood Turtles (*Glyptemys insculpta*) exhibiting varying degrees of symptoms for mycotic shell disease on the carapace: (A) severe, (B) mild, (C) no symptoms. Turtle A also has a VHF radio-transmitter affixed with white epoxy putty to the posterior right of its carapace.

Table 2.1. Parameters used in population viability analysis assessing the impact of headstarting to recover a Wood Turtle population after the large decline in the mid 1990s (Figure 2.1, Brooks *et al.* unpubl. data). The estimated population values in scenario “No Management” are derived from metapopulation-specific life-history data. The estimated population values in scenario “Headstarting” are the predicted changes to “No Management” values incurred through implementing a headstarting program. Both models were based on metapopulation-specific data for 2002.

Parameter	Estimated Population Value		Change in Estimated Population Value Between Models
	No Management Model	Headstarting Model	
Egg Mortality Rate (%)	70	50	-20
Hatchling Mortality Rate (%)	90	25	-65
Juvenile Mortality Rate (%)	30	25	-0.05
Age at Maturity (years)	16	11	-5

Table 2.2. Models analyzed using a Jolly-Seber (Jolly 1965, Seber 1965) model through POPAN (Schwarz and Arnason 1996) in Program MARK (White and Burnham 1999) to estimate adult population size and apparent adult survival across PopA and PopB for 1991-2018. Three models were fitted per “Population” where probability of entry (pent) was held always time-dependent (t) while apparent survival (phi) and capture probability (p) were either constant (.) or time-dependent. I chose models based on QAIC_c (quasi-Akaike’s information criterion, Akaike 1974) then QDeviance. I also scrutinized all models ensure they were biologically valid and reflected the known declines in population size (time-dependent apparent survival) and known yearly variation in survey effort (time-dependent variation in capture probability). Chosen models for each “Population” are indicated in bold.

Population	Model	QAIC _c	Likelihood	K	QDeviance
PopA Adults (1991-2017)	POPAN: {phi(.),p(t),pent(t)}	1006.3515	1.0000	34	991.1633
	POPAN: {phi(t),p(t),pent(t)}	1030.8552	<0.0001	54	963.4053
PopA Adults (1997-2017)	POPAN: {phi(.),p(.),pent(t)}	1118.0843	<0.0001	10	1167.3389
	POPAN: {phi(.),p(.),pent(t)}	439.1338	1.0000	10	201.9906
	POPAN: {phi(.),p(t),pent(t)}	449.8750	0.0047	27	170.3656
PopA Males (1997-2017)	POPAN: {phi(t),p(t),pent(t)}	479.3406	<0.0001	41	157.3777
	POPAN: {phi(.),p(.),pent(t)}	327.1487	1.0000	12	160.2827
	POPAN: {phi(.),p(t),pent(t)}	341.9630	<0.0001	26	134.1912
PopA Females (1997-2017)	POPAN: {phi(t),p(t),pent(t)}	396.0075	<0.0001	41	127.0222
	POPAN: {phi(.),p(.),pent(t)}	498.9787	1.0000	12	238.1916
	POPAN: {phi(.),p(t),pent(t)}	516.7561	0.0001	29	203.9009
PopB Adults (1991-2017)	POPAN: {phi(t),p(t),pent(t)}	567.3625	<0.0001	44	186.6224
	POPAN: {phi(.),p(t),pent(t)}	1038.4182	1.000	36	348.0080
	POPAN: {phi(t),p(t),pent(t)}	1082.0422	<0.0001	60	334.5239
PopB Adults (1997-2017)	POPAN: {phi(.),p(.),pent(t)}	1216.8213	<0.0001	11	580.8177
	POPAN: {phi(.),p(t),pent(t)}	506.3326	1.0000	29	175.2083
	POPAN: {phi(t),p(t),pent(t)}	541.2371	<0.0001	46	167.9734
PopB Males (1997-2017)	POPAN: {phi(.),p(.),pent(t)}	543.3120	<0.0001	13	248.2229
	POPAN: {phi(.),p(.),pent(t)}	368.9032	1.0000	12	191.5053
	POPAN: {phi(.),p(t),pent(t)}	373.2203	0.1035	27	152.7429
PopB Females (1997-2017)	POPAN: {phi(t),p(t),pent(t)}	430.0241	<0.0001	44	140.6934
	POPAN: {phi(.),p(t),pent(t)}	418.7866	1.0000	34	120.1985
	POPAN: {phi(.),p(.),pent(t)}	438.8669	<0.0001	14	188.7229
	POPAN: {phi(t),p(t),pent(t)}	462.5575	<0.0001	51	115.8350

Table 2.3. Confirmed reproductive status of headstarted turtles at both PopA and PopB. Age at First Confirmed Reproduction denotes the age at which the turtle was confirmed to be reproductively active; however, their first reproduction could have occurred earlier. Males were confirmed mature only by observations of mating, and females were confirmed mature only by observations of nesting. Table is sorted by Sex, then by Cohort Year.

