North Carolina Conservation Plan for Five Rare Aquatic Species Restricted to the Neuse and Tar-Pamlico River Basins:

Dwarf Wedgemussel (*Alasmidonta heterodon*),
Yellow Lance (*Elliptio lanceolata*),
Tar River Spinymussel (*Parvaspina steinstansana*),
Carolina Madtom (*Noturus furiosus*),
and
Neuse River Waterdog (*Necturus lewisi*)

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Cover photos: Dwarf Wedgemussel (*Alasmidonta heterodon*) – Top row, left
Yellow Lance (*Elliptio lanceolata*) – Top row, center
Tar River Spinymussel (*Parvaspina steinstansana*) – Top row, right
Carolina Madtom (*Noturus furiosus*) – Bottom row, left
Neuse River Waterdog (*Necturus lewisi*) – Bottom row, right

Cover photos taken by NCWRC Eastern Region Aquatic Wildlife Diversity Staff

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EXECUTIVE SUMMARY

The North Carolina Wildlife Resources Commission developed this conservation plan to direct management activities for three freshwater mussel species (Dwarf Wedgemussel (*Alasmidonta heterodon*), and Yellow Lance (*Elliptio lanceolata*), Tar River Spinymussel (*Parvaspina steinstansana*)), one freshwater fish species (Carolina Madtom (*Noturus furiosus*)), and one aquatic salamander species (Neuse River Waterdog (*Necturus lewisi*)) known in North Carolina from the Neuse and Tar-Pamlico river basins. Historically, these species inhabited waterways from the headwaters to lower reaches of both river basins. Each species requires slightly different habitat requirements; however, they all require high-quality waterways containing cool, well oxygenated and unpolluted water. Waterways must contain adequate suitable habitat, including constant flow, natural flow regime, unembedded substrate, and stable instream habitat. Direct threats to these species include pollution (chemical and thermal), unnatural flow conditions, dams, sedimentation, unstable or fragmented habitat, invasive species, and diseases.

The Dwarf Wedgemussel and Tar River Spinymussel were listed as state endangered in 1977 and listed as federally endangered in 1990 and 1985, respectively. The Yellow Lance was listed as state endangered in 1977, downlisted to state threatened in 1990, and uplisted to state endangered in 2001. It was listed as federally threatened in 2018. The Carolina Madtom was state listed as special concern in 1977, modified to state special concern (Neuse River basin only), and uplisted to state threatened in 2006. The Neuse River Waterdog is state listed as a Species of Special Concern in 1990. In 2010, Yellow Lance, Carolina Madtom, and Neuse River Waterdog were petitioned for federal listing under the Endangered Species Act of 1973. The goal of this conservation plan is to prevent the extinction of these species and promote population viability within North Carolina for the next 100 years. Species specific conservation objectives and research needs are outlined within each species account. However, a general theme can be found for these species and focuses on identifying and reducing threats, promoting population viability, habitat protection, population monitoring, research, and partnerships. Establishing and maintaining partnerships between North Carolina Wildlife Resources Commission staff and other state agencies, federal agencies, universities, non-profit organizations, companies, local governments, and citizens are essential to the implementation of this conservation plan. The management of these species will require collaborative stakeholder efforts to protect sensitive habitats and maintain high-quality water resources throughout the Neuse and Tar-Pamlico River basins.

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INTRODUCTION

This conservation plan outlines recovery action needs of five aquatic species within the Neuse and Tar-Pamlico River basins in North Carolina. The species covered in this conservation plan include three freshwater mussels; Dwarf Wedgemussel (*Alasmidonta heterodon*), Yellow Lance (*Elliptio lanceolata*), Tar River Spinymussel (*Parvaspina steinstansana*); one freshwater fish; Carolina Madtom (*Noturus carolinensis*) and an aquatic salamander; Neuse River Waterdog (*Necturus lewisi*). The Dwarf Wedgemussel and Tar River Spinymussel are listed as state and federally endangered. The Yellow Lance is listed as state endangered and federally threatened, Carolina Madtom as state threatened, and Neuse River Waterdog as Special Concern; however, the latter two species were petitioned in 2010 for federal listing under the Endangered Species Act of 1973 and are being evaluated to determine their federal conservation status.

