EFFECTIVENESS OF HEAD-STARTING AS A MANAGEMENT TOOL FOR ESTABLISHING A VIABLE POPULATION OF BLANDING'S TURTLES

by

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(Under the Direction of Richard B. Chandler and Tracey D. Tuberville)

ABSTRACT

Blanding's turtles (*Emydoidea blandingii*) are facing a variety of anthropogenic threats that interfere with their natural life history cycle, decreasing population sustainability. To combat diminished populations, a repatriation project is underway using annual translocations of hatchlings from a donor site to the Assabet River National Wildlife Refuge. To increase first-year survivorship (which is typically low in turtles), 52% of translocated hatchlings were head-started (raised in captivity for nine months post-hatching). We conducted mark-recapture efforts and used a Cormack-Jolly-Seber (CJS) analysis to estimate apparent survival of direct-released hatchlings (released shortly after hatching) and head-started hatchlings. We examined post-release site fidelity and habitat selection by monitoring a subset of individuals through radio telemetry. We found that head-starts first year post-release apparent survival was nearly six times higher than direct-releases. Both direct-releases and head-starts displayed site fidelity when there was a variety of habitat types available at the release site to select from.

INDEX WORDS: *Emydoidea blandingii*, Blanding's turtle, head-starting, translocation, mark-recapture, Cormack-Jolly-Seber, site fidelity, habitat selection.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

There are currently 335 turtle species across the globe, 40% of which are considered threatened (Critically Endangered, Endangered, or Vulnerable) according to the 2013 IUCN Red List (Turtle Taxonomy Working Group 2014). The threat of extinction is common across taxa, with 25% of mammal species and 13% of bird species listed as threatened (IUCN 2015). The current global status of turtles is driven in part by the global decline of freshwater turtle species, of which over 40% are threatened (Olson and Kiester 2011). The decline of freshwater turtle species has been well documented (Buhlmann et al. 2009, Garber and Burger 1995, Lovich and Ennen 2013, Olson and Kiester 2011). A main factor contributing to their vulnerability are their suite of life history characteristics. Many turtle species have evolved to exhibit delayed sexual maturity, low adult mortality, lengthened generation times, and increased rates of mortality in eggs and hatchlings (Congdon et al. 1993, Congdon et al. 1994). To maintain self-sustaining populations, turtles must have high adult survivorship and long life spans (Heppell 1998), as well as high juvenile survivorship (Congdon et al. 1994). These requirements make them vulnerable to changes in their environment, with just a 3% decrease in adult annual survival potentially resulting in reduced population viability (Gibbs and Shriver 2002). These characteristics also make turtle populations slower to recover from populations declines than other, shorter-lived species (Chaloux 2011).

Turtles are facing a variety of threats that negatively affect their natural life histories and place added stress on populations. The main anthropogenic factors affecting turtles are habitat loss/degradation, abnormally high rates of mortality related to human activities (i.e. traffic mortalities, bycatch in fishing nets, greater densities of natural predators near developed areas), and illegal/unregulated collection for food consumption/pet trade (Chaloux 2011). Loss of both wetland and upland nesting habitat has been well documented for many freshwater turtle species. Spotted Turtles (*Clemmys guttata*) in Ohio have been found to be vulnerable to habitat loss and fragmentation associated with developments, as well as habitat degradation through the proliferation of invasive species (Lewis et al. 2004). Habitat degradation caused by the oil industry was responsible for decreases in turtle abundance of four freshwater species in West Africa (Luiselli et al. 2006). Bog Turtle (*Glyptemys muhlenbergii*) habitat in the northeastern United States was negatively affected by the proliferation of invasive flora that were boosted by nutrient enrichment from manure and run-off from livestock operations (Tesauro and Ehrenfeld 2007). Isolated, remnant wetland habitats have lower levels of turtle species richness when compared to connected wetlands (Galat et al. 1998). However, even when habitat is protected, self-sustaining populations may not persist when other threats exist (Browne and Hecnar 2007).

Many turtles show periods of high seasonal mobility in search of food or suitable nesting habitats (Bodie and Semlitsch 2000). These periods of mobility can increase their probability of encountering roads, and lead to road mortality rates that cause population declines in areas where traffic densities are high (Gibbs and Shriver 2002). Given that adult female turtles may make at least one overland migration each year to find suitable nesting habitat, they may be at greater risk for road mortalities and their loss can jeopardize recruitment into self-sustaining populations.

There are additional anthropogenic activities that can have negative effects on turtle populations, such as the accidental bycatch of turtles during fishing related activities, subsidizing of natural predators of turtles, and collection of turtles for food consumption/pet trade. Human elimination of large predators from an ecosystem can increase the abundance of mesopredators (Soule et al. 1988), which prey on turtle nests. Mesopredators have also become more abundant due to anthropogenic changes to natural ecosystems through the creation of edge habitat and food supplementation (Bernstein 2015). The loss of turtle nesting habitat can also increase nest predator efficiency by concentrating larger numbers of nesting females into smaller nesting areas (Roosenburg et al. 2014). Turtles have long been harvested by humans for either consumption or the pet trade (Caputo et al. 2005, Chapin and Meylan 2010, Gallego-Garcia and Castano-Mora 2008, Platt et al. 2008, Velo-Anton et al. 2011). Finally, incidental bycatch mortality of freshwater turtles has been observed with a variety of passive fishing techniques (Barko et al. 2004), including the use of crab traps (Dorcas et al. 2007), hoop nets (Larocque et al. 2012a), and fyke nets (Larocque et al. 2012b).

Combined these threats have the potential to limit the viability of turtle populations. Recovery of diminished or vulnerable populations may require remedying any sources of adult mortality, restoring degraded habitats and acquiring/protecting suitable habitat, and ensuring self-sustaining levels of survivorship of nests and hatchlings. The loss, alteration, degradation, and fragmentation of habitat are among the largest factors related to the decline of turtles and other reptiles (Gibbons et al. 2000). As such, the restoration and management of turtle habitats can maintain or increase turtle populations (Sirous et al. 2014). Due to their longevity, turtle populations can sustain short periods with little recruitment, but may soon disappear with low adult survivorship (Burke 2015). Therefore, the protection of adult and larger juvenile turtles is one of the quickest and most efficient ways to reverse the decline of a population (Frazer 1992). The protection of turtle nests should be of high priority, as high predation of turtle nests can limit recruitment and skew populations toward older age classes (Browne and Hecnar 2007).

Once the sources of population decline have been addressed, there are several management strategies that can be used to augment and recover diminished populations, including population manipulations, such as nest protection, head-starting programs, and/or repatriations/translocations (Seigel and Dodd 2000). High levels of nest predation may allow for limited recruitment of young turtles into adult populations (Congdon et al. 1983). The immediate placement of screened predator exclosures above nest chambers have been found to stop the majority of predation of turtle nests (Standing et al. 2000). Nest monitoring and protection efforts may be an effective tool at helping to increase turtle populations if conducted for a sufficient period of time and if other population stressors have been addressed (Dutton et al. 2005). These manipulations require adequate planning to lessen the risk of disease, maintain genetic diversity, and limit the impact to other native species that are present (Buhlmann et al. 2015). Head-starting programs and/or translocations may be a management tool that can be used to help speed the recovery time of diminished populations, or to establish new populations at suitable sites.

Head-starting involves captive rearing of wild-caught hatchlings to a larger body size to help them avoid high mortality rates in their first year, which is common in many turtle species (Heppell et al. 1996). Head-starting may help counteract high rates of adult mortality caused by human influences by increasing juvenile survival. Several studies have attributed higher survival rates among head-started turtles to their larger body size upon release as a result of accelerated growth rate or extended growth period while in captivity (Haskell et al. 1996; Vander Haegen et al. 2009). Research had also been conducted to investigate the success of freshwater turtle reintroduction projects, including those with a head-starting component involving the Northern Red-bellied Cooter (*Pseudemys rubriventris*; Haskell et al. 1996), European Pond Turtle (*Emys* *orbicularis*; Mitrus 2005); and Western Pond Turtle (*Actinemys marmorata*; Spinks et al. 2003 and Vander Haegen et al. 2009), but further research that can contribute to the current base of knowledge regarding the success of head-starting efforts involving freshwater turtle species may lend additional credence to its use as a management tool.

Translocations are the intentional release of animals to "establish, reestablish, or augment a population" (Griffith et al. 1989). This strategy is increasingly being adopted as a management technique (Attum et al. 2013) across taxa, as the number of reintroductions from 1902 - 2005 have grown 97% for invertebrates, 77% for mammals, 77% for reptiles and amphibians, 61% for birds, and 55% for fish (Seddon et al. 2007). The success rates for translocations had appeared to be higher for birds and mammals than reptiles and amphibians (Dodd and Seigel 1991), but comparatively little research has been conducted on reptile and amphibian translocation projects (Tuberville et al. 2005). There have been several studies conducted on the translocation of turtles over the last 10 years, such as Agassiz's Desert Tortoise (*Gopherus agassizii*; Nussear et al. 2012), Alligator Snapping Turtle (*Macrochelys temminckii*; Bogosian 2010 and Moore et al. 2014), Blanding's Turtle (*Emydoidea blandingii;* Buhlmann et al. 2015), Ornate Box Turtle (*Terrapene ornata ornata*; Sosa and Perry 2015), European Pond Turtle (Mignet et al. 2014), Gopher Tortoise (*Gopherus polyphemus*; Tuberville et al. 2005 and Tuberville et al. 2008), Hermann's Tortoise (*Testudo hermanni*; Lepeigneul et al. 2014), Musk Turtle (*Sternotherus odoratus*; Attum et al. 2013), and Red-eared Slider (*Trachemys scripta*; Attum and Cutshall 2015). Many of the translocations listed above involve the transporting of subadult or adult individuals from areas at which they had established home ranges. It is important to note that in our research, translocations in this context refer to hatchlings that are being moved from a site at which they were born, but to which they had limited exposure. Research has shown that while translocations often generate public interest, the effectiveness of translocations of reptiles has yet to be demonstrated on a large scale (Dodd and Seigel 1991), creating the opportunity for further research to be conducted.

Study Animal

The Blanding's Turtle (*Emydoidea blandingii*) is a member of Family *Emydidae*, a large and diverse group of freshwater and terrestrial turtles. The *Emydidae* Family includes species such as the Eastern Box Turtle (*Terrapene carolina carolina*), Diamondback Terrapin (*Malaclemys terrapin*), Painted Turtle (*Chrysemys picta*), and Spotted Turtle (Iverson 2011). Previous research (Bickham et al. 1996, Burke at al. 1996, Feldman and Parham 2002, Wiens et al. 2010) has shown the Blanding's Turtle to be closely related to the European Pond Turtle and Western Pond Turtle, though the three species are highly distinct morphologically (Fritz et al. 2011).

The Blanding's Turtle is a semi-aquatic freshwater turtle species that is easily identified by its distinctive bright yellow chin and throat. It also possesses a highly-domed carapace, which is marked with yellow flecking. An adult Blanding's Turtle has an average carapace length of 15 - 24 centimeters, and a body mass of 750 – 1400 grams (Congdon et al. 2008). They typically inhabit wetlands modified by American Beaver (*Castor canadensis*), marshes, ponds, slow-moving streams, swamps, and vernal pools (Congdon et al. 2008). Blanding's Turtles make seasonal overland movements during periods of nesting or when traveling to and from ephemeral wetlands (Grgurovic 2007).

The Blanding's Turtle has a potential lifespan of over 70 years and exhibits delayed sexual maturity, not reproducing until 14 to 20 years of age (Brecke and Moriarty 1989;

Congdon and van Loben Sels 1993). A previous long-term study found that they have a 96% annual survival rate as adults, but as juveniles (2 – 13 years) and hatchlings (0-1 years) their annual rates of survival are only 78% and 26%, respectively (Congdon and van Loben Sels 1993). In addition to the effect of low juvenile and hatchling survival, recruitment rates are also dampened by low nesting success, with some years resulting in 100% nest failure (Congdon and van Loben Sels 1993). The combination of delayed sexual maturity coupled with a low recruitment rate results in a generation time of almost 40 years (Congdon et al. 1993). The combination of delayed sexual maturity, low annual fecundity, and low rates of recruitment are natural impediments to population growth and recovery (Congdon and van Loben Sels 1993; Congdon et al. 1993). Increased adult mortality caused by human activities place the Blanding's Turtle at greater risk of population declines and extirpations.

The Blanding's Turtle ranges across parts of the northern United States and southeastern portions of Canada, with some form of protection throughout its range. The population in the northeastern United States is geographically isolated from the larger population, which occurs in the Midwest and Great Lakes Region. While populations throughout the Midwest are somewhat contiguous, populations in the northeastern United States and southeastern Canada have become fragmented.

Within New England, there are approximately 180 known records for Blanding's Turtles, but most of these are represented by only one or a few animals and many were observations of turtles crossing roads (Compton 2007). Nine sites in New England had documented 10-50 turtles and only three had more than 50 animals known (Compton 2007). Thus, most of the New England populations do not appear to represent long-term viable populations. Therefore, simply proposing to protect existing sites may not be enough to maintain this species as a viable component of the New England landscape (Buhlmann et al. 2015). In particular, the population of Blanding's Turtles at Great Meadows NWR has declined by more than 60% in the past 40 years, with road mortality of nesting females the most likely driving factor (Windmiller et al. 2015). Biologists throughout the northeast have expressed concern for the conservation status of this species, and have suggested that the species warrants consideration for federal listing (Compton 2007).

Only a single site in Massachusetts is considered to support a long-term viable population under current conditions (Buhlmann et al. 2015). However, Assabet River National Wildlife Refuge (NWR), a large tract of protected land situated in a densely-populated area in eastern Massachusetts, has been the site of a Blanding's Turtle repatriation project initiated in 2007 to establish an additional viable population (Buhlmann et al. 2015). Assabet River NWR envelopes nearly 900 hectares of land that is located roughly 40 kilometers (km) west of Boston, Massachusetts. The land was historically used for agricultural purposes, before being purchased by the U.S. Army in 1942 to be used as a military training ground as part of the U.S. Army’s Fort Devens complex. The refuge was established in 2000 after the land was transferred to the U.S. Fish & Wildlife Service.

An initial environmental assessment identified Assabet River NWR as an ideal site for a Blanding’s Turtle repatriation project due to its suitable habitat and close proximity to Massachusetts’ only stable population of Blanding’s turtles, which is located just 16 km away (USFWS 2007). Although the habitat on the refuge appears to be suitable for Blanding’s Turtles and multiple small populations of Blanding’s Turtles occur within the same watershed, previous surveying efforts at the site concluded there were no existing Blanding’s Turtle population present at Assabet River NWR (Aneptek 1991; Butler 1992; Buhlmann and Gibbons 2006).

Even though human activities are abated, natural recolonization of the area is unlikely due to high levels of habitat fragmentation that have created barriers to dispersal. The presence of suitable habitat on a protected site within the historic range of the Blanding's Turtle presented the opportunity for a reintroduction project at Assabet River NWR. The aforementioned environmental assessment (USFWS 2007) used a Population Viability Analysis (PVA) to evaluate the relative benefits and risks of releasing different life stages of Blanding's Turtles at Assabet River NWR. The life stages examined included adults, subadults, head-started hatchlings (raised in captivity for the first 9 months post-hatching) and direct-released hatchlings (released in autumn shortly after hatching; Buhlmann et al. 2015). Given that translocated adults are more likely to disperse from release sites than younger age classes, head-starts and direct-releases were chosen for the reintroduction project. The use of head-started hatchlings, which should experience survival and growth at greater rates than their direct-release counterparts, should shorten the time period for population establishment.

This thesis research compares survival of head-started and direct-released hatchlings and habitat selection and site fidelity of hatchlings. This information can be used to help assess the success of the repatriation project and inform population models used to predict future population growth and determine number of releases necessary. Survival and growth of head-started hatchlings is presumably significantly higher than in direct-released hatchlings, but experimentally quantifying the effectiveness of head-starting on survival and growth rates is important because head-starting is both cost- and labor-intensive. A greater understanding of habitat selection by young turtles and the amount of post-release site fidelity demonstrated by hatchling turtles is important for identifying suitable habitat and possible release sites. Given that 40% of the world's turtle population is considered threatened (Turtle Taxonomy Working

Group 2014), my research evaluating the effectiveness of head-starting and translocations of Blanding's Turtles at Assabet River NWR will help contribute to the body of knowledge regarding the use of population manipulations to address declining turtle populations.

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CHAPTER 2

SURVIVAL IN HEAD-STARTED & DIRECT-RELEASED BLANDING'S TURTLE HATCHLINGS

Introduction

Freshwater turtle species are increasingly at risk of extinction (Buhlmann et al. 2009a), with over 40% of them listed as threatened (Olson and Kiester 2011). High adult survivorship and long lifespans are key components of maintaining self-sustaining populations (Heppell 1998). The natural life histories for many turtle species consist of delayed sexual maturity, lengthened generation times, low adult mortality, and low juvenile survival (Congdon et al. 1993, Congdon et al. 1994). First-year survivorship in particular has been found to be significantly lower than that of subadults and adults, across a range of turtle species (Iverson 1991). An examination of freshwater turtle survivorship by Paterson et al. (2014), found that only 37.5% of Blanding's Turtle (*Emydoidea blandingii*) hatchlings and 14.3% of Wood Turtle (*Glyptemys insculpta*) hatchlings survived from the time of hatchling emergence through the selection of an overwintering site. First-year survivorship of Chicken Turtles (*Deirochelys reticularia*) has ranged between 7-43% (Buhlmann et al. 2009b), while juvenile (those between yearling and subadult life stages) Painted Turtles (*Chrysemys picta*) and Mary River Turtles (*Elusor macrurus*) exhibited survival rates of 46% (Mitchell 1988) and 50% (Micheli-Campbell et al. 2013), respectively.

Hatchling and juvenile turtles likely demonstrate lower survivorship than adults due to a greater risk of predation. Hatchling turtles are believed to be subject to intense predation pressures until they grow to a size where they are no longer subject to predation by gape-limited predators (Britson and Gutzke 1993). Juvenile freshwater turtles have been observed being preyed upon by amphibians, birds, fish, insects, and mammals (Haskell et al. 1996; Jones and Sievert 2012; Lefevre and Brooks 1995; Standing et al. 1997, Tuttle and Carroll 2005). The wide suite of predators of hatchling and juvenile turtles due to their small size upon hatching makes it likely that the majority of freshwater turtles will exhibit low juvenile survival.

One of the possible management strategies to aid in the recovery of a previously diminished population is to address low juvenile survival through head-starting. Head-starting is a conservation technique aimed at increasing juvenile survival in species that typically experience high mortality amongst juveniles (Sacerdote-Velat et al. 2014), by the captive rearing of wild-caught individuals through their most vulnerable period (Heppell et al. 1996). Head-starting not only protects hatchlings from predation during the time they are in captivity, but also helps increase the growth of hatchlings to a size at which they will be less vulnerable to their natural predators (Frazer 1992). Turtles and other reptiles appear to be well-suited for mass-rearing programs because they require little to no parental care (Heppell et al. 1996).

An increasing number of studies have investigated the effectiveness of head-starting freshwater and terrestrial turtles (Buhlmann et al. 2015; Haskell et al. 1996; Hazard et al. 2015; Masin et al. 2015; Michell and Michell 2015; Mitrus 2005; Moore 2013; Nagy et al. 2015; Penaloza et al. 2015; Spinks et al. 2003; Vander Haegen et al. 2009), many of which found that head-starts experienced high rates of survival. Among members of the *Emydidae* Family, head-starting of European Pond Turtles (*Emys orbicularis*) resulted in the attainment of sexual maturity several years earlier than their wild counterparts (Masin et al. 2015), while head-starting of Western Pond Turtles (*Actinemys marmorata*) was found to be a viable strategy for increasing recruitment (Spinks et al. 2003; Vander Haegen et al. 2009). Head-starting projects in the northeastern United States have found that Wood Turtle head-starts demonstrated 100% survivorship for two years post-release (Michell and Michell 2015), while first year post-release survivorship of head-started Northern Red-bellied Cooters (*Pseudemys rubriventris*) was related to size at release, with the largest head-starts exhibiting the highest survival (Haskell et al. 1996). The researchers in all freshwater head-starting projects agreed that head-starting can increase abundance, but should not be used as the only management tool to remedy population declines. So while increasing first-year survival may not fully compensate for population effects of high adult mortality, large enough quantities of captive-reared cohorts can serve to boost recovering populations once other causes of the decline have been remedied (Heppell et al. 1996; Heppell 1998).