Notch	Cohort/Release Year	Population	Sex	Age at First Confirmed Reproduction	Size at First Confirmed Reproduction (MaxCL [mm])	Notes
600	2003/2005	B	M	12	192	Observed mating
465	2008/2010	B	M	10	172	Observed mating
446	2005/2008	B	F	11	167	Clutch size = 9; 7 hatched
602	2006/2009	A	F	11	171	Clutch size = 7; 7 hatched
477	2007/2010	B	F	10	166	UNK clutch information
478	2007/2010	B	F	10	166	UNK clutch information

Table 2.4. Wood Turtle (*Glyptemys insculpta*) life-history parameters for the Base Model in a population viability analyses using VORTEX. The Base Model represents current population demographics. Nest survivorship values are excluded given all eggs are artificially incubated ex situ. Parameter values are from this study, unless otherwise noted. Values were calculated based on previous studies, and are conservative estimates.

Parameter	Values		Source / Explanation
	PopA	PopB	
Years Projected	36		50 years of headstarting (2003-2053)
Iterations per Model	1000		
Initial Population Size	18	119	
Male Age Distribution (1-5y)	2	37	
Male Age Distribution (6-10y)	2	5	
Male Age Distribution (11-60y)	3	8	
Female Age Distribution (1-5y)	2	37	
Female Age Distribution (6-10y)	2	8	
Female Age Distribution (11-60y)	6	24	
Carrying Capacity	none		Population growth likely not constrained by carrying capacity (Brooks <i>et al.</i> 1991) Galbraith (1991)
Breeding System	polygynous		
Adult Females Breeding (%)	47	64	
Adult Males Breeding (%)	75		Ernst and Lovich (2009)
Max Number of Clutches Per Year	1		
Clutch Size (SD)	6.63(3)		Mean number of eggs hatched per clutch
Maximum Clutch Size (eggs)	12		
Male Age at Sexual Maturity	10		
Female Age at Sexual Maturity	10		
Maximum Age of Reproduction	70		Jones (2009)
Maximum Lifespan	70		Jones (2009)
Sex Ratio (Eggs)	1:1		assumed
Average Headstarted Turtles Released (2013-2016)	13	59	
Mortality Rates (%)			
<i>Juveniles in the Headstarting Program</i>			

From Age 0 to 1 (only non-HS hatchlings from clutches which were missed) ¹	99	99	82% egg mortality rate (Brooks <i>et al.</i> unpubl) and 95% egg collection rate (rate will decrease with increased recruitment)
From Age 1 to 2 (only non-HS hatchlings from clutches which were missed) ²	88	88	Juvenile mortality rates (65%) doubled as an estimate for modelling added mortality of non-HS hatchlings to naturally reach size of HS within the model
From Age 2 to 3	68	48	
From Age 3 to 4	38	36	
From Age 4 to 5	40	39	
From Age 5 to 6	33	15	
From Age 6 to 7	30	27	
From Age 7 to 10	25	15	
<i>Juveniles from the Non-Headstarting Program</i>			
From Age 0 to 1 (No Management)	94		82% egg mortality rates (Brooks <i>et al.</i> unpubl) combined with 65% juvenile mortality rate
From Age 0 to 1 (Nest Protection)	81		54% egg mortality rates (Brooks <i>et al.</i> unpubl) combined with 65% juvenile mortality rate
From Age 0 to 1 (Egg Incubation)	72	75	20% (PopA) and 29% (PopB) incubation mortality rates combined with 65% juvenile mortality rate
From Age 1 to 10 (Non-HS)	65		Coarse estimation - released 51 hatchlings in 2009, only 1 survived
<i>Adults (includes both headstarted and non-headstarted turtles)</i>			
From Age 11 to 70 (Adult Males)	7.5	8.0	
From Age 11 to 70 (Adult Females)	11.0	7.0	

¹Most eggs are collected for the headstarting program however some nests are missed. This parameter models the survival of these nests by incorporating non-management egg mortality rate (82%), juvenile mortality rate (65%), and egg collection rate (95%). This survival does not affect turtles in captivity as those survival rates are modeled through average headstarts released (2013-2016).