SPECIES ACCOUNTS

Dwarf Wedgemussel (*Alasmidonta heterodon*)

Biological Information

<u>Description and Taxonomic Classification</u>

The Dwarf Wedgemussel (*Alasmidonta heterodon* Lea 1830) is a state and federally endangered freshwater mussel that historically inhabited numerous waterways along the Atlantic Slope. The Dwarf Wedgemussel is a member of the genus *Alasmidonta*, which includes 12 species that typically have a thin shell, a well-developed posterior ridge, weak to moderate pseudocardinal teeth, and weak to absent lateral teeth (Turgeon et al. 1998; Williams et al. 2008). The Dwarf Wedgemussel is easily distinguished from the other *Alasmidonta* species by the presence of two weak lateral teeth on the right valve. The external surface of the shell (periostracum) is often green to olive with variable rays, and the inside of the shell (nacre) is white to bluish white. Adults are sexually dimorphic and reach a maximum length of < 60 mm. Females have a shell that is laterally inflated, which results in a steep posterior slope and truncated appearance. In comparison, males have a shell that is compressed, lacking a steep posterior slope, and an elongate oval shell outline. **Etymology:** *heterodon*, referring to the fact that Dwarf Wedgemussel is the only North American freshwater mussel that typically has two lateral teeth on the right valve and one on the left (Fuller 1977).

Taxonomic Hierarchy (Integrated Taxonomic Information System 2017):

Kingdom:	Animalia
Phylum:	Mollusca
Class:	Bivalvia
Order:	Unionoida
Family:	Unionidae
Genus:	*Alasmidonta*
Species:	*Alasmidonta heterodon*

Distribution and Population Status

The historical distribution of Dwarf Wedgemussel ranged from North Carolina to New Brunswick, Canada (USFWS 1993). Currently, the population in Canada is considered extirpated, and the remaining populations occur in isolated locations between New Hampshire and North Carolina. Despite this species' apparently large range, Dwarf Wedgemussel has a very disjunct distribution consisting of small, relict populations. In North Carolina, Dwarf Wedgemussel is restricted to the Piedmont and western edge of the Coastal Plain within the Neuse and Tar-Pamlico River basins (Figure 1). Neuse River basin occurrence records exist for Buffalo Creek, Eno River, Little Creek, Little River, Middle Creek, Moccasin Creek, Neuse River, Swift Creek, Turkey Creek, and White Oak Creek. The Neuse River basin population of Dwarf Wedgemussel is highly fragmented, extremely small, and at-risk of extirpation. In the Tar-Pamlico River basin, it historically occurred in Bens Creek, Cedar Creek, Crooked Creek, Cub Creek, Fox Creek, Isinglass Creek, Little Shocco Creek, Long Branch, Maple Branch, Norris Creek, North Fork Tar River, Red Bud Creek, Rocky Swamp, Ruin Creek, Shelton Creek, Shocco Creek, Stony Creek, Tabbs Creek, Tar River, unnamed tributary to Cub Creek, and an unnamed tributary to Little Fishing Creek. The Tar-Pamlico River basin population is also fragmented; however, the watershed remains a stronghold for the species within North Carolina.

Surveys focused specifically on Dwarf Wedgemussel in North Carolina are somewhat limited because many freshwater mussel surveys assess freshwater mussel diversity rather than the status of a single species. As such, numerous freshwater mussel surveys have been conducted throughout the Neuse and Tar-Pamlico River basins (Figure 1). To date, Dwarf Wedgemussel has been collected within 18 watersheds (i.e., 10-digit hydrologic units) in North Carolina. Within the past decade (2008 – 2017), Dwarf Wedgemussel has been collected from only 1 of 8 watersheds (13%) and 6 of 10 watersheds (60%) within the Neuse and Tar-Pamlico River basins, respectively. 2008 – 2007

The status of Dwarf Wedgemussel was listed as "Endangered" by Fuller (1977) due to dwindling populations and rarity. The Dwarf Wedgemussel was listed as state endangered in 1977. In 1986, Master submitted the results of a global status survey and strongly recommended that Dwarf Wedgemussel be listed as "Endangered". Subsequently, on March 14, 1990, the U.S. Fish and Wildlife Service made a final ruling that the Dwarf Wedgemussel be listed as a threatened species with protection provided by the Endangered Species Act of 1973 (USFWS 1993). The findings of the U.S. Fish and