Study Animal

The Blanding's Turtle (*Emydoidea blandingii*) is a semi-aquatic freshwater turtle species that is found across parts of the northern United States and southeastern portions of Canada, with some form of protection throughout its range. The Blanding's Turtle epitomizes the life history traits of many other long-lived freshwater turtles, not reaching sexual maturity until 14 to 20 years of age and a potential lifespan of over 70 years (Brecke and Moriarty 1989; Congdon and van Loben Sels 1993). As with many other freshwater turtle species, there is low adult mortality and higher mortality among younger turtles. Blanding's Turtle adults are estimated to possess a 96% annual survival rate as adults, but hatchling and juvenile annual rates of survival are only estimated to be 26% and 78%, respectively (Congdon et al. 1993). Low nest and hatch success, with the potential for 100% nest failure in some years, and low hatchling and juvenile survival can dampen recruitment rates (Congdon and van Loben Sels 1993). The combination of delayed sexual maturity coupled with a low recruitment rate results in a generation time of almost 40 years (Congdon et al. 1993).

As with other freshwater species, Blanding's Turtles have experienced population declines due to the interference of their life history cycle by human actions. Head-starting of hatchlings, which increases 1st-year survival and possibly leads to the earlier onset of sexual maturity, may be an effective way to recover diminished populations or to establish new ones. As such, we undertook an opportunity to test the effectiveness of head-starting as a way to establish a new population of Blanding's Turtles on a protected site, as established in Buhlmann et al. (2015).

The objectives of the study were to compare post-release survivorship of direct-release (released after hatching in August-September) and head-started hatchlings (raised in captivity for 9 months after hatching) through a mark-recapture effort, and subsequently model post-release survivorship. The survivorship data here builds on a project that was started in 2007, based on the modeling results (USFWS 2007) and goals and methodologies in Buhlmann et al. (2015).

Methods

Donor site

The donor site is a 674 hectare (ha) protected area owned and managed by the U.S. Fish & Wildlife Service (USFWS). It is located roughly 60 kilometers (km) west of Boston, Massachusetts and lies within the Nashua River watershed. The site supports a variety of wetland and terrestrial habitats, including seasonal and permanent wetlands, riparian zones, upland forest, and open fields. Prior to its acquisition by the USFWS in 1974, the land was owned by the U.S. military and subject to clearing of large patches of land. The resulting open canopy patches provided important nesting habitat for a variety of turtle species, including Blanding's Turtles. From 1987-2015 (Butler personal comm.), researchers have protected Blanding's Turtle nests every year at this site, which is thought to support the largest population of Blanding's Turtles in New England, with a population consisting of at least 500 individuals (Mockford et al. 2007).

Recipient site

Assabet River National Wildlife Refuge (NWR) is an 880 ha protected area located approximately 40 km west of Boston, Massachusetts and it is also approximately 16 km southeast of the donor site. The land was primarily managed for agriculture prior to its purchase by the U.S. Army in 1942 as a military training ground as part of the U.S. Army's Fort Devens complex. The land was transferred to the USFWS and the refuge was established in 2000. Wetlands comprise 20% of the total refuge area (USFWS 2007) and there are numerous vernal pools and permanent shrub wetlands that provide the habitat types necessary to support Blanding's Turtles year-round. The array of wetland and terrestrial habitats, coupled with the size of the refuge, and minimal automobile traffic, suggests that the refuge could support a sizeable population of Blanding's Turtles (Buhlmann et al. 2015). Although the habitat on the refuge appears to be suitable for Blanding's Turtles, previous surveys of the area have not found any existing populations, possibly due to prior human activities occurring at the site and barriers to natural recolonization.

Two wetlands located in the northeast corner of the refuge appear to possess favorable Blanding's Turtle habitat and were selected as release sites for this study. Taylor Brook Wetland (approximately 2.5 ha) is an herbaceous emergent marsh with sedges (*Carex spp.*), water lilies (*Nymphaeaceae spp.*), and duckweed (*Lemnaceae spp.*). This marsh forms the headwaters of Taylor Brook. Water levels, which often exceed two meters (m) in some areas in the spring, vary seasonally but the wetland is often kept flooded by resident American Beaver (*Castor canadensis*) that maintain a dam downstream. Pump Station Wetland (approximately 16.3 ha) is a scrub-shrub, marsh habitat with an unconsolidated bottom that is currently influenced by beaver activity, similar to the wetlands at the donor site. It is dominated by shrubby, woody vegetation, including Speckled Alder (*Alnus rugosa*), Buttonbush (*Cephalanthus occidentalis*), Leatherleaf (*Chamaedaphne catyculata*), Red Maple (*Acer rubrum*), Swamp Loosestrife (*Decodon verticillata*), and Sweet Gale (*Myrica gale*). There are large areas with significant amounts of *Sphagnum*, as well as patches of open water with Duckweed-covered pockets (Buhlmann et al. 2015). Taylor Brook Wetland and Pump Station Wetland are separated from each other by a dirt trail less than ten meters wide.

Nest protection and collection of hatchlings from the donor site

Blanding's Turtle nests at the donor site have been protected from predators since 1987 (B. Butler, personal comm.), but we only report here on the efforts of 2006-2013. During late May through late June, we conducted nightly searches (between the hours of 17:00-23:30) of known nesting areas for gravid females. Once females completed nesting, we placed a metal predator-proof exclosure over the nest. During August – September, we performed daily checks of nests for signs of hatchling emergence. When hatchlings were seen emerging from the nest chamber, we collected live hatchlings and retrieved any unhatched eggs and incubated them in captivity.

We processed all hatchlings shortly after they emerged, recording carapace length at the midline (MCL) to the nearest millimeter (mm) and mass to the nearest 0.1 gram (g) and noting any scute abnormalities. We marked turtles by cutting triangular notches into the marginal scutes with cuticle scissors using a standard shell-notching identification system (Cagle 1939; Figure 2.1). We assigned individual codes at hatching to those turtles that were designated for head-starting. For the 2007-2009 cohorts, we also assigned individual codes to direct-release hatchlings at time of hatching. However, during 2011-2013, direct-release hatchlings were assigned only a cohort mark at hatching and did not receive an individual mark until subsequently recaptured.

Head-starting husbandry

The hatchlings designated for head-starting were raised in captivity, with the majority being reared for roughly nine months (August/September – May/June). The 2006-2008 head-start cohorts were reared by researchers from the U.S. Fish & Wildlife Service and Oxbow Associates, Inc., the private consulting company that had been responsible for nest protection efforts at the donor site since 1987. The majority of head-started hatchlings from the 2009-2013 cohorts have been cared for by the Bristol County Agricultural High School, a local technical school with a natural resource management focused curriculum. Regardless of cohort and rearing location, hatchlings were kept active throughout the winter by being kept indoors in warm water and fed at least five times per week. Detailed husbandry details can be found in Buhlmann et al. (2015). As the head-starting program expanded, we recruited additional partners

– primarily schools and non-profit organizations – to foster environmental education and to engage the surrounding communities. In most cases these additional partners reared only a few head-starts each year, and were reared as described by Windmiller and Berkholtz (2012), which was very similar to the protocols described by Buhlmann et al. (2015).

Hatchling treatment and releases

As part of a larger study to evaluate the effectiveness of head-starting as a management tool for Blanding's Turtles (Buhlmann et al. 2015), we collected 2049 hatchlings from protected nests at the donor site from 2006 through 2014. To minimize impacts to the donor population, we directly-released 50% of the hatchlings from each nest back into the donor site wetland shortly after hatching (August – October; Buhlmann et al. 2015). Prior to release, all hatchlings were given a cohort mark (indicating hatch year) for identification in future donor site monitoring. Hatchlings released at the donor site are not considered further here. The remaining 50% of hatchlings from each donor site nest were retained for release at the recipient site. In most years, half of the recipient site individuals were directly-released shortly after hatching and the other half were first head-started indoors for nine months and released the following spring. However, in 2006 and 2010 all recipient site designated hatchlings were head-started.

Two direct-released cohorts (2007, 2008) and two head-started cohorts (2006, 2007) were released into Taylor Brook Wetland. All remaining direct-released (2009, 2011-2013) and head-started (2008-2013) cohorts were released into Pump Station Wetland. Release locations were recorded using hand-held GPS units (Garmin eTrex, Olathe, Kansas) with a positional accuracy of approximately ±5m. We released all direct-released hatchlings during August-October shortly after hatching. Most head-starts were released during May - June when they were approximately

9 months old and the risk of exposure to freezing temperatures had passed (Buhlmann et al. 2015). However, all 2006 head-starts and a total of seven head-starts from the 2009 and 2010 cohorts were held until the following fall when they were approximately a year old to allow them extra time to attain a size large enough to receive a radio transmitter at release (see Chapter 3).

Mark-recapture efforts at the recipient site

We trapped at the recipient site each year from 2011 - 2014 to estimate survival of direct-released and head-started hatchlings, and designated the release date for each individual as its initial capture for the mark-recapture analysis. We used several sizes of aquatic hoop traps baited with sardines (Ream and Ream, 1966): extra-large (91 cm diameter, 5 cm mesh, Memphis Net & Twine, Memphis, Tennessee), large (76 cm diameter, 2.5 cm mesh, Memphis Net & Twine, Memphis, Tennessee), medium (61 cm diameter, 2.5 cm mesh, Memphis Net & Twine, Memphis, Tennessee), and small (31 cm diameter, 0.6 cm mesh, Promar, Gardena, California). We checked traps every 24 – 48 hours, depending on their location, as water levels could fluctuate in areas influenced by beaver activity. Trap effort varied among years. We collected morphometric data (MCL and mass) for each recapture event. The minimum and average MCL at time of release was calculated for all turtles that were recaptured to serve as a minimum size requirement for head-starting managers, as well as the minimum and average MCL at time of first capture for all turtles that were recaptured to serve as a reference to managers as to how many years post-release to begin future trapping efforts.

Statistical analysis

We tested whether there was a significant difference in release size (MCL) between all direct-released and head-started hatchlings by performing an unpaired 2-sample t-test in program R (R Core Team 2014). We also analyzed whether there was a significant difference in mean MCL among cohorts of head-starts by performing a one-way Analysis of Variance (ANOVA) in program R to determine whether the growth during the head-starting period differed among years. We used the Tukey's Honest Significant Difference (HSD) post-hoc test in program R to determine which pairs of head-starts differed significantly in size.

Survival analysis

We estimated post-release survival in two ways. First, we estimated the minimum first-year post-release survival of each of the 2007-2011 cohorts and each treatment (direct-released and head-started) from the raw 2011-2014 recapture data. For each cohort-treatment group, we divided the number of individuals recaptured by the number of individuals released as part of the corresponding cohort-treatment group.

Next, we used the trapping data from 2011-2014 to construct mark-recapture histories for each individual, which we used to estimate apparent survival using a modified Cormack-Jolly-Seber open population model (CJS; Cormack 1964; Jolly 1965; Seber 1965) implemented using JAGS and the R package "rjags" (R Core Team 2014). The CJS analysis estimates apparent survival, which does not distinguish between mortality and emigration, producing a lower estimate than that of true survival (Schaub and Royle 2013). Estimates of apparent survival account for the fact that individuals may go undetected. Encounter probability in this context is defined as the probability of encountering an individual at a given trap on a given day. We used trap-level data, rather than trap-array-level data, because our trapping effort varied in space and time. Our model included six apparent survival (ϕ) parameters for the direct-releases and the head-starts for year 1, year 2, and year ≥ 3 post-release. It included three encounter probability parameters based on the age and likely size of the turtle: direct-release 1st-year post-release (p_1); direct-release 2nd-year post-release and head-start 1st-year post-release (p_2); and direct-release ≥ 3rd-year post-release and head-start ≥ 2nd-year post-release (p_3). Thus, the model included a total of 9 parameters. We did not include data from the 2006, 2012, or 2013 cohorts due to low encounter probabilities of those cohorts during sampling periods.

Results

Nest protection, collection of hatchlings and hatchling releases

We protected an average of 43.4 nests per year (range 17-87) at the donor site for a total of 347 nests during 2006-2013 (Table 2.1). Of the 2049 hatchlings, 1208 were directly-released at the donor site, 401 were directly-released at the recipient site, and 440 were head-started and subsequently released at the recipient site. On average, 50 direct-releases (range 0-154) and 55 head-starts (7-120) were released at the recipient site each year (Figure 2.2).

Trapping effort and success

Trapping effort and success varied among years, but was substantially greater in 2013-2014 (Table 2.2). In total, there were 180 unique individuals (23 direct-released hatchlings and 157 head-started hatchlings) recaptured out of 841 hatchlings that had been released to the recipient site. There were individuals from three direct-release cohorts (2008, 2011, 2012) and seven head-start cohorts (2007-2013) trapped and measured during the recapture events (Figure 2.3).

Body sizes at release and recapture

Head-starts were significantly larger at release than were direct-releases (df = 455.781, t = -43.499, $p < 0.001$), with a mean MCL of 66.2 mm compared to 35.3 mm. Head-start MCL at release also varied significantly among cohorts (df = 7, f-value = 40.3, $p < 0.001$), ranging from mean of 49.4 mm in 2007 to 80.4 mm in 2013 (Figure 2.4). Post-hoc comparisons revealed over 50% of the cohorts were significantly different in MCL at time of release (Table 2.3).

The average MCL at time of release of the direct-releases that were recaptured in traps at was 35.3 millimeters, ranging from the 2011 cohort average MCL of 34.8 mm to 38 mm. The average MCL at time of release of the head-starts that were recaptured in traps was 70 mm, ranging from 40.9 mm to 105.1 mm.

The minimum body size (MCL) at first recapture was 49.3 mm (2011 cohort individual captured in the year following its release) for direct-releases and 56.5 mm (2012 cohort individual captured in the year following its release) for head-starts. The average body size (MCL) of the direct-releases (N=23) at first recapture was 79.9 mm; average body size of the head-starts (N=154) at first recapture was 97.7 mm (Table 2.4). There were 3 head-starts that did not have their MCL taken during their first capture, so N=154 instead of N=157. Of the turtles that were recaptured, over 90% of them had a MCL that was at least 70 mm at first recapture (Figure 2.5).

Survival estimates

Based on trapping data, minimum first year post-release survival of direct-releases confounded with the trapping effort is low and varies among years (Figure 2.6). We did not recapture any direct-releases from the 2007, 2009, or 2013 cohorts, although individuals from the 2013 cohort were likely too small to be caught in our aquatic hoop traps. Only 14.7%, 12.8%, and 4% of direct-releases have been recaptured from the 2008, 2011, and 2012 cohorts, respectively (Figure 2.6). Minimum first year post-release survival of head-starts also varied among years, ranging from 0% (2006 cohort) – 71% (2008 cohort), but overall was higher than for direct-releases (Figure 2.6). Excluding the cohorts with very few to no recaptures (2006, 2012, 2013), minimum first year post-release survival was 9.8% for direct-releases and 55.1% for head-starts (Table 2.5).

Based on the CJS models, mean first year post-release apparent survival for head-starts was 72% (95% CI = 61, 85), which was significantly higher than the 12% estimated for direct-releases (95% CI = 7, 18). However, there was no significant difference in apparent survival between head-starts and direct-releases after the first year post-release, as the 95% confidence intervals overlapped (Table 2.6; Figure 2.7). Encounter probability increased with time since release, ranging from 0.001 (p_1) to 0.003 (p_3) (Table 2.6).

Discussion

The number of individuals from each cohort that were trapped during each season provided insight into when trapping efforts are most likely to be successful given the number of years since the individuals were released. The 2011 and 2013 trap efforts did not result in the recapture of any turtles that were released during that same year, the 2012 trap effort resulted in the recapture of a single head-start from the 2011 cohort (released in 2012), and the 2014 trap effort resulted in the capture of four individuals from the 2013 cohort (released in 2014). These results suggest that trapping for head-started individuals in the same year in which they are released is likely to result in little-to-no trapping success. However, conducting a trap effort during the 2nd year post-release should result in a much higher rate of capture of head-started hatchlings. The 2013 and 2014 trap efforts resulted in many captures of individuals that had been released in 2011 and 2012, suggesting that trap efforts for head-started turtles should not begin until the 2nd year post-release and trap success should increase in subsequent years post-release. The trapping of direct-release hatchlings produced similar results regarding increasing trap success as years post-release increases. Our results are similar to those of other studies, which have also found that a size bias, favoring larger individuals, may be present when trapping turtles (Ream and Ream, 1966; Bodie and Semlitsch, 2000). The body size of turtles at the time of their first capture also suggest that Blanding's turtles must be of a certain size before they are likely to be trapped. There were similarities between both the head-start hatchlings and the direct-release hatchings, in that the minimum MCL at first-capture was 56.5 mm and 49.3 mm, respectively. These results suggest that recaptures are unlikely when attempting to capture individuals that are likely to be smaller than the minimum MCLs recaptured during our study.

One goal of this study was to identify if there is a minimum size that needs to be attained by the time of release for a head-started hatchling to have increased rates of survival. Among head-starts that were later recaptured, the shortest MCL at time of release was 40.9 mm. The minimums were quite smaller from the average body measurements at time of release amongst head-starts that were later recaptured, with the average MCL at time of release of a turtle that was later recaptured was 70 mm. This suggests that head-start survival is likely to be lowest amongst those with body sizes at time of release under 40.9 mm MCL, with increased rates of survival among larger head-starts. These results are similar to those involving another freshwater turtle found in Massachusetts, in which Northern Red-bellied Cooter head-starts that were released at a carapace length of less than 40 mm had little chance of surviving (Haskell et al. 1996).

Mean first-year post-release apparent survival was six times higher for head-starts (0.72) than direct-releases (0.12). The mean 72% head-start apparent survival was also nearly three times higher than first-year Blanding's turtle survival as reported by Congdon et al. (1993). Our results are similar to that of Mitrus (2005), who found that head-started European Pond Turtles demonstrated first-year survivorship rates over five times that of their wild counterparts. The average MCL of head-starts released to the recipient site (66.2 mm) was 1.9x larger than that of the average MCL of direct-releases released to the recipient site (35.3 mm). The larger size of our head-starts at time of release most likely made them less vulnerable to their natural predators (Frazer 1992).

Mean direct-release first-year post-release survival (0.12) was somewhat lower than that reported by Congdon et al. (1993), but the direct-release apparent survival estimates may be conservative as the encounter probability for direct-releases was lower than that for head-starts. The smaller size of the direct-releases may have been the main contributor to fewer encounters, as Congdon et al. (1993) hypothesized that juvenile turtles are not susceptible to trapping due to their secretive ways. One criticism of head-starting is that the head-started individuals become accustomed to people and so are more readily trapped, however, Spinks et al. (2003) and Mitrus (2005) found that there was not a significant difference in recapture rates between head-started turtles and wild turtles. Dispersal is also unlikely to affect recapture, as previous investigations of juvenile Blanding's turtles have shown mostly short migrations, with nearly 70% dispersing less than 100m and less than 1% dispersing more than 500m (McMaster and Herman, 2000). This suggests that the greater amount of head-starts that were recaptured over direct-releases is more likely attributable to greater survival among the head-starts, instead of a trapping bias. Even with a conservative estimate of apparent survival of direct-releases, the head-starts demonstrated significantly higher rates of apparent survival in the first year post-release.