²Increasing non-HS hatchling mortality rate to include added mortality to reach the size class of headstarted turtles post-release is necessary so that the PVA can model both non-HS hatchlings from clutches that were not collected and headstarted turtles as a single population.

Table 2.5. Elasticity analyses to evaluate deviations in the intrinsic rate of population increase (r) of the “Headstarting Program” and “No Management Program” base models resulting from a variety of parameter changes, as noted. Given that PopA is the closest to extirpation, parameters within this table are sorted by largest population growth rate to the smallest population growth rate for the PopA Headstarting Program.

Parameter Change	Headstarting Program Population Growth Rate		No Management Program Population Growth Rate	
	PopA (r)	PopB (r)	PopA (r)	PopB (r)
All Juvenile Mortality Rates Decrease 25%, 15% Minimum ¹	0.0352	0.0339	-0.0287	-0.0724
Split Release Scenario ²	0.0234	-0.0069	NA	NA
All Juvenile Mortality Rates Decrease 10%, 15% Minimum ¹	0.0157	0.0200	-0.0577	-0.1077
Adult Mortality Rates Decrease to 5%	0.0029	0.0104	-0.0515	-0.1007
Age at Sexual Maturity Decreases by 2 Years	-0.0009	0.0090	-0.1074	-0.1163
Stable Age Distribution ³	-0.0016	0.0065	-0.1976	-0.1844
Proportion of Adult Females Breeding Annually Increases to 70%	-0.0018	0.0069	-0.1068	-0.1326
Lifespan Increases to 90 Years	-0.0018	0.0093	-0.1258	-0.1299
Fertilized Eggs/Clutch Decreases 25%	-0.0020	0.0069	-0.1442	-0.1389
Adult Mortality Rates are 10%	-0.0021	-0.0044	-0.1250	-0.1754
Lifespan Decreases to 50 Years	-0.0021	0.0090	-0.1466	-0.186
Proportion of Adult Females Breeding Annually Decreases to 30%	-0.0022	0.0069	-0.1527	-0.1496
Fertilized Eggs/Clutch Increases 25%	-0.0022	0.0071	-0.1120	-0.1295
BASE MODEL	-0.0023	0.0069	-0.1276	-0.1337
Age at Sexual Maturity Increases by 2 Years	-0.0025	0.0055	-0.1482	-0.1410
Adult Mortality Rates are 15%	-0.0032	0.0012	-0.2012	-0.2537
All Juvenile Mortality Rates Increase 10%, 95% Maximum ¹	-0.0191	-0.0122	-0.1436	-0.1420
All Juvenile Mortality Rates Increase 25%, 95% Maximum ¹	-0.0850	-0.3820	-0.1568	-0.1451

¹Not including age 0 to age 1 for Headstarting Program given eggs are collected

²Splitting the number of headstarted released at both PopA and PopB, thus 36 headstarted turtles released both PopA and PopB

³Stable Age Distribution as calculated in VORTEX (Lacy *et al.* 2005)

Table 2.6. Demographic parameters of the Recommended Management Plan Model for use in VORTEX. Demographic parameters are a combination of Base Model demographic parameters estimated using population specific data, supplemented with data from the literature if necessary (see Table 2.4) and hypothetical changes through the incorporation of the Recommended Management Plan. The Recommended Management Plan incorporated a predator-removal management strategy to current management which hypothetically should increase survivorship of all life-stages excluding eggs incubated ex-situ and headstarted turtles in captivity. Demographic parameters were calculated based on previous studies, and are conservative estimates unless otherwise noted. Note this model is based off hypothetical changes to demographic parameters and not population specific parameters.

Parameter Change	Values		Explanation for Change and Source
	PopA	PopB	
Mortality Rates (%)			
<i>Juveniles</i>			
From Age 0 to 1 (No Management)	74	94 ¹	Increased survivorship due to predator-removal
From Age 0 to 1 (Nest Protection)	64	84 ¹	54% egg mortality rates (Brooks <i>et al.</i> unpubl) combined with 22% juvenile mortality rate (Congdon <i>et al.</i> 1993)
From Age 0 to 1 (Ex-Situ and Release)	38	65 ¹	20% (PopA) and 29% (PopB) incubation ex-situ mortality rates combined with 22% juvenile mortality rate (Congdon <i>et al.</i> 1993)
From Age 1 to 10 for Non-Headstarted Juveniles	22		Increased non-headstarted juvenile survival to published values from Congdon <i>et al.</i> (1993)
From Age 2 to 4 for Headstarted Juveniles	20		Increased values for increases in size/age
From Age 5 to 7 for Headstarted Juveniles	15		Increased values for increases in size/age
From Age 8 to 10 for Headstarted Juveniles	10		Increased values for increases in size/age
<i>Adults</i>			
Headstarted and Non-headstarted Adult Males age 11+	5		Slightly higher than 3% reported by Schneider <i>et al.</i> (2018)
Headstarted and Non-headstarted Adult Females age 11+	5		Slightly higher than 3% reported by Schneider <i>et al.</i> (2018)
Headstarts Released (If Applicable)	36	36	Split Release Scenario