Subsequent apparent survival is statistically the same between direct-released and head-started hatchlings, as evidenced by the 95% confidence intervals. Despite low first-year post-release apparent survival for direct-releases, they appear to have much higher survival in subsequent years, as several of them were encountered several years after release. Mean apparent survival for head-starts and direct-releases ≥ 2^{nd}-year post-release was found to be greater than that of mean survival rates (78%) of juvenile (age 1-13) Blanding's turtles found by Congdon et al. (1993).

Buhlmann et al. (2015) developed an original population model to "estimate the number of hatchlings and duration of repetitive introductions that would be necessary to establish a stable population of Blanding's Turtles on the recipient site and predict the relative efficiency of different release strategies, including direct release of hatchlings vs. release of head-started hatchlings." The survival estimates based on 2011-2014 recapture data can now be used to reevaluate the number of hatchlings and duration of reintroductions that are needed to establish a stable population of Blanding's Turtles at the Assabet River NWR. The original model was constructed using life-history parameters from the literature and unpublished field data, and it conservatively assumed that head-start survival was similar to wild-recruited turtles. This study shows that head-starts survive at a rate six times greater than their non-head-started counterparts in their first year post-release. A revaluation of the original model should reflect the higher observed survival rate of head-starts, which will dictate a smaller number of head-started hatchlings and a shorter duration of releases to establish a stable population of Blanding's Turtles at Assabet River NWR.

Head-starting will only be effective for establishing a new population or augmenting an existing population if potential sources of human-caused mortality (particularly adults) has been removed (Heppell et al. 1996; Heppell 1998). Turtle populations may remain self-sustaining for many years even with little recruitment, but can soon disappear with low adult survivorship (Burke 2015). Therefore, the protection of adult and larger juvenile turtles is one of the quickest and most efficient ways to reverse the decline of a population (Frazer 1992). The establishment of a self-sustaining population at Assabet River NWR is likely to be successful because the refuge offers protection from potential anthropogenic sources of mortality (i.e. habitat fragmentation and loss, road mortality, etc.).

The majority (74%) of head-starts were raised by a single school (Bristol County Agricultural High School) according to an established protocol (Buhlmann et al. 2015), which helped to ensure consistent quality in husbandry. Collaborating with this dedicated teacher and students, who provide consistent care and monitoring, has been the key to a successful head-starting program (Buhlmann et al. 2015). The use of other schools and organizations to raise the remaining head-starts has been very useful for generating community support and awareness for the project.

The creation of a self-sustaining population of freshwater turtles through the use of head-starting is dependent on a long-term partnership between committed partners using an established protocol (as outlined in our project) and long-term monitoring. Collaboration among numerous and varied partners aided in the generation of support for the project and helped to assure the project's long-term viability (Buhlmann et al. 2015). Our project drew upon a diverse group of collaborators that had a wide variety of expertise that allowed for sharing of responsibilities that made long-term monitoring of the turtles manageable. This successful collaboration among numerous partners that requires long-term monitoring should serve as a model for other freshwater turtle head-starting projects.

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Table 2.1. Number of Blanding's turtle nests protected at the donor site (Oxbow National Wildlife Refuge) each year during 2006-2013 and the number of hatchlings released at the donor site and recipient site (Assabet River National Wildlife Refuge).

Cohort	Nests Protected at the Donor Site	Direct-releases Released at the Donor Site	Direct-releases Released at the Recipient Site	Head-starts Released at the Recipient Site
2006	30	165	0	7
2007	40	208	25	22
2008	26	67	34	31
2009	28	76	21	47
2010	17	55	0	54
2011	66	189	94	91
2012	87	300	154	120
2013	53	148	73	68
Total	347	1208	401	440

Table 2.2. Trapping effort conducted at the recipient site (Assabet River National Wildlife Refuge) during 2011-2014 and the resulting number of captures and number of individual Blanding's turtles trapped.

Trap Year	Trapping Duration	No. of Traps	Trap Nights	Unique Head-starts Captured	Unique Direct-releases Captured	Unique Individuals Captured	Total No. of Captures
2011	25 May – 29 July	22	292	3	0	3	3
2012	16 July – 14 September	19	412	10	1	11	12
2013	3 May – 20 July	46	1225	82	4	86	225
2014	10 May – 8 August	29	1127	124	21	145	448

Table 2.3. Results of the Tukey's Honest Significant Difference (HSD) post-hoc test in differences between mean body size (midline carapace length; MCL) at time of release at the recipient site (Assabet River National Wildlife Refuge) between 2006-2013 Blanding's turtle head-start cohorts.

Cohorts	Mean Difference	Significance	95% Confidence Interval	
			Lower bound	Upper bound
2007 – 2006	-0.349	1.000	-15.674	14.977
2008 – 2006	14.667	0.053	-0.111	29.446
2009 – 2006*	19.831	0.001	5.523	34.138
2010 – 2006	13.412	0.079	-0.775	27.599
2011 – 2006*	30.626	0.000	16.774	44.478
2012 – 2006	7.758	0.673	-5.974	21.49
2013 – 2006*	20.876	0.000	6.857	34.894
2008 – 2007*	15.016	0.000	5.171	24.861
2009 – 2007*	20.179	0.000	11.056	29.302
2010 – 2007*	13.761	0.000	4.828	22.693
2011 – 2007*	30.975	0.000	22.585	39.365
2012 – 2007	8.107	0.055	-0.084	16.297
2013 – 2007*	21.224	0.000	12.562	29.887
2009 – 2008	5.163	0.535	-3.008	13.335
2010 – 2008	-1.255	1.000	-9.213	6.703
2011 – 2008*	15.959	0.000	8.615	23.303
2012 – 2008	-6.909	0.064	-14.025	0.206
2013 – 2008	6.208	0.211	-1.445	13.862
2010 – 2009	-6.419	0.104	-13.464	0.626
2011 – 2009*	10.795	0.000	4.452	17.139
2012 – 2009*	-12.073	0.000	-18.15	-5.996
2013 – 2009	1.045	1.000	-5.654	7.744
2011 – 2010*	17.214	0.000	11.148	23.281
2012 – 2010	-5.654	0.061	-11.441	0.133
2013 – 2010*	7.464	0.011	1.026	13.901
2012 – 2011*	-22.868	0.000	-27.777	-17.959
2013 -2011*	-9.751	0.000	-15.412	-4.089
2013 – 2012*	13.118	0.000	7.757	18.478
*The mean difference is significant at the 0.05 level between the cohorts.				

Table 2.4. The mean midline carapace length (MCL) and MCL range for Blanding's turtle direct-releases (N=23) and head-starts (N=154) at their first recapture at the recipient site (Assabet River National Wildlife Refuge) during trapping efforts. For head-starts, N=154 instead of N=157 because three head-starts did not have their MCL taken during their first captures.

Cohort	Treatment	No. of Turtles Recaptured	Mean MCL (mm)	MCL (mm) Range
2006	Direct-release	N/A	N/A	N/A
	Head-start	0	N/A	N/A
2007	Direct-release	0	N/A	N/A
	Head-start	6	133.8	112.5 – 149.0
2008	Direct-release	5	113.0	106.0 – 117.8
	Head-start	22	119.9	98.5 – 144.0
2009	Direct-release	0	N/A	N/A
	Head-start	30	107.9	81.1 – 136.5
2010	Direct-release	N/A	N/A	N/A
	Head-start	26	92.5	74.5 – 116.0
2011	Direct-release	12	75.5	49.3 – 81.5
	Head-start	50	90.2	63.2 – 111.0
2012	Direct-release	6	61.1	51.0 – 68.0
	Head-start	18	72.1	56.5 – 100.0
2013	Direct-release	0	N/A	N/A
	Head-start	2	80.5	66.0 – 95.0
Total		177		

Table 2.5. Number of individual Blanding's turtles from the 2007-2011 cohorts released at the recipient site (Assabet River National Wildlife Refuge) and the percent subsequently recaptured during 2011-2014 trapping efforts. Minimum 1st-year post-release survival is the percentage of individuals recaptured.

	Direct-releases		**Head-starts**	
Cohort	**No. Released (No. Recaptured)**	**Minimum 1st-Year Post-Release Survival**	**No. Released (No. Recaptured)**	**Minimum 1st-Year Post-Release Survival**
2007	25 (0)	0%	22 (6)	27.3%
2008	34 (5)	14.7%	31 (22)	71.0%
2009	21 (0)	0%	47 (31)	66.0%
2010	0 (0)	N/A	54 (26)	48.1%
2011	94 (12)	12.8%	91 (50)	54.9%
Total	174 (17)	9.8%	245 (135)	55.1%

Table 2.6. Encounter probability (p) and apparent survival (ϕ) parameter estimates from the Cormack-Jolly-Seber analysis based on 2011 – 2014 trapping efforts for Blanding's turtles at the recipient site (Assabet River National Wildlife Refuge). The encounter probability groups are correlated to the following survival parameters: p_1 (ϕ_4), p_2 (ϕ_1, ϕ_5), p_3 (ϕ_2, ϕ_3, ϕ_6).

Parameter estimates (Posterior summaries)					
Parameter	**Description**	**Mean Estimate**	**Standard Deviation**	**95% Confidence Interval**	
				Lower bound	**Upper bound**
p_1	Encounter Probability: Group 1	0.0012	8.00E-05	0.0011	0.0014
p_2	Encounter Probability: Group 2	0.0022	1.86E-04	0.0018	0.0026
p_3	Encounter Probability: Group 3	0.0031	2.08E-04	0.0027	0.0035
ϕ_1	Direct-release apparent survival: year 1 post-release	0.1201	2.78E-02	0.0718	0.1803
ϕ_2	Direct-release apparent survival: year 2 post-release	0.9015	8.71E-02	0.6754	0.9971
ϕ_3	Direct-release apparent survival: ≥ year 3 post-release	0.9079	6.25E-02	0.7600	0.9944
ϕ_4	Head-start apparent survival: year 1 post-release	0.7203	6.09E-02	0.6136	0.8556
ϕ_5	Head-start apparent survival: year 2 post-release	0.8986	6.85E-02	0.7438	0.9950
ϕ_6	Head-start apparent survival: ≥ year 3 post-release	0.7812	3.79E-02	0.7050	0.8539

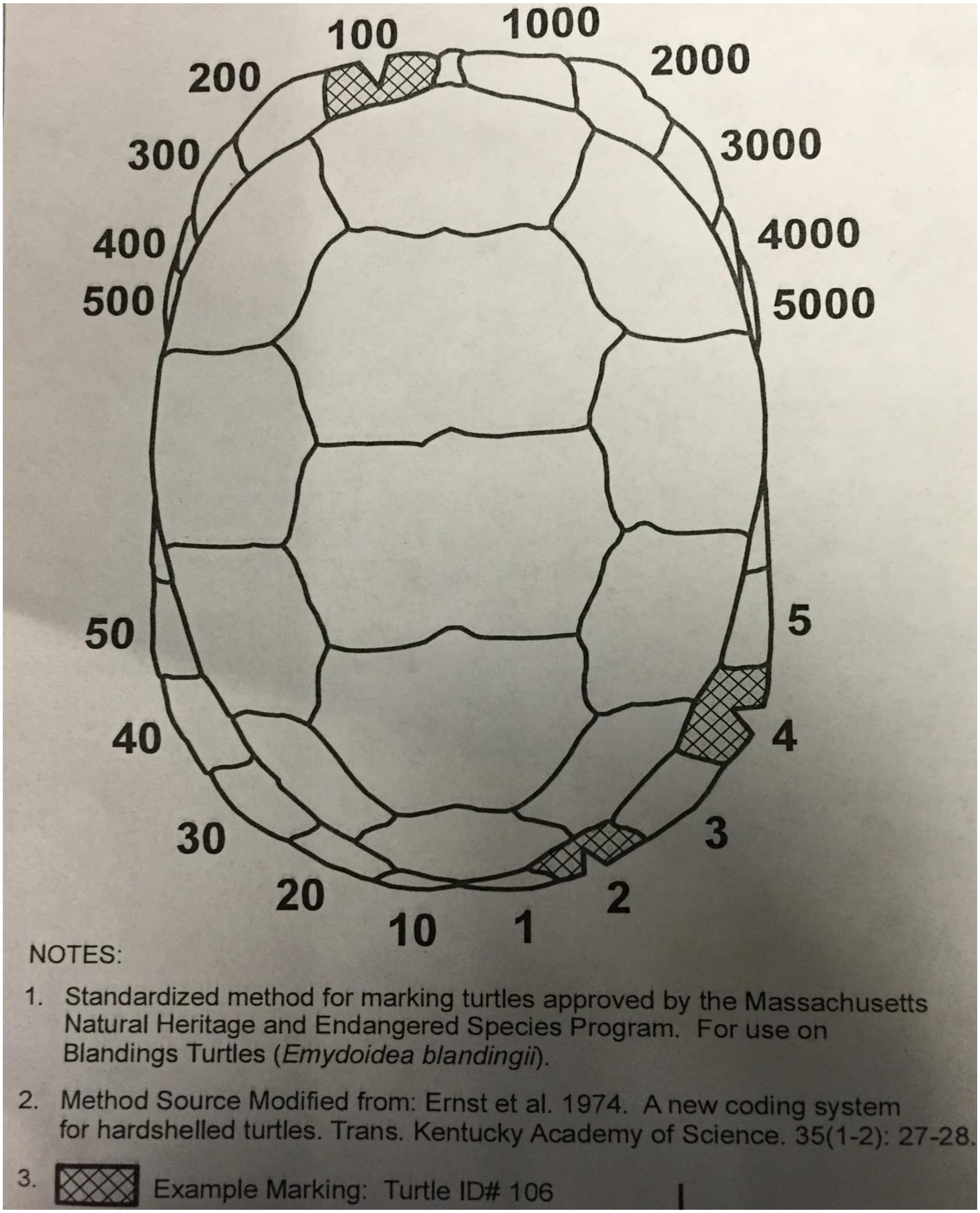

Figure 2.1. Blanding's Turtle shell marking system used in Massachusetts.

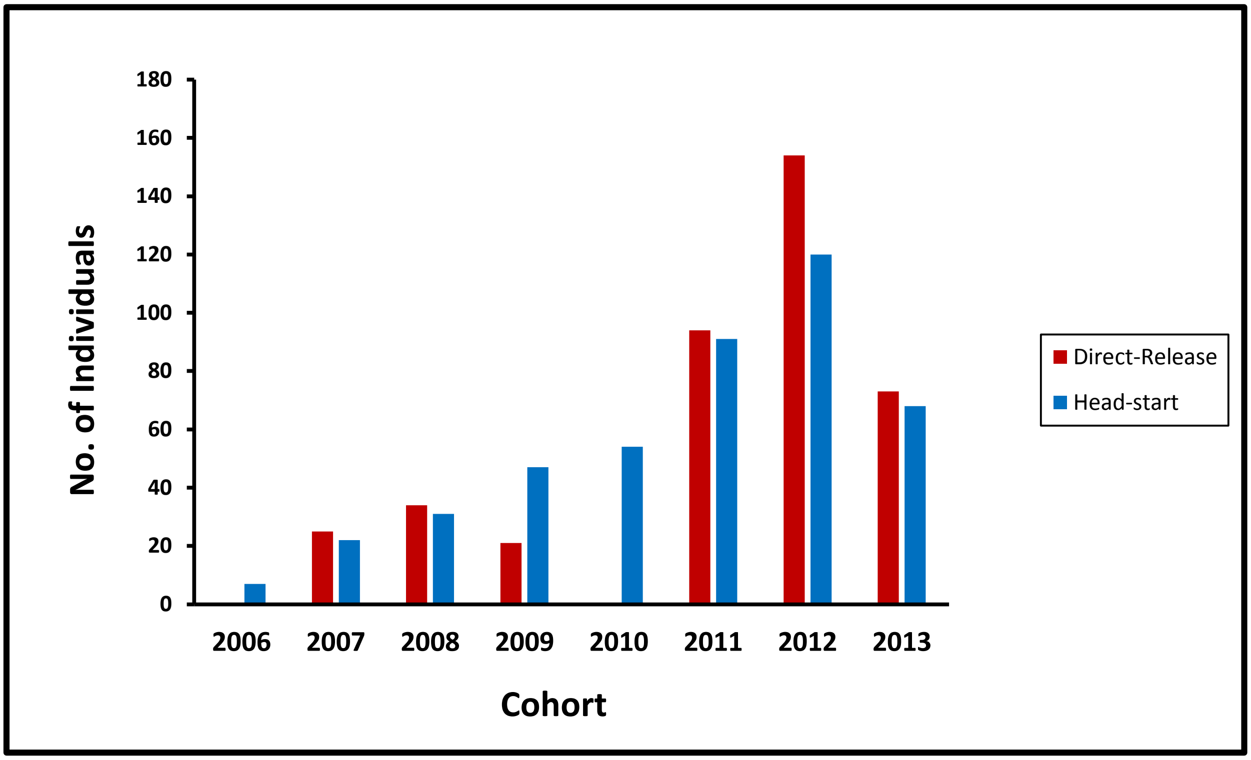

Figure 2.2. Number of direct-release hatchlings and head-start Blanding's turtles from the 2006-2013 cohorts released at the recipient site (Assabet River National Wildlife Refuge).

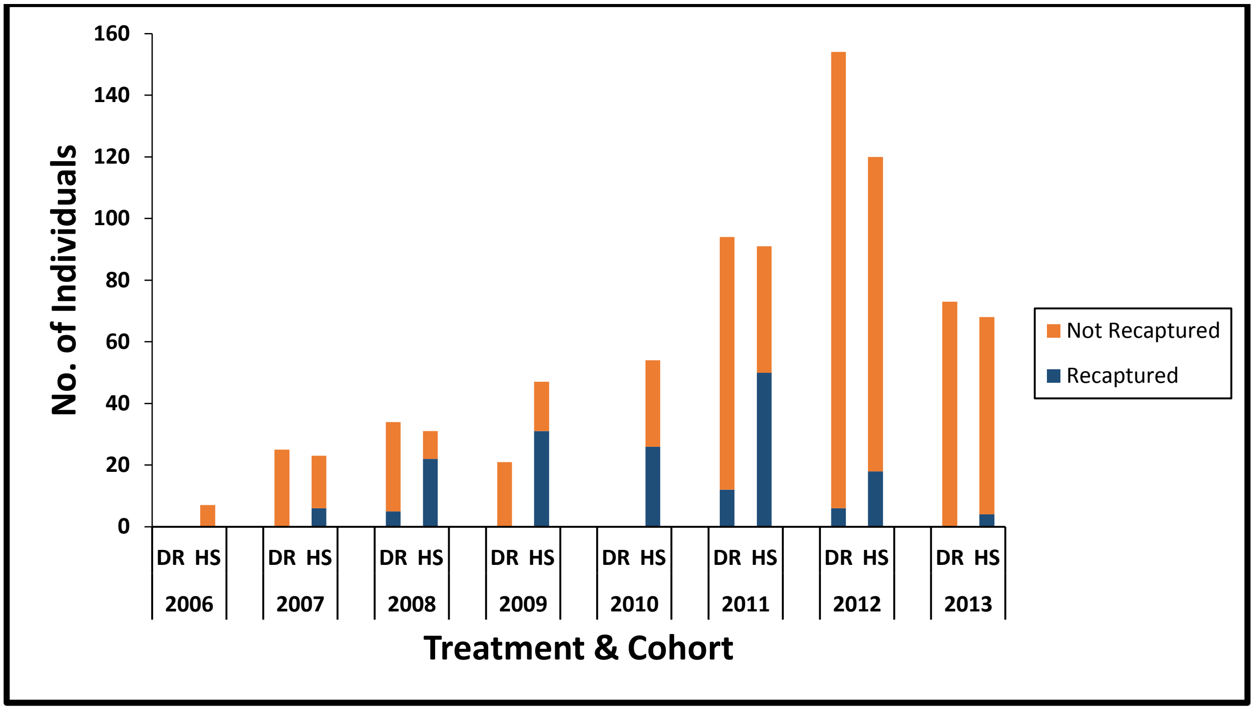

Figure 2.3. Proportion of 2006-2013 Blanding's turtle direct-release (DR) and head-started (HS) hatchlings released at the recipient site (Assabet River National Wildlife Refuge) and subsequently recaptured during 2011-2014 trapping efforts. No hatchlings were direct-released in 2006 or 2010.

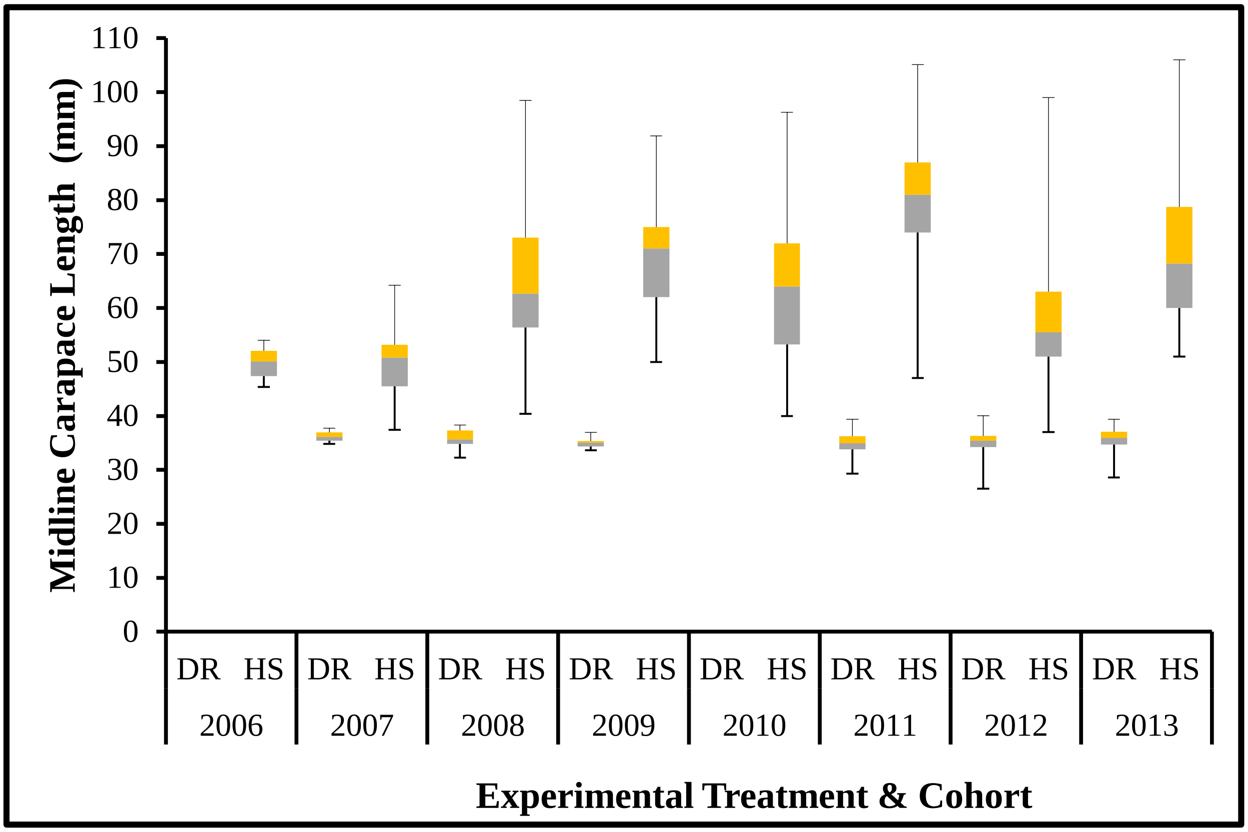

Figure 2.4. Midline carapace length (± 1 SE) at time of release for Blanding's turtle direct-release (DR) and head-start (HS) hatchlings released at the recipient site (Assabet River National Wildlife Refuge).

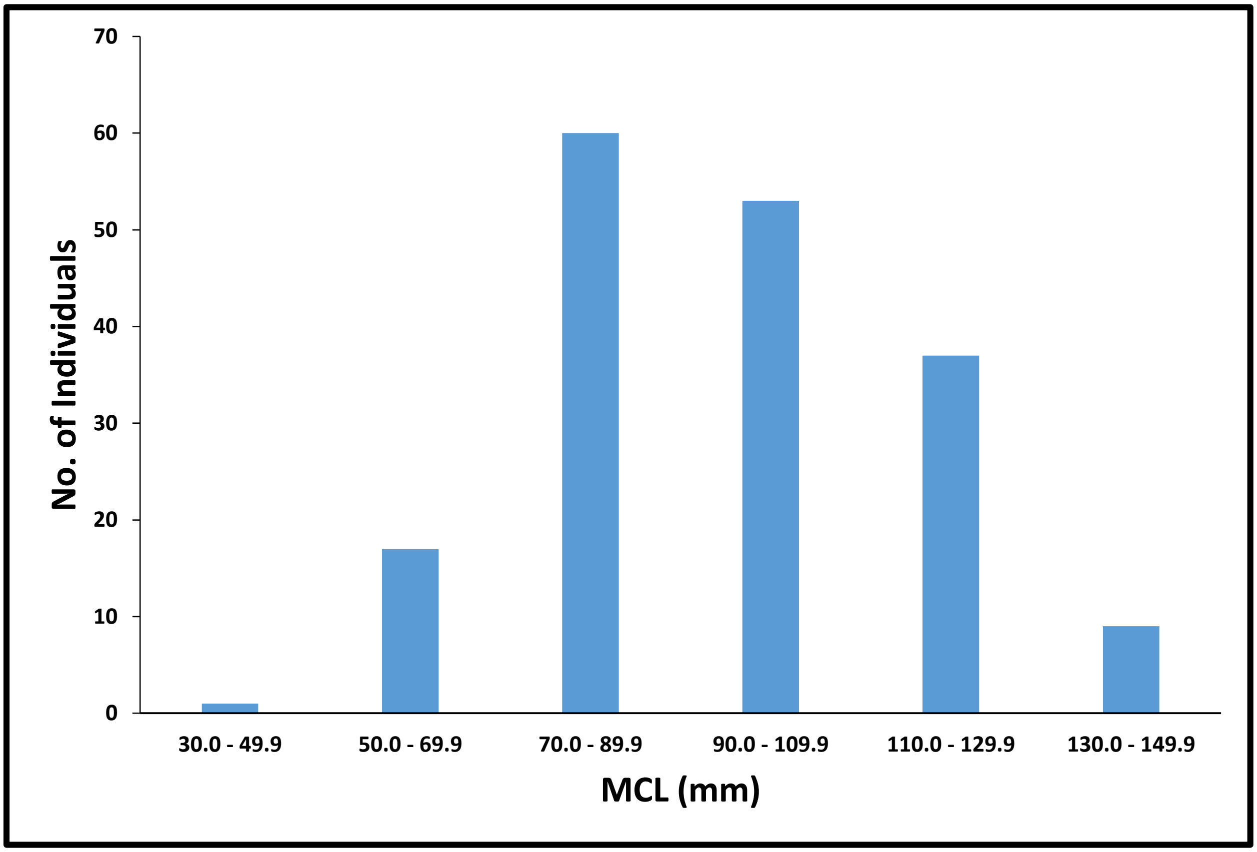

Figure 2.5. Midline carapace length (MCL) of Blanding's turtles at the time of their first recapture at the recipient site (Assabet River National Wildlife Refuge), from 2011 - 2014.

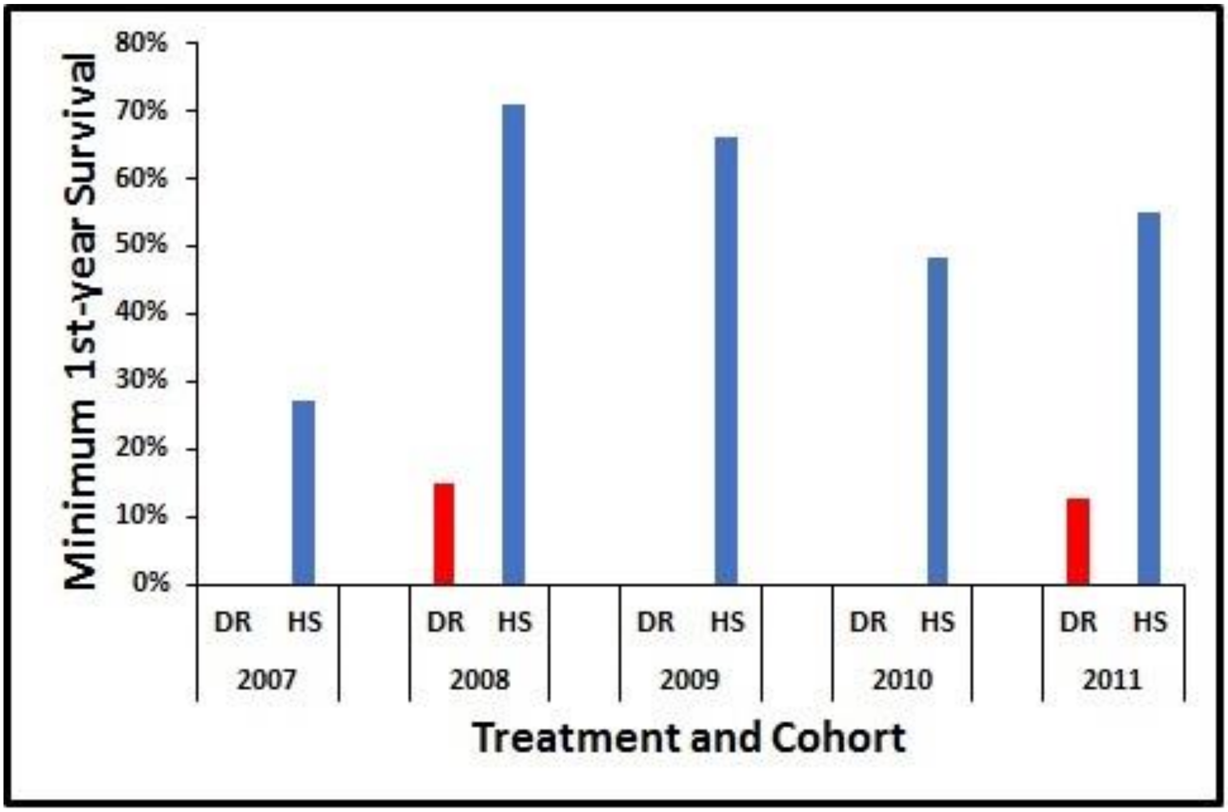

Figure 2.6. The minimum percent 1^{st}-year post-release survival for direct-release and head-started Blanding's turtles at the recipient site (Assabet River National Wildlife Refuge) based on the 2011-2014 recapture data. Survival was not estimated for the 2006, 2012, and 2013 cohorts due to low encounter probabilities for those cohorts during the sampling periods.

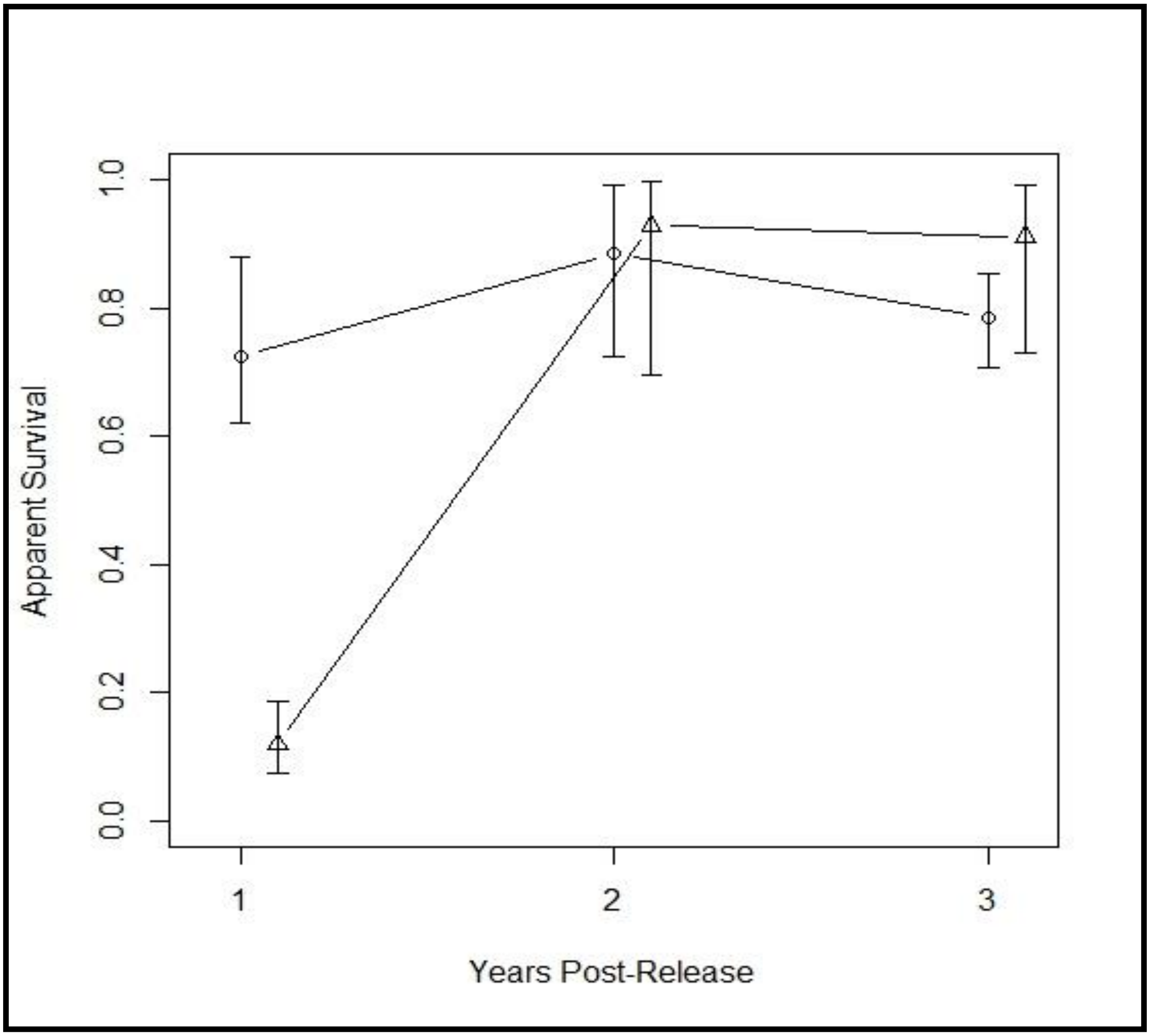

Figure 2.7. Post-release apparent survival probabilities of head-start (o) and direct-release (Δ) Blanding's turtles based on 2011-2014 trapping of the recipient site (Assabet River National Wildlife Refuge). Error bars indicate 95% confidence intervals.

CHAPTER 3

POST-RELEASE SITE FIDELITY & HABITAT SELECTION OF HEAD-STARTED & DIRECT-RELEASED BLANDING'S TURTLES

Introduction

Translocations, which are the deliberate release of individuals of a species at sites within their range but different from their capture locations (Tuberville 2008), are increasingly being adopted as a management technique (Attum et al. 2013) to augment, establish, or reestablish populations (Griffith et al. 1989). The need for translocations can range from augmentation of diminished populations, recolonization of previously-occupied areas, or protection of imperiled species at-risk from human development and/or human-wildlife conflicts (Nussear et al. 2012). The quantity of translocations and their effectiveness appear to differ across taxa. The majority of translocations have involved native game birds and mammals, which resulted in an overall success rate of 86% (Griffith et al. 1989), while Dodd and Siegel (1991) questioned the effectiveness of translocations of reptiles and amphibians, with a demonstrated success rate of only 19% (Dodd and Siegel 1991).

The range of success of translocation projects is driven by varying degrees of post-release survivorship, site fidelity, and habitat selection. Failed releases of captive-reared Ring-necked Pheasants (*Phasianus colchicus*) were attributable to high rates of dispersal from the release site and low survivorship (Wilson et al. 1992). Conversely, translocated Delmarva Fox Squirrels (*Sciurus niger cinereus*) that displayed high rates of site fidelity were found to have similar rates of mortality to non-translocated individuals (Bendel and Therres 1994). Selecting release sites with appropriate habitat may lead to higher retention of individuals to the release site and higher rates of survival.

Translocations of Eastern Box Turtles (*Terrapene carolina*; Hester et al. 2008), Three-toed Box Turtles (*Terrapene carolina triunguis*; Rittenhouse et al. 2007) and Gopher Tortoises (*Gopherus polyphemus*; Tuberville et al. 2005) have shown that translocated individuals are more likely to range over larger distances and leave the release site than non-translocated turtles. This may be due to translocated individuals showing an inability to adapt their predisposed foraging behaviors to local conditions (Rittenhouse et al. 2008). To counter the high rates of dispersal from the release sites, soft-releases occurring at the beginning of the aestivation season were found to limit the movements of Spur-thighed Tortoises (*Testudo graeca*; Attum et al. 2011).

Increasingly, studies have been conducted on the post-release habitat use and movement patterns of translocated freshwater turtles (Attum et al. 2013; Bertolero and Oro 2009; Bogosian 2010; Buhlmann et al. 2015; Cadi and Miquet 2004; Mignet et al., 2014; Moore et al., 2013; Saumure et al. 2010; Shen et al. 2010). Several studies have examined post-release survival, while others have monitored post-release movements. A study of translocated Mediterranean Pond Turtles (*Mauremys leprosa*) showed that they exhibited good post-release growth and physical condition, but exhibited high temporary emigration and had reduced survivorship compared to that of non-translocated individuals (Bertolero and Oro 2009). Studies of translocated Alligator Snapping Turtles (*Macrochelys temminckii*) showed that they were adept at foraging in novel habitats and locating suitable overwintering sites in Louisiana (Bogosian 2010), and successfully overwintered and reproduced in Oklahoma (Moore et al. 2013). Translocated, semi-aquatic Big-headed Turtles (*Platysternon megacephalum*) in China displayed relative site fidelity by remaining close to their release site (Shen et al. 2010). Attum et al. (2013) theorized that translocated highly aquatic turtle species may demonstrate site fidelity to their release site due to the high cost of dispersal to new wetlands based on an examination of translocated Musk Turtles (*Sternotherus odoratus*). Translocated Wood Turtles (*Glyptemys insculpta*) exhibited post-release movements that suggested the movements were meant to maximize the likelihood of locating resources in a new environment (Saumure et al., 2010). Translocated European Pond Turtles (*Emys orbicularis*) showed that they demonstrated post-release site fidelity (Mignet et al. 2014), and also experienced high survivorship and exhibited nesting behaviors (Cadi and Miquet 2004). The majority of these recent studies have focused on the effectiveness of translocations of adults of freshwater species, while little information is available regarding the translocation of juvenile freshwater turtles. It is important to note that in our research, translocations in this context refer to hatchlings that are being moved from a site at which they were born, but to which they had limited exposure.