¹PopB estimate is 20% higher to account for eggs being removed from PopB for supplementing PopA

General Conclusion

In Chapter I, I experimentally tested a fundamental assumption of headstarting by simultaneously releasing three cohorts of Wood Turtles (*Glyptemys insculpta*) with varying degrees of headstarting to determine if raising turtles to larger body sizes increase post-release survivorship. I showed that headstarting turtles to a larger body size confers a survival advantage. I modelled these results and predicted that this survival advantage should increase population growth rate relative to wild recruitment. I provided support for headstarting as an effective conservation strategy, with the caveat that all headstarting projects must be paired with management plans that maintain high adult and juvenile survival.

In Chapter II, I quantitatively assessed the effectiveness of a 15-year Wood Turtle headstarting program. I modelled population-specific demographic parameters to evaluate recovery efforts to date. I report that both study populations of Wood Turtles have shown some evidence of recovery, though the long-term survival of the both populations will require additional intervention, including continued headstarting, predator reduction, and disease management. Headstarting alone is not enough to save both populations from local extinction as these populations face multi-faceted complex problems for which management is challenging. This highlights the importance of using headstarting only as a supplementary strategy in stable environments. I made 7 recommendations to further enhance the success of this project.

Opportunistically, I also illustrated the limitations of modeling. Brooks *et al.* (unpubl. data) predicted population recovery if a headstarting program was initiated. In Chapter I, using the best data available at the time, I also predicted population recovery using headstarting. In Chapter II, I analyzed the long-term dataset and reported that both adult and juvenile survival were slightly

lower than previously modelled by both me and Brooks *et al.* (unpubl. data). After incorporating these seemingly minute changes, I predicted that headstarting alone was not enough to save both populations from local extinction. This disparity of results between chapters provides support for two often repeated 21st century concepts in wildlife biology. The first is that population models are simply hypotheses that need to be tested with field data (Bennett *et al.* 2017). Small changes between models and reality may have cumulative effects which can drastically influence growth rate projections. Models have the potential to misinform management plans and waste valuable conservation funds while further endangering species-at-risk if deployed without caution (Shrader-Frechette 2004, Conroy *et al.* 2006). This caveat must be recognized as we use population models to inform management plans. The second is that long-term monitoring programs such as this one are essential in providing extensive datasets where models and conservation initiatives such as headstarting can be thoroughly evaluated.