A crucial aspect of translocation success is the likelihood of the translocated individual to remain within the vicinity of the release site, or demonstrate release site fidelity (Attum et al. 2013; Bendel and Therres 1994). By remaining at or near the initial release site, individuals avoid the costs associated with seeking out new habitat (Lewis 1995). These costs can be in the form of increased risks of predation and energy, and time spent searching for new habitat and food (Fahrig 2007; Switzer 1993). The presence of desirable habitat at the release site should increase release site fidelity and lead to higher translocation success (Attum et al. 2013). Translocated adult individuals are more likely to disperse from release sites than translocated juveniles (Tuberville et al. 2005). Adult turtles may be more inclined to make greater movements, either searching for mates (Morreale et al. 1984) or for adult females, nesting-associated movements (Litzgus and Mousseau 2004). The importance of high site fidelity to the release site emphasizes the need to ensure the age of the translocated individuals has been taken into consideration.

Habitat selection data is becoming increasingly important for the conservation of threatened animal populations (Janiszewski et al. 2014). The selection of habitat can be driven by behavior (Roever et al. 2014), environmental cues (Graeter et al. 2008), landscape-scale features (Croak et al. 2012), or the desire to maximize individual lifetime fitness (Fretwell and Lucas 1970). For translocation to be successful, there must be suitable habitat available for the individuals to select from. Turtles demonstrate habitat preferences that differ by age, season, sex, and species (Buhlmann and Gibbons 2001; Bury and Germano 2003; Carter et al. 1999; Rowe 2003; Tran et al. 2007). The presence of abundant food, cover and areas of relief from predators, nesting habitat, and proximity to other desirable habitats are all important components when considering how turtles select their habitat.

Study Animal

The Blanding's Turtle (*Emydoidea blandingii*) is a semi-aquatic freshwater turtle species that is threatened across much of its range, where it occurs in parts of the northern United States and southeastern portions of Canada. They often inhabit American Beaver (*Castor canadensis*)-impacted wetlands, marshes, ponds, slow-moving streams, swamps, and vernal pools (Congdon et al. 2008). Shrub swamps have also been found to be a common preferred habitat of Blanding's Turtles (Chaloux 2011; Crockett 2008; Grgurovic 2007; Hartwig 2004; Piepgras and Lang 2000). Adult Blanding's Turtles in Massachusetts have been found to select areas of deep water (greater than 0.5m) with vegetative structures throughout the water column (27.5-92.5%

cover) present. Blanding's turtles must make seasonal overland movements during periods of nesting or when traveling to and from ephemeral wetlands (Grgurovic 2007). The upland habitat accessed during these movements are terrestrial habitats not associated with water (Edge et al. 2010).

Blanding's Turtles in the northeastern part of their range usually live in areas that contain a cluster of wetlands that are in close proximity (Chaloux 2011). This allows them to meet their life history needs to feed, nest, overwinter, reproduce, and thermoregulate (Hartwig 2004). They will typically move in the spring from hibernaculum locations in permanent wetlands to vernal pools, where there is a higher seasonal abundance of food, such as tadpoles and macroinvertebrates. In early summer, the females make overland movements to nesting habitats, sometimes traveling over 1 kilometer (Butler 1997; Congdon et al. 1983; Grgurovic and Sievert 2005; Joyal et al. 2000; Kiviat 1997; and Piepgras and Lang 2000). After the vernal pools have dried up, Blanding's Turtles typically migrate back to larger, permanent wetlands. Studies have found that some Blanding's Turtles overwinter in the same wetlands that they occupied during the summer (Rowe and Moll 1991). Permanent wetlands within a cluster of seasonal pools may increase the viability of stable populations by reducing the vulnerabilities associated with making large overland movements.

Hatchling and juvenile Blanding's Turtles have been found to inhabit areas with dense vegetative cover, particularly favoring areas with an abundance of Sphagnum Moss (*Sphagnum spp.*; McMaster and Herman 2000; USFWS unpublished data). Areas of shallow water with surrounding emergent root masses may reduce intraspecific competition for food with larger turtles and also offer cover from predators (Pappas and Brecke 1992). Juvenile Blanding's Turtles typically have smaller home ranges than adults, but they do sometimes make long-distance movements between seasonal and permanent wetlands (Chaloux 2011; Piepgras and Lang 2000). Little more is known about the habitat preferences of juvenile Blanding's Turtles. Juvenile turtles do not occupy the same habitats as adults, which are the group most often studied (Congdon et al. 1983; Gibbons 1968; Graham and Doyle 1977; Kofron and Schreiber 1985; Pappas and Brecke 1992; Ross 1989). Further investigation into the habitat selection preferences of juvenile turtles is necessary to ensure release sites for translocations will meet the needs of all age classes of the focal turtle species.

As with other freshwater species, Blanding's Turtles have experienced a population decrease due to anthropogenic impacts such as habitat fragmentation. Habitat fragmentation may be difficult to reverse in developed landscapes and successful Blanding's Turtle population recovery may require other management tools, such as translocations to augment or re-establish populations in protected areas with limited human influence. Translocation of juvenile turtles from stable populations using head-started hatchlings may be an effective way to recover an existing population or jump-start a new population, and can be viewed as successful if efforts result in self-sustaining populations (Griffith et al. 1989). Our research tested the effectiveness of head-starting and translocation as a way to establish a new population of Blanding's Turtles on a protected site (Buhlmann et al. 2015). Translocation of hatchlings from an area which they had limited exposure prior to translocation may increase the odds of to Survivorship of translocated, head-started Blanding's Turtle hatchlings has been evaluated (Green Thesis Chapter 2), and in this study we investigated post-release habitat selection and site fidelity of translocated Blanding's Turtle hatchlings.

Methods

Donor Site

The donor site is a 674 hectare (ha) protected area owned and managed by the U.S. Fish & Wildlife Service (USFWS). It is located roughly 60 kilometers (km) west of Boston, Massachusetts and lies within the Nashua River watershed. The site supports a variety of wetland and terrestrial habitats, including seasonal and permanent wetlands, riparian zones, upland forest, and open fields. Prior to its acquisition by the USFWS in 1974, the land was owned by the U.S. military and subject to clearing of large patches of land. The resulting open canopy patches provided important nesting habitat for a variety of turtle species, including Blanding's Turtles. Since 1987 (Butler personal comm.), researchers have protected Blanding's Turtle nests every year at this site, which is thought to support the largest population of Blanding's Turtles in New England, with a population consisting of at least 500 individuals (Mockford et al. 2007).

Recipient Site

Assabet River National Wildlife Refuge (NWR) is an 880 ha protected area located approximately 40 km west of Boston, Massachusetts and 16 km southeast of the donor site. The land was primarily managed for agriculture prior to its purchase by the U.S. Army in 1942 as a military training ground as part of the U.S. Army's Fort Devens complex. The land was transferred to the USFWS and the refuge was established in 2000. Wetlands comprise 20% of the total refuge area (USFWS 2007) and there are numerous vernal pools and permanent shrub wetlands that represent the habitat types necessary to support Blanding's Turtles year-round. The array of wetland and terrestrial habitats, coupled with the size of the refuge, and minimal automobile traffic, suggests that the refuge could support a sizeable population of Blanding's Turtles (Buhlmann et al. 2015). Two wetlands that are located in the northeast corner of the refuge and that appear to possess favorable Blanding's Turtle habitat were selected as release sites (Figure 3.1). Taylor Brook Wetland (approximately 2.5 ha) is an herbaceous emergent marsh with sedges (*Carex spp.*), water lilies (*Nymphaeaceae spp.*), and duckweed (*Lemnaceae spp.*). This marsh forms the headwaters of Taylor Brook. Water levels, which often exceed two meters (m) in some areas in the spring, vary seasonally but the wetland is often kept flooded by resident American Beaver (*Castor canadensis*) that maintain a dam downstream. Pump Station Wetland (approximately 16.3 ha) is a scrub-shrub, marsh habitat with an unconsolidated bottom that is currently influenced by beaver activity, similar to the wetlands at the donor site. It is dominated by shrubby, woody vegetation, including Speckled Alder (*Alnus rugosa*), Buttonbush (*Cephalanthus occidentalis*), Leatherleaf (*Chamaedaphne catyculata*), Red Maple (*Acer rubrum*), Swamp Loosestrife (*Decodon verticillata*), and Sweet Gale (*Myrica gale*). There are large areas with significant amounts of *Sphagnum*, as well as patches of open water with Duckweed-covered pockets (Buhlmann et al. 2015). Taylor Brook Wetland and Pump Station Wetland are separated from each other by a dirt trail less than ten meters wide. The array of wetland and terrestrial habitats, coupled with the size of the refuge, and minimal automobile traffic, suggests that the refuge could support a sizeable population of Blanding's Turtles (Buhlmann et al. 2015). Although the habitat on the refuge appears to be suitable for Blanding's Turtles, previous surveys of the area have not found any existing populations, possibly due to the prior human activities occurring at the site.

Hatchling treatment and releases

As part of a larger study to evaluate the effectiveness of head-starting as a management tool for Blanding's Turtles (Buhlmann et al. 2015; Green Thesis Chapter 2), we have collected 2049 hatchlings from protected nests at the donor site since 2006. To minimize impacts to the donor population, we directly-released half of the hatchlings from each nest back into the donor site wetland within 1-4 weeks of hatching (August – October; Buhlmann et al. 2015). All hatchlings released at the donor site are not considered further here. The remaining hatchlings were retained for release at the recipient site. In most years, half of the recipient site individuals were directly-released shortly after hatching and the other half were first head-started indoors and released the following spring, except for the 2006 and 2010 cohorts which were all head-started and released the following spring. A total of 440 head-starts have been released at Assabet River NWR between 2007-2014, a subset of which we subsequently monitored using radio-telemetry.

Two head-start cohorts (2006, 2007) and two direct-release cohorts (2007, 2008) were released into Taylor Brook Wetland. All remaining head-start (2008-2013) cohorts and direct-release (2009, 2011-2013) cohorts were released into Pump Station Wetland. Coordinates from release locations were recorded using hand-held GPS units (Garmin eTrex, Olathe, Kansas) with a positional accuracy of approximately ±5m. We released the majority of head-starts in May or June of each year when they were approximately 9 months old and the risk of exposure to freezing temperatures had passed (Buhlmann et al. 2015). However, all 2006 head-starts and seven head-starts from the 2009 and 2010 cohorts were held until the following fall when they were approximately a year old to allow them extra time to attain a size large enough to receive a radio transmitter at release.

Post release monitoring using radio telemetry

A subset of head-started turtles from each cohort was radio-tracked. Most head-started hatchlings were large enough at release to monitor via radio-telemetry; however, some head-started individuals included in our tracking dataset were too small at release and were not tracked until recaptured at a larger size. Likewise, all radio-tracked direct-release hatchlings were only tracked once they were recaptured in a subsequent year at a size large enough to receive a radio-transmitter. From 2009-2012, we only placed transmitters on turtles weighing a minimum of 115 grams (g), to ensure the total weight of the transmitter did not exceed 7% of the turtle's total body weight. From 2012-2014, we increased the minimum weight needed to receive a transmitter to 150 g to decrease the percentage of the transmitter's weight on the turtle's total body mass. We affixed VHF radio-transmitters (model R1680, 4 g, 6-month battery life, Advanced Telemetry Systems, Isanti, Minnesota) to one side of the posterior carapace using waterproof epoxy (WaterWeld, J-B Weld, Sulphur Springs, Texas).

During April – October of 2011-2014, all transmittered turtles were tracked on a weekly basis to determine which wetland each individual occupied. Transmittered turtles were also captured once in the spring (April – June) and once in the fall (September – October) to obtain the exact location of the animals, take morphometric measurements to monitor growth, and to replace the transmitters. Location was recorded in UTM, NAD83 using handled GPS units (Garmin eTrex, Olathe, Kansas) with a positional accuracy of approximately ±5m.

Post-release trapping and incidental encounters

As part of our long-term monitoring of the recipient site population, we trapped annually from 2011-2014 using aquatic hoop traps to monitor post-release survival (Green Thesis Chapter

2). Because we were only able to radio-track a small proportion of individuals released, we also included all these recapture events in our evaluation of site fidelity. Individuals were also occasionally incidentally encountered (hand-captured in a wetland or on a dirt trail adjacent to the release wetlands) and these individuals were also incorporated into our assessment of site fidelity. At each capture location we also recorded water depth and habitat parameters (see below).

Habitat map creation and verification

For each capture (including both transmittered and non-transmittered turtles), we characterized the habitat within a 5 m radius of the capture location. We recorded the following habitat parameters: water depth (to the nearest 1 cm for depths 0-100cm; or greater than 100cm), canopy height (to the nearest m), identification of the three to four most dominant vegetative species in the surrounding area, the percentage of each surrounding habitat type (i.e. percent tree canopy cover, percent open water, percent shrub cover, etc.), and identification of the major wetland type (i.e. shrub wetland, vernal pool, etc.).

The data gathered at each of these radioed captures was used to inform the creation of a habitat map for the two release wetlands at the recipient site. This map was hand-digitized in ArcMap 10.1 by overlaying polygons that represented vegetation communities on a digital image (USGS Color Ortho Imagery, 0.3 m pixel resolution, 2013, MassGIS).

Data processing and analysis

We imported all encounter locations into ArcMap 10.1, including those associated with radio-tracking and transmitter replacement, trap captures, and incidental encounters. Using the

Measure tool, we calculated for each individual the distance between the release point and all subsequent encounters. These data were used to identify the minimum and maximum distance each individual traveled from its initial release location. The minimum and maximum distances traveled from release site were tested for significant differences between the two release sites (Taylor Brook Wetland vs. Pump Station Wetland) using an unpaired, two-tailed, unequal variance t-test (Microsoft Excel 2010).

We also overlaid radio telemetry and incidental encounter records on the habitat map and identified the habitat type associated with each encounter location. We then calculated the number of radio telemetry encounters in each habitat type as a measure of (observed) habitat use. Using the habitat map, we calculated the area of each habitat type using the Calculate Geometry tool in ArcMap 10.1. We calculated the expected number of observations assuming no habitat selection (i.e., that habitat is used in direct proportion to its availability) by calculating the percent area of each habitat type by the total number of observations. We then tested for habitat selection by comparing the expected and observed habitat use by using a chi-square analysis (Microsoft Excel 2010).

Results

We released 841 Blanding's Turtles at the recipient site during 2007-2014, 401 direct-release hatchlings and 440 head-starts, which were 9-12 months old (Figure 3.2). There were 59 direct-releases (2007, 2008 cohorts) and 29 head-starts (2006, 2007 cohorts) released into Taylor Brook Wetland. There were 342 direct-releases (2009, 2011-2013 cohorts) and 411 head-starts (2008-2013 cohorts) released into Pump Station Wetland.

Site Fidelity

Trapping effort and success varied among years, but was substantially greater in 2013-2014 (Table 3.1). In total, there were 180 unique individuals captured, 157 head-starts and 23 direct-releases. All of the individuals that were recaptured were trapped in the Pump Station Wetland. Despite a trap effort in the Taylor Brook Wetland for the 2006 and 2007 head-start cohorts (29 individuals) and the 2007 and 2008 direct-release cohorts (59 individuals) originally released in Taylor Brook Wetland, no turtles were recaptured there (Figure 3.3). Conversely, five individuals from the 2008 direct-release cohort and 6 individuals from the 2007 head-start cohort were recaptured in the Pump Station Wetland after being originally released in the Taylor Brook Wetland. As a sign of post-release site fidelity, 18 direct-release individuals and 151 head-start individuals that had been released to the Pump Station Wetland were recaptured there during trapping efforts (Figure 3.3). Conversely, nearly 15% of the direct-releases from the 2008 cohort and just over 27% of the head-starts from the 2007 cohort were recaptured in the Pump Station Wetland, indicating that post-release emigration from the Taylor Brook Wetland has occurred. There have been 342 direct-release individuals released to the Pump Station Wetland and 5.3% of them have been recaptured in there. There were also 411 head-starts released to the Pump Station Wetland and 37% of them have been recaptured there.

There were eight incidental encounters from 2010-2014. Four of the individuals had been head-started, one from the 2009 cohort and three from the 2011 cohort. All four had been released in Pump Station Wetland; three were subsequently encountered there and the fourth was encountered walking on a dirt trail adjacent to the same wetland. All four were also captured in Pump Station Wetland during the 2011-2014 trapping effort.

The other four individuals that were incidental encounters had been direct-released: one from the 2008 cohort, one from the 2011 cohort, and two from the 2012 cohort. The 2008 cohort individual had originally been released in Taylor Brook Wetland, but it was encountered in Pump Station Wetland. This individual was subsequently monitored with radio telemetry and remained in Pump Station Wetland for the duration of its tracking until it was predated in 2014. Since they had been batch notched at their initial release, the exact release coordinates for the three direct-release turtles from the 2011 and 2012 cohorts are unknown. They all had been released somewhere in Pump Station Wetland; two were incidentally encountered there and the third was encountered walking along the same dirt trail as the incidentally encountered head-start described above.

We radio-tracked a total of 35 individuals during 2009-2014 (Table 3.2). We radio-tracked 32 head-starts (nearly 5% of all released head-starts); 21 were outfitted with transmitters at their initial release and eleven received transmitters after their initial release after being captured in aquatic hoop traps between 2011-2014 (Figure 3.4). Of the 32 head-started individuals: two were initially released without transmitters in the Taylor Brook Wetland in 2008, but were later recaptured in Pump Station Wetland and outfitted with transmitters; the other 30 head-started individuals were all released into Pump Station Wetland.

None of the 401 direct-releases received transmitters at their initial release, but three were outfitted with transmitters at recapture (two via aquatic hoop traps, one incidentally). All three radio-tracked direct-releases had been initially released into Taylor Brook Wetland in 2008, but were recaptured in subsequent years in Pump Station Wetland and then radio-tracked.

Of the 35 radio-tracked individuals, 71.4% exhibited fidelity to their release wetland. Two head-starts and three direct-releases are known to have successfully migrated from their initial release at Taylor Brook Wetland to Pump Station Wetland. Conversely, one transmittered head-start (#3307) released into Pump Station Wetland subsequently traveled to Taylor Brook Wetland in the months following its initial release (Figure 3.5), where it attempted to overwinter, but did not survive. Another transmittered head-start (#3295) that had been released into Pump Station Wetland without a radio in 2009 but then received a radio in 2012 after being captured in an aquatic hoop trap in Pump Station Wetland. It remained in Pump Station Wetland until the week of 12 June 2014, when it suddenly disappeared from Pump Station Wetland and was found predated on 19 June 2014 in an unnamed brook over 1300 m away (Figure 3.5).

The minimum distance that a turtle was recorded traveling from its initial release site was 17 meters and the maximum distance that a turtle was recorded traveling from its initial release site was 300 meters. The average minimum distance traveled from a radioed turtle's release site was 96.0 meters and the average maximum distance traveled from a turtle's release site was 126.9 meters. The turtles that were released in Taylor Brook Wetland had higher minimum and maximum distances traveled from release site than those released in Pump Station Wetland (Figure 3.6). A two-tailed, unpaired t-test showed that there was a significant difference ($p < 0.05$) between the minimum and maximum distances traveled from release site between those individuals released in Taylor Brook (N=5) vs. those released in Pump Station Wetland (N=30).

There were 3 instances (head-starts #3460, #3780, and #3818) of only the transmitter being recaptured within five meters of the turtle's original release location, and these data were not included in the range or average reporting as they most likely were the result of a radio transmitter falling off shortly after attachment. The final supposed movement for head-start #3295 (see above) was also not used in the range or average reporting as the turtle had no history of making such a large movement, and did so in less than a week's time. The evidence of predation coupled with the unusual movement, make it likely that the turtle was moved there by a predator rather than moving such a great distance under its own locomotion.

Habitat Selection

The habitat data that was collected during each capture was used to form seven habitat classifications (Table 3.3) that were present in Taylor Brook Wetland and Pump Station Wetland (Figure 3.7). The most abundant habitat types available are Shrub (Figure 3.8), Open Water, and White Pine Forest-Flooded, with Brook (Figure 3.9), Tree, Open Water Channel, and Dirt Trail (located between Taylor Brook Wetland and Pump Station Wetland) being the least abundant.

There were 77 locations gathered from turtles equipped with radio transmitters (Figure 3.10). The largest percentage of these encounters (Figure 3.11) occurred in the Shrub habitat, followed by the Tree, Open Water Channel, and Brook habitats. There were zero encounters of transmittered individuals in the Dirt Trail, Open Water or White Pine Forest-Flooded habitats.

A chi-square analysis was done to compare observed and predicted values of number of radioed turtle encounters in each habitat type with the actual data. The Brook and Dirt Trail habitats were not included in the chi-square analysis because they were not found in the Pump Station Wetland in which the overwhelming majority of radio-telemetry encounters occurred. The chi-square test (degrees of freedom = 4, test-statistic = 99.0, p-value < 0.001), meaning that there was a significant difference between the number of actual vs. predicted encounters in the various habitat types. The actual vs. expected data showed that the radioed turtles displayed a high selection for Scrub habitat, a high avoidance to Open Water and White Pine Forest-Flooded, a slight avoidance of Tree habitat, and a slight selection of Open Water Channel habitat (Table 3.4).

Trapping efforts from 2011-2014 occurred in both Taylor Brook Wetland and Pump Station Wetland (Figure 3.12), with 116 different trap locations used. There were also four defined clusters of Tree habitat, in which two clusters had channels which allowed for trapping and two were un-trappable. All successful trapping captures occurred in the Shrub, Tree, and Open Water Channel habitat; there were no trapping captures in the Brook, Open Water, or White Pine Forest-Flooded habitats.

There were eight incidental encounters (four head-starts, four direct-releases) at the recipient site from 2010-2014 (Figure 3.13). Five encounters occurred in the Shrub habitat, followed by two in the Dirt Trail habitat and one in the Open Water Channel habitat. Both of the individuals found in the Dirt Trail habitat were most likely using it to cross between Taylor Brook and Pump Station Wetlands, though it is not know which wetland either turtle was entering or exiting.

Discussion

The turtles released in Pump Station Wetland exhibited high post-release site fidelity, while turtles released in Taylor Brook Wetland exhibited high rates of emigration to Pump Station Wetland. Prior studies of translocated turtle species (Bertolero and Oro 2009; Hester et al. 2008; Rittenhouse et al. 2007; Tuberville et al. 2005) have shown that translocated individuals display a higher tendency to make larger movements and leave the release site than non-translocated turtles, suggesting that it was not unusual that the turtles initially released in Taylor Brook Wetland would leave their release site. Portions of Taylor Brook Wetland and Pump Station Wetland are separated by a dirt trail that is only several meters wide meaning that the turtles that did emigrate from Taylor Brook Wetland did not make relatively large movements away from the release site. The turtles may have made remained on site (though not in their release wetland) to avoid the costs, such as greater risk of predation and energy loss searching for new habitat (Fahrig 2007; Switzer 1993), associated with searching for new habitat (Lewis 1995). Another factor in the emigration to Pump Station Wetland, as well as the high rate of site fidelity displayed by individuals released in Pump Station Wetland, may be the available habitat at the two release sites. Pump Station Wetland has a high abundance of shrub habitat, which other head-started Blanding's turtles in eastern Massachusetts have shown a preference for when selecting habitat post-release (Windmiller et al. 2015). Blanding's turtles in Massachusetts are likely to occur in areas with deep water and vegetative structures (Grgurovic 2007), and Pump Station Wetland offers a higher density of vegetative cover than Taylor Brook Wetland and also retains higher water levels than Taylor Brook Wetland throughout the summer. Higher translocation success and increased release site fidelity occurs when there is desirable habitat at the release site (Attum et al. 2013), suggesting that Pump Station Wetland appears to possess desirable habitat that is causing turtles released there to remain and those released nearby to emigrate to it.

Turtles released in Pump Station Wetland have displayed high levels of selection and avoidance of certain habitat types. The Shrub habitat has been highly selected at rates disproportionate to its availability within the wetland. Conversely, the Open Water and White Pine Forest-Flooded habitats have been completely avoided, despite being the 2nd and 3rd most abundant habitat types available in Pump Station Wetland. As mentioned above, head-started Blanding's turtles in Massachusetts have shown a preference for shrub habitat (Windmiller et al. 2015). The direct-release and head-start turtles at the recipient site have not yet reached the size of adult turtles, and juvenile turtles may occupy different habitats from adults (Congdon et al.

1983; Gibbons 1968; Graham and Doyle 1977; Kofron and Schreiber; Pappas and Brecke 1992; Ross 1989). The Shrub habitat at the recipient site contains numerous patches of *Sphagnum*, which hatchling and juvenile Blanding's Turtles have been known to favor (Green personal observation; McMaster and Herman 2000). Large portions of the Shrub habitat contains narrow channels with shallow water and vegetative cover throughout. Areas of emergent vegetative flora in shallow water may be favored by juvenile turtles because they can provide cover from predators and reduce intraspecific competition for food with larger turtles (Pappas and Brecke 1992). The lack of vegetation to provide food and cover and areas of open water that leave them vulnerable to predation may explain the complete lack of turtle encounters in the Open Water and White Pine Forest-Flooded habitats.

Direct-released and head-started turtles have been equipped with radio transmitters to better understand post-release habitat selection. The data we gathered from monitoring the radioed turtles allowed us to recognize that those turtles initially released in Taylor Brook Wetland were not remaining in that wetland, but rather emigrating to Pump Station Wetland. We were able to identify that the turtles were selecting for habitat within Pump Station Wetland that was not available in Taylor Brook Wetland. This led to all subsequent releases of turtles having occurred in Pump Station Wetland. As we radio-tracked the turtles within Pump Station Wetland, habitat selection within the wetland was observed. The majority of these encounters occurred in the Shrub habitat, often in or near shallow channels that were bordered by areas of *Sphagnum*. The encounter locations appeared to offer young turtles sufficient cover and places to rest/bask, and so these areas were then chosen as the subsequent release sites within Pump Station Wetland. Higher rates of release site fidelity are observed when there is desirable habitat at the release site (Attum et al. 2013), so we used the radio-tracking data to inform where the turtles' desired habitats were and conduct our releases in those areas to maximize post-release site fidelity.

The successful translocation of freshwater turtles requires a release site with a variety of suitable habitat types for the turtles to select from. In order to meet all their life history needs such as feeding, overwintering, and thermoregulating (Hartwig 2004), Blanding's Turtles often live in areas that contain a cluster of wetlands that are in close proximity (Chaloux 2011). The habitats preferred by Blanding's Turtles can range from beaver-impacted wetlands, marshes, ponds, slow-moving streams, shrub-swamps, and vernal pools (Chaloux 2011; Congdon et al. 2008; Crockett 2008; Grgurovic 2007; Hartwig 2004; Piepgras and Lang 2000). These varied habitats likely offer unique advantages that turtles make use of throughout the year, but a single habitat type may not contain all the preferred components to support a turtle throughout the year. The translocated turtles at the Assabet River NWR did not remain in their initial release location, but were able to travel unimpeded to the nearby Pump Station Wetland, which itself contained a variety of habitats. As such, translocations that have a variety of habitat types that the turtles are able to easily access should increase the probability that translocated individuals will remain at or near the release site.

The creation of a self-sustaining population of freshwater turtles through the use of translocation is dependent on long-term monitoring and adaptive management. We were able to adapt our release strategy based on the data that was gathered from several seasons of radio-tracking turtles after their release. Post-release site fidelity has improved greatly since taking into account the habitat that was selected by the radioed turtles. The identification of the habitats being selected within the release wetlands should also serve to boost survival rates, as turtles are being released to areas of preferred habitat right away. The data that we have collected on post-release site fidelity and habitat selection of translocated turtles should serve as a model for future translocation projects involving freshwater turtles.

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Table 3.1. Trapping effort conducted at the recipient site (Assabet River National Wildlife Refuge) during 2011-2014 and the resulting number of captures and number of individual Blanding's turtles trapped.

Trap Year	**Trapping Duration**	**No. of Traps**	**Trap Nights**	**Unique Head-starts Captured**	**Unique Direct-releases Captured**	**Unique Individuals Captured**	**Total No. of Captures**
2011	25 May – 29 July	22	292	3	0	3	3
2012	16 July – 14 September	19	412	10	1	11	12
2013	3 May – 20 July	46	1225	82	4	86	225
2014	10 May – 8 August	29	1127	124	21	145	448

Table 3.2. List of turtles that were tracked with radio telemetry at the recipient site (Assabet River National Wildlife Refuge) during the study from May 2009 – October 2014.

ID	Cohort	Treatment	Tracking Duration	Fate (As of 10/2014)	Radio Attached at Initial Release	Release Wetland
3222	2007	Head-start	10/27/13-10/31/14	Alive	No	Taylor Brook
3226	2007	Head-start	7/18/11-4/19/13	Dead	No	Taylor Brook
3240	2008	Direct-release	6/1/14-10/31/14	Alive	No	Taylor Brook
3253	2008	Head-start	5/28/09-5/25/10	Dead	Yes	Pump Station
3258	2008	Direct-release	6/3/14-10/31/14	Alive	No	Taylor Brook
3262	2008	Head-start	7/29/11-5/18/12	Dead	No	Pump Station
3264	2008	Head-start	7/20/12-10/5/12	Unknown	No	Pump Station
3282	2008	Head-start	8/6/12-4/24/13	Unknown	No	Pump Station
3293	2008	Head-start	8/6/12-10/4/12	Unknown	No	Pump Station
3294	2008	Direct-release	10/18/10-6/8/12, 5/9/13-10/8/14	Dead	No	Taylor Brook
3295	2008	Head-start	8/3/12-6/19/14	Dead	No	Pump Station
3307	2009	Head-start	9/15/10-4/28/11	Dead	Yes	Pump Station
3324	2009	Head-start	7/27/12-10/31/14	Alive	No	Pump Station
3326	2009	Head-start	8/3/12-4/17/13	Unknown	No	Pump Station
3331	2009	Head-start	9/15/10-5/17/12	Unknown	Yes	Pump Station
3332	2009	Head-start	5/21/10-6/5/12	Dead	Yes	Pump Station

3344	2009	Head-start	7/15/11-5/18/14	Unknown	No	Pump Station
3357	2009	Head-start	8/30/10-5/2/11	Dead	No	Pump Station
3373	2009	Head-start	8/30/10-5/2/12	Unknown	Yes	Pump Station
3392	2010	Head-start	8/30/11-5/17/12	Unknown	Yes	Pump Station
3398	2010	Head-start	8/30/11-6/5/12	Dead	Yes	Pump Station
3400	2010	Head-start	8/30/11-5/17/12	Dead	Yes	Pump Station
3436	2011	Head-start	6/6/12-9/25/12	Unknown	Yes	Pump Station
3441	2011	Head-start	6/6/12-5/27/14	Unknown	Yes	Pump Station
3450	2011	Head-start	6/6/12-10/6/14	Dead	Yes	Pump Station
3460	2011	Head-start	6/11/12-10/4/12	Unknown	Yes	Pump Station
3473	2011	Head-start	6/6/12-9/25/12	Unknown	Yes	Pump Station
3479	2011	Head-start	6/6/12-10/2/12	Unknown	Yes	Pump Station
3602	2011	Head-start	5/23/12-2012 Unknown	Unknown	Yes	Pump Station
3606	2011	Head-start	5/23/12-9/25/12	Unknown	Yes	Pump Station
3622	2011	Head-start	5/23/12-9/25/12	Unknown	Yes	Pump Station
3667	2011	Head-start	6/6/12-10/2/12	Unknown	Yes	Pump Station
3736	2012	Head-start	5/27/13-10/21/13	Unknown	Yes	Pump Station
3780	2013	Head-start	6/2/14-10/6/14	Unknown	Yes	Pump Station
3818	2013	Head-start	6/23/14-10/8/14	Unknown	Yes	Pump Station

Table 3.3. Description and relative abundance of available habitat types at the Pump Station Wetland complex at the recipient site (Assabet River National Wildlife Refuge).

Habitat Type	Description	Area (hectares)
Shrub	Dominated by shrubs like Sweet Gale, Buttonbush, Leatherleaf; large areas of Sphagnum often found in these areas; sometimes contain areas of deeper water (up to two meters)	5.36
Open Water	Dominated by deep water (over two meters) with limited amounts of Lilies and Sedge Grass clumps	4.28
White Pine Forest-Flooded	Dominated by deep, open water (up to two meters), with dead White Pine trees and Sedge Grasses	3.87
Brook	Mix of open water (up to two meters deep) with large quantities of Sedge Grasses and Cattails	2.52
Tree	Dominated by trees like Red Maple, White Pine, and Poison Sumac; large areas of Sphagnum often found in these areas; contain more shallow channels of water (less than 1 meter)	2.12
Open Water Channel	Deep water (over two meters) found in the center, but edge portions are dominated by shallower water (less than 0.5 meters) with Swamp Loosestrife and/or other shrubs	0.68
Dirt Trail	Walking path located between Pump Station Wetland and Taylor Brook; limited ground cover, but high canopy (> 3 m) cover	0.17

Table 3.4. Predicted number of Blanding's turtle locations at the recipient site (Assabet River National Wildlife Refuge) gathered in each habitat type based on the amount of habitat available vs. the actual number of Blanding's turtle locations.

Habitat Type	Predicted No. of Radioed-turtle Locations	Actual No. of Radioed-turtle Locations
Shrub	25	62
Open Water	20	0
White Pine Forest-Flooded	18	0
Tree	10	7
Open Water Channel	3	7

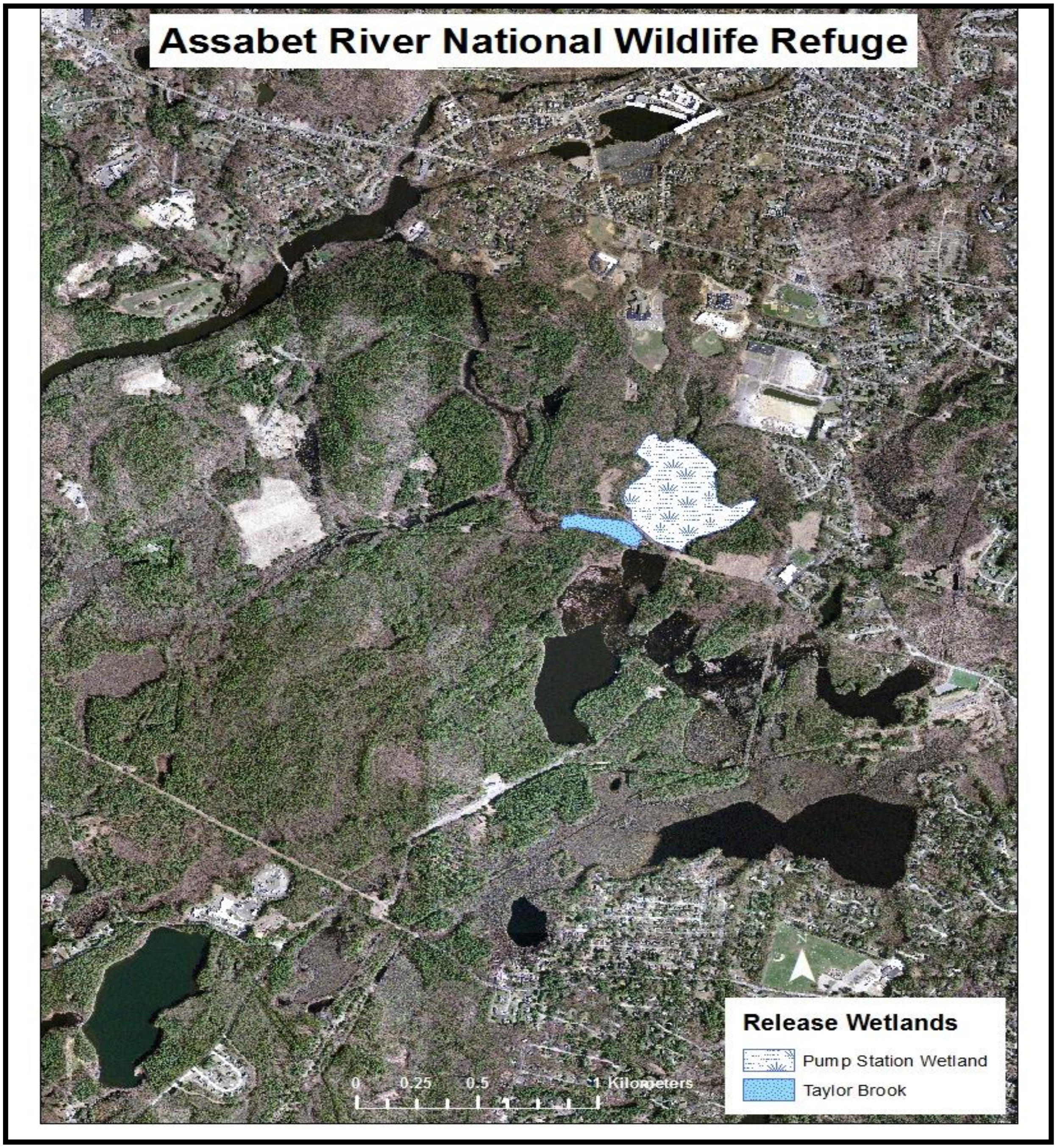


Figure 3.1. The recipient site is the Assabet River National Wildlife Refuge, an 880 ha protected area located approximately 40 km west of Boston, MA. The translocation of Blanding's Turtles has occurred in two wetlands located in the northeast corner of the refuge property, Pump Station Wetland and Taylor Brook Wetland.

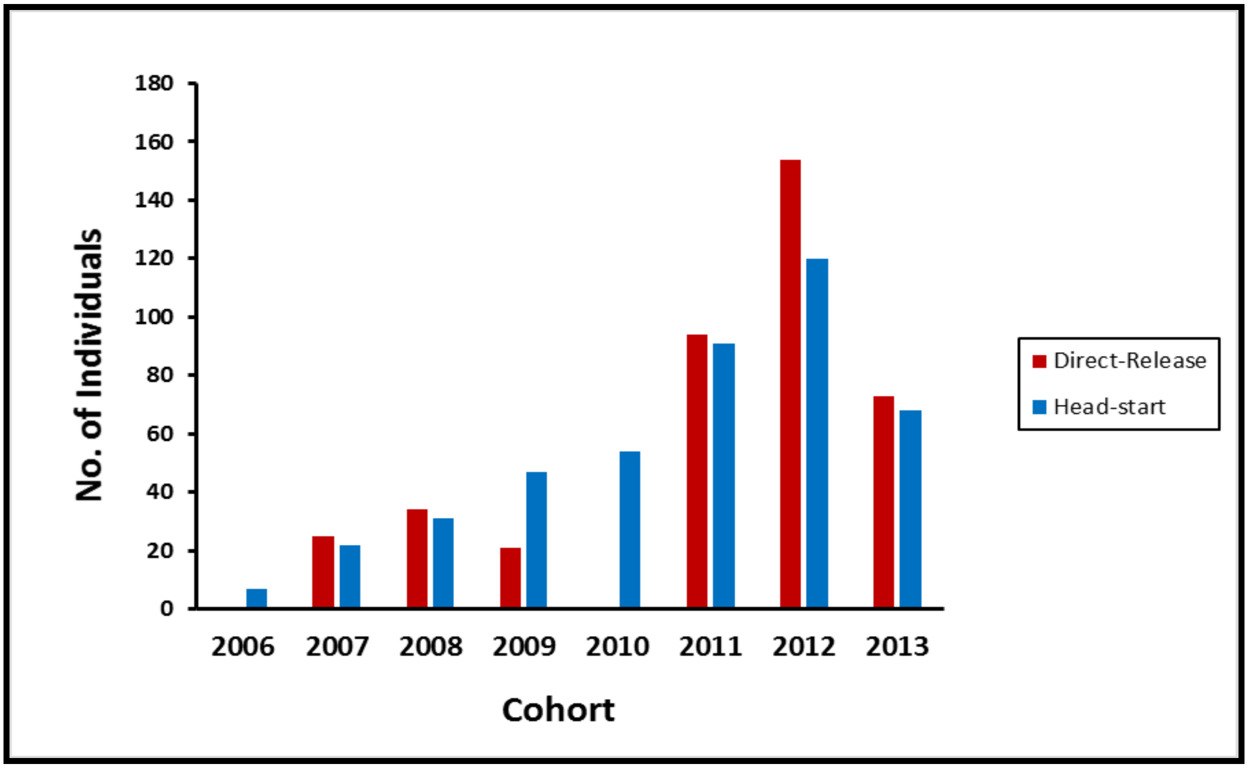


Figure 3.2. Number of direct-release hatchlings and head-started Blanding's turtles from the 2006-2013 cohorts released at the recipient site (Assabet River National Wildlife Refuge).