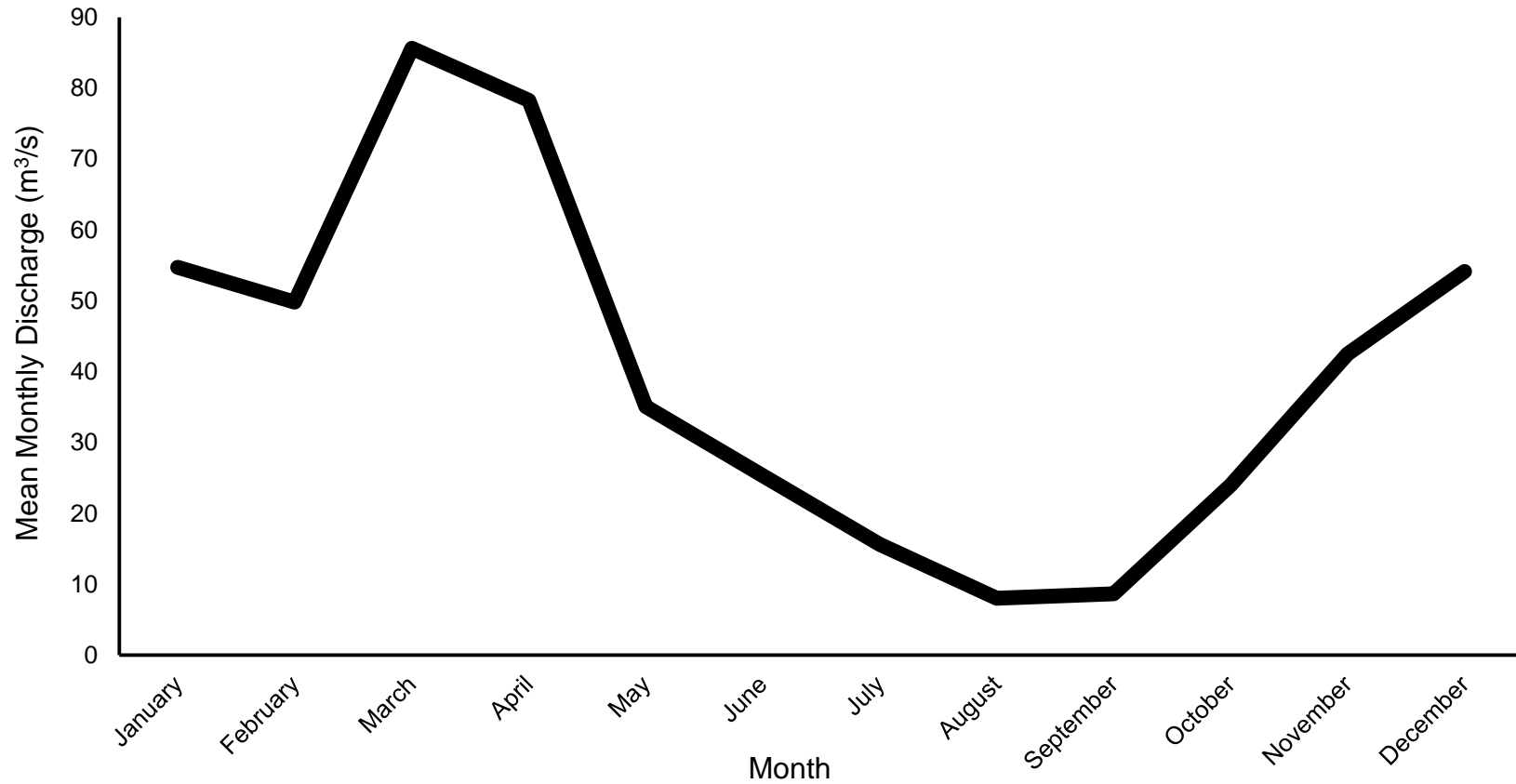
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APPENDIX A.

SEASONAL WATER FLOW RATES

Mean monthly water discharge rates (meters³/second) for 2003-2017 from an Environment Canada hydrometric station located in proximity to both PopA and PopB. Name of hydrometric station is withheld for species protection.



APPENDIX B.

POPA CAPTURE HISTORIES OF HEADSTARTED TURTLES

Summary of capture histories for eggs collected, hatchlings reared, and headstarted turtles released from/into PopA for 2003-2017.

Capture histories were manipulated for my analysis and the explanations and details of these manipulations are available in *Methods –*

Data Analysis – Population Demographics of Headstarted Turtles.

<i>Cohort</i>	<i>Eggs</i>	<i>Hatchlings</i>	<i>Hatchlings Headstarted</i>	<i>Total Headstarts Released from PopA</i>	<i>Total Headstarts Released</i>	<i>Total Captures Years Post-Release</i>											
						<i>1</i>	<i>2</i>	<i>3</i>	<i>4</i>	<i>5</i>	<i>6</i>	<i>7</i>	<i>8</i>	<i>9</i>	<i>10</i>	<i>11</i>	<i>12</i>
2003	11	4	4	3	3	3	2	2	1	1	1	1	1	1	1	1	1
2004	14	8	8	6	6	2	2	1	1	1	1	1	1	1	1	1	0
2005	0	0	0	0	0	0											
2006	20	16	16	12	12	10	8	6	4	2	1	1	1				
2007	37	28	14	13	13	4	2	1	0								
2008	0	0	0	0	0	0											
2009	24	21	21	9	9	5	3	0									
2010	20	17	17	17	17	10	7	5	4	3							
2011	27	25	25	25	25	5	1	0									
2012	2	0	0	0	0	0											
2013	19	15	15	13	22	3	1										
2014	16	10	10	9	9	2	0										
2015	10	10	10	7	7	0											
2016	20	18															
2017	26	25															
SUM	246	197	140	114	123	44	26	15	10	7	3	3	3	2	2	1	1

APPENDIX C.