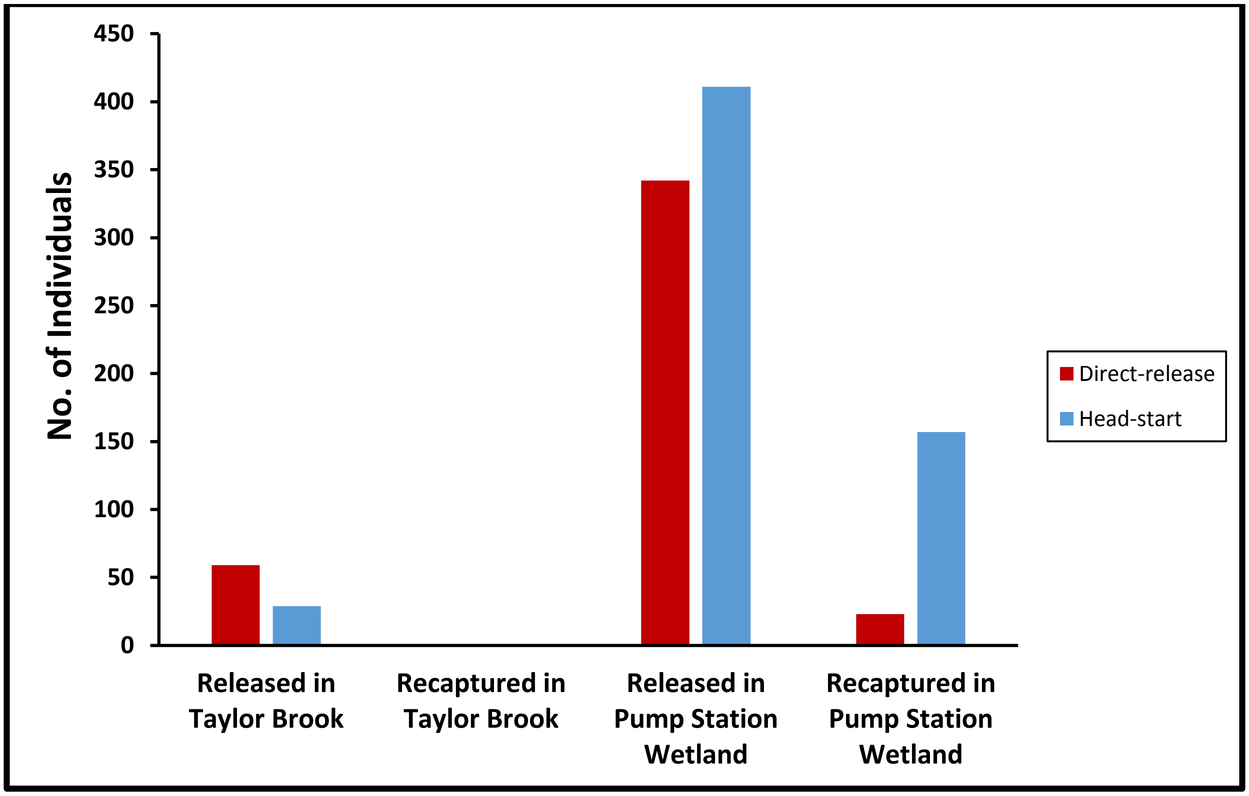
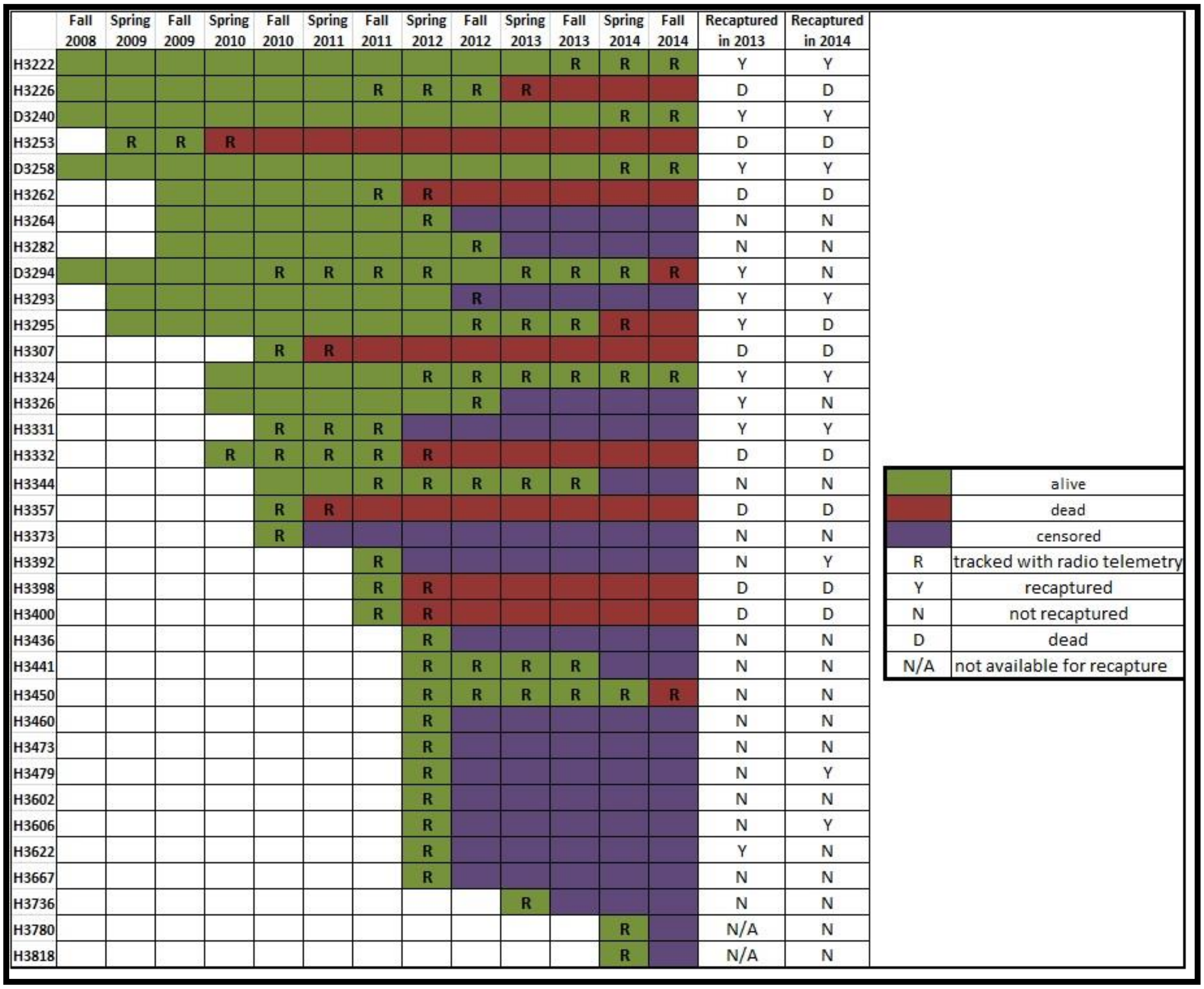


Figure 3.3. Number of direct-release and head-start turtles released and recaptured in Taylor Brook and Pump Station wetlands during 2011-2014 trapping efforts at the recipient site (Assabet River National Wildlife Refuge).



	Fall 2008	Spring 2009	Fall 2009	Spring 2010	Fall 2010	Spring 2011	Fall 2011	Spring 2012	Fall 2012	Spring 2013	Fall 2013	Spring 2014	Fall 2014	Recaptured in 2013	Recaptured in 2014
H3222											R	R	R	Y	Y
H3226							R	R	R	R				D	D
D3240												R	R	Y	Y
H3253		R	R	R										D	D
D3258												R	R	Y	Y
H3262							R	R						D	D
H3264								R						N	N
H3282									R					N	N
D3294					R	R	R	R		R	R	R	R	Y	N
H3293									R					Y	Y
H3295									R	R	R	R		Y	D
H3307					R	R								D	D
H3324								R	R	R	R	R	R	Y	Y
H3326									R					Y	N
H3331					R	R	R							Y	Y
H3332				R	R	R	R	R						D	D
H3344							R	R	R	R	R			N	N
H3357					R	R								D	D
H3373					R									N	N
H3392							R							N	Y
H3398							R	R						D	D
H3400							R	R						D	D
H3436								R						N	N
H3441								R	R	R	R			N	N
H3450								R	R	R	R	R	R	N	N
H3460								R						N	N
H3473								R						N	N
H3479								R						N	Y
H3602								R						N	N
H3606								R						N	Y
H3622								R						Y	N
H3667								R						N	N
H3736										R				N	N
H3780												R		N/A	N
H3818												R		N/A	N

(green)	alive
(red)	dead
(purple)	censored
R	tracked with radio telemetry
Y	recaptured
N	not recaptured
D	dead
N/A	not available for recapture

Figure 3.4. Head-started and directly-released Blanding's turtles released at Assabet River National Wildlife Refuge and radio-tracked for at least a portion of the period of May 2009 – October 2014. There were 21 head-started turtles that were outfitted with transmitters at time of their initial release. Eleven head-starts and three direct-release hatchlings received transmitters after being subsequently recaptured. Green cells correspond to periods when the turtle was known to be alive and red when a turtle was known to have been dead. Purple cells are censored periods when the radio transmitter either fell off the turtle or stopped working, making the turtle's fate unknown.

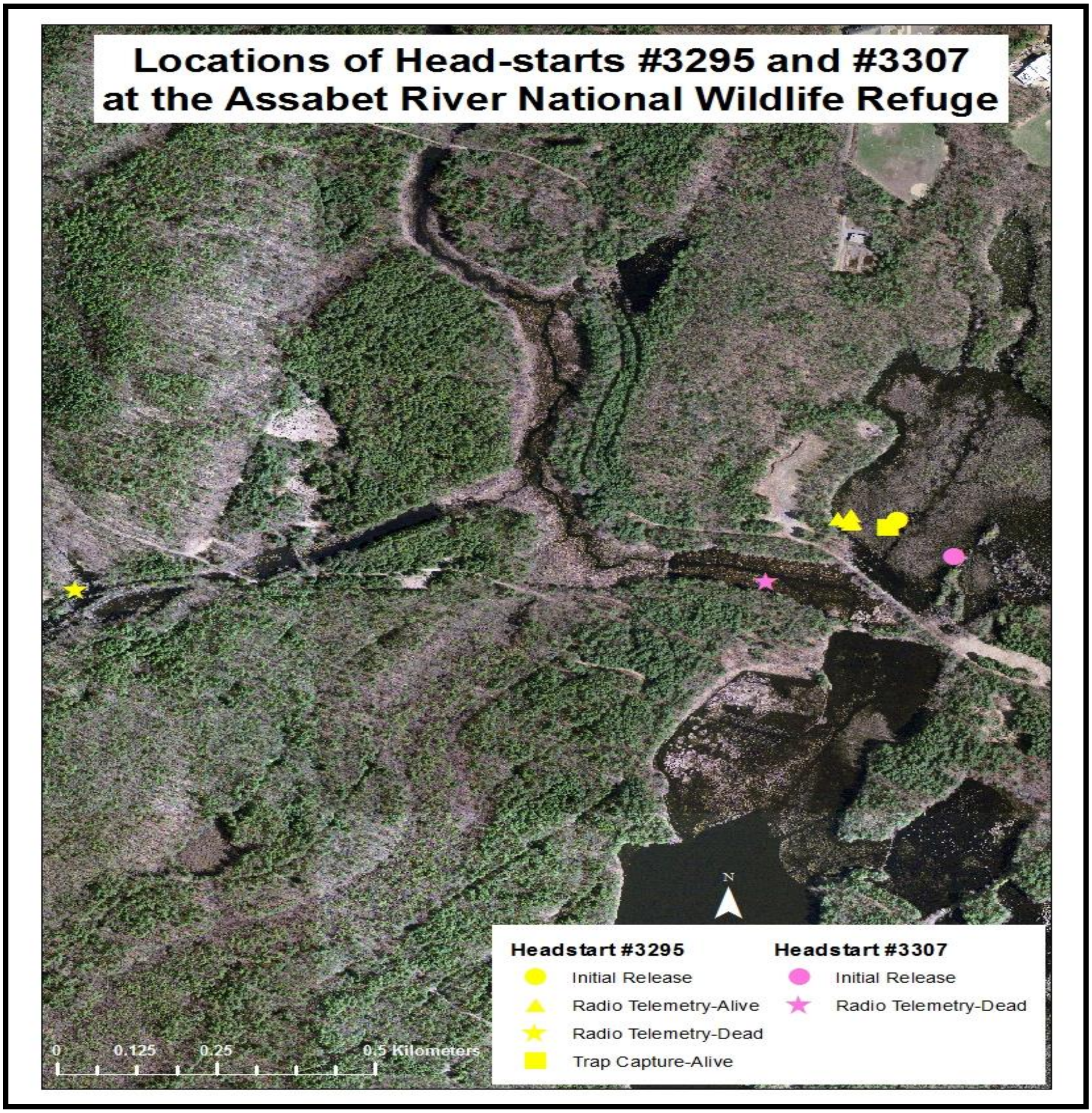


Figure 3.5. Release and capture locations for head-starts #3295 (yellow markers) and #3307 (pink markers), which are the only two head-started turtles known to have dispersed out of their initial release sites in the Pump Station Wetland during the study period of 2007-2014.

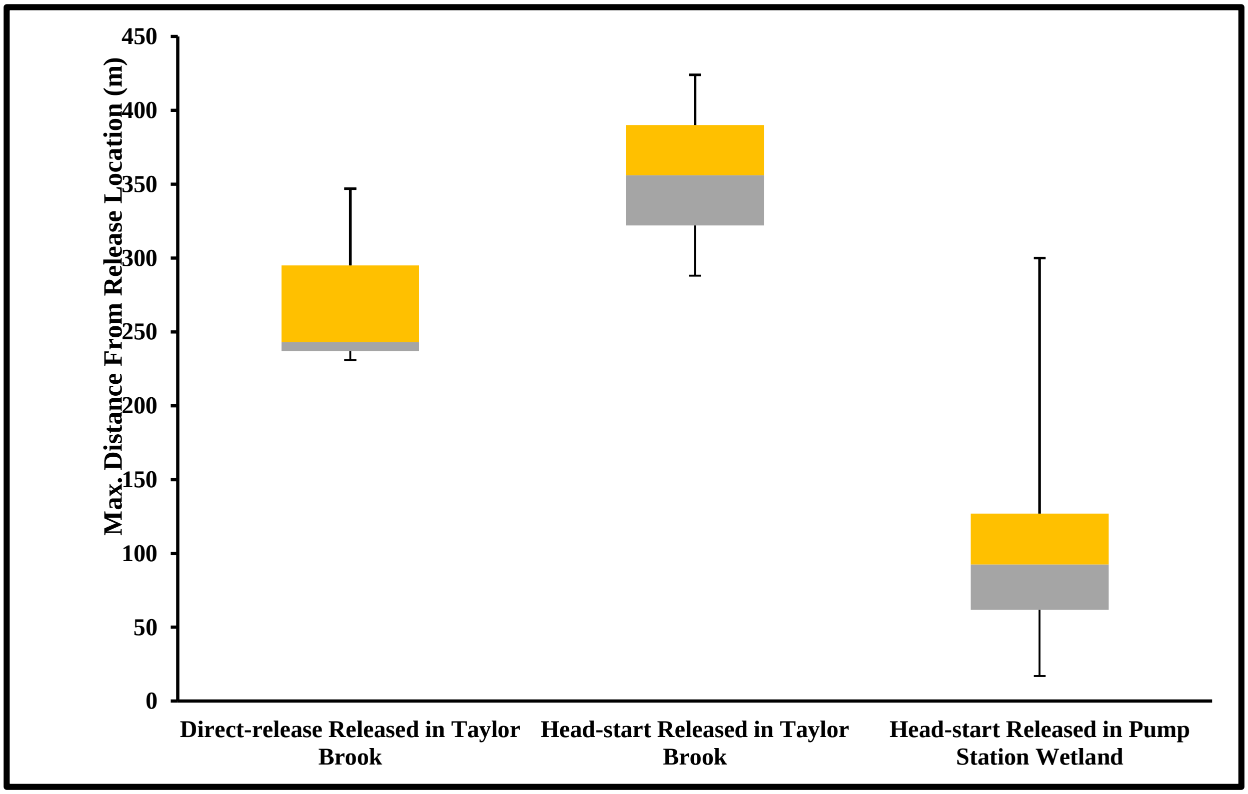


Figure 3.6. The maximum distances radioed Blanding's turtles traveled from their initial release sites at the Assabet River National Wildlife Refuge during the study period of 2009-2014. Both direct-releases and head-starts were initially released in either Taylor Brook Wetland or Pump Station Wetland.

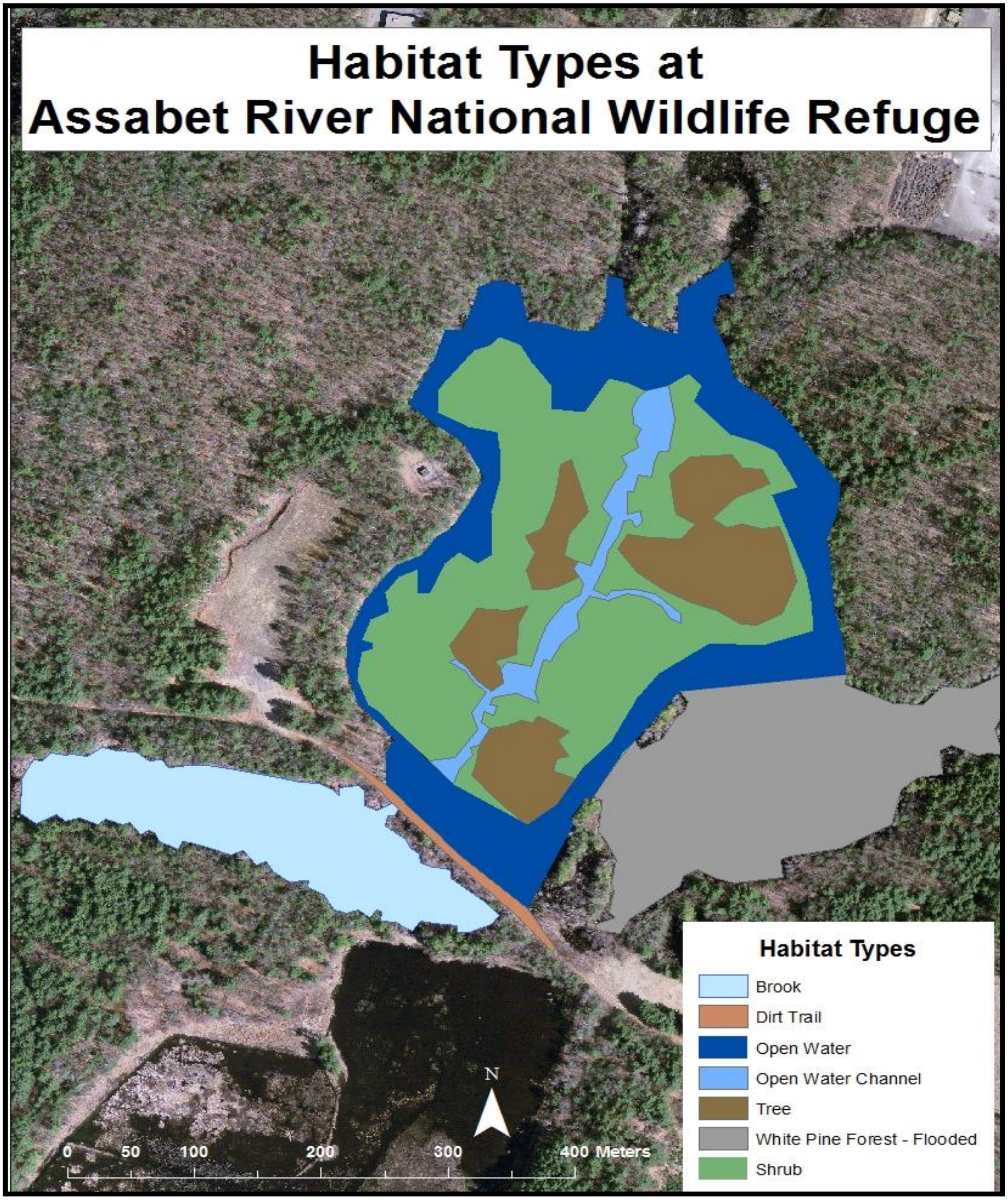


Figure 3.7. Map of available habitat types in the two wetlands at the recipient site (Assabet River National Wildlife Refuge) that served as the release sites for head-started and directly-released Blanding's turtles.