POPB CAPTURE HISTORIES OF HEADSTARTED TURTLES

Summary of capture histories for eggs collected, hatchlings reared, and headstarted turtles released from PopB between 2003-2017.

Capture histories were manipulated for our analysis and these explanation and details of this manipulation is available in *Methods –*

Data Analysis – Population Demographics of Headstarted Turtles.

Cohort	Eggs	Hatchlings	Hatchlings Headstarted	Total Headstarts Released from PopB	Total Headstarts Released	Total Captures Years Post-Release											
						1	2	3	4	5	6	7	8	9	10	11	12
2003	29	27	27	22	22	11	4	4	3	3	3	3	3	3	3	3	3
2004	15	10	10	7	7	5	4	2	0								
2005	16	16	16	10	10	6	4	3	3	3	3	3	3	2			
2006	36	10	10	6	6	0											
2007	17	16	16	12	12	3	2	2	2	2	2	2					
2008	40	23	23	20	20	6	5	4	4	3	3	3					
2009	65	51	10	8	8	4	2	1	1	1	1						
2010	54	40	40	32	32	21	11	11	8	6							
2011	45	29	29	28	28	8	4	3	1								
2012	59	7	7	7	7	5	3	3									
2013	76	58	58	47	38	25	18										
2014	103	91	91	87	87	45											
2015	77	62	62	53	53	34											
2016	96	83															
2017	95	60															
SUM	823	583	399	339	330	173	57	33	22	18	12	11	6	5	3	3	3

APPENDIX D.

MORTALITIES OBSERVED WITHIN POPA AND POPB

Mortalities observed and general demographic data of the dead turtles from PopA and PopB from 2004 to 2017. Only turtles confirmed dead are included, thus this table does not include instances where a radio-transmitter was found but no turtle was found, hence lost turtles are not included. Table is sorted by *Population* (PopA then PopB), then *Capture Date*.

Population	Sex	Age	Capture Date	Suspected Cause of Death	Notes
PopA	Male	Adult	2004-05-07	Unk	Under woody branch, no soft tissue left. 11th and 12th marginal scutes broken
PopA	Male	Adult	2005-06-02	Unk	Suspected to have died 4 or 5 years ago, shell looks in good condition no wounds
PopA	Unk	3	2007-08-14	Predation	Tracked VHF telemetry signal to a hole in a tree
PopA	Female	Adult	2009-09-02	Agriculture	Very obviously killed by haying equipment
PopA	Unk	3	2010-09-15	Unk	No notes
PopA	Unk	4	2010-03-11	Predation	Found shell cut in half with scutes, other broken parts of shell, and transmitter scattered nearby
PopA	Male	Adult	2011-04-27	Predation	Soft tissue left inside the body cavity
PopA	Unk	5	2011-06-21	Vehicle	Suspected of being run over by a gravel truck
PopA	Unk	3	2012-03-15	Unk	Found in the water
PopA	Unk	3	2012-03-15	Unk	No notes
PopA	Unk	2	2013-07-22	Predation	Found on plastron with head and limbs missing but the tail is still intact. Antenna is missing from the transmitter
PopA	Unk	6	2013-07-08	Unk	No soft tissue left
PopA	Unk	7	2013-05-08	Agriculture	Found turtle shell cut in half in agricultural land
PopA	Unk	7	2013-05-29	Predation	Head severed at the neck (still hanging on)
PopA	Unk	4	2013-06-24	Predation	Head and all limbs missing
PopA	Unk	4	2013-04-17	Predation	Hind limbs missing
PopA	Unk	3	2014-09-08	Predation	Piece of shell attached to transmitter with bone
PopA	Female	Adult	2014-05-16	Unk	Some soft tissue left, heavily decomposed with shell intact
PopA	Unk	8	2014-07-30	Predation	No soft tissue left, chew marks and scutes missing on left side of carapace near bridge
PopA	Unk	2	2015-08-04	Unk	In cedar bush, soft tissue completely intact. No chew marks
PopA	Female	Adult	2015-12-04	Agriculture	Badly damaged shell found between rows in wheat field. Suspected of going through combine

APPENDIX D cont'd

MORTALITIES OBSERVED WITHIN POPA AND POPB

Population	Sex	Age	Capture Date	Suspected Cause of Death	Notes
PopA	Unk	2	2015-07-08	Predation	Head missing but most tissue intact. No chew marks. Covered in maggots
PopA	Unk	2	2015-07-08	Unk	Found 1 m from transmitter. Soft tissue intact. No bite marks on shell
PopA	Unk	3	2016-04-20	Unk	Some skin and back part of body still present
PopA	Female	Adult	2016-04-19	Unk	Found shell scattered in pieces on floodplain
PopA	Unk	3	2016-07-06	Unk	No notes
PopA	Unk	Hatchling	2016-08-18	Predation	25cm below the ground in a small burrow. Likely a small mammal predation
PopA	Unk	1	2016-07-18	Predation	Missing head and all limbs
PopA	Male	Adult	2017-05-19	Unk	Died at least a couple years ago
PopA	Unk	Hatchling	2017-08-21	Predation	Found transmitter deep in small mammal burrow, very likely dead therefore included as dead
PopA	Unk	Hatchling	2017-09-02	Predation	Found beside small mammal burrow
PopA	Unk	7	2017-06-09	Predation	Missing head and forepart of limbs
PopA	Female	11	2017-07-03	Personal Lawn Mower	Found on carapace in recently mowed area. Head soft tissue slightly intact though has a strange fracture through the skull; broken left back leg and broken shell; looks fresh.
PopA	Unk	1	2017-07-30	Predation	Found on carapace all limbs and head chewed off, in fallen trees
PopB	Unk	2	2005-07-20	Predation	Missing one forelimb, VHF radio antenna damaged
PopB	Female	Adult	2006-05-07	Unk	Left side of carapace cracked
PopB	Unk	< 5	2006-05-07	Unk	No notes
PopB	Female	Adult	2006-05-17	Unk	No notes
PopB	Unk	3	2006-05-26	Unk	No notes
PopB	Unk	3	2006-06-23	Predation	Shell with little to no soft tissue, researchers suspected coyote predation as shell had bite marks
PopB	Male	Adult	2006-06-26	Unk	First capture since 1993, no other specified notes.
PopB	Unk	3	2006-06-26	Unk	No notes
PopB	Unk	3	2006-06-28	Drowning	Found far downstream from release spot
PopB	Unk	4	2007-05-28	Predation	Found transmitter with parts of shell still attached with chew marks on VHF radio antenna
PopB	Unk	4	2007-06-19	Predation	Found on carapace
PopB	Unk	4	2007-06-21	Unk	Found shell underwater broken into pieces
PopB	Unk	3	2007-07-03	Predation	Found on carapace under vegetation

APPENDIX D cont'd

MORTALITIES OBSERVED WITHIN POPA AND POPB

Population	Sex	Age	Capture Date	Suspected Cause of Death	Notes
PopB	Unk	4	2008-04-23	Predation	Transmitter base ripped off from shell and tail tangled around the branches of a shrub (almost tied in knots); turtle facing towards ground and semi-suspended in air; skin fairly pliable
PopB	Unk	4	2009-04-30	Predation	Found on carapace missing forelimbs and head
PopB	Unk	5	2009-04-30	Predation	Found near a log amongst cedars missing head and most of its limbs
PopB	Unk	< 4	2009-05-12	Predation	Missing head, left forelimb, left hind limb, and most of tail
PopB	Unk	6	2009-06-01	Predation	Missing had and most limbs, only a bit of the left front foot remaining
PopB	Unk	3	2009-08-05	Unk	No damage to soft tissue or shell. Slight decomposition at end of claws
PopB	Unk	4	2009-08-05	Predation	Missing all limbs and head
PopB	Unk	4	2011-04-18	Unk	Found shell under some brush
PopB	Male	Adult	2012-03-18	Unk	Found dead in March during 2012 heat wave
PopB	Unk	3	2013-04-15	Predation	Missing head and limbs
PopB	Male	Adult	2013-04-26	Unk	No soft tissue left
PopB	Unk	3	2013-04-26	Predation	Missing head, limbs and tail but body cavity intact
PopB	Unk	< 4	2013-04-26	Predation	Missing limbs
PopB	Male	Adult	2013-05-02	Predation	Missing forepart of bone on all 4 limbs. Minimal soft tissue remaining
PopB	Unk	3	2013-05-02	Predation	Missing head and limbs, soft tissue remaining within the body cavity
PopB	Unk	3	2013-05-02	Predation	Found dead in tree hollow
PopB	Unk	3	2013-05-09	Predation	Missing head and limbs, soft tissue within body cavity intact
PopB	Unk	4	2013-05-09	Unk	Minimal soft tissue remaining
PopB	Unk	3	2013-05-13	Unk	Missing head and limbs, soft tissue within body cavity intact
PopB	Unk	2	2013-07-09	Predation	Missing head and limbs also missing part of tail
PopB	Unk	3	2013-08-19	Predation	Missing head and limbs but body cavity intact
PopB	Unk	3	2014-04-28	Drowning	Found dead in water, turtle still intact. Appears to have drowned.
PopB	Unk	3	2014-05-07	Unknown	No soft tissue remaining
PopB	Unk	6	2014-05-07	Unk	Found on floodplain
PopB	Unk	3	2014-05-11	Unk	No notes
PopB	Female	Adult	2014-05-30	Vehicle	Dead on side of road, carapace badly fractured. Likely vehicle mortality
PopB	Unk	4	2015-04-28	Unk	Found pieces of scutes and bones in pile; unusual way to find a dead turtle; maybe crushed by a vehicle

APPENDIX D cont'd

MORTALITIES OBSERVED WITHIN POPA AND POPB

Population	Sex	Age	Capture Date	Suspected Cause of Death	Notes
PopB	Male	Adult	2015-05-06	Unk	No soft tissue left
PopB	Unk	5	2015-06-22	Unk	No soft tissue left
PopB	Unk	3	2016-04-18	Predation	Brought into rehab as turtle was unresponsive; died in captivity possibly due to infection from bite mark on tail
PopB	Unk	3	2016-04-29	Predation	Some soft tissue remaining
PopB	Unk	3	2016-05-16	Predation	Missing head and limbs
PopB	Unk	3	2016-05-23	Drowning	Found >40 kilometres downstream of release spot 11 months post-release; likely got washed down in spring flood
PopB	Unk	2	2016-07-11	Predation	Found on carapace, missing head and limbs
PopB	Unk	1	2016-07-17	Predation	Missing head and limbs
PopB	Unk	2	2016-07-21	Predation	Missing forepart of limbs
PopB	Female	11	2016-08-03	Predation	Minimal soft tissue left; vegetation was crushed around where she was found; transmitter antenna is damaged.
PopB	Unk	Hatchling	2016-08-08	Predation	No soft tissue left
PopB	Unk	2	2016-08-10	Predation	Found in the water, missing head and limbs, heavy decomposition of soft tissue within the body cavity
PopB	Female	Adult	2017-01-21	Predation	Found on carapace on snow, all limbs missing but head and tail intact
PopB	Unk	3	2017-01-21	Predation	Found on plastron, missing head and limbs
PopB	Female	Adult	2017-03-15	Predation	Found dead on carapace 2.7 m up in a Sugar Maple tree; covered in raccoon hairs
PopB	Male	Adult	2017-04-21	Predation	Front limbs gone, some red blood stains in area; eyes white and puffy; CWHC confirmed predation as hemorrhage and inflammation indicated damage to limbs occurred while the turtle was alive
PopB	Unk	2	2017-04-23	Predation	Found on plastron, head and limbs missing, minimal amount of soft tissue left
PopB	Unk	2	2017-04-24	Predation	Found small white hairs everywhere
PopB	Unk	2	2017-04-24	Unk	No notes
PopB	Unk	3	2017-04-24	Predation	Missing head and forepart of both fore and rear limbs; found near Raccoon tracks
PopB	Unk	5	2017-04-25	Predation	Found on carapace in small creek; missing head and limbs
PopB	Female	Adult	2017-05-26	Predation	Left forelimb and head missing, bite mark in back right leg; kill was fresh as back right leg was still moving when researchers found her; had eggs, none viable when incubated
PopB	Unk	2	2017-05-26	Predation	Missing head and forepart of limbs
PopB	Unk	3	2017-05-26	Predation	Missing head and limbs
PopB	Unk	2	2017-06-08	Predation	Found on carapace, no soft tissue left
PopB	Female	14	2017-06-21	Predation	Found on carapace, missing head and limbs, minimal soft tissue left within body cavity, late stages of decomposition
PopB	Unk	8	2017-07-03	Unk	minimal soft tissue left

APPENDIX D cont'd

MORTALITIES OBSERVED WITHIN POPA AND POPB

Population	Sex	Age	Capture Date	Suspected Cause of Death	Notes
PopB	Male	Adult	2017-07-03	Predation	Alive, missing both front limbs; bone protruding out of skin; brought into captivity.; deemed unreleasable.
PopB	Unk	2	2017-07-21	Predation	Missing head and limbs but tail intact
PopB	Unk	5	2017-07-21	Predation	Found on plastron, minimal soft tissue left
PopB	Unk	6	2017-10-02	Predation	Left back leg is red (blood pooling subcutaneously) and bite mark on heel of foot; turtle seems unresponsive; died in captivity