Figure 3.8. Shrub habitat at Pump Station Wetland at the recipient site (Assabet River National Wildlife Refuge). The shrub habitat is dominated by shrubs like Sweet Gale, Buttonbush, Leatherleaf; large areas of *Sphagnum* are often found in this habitat; and it sometimes contain areas of deeper water (up to two meters).

Figure 3.9. Taylor Brook Wetland at the recipient site (Assabet River National Wildlife Refuge). This brook habitat is a mix of open water (up to two meters deep) with large quantities of Sedge Grasses and Cattails.

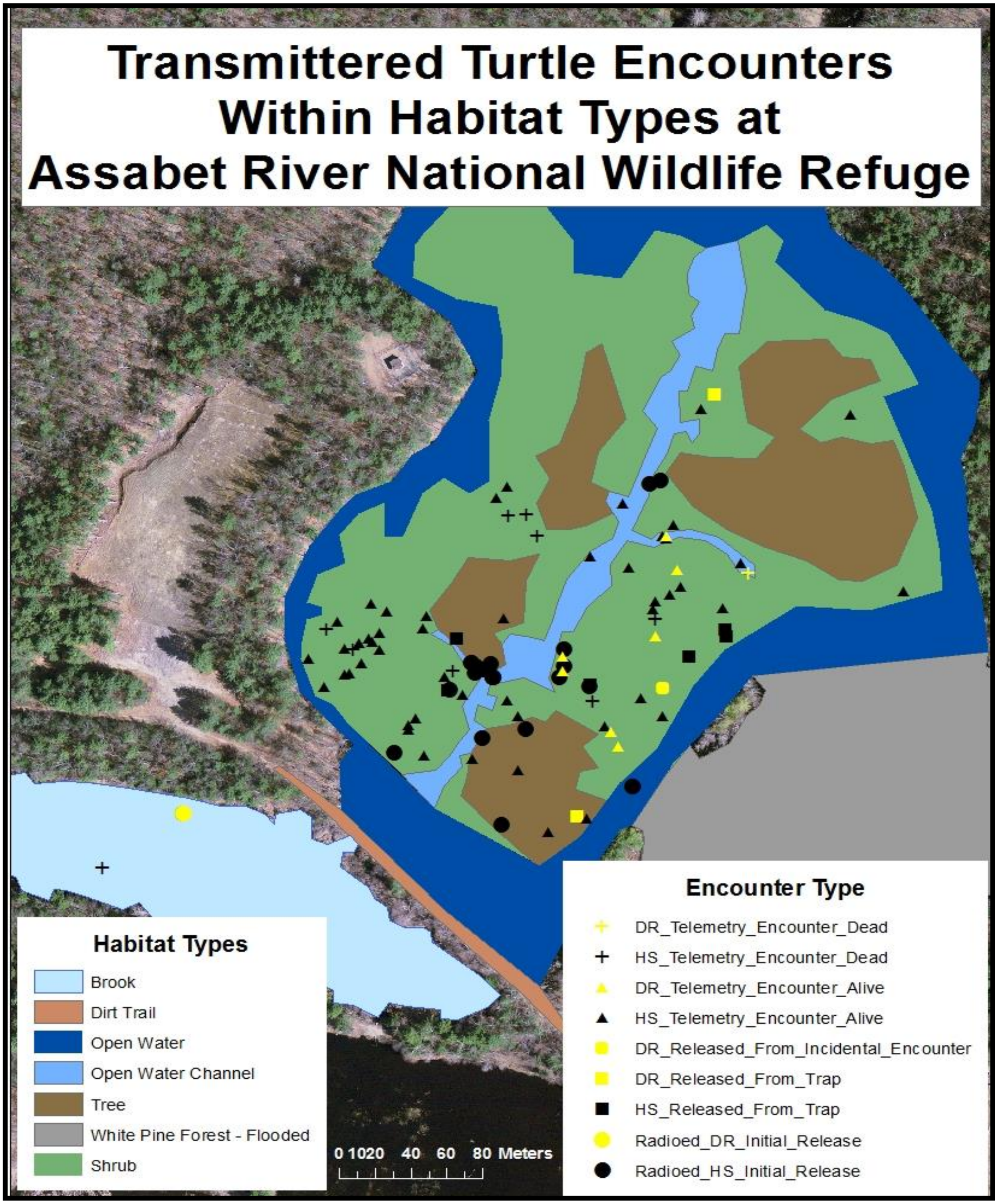


Figure 3.10. Type of encounters of radioed Blanding's turtles within each habitat type in the two release wetlands at the recipient site (Assabet River National Wildlife Refuge).

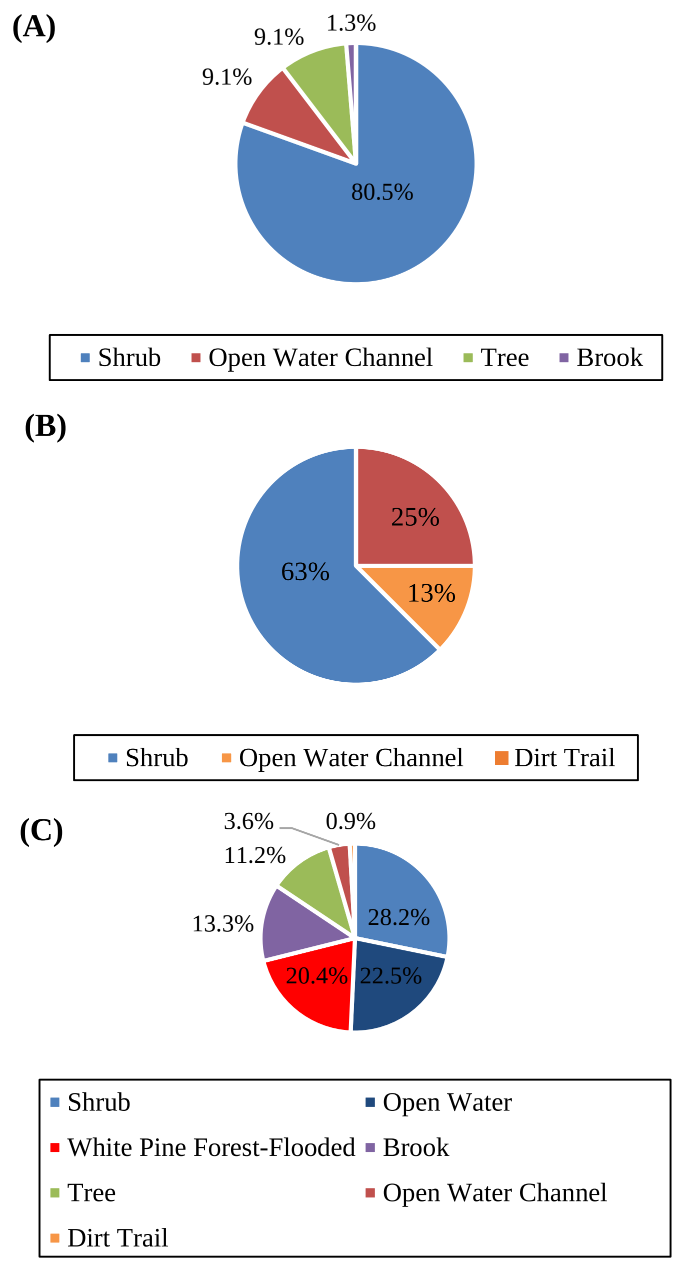


Figure 3.11. Percentage of (A) radio telemetry and (B) incidental encounter locations gathered in each (C) available habitat type at the recipient site (Assabet River National Wildlife Refuge).

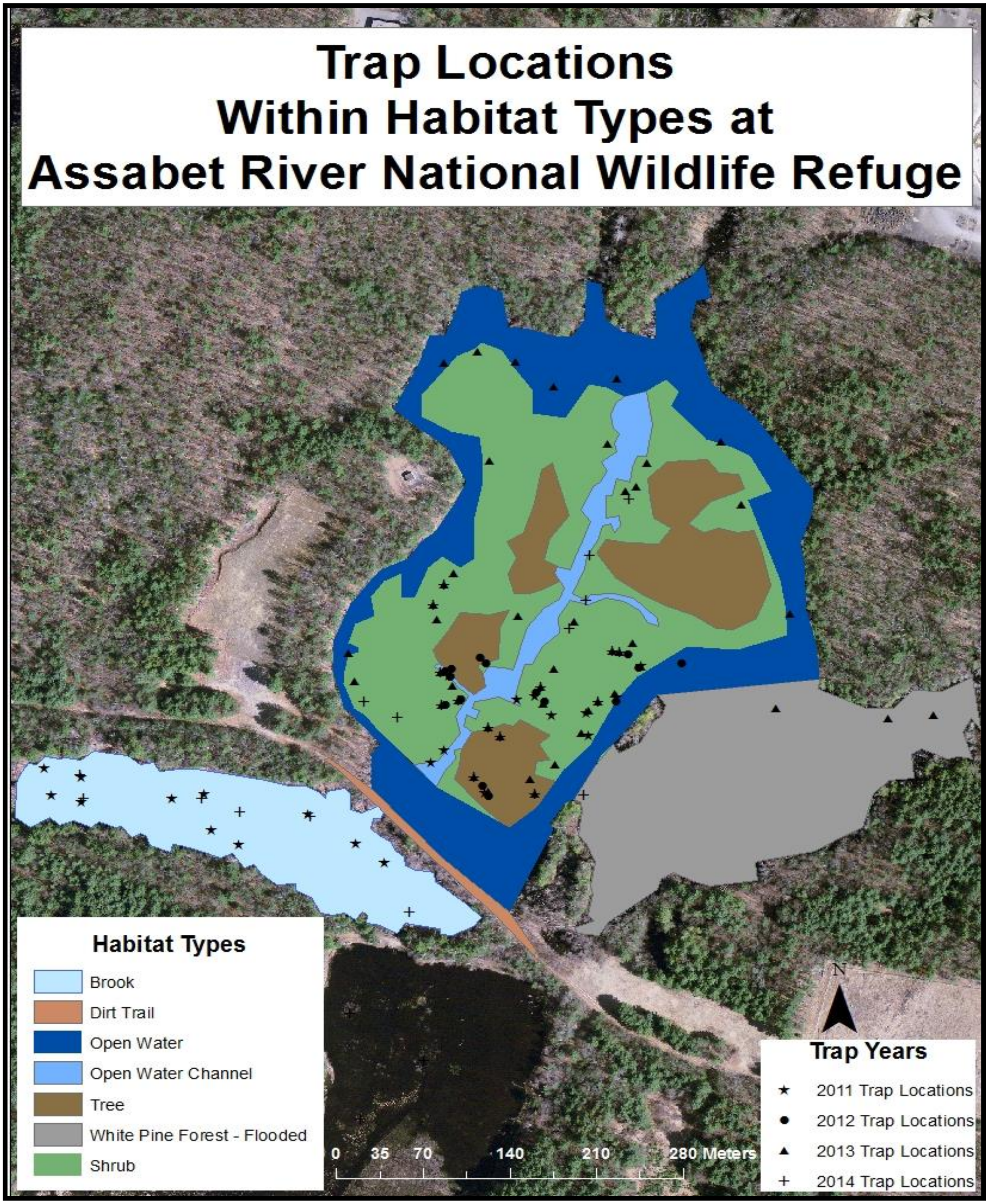


Figure 3.12. Trap locations within habitat types in the Taylor Brook and Pump Station Wetlands at the recipient site (Assabet River National Wildlife Refuge) from 2011-2014.

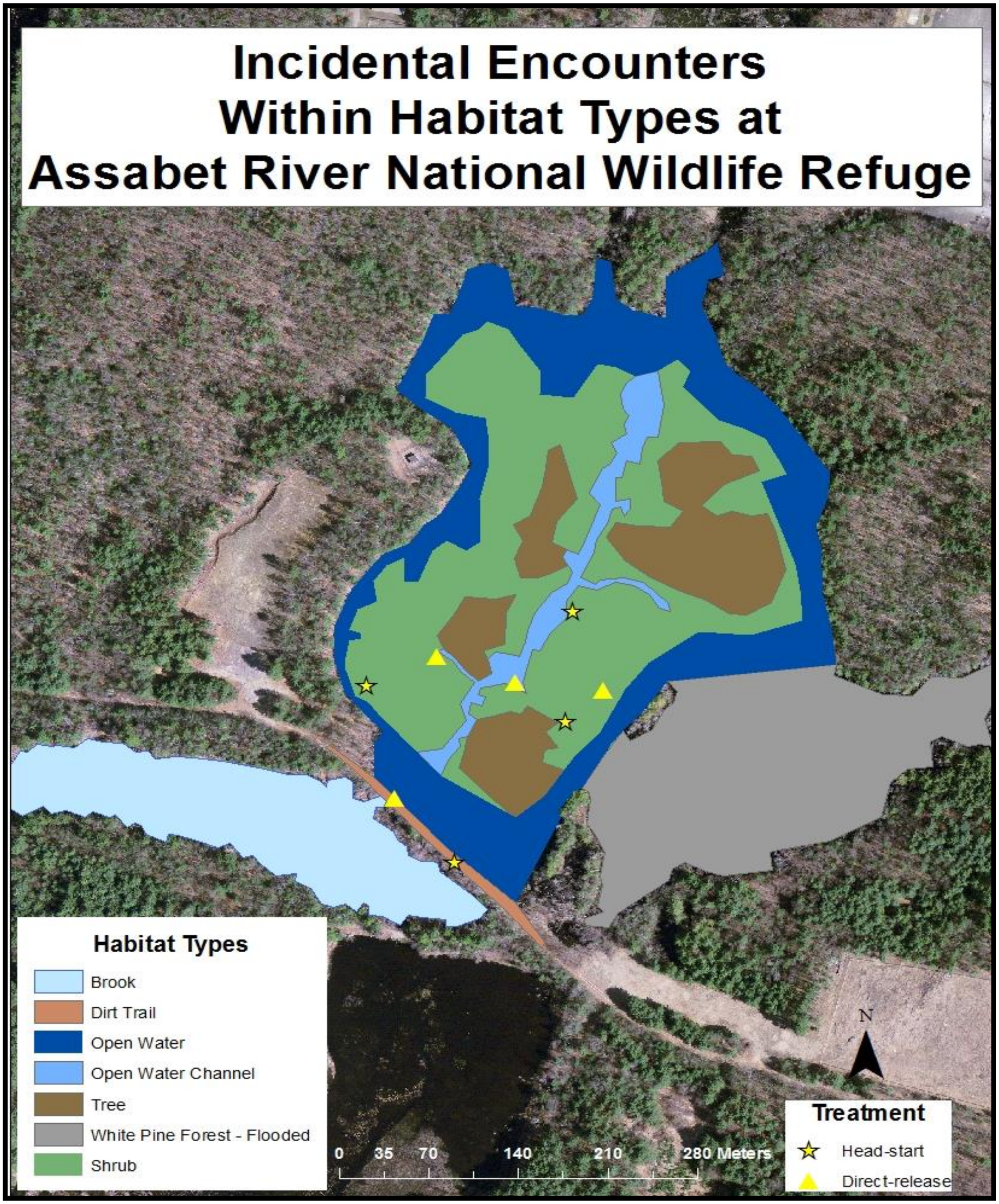


Figure 3.13. Incidental encounters at the recipient site (Assabet River National Wildlife Refuge) within habitat types from 2010-2014.

CHAPTER 4

CONCLUSIONS

Freshwater turtles are increasingly being adversely impacted by human activity, with 2 out of every 5 species listed as threatened (Olson and Kiester 2011). To combat diminished populations, population manipulations such as head-starting and/or translocations are available (Seigel and Dodd 2000) once the potential sources of human-caused mortality have been removed (Heppell et al. 1996; Heppell 1998). There has been an increasing amount of information on the effectiveness of head-starting and translocations of turtles, but further research is needed to alleviate the concerns that these are ineffective management strategies (Frazer 1992).

Head-starting has been shown to result in high levels of survivorship across a range of freshwater turtle species (Haskell et al. 1996; Michell and Michell 2015; Vander Haegen 2009). My results provided similar support in favor of head-starting, as head-started Blanding's Turtle hatchlings survived at a rate approximately six times that of direct-released hatchlings during their first-year post-release to the Assabet River NWR. Fostering higher survivorship amongst hatchling and juvenile turtles should allow for faster recruitment into a diminished population and/or faster establishment of a new population.

Future research on head-starting should examine the long-term success of head-starting projects. This can include whether head-started turtles display similar rates of survival as their wild counterparts once they have reached maturity. The age at sexual maturity should be investigated as well, to determine whether the onset of reproductive activities occurs earlier amongst head-starts. The earlier onset of sexual maturity amongst head-started European Pond Turtles has already been observed (Masin et al. 2015), but investigation into whether this is true in other freshwater turtle species is needed.

Translocations were previously believed to be ineffective with reptiles and amphibians (Dodd and Siegel 1991), but recent research has shown it to be successful amongst turtles (Cadi and Miquet 2004; Mignet et al. 2014; Moore et al. 2013). Little information was available on the effectiveness of translocated juvenile turtles, and so the translocation of head-started and direct-released Blanding’s turtle hatchlings to the Assabet River NWR should aid in the greater understanding of juvenile turtle translocation success. My results showed that translocation of juvenile turtles was a success in that they displayed fidelity to the refuge at which they were released. They did not all exhibit fidelity to the wetland at which they were released, suggesting that there needs to be a variety of habitat types available from which to select from to ensure that they stay at/or near their release site. This is important for translocations that may be occurring in heavily fragmented areas where dispersal from the release site could result in mortality. To ensure that translocated individuals have the appropriate habitat to select from, greater understanding of habitat selection of head-started and/or juvenile freshwater turtles is needed. Translocated Blanding’s turtle head-starts overwhelmingly selected for shrub habitat at rates that were disproportionate to its availability. Insight into the habitat preferences of juvenile freshwater turtles should aid in the identification of suitable release sites that increase the success of translocation projects, including those that are translocating head-started individuals.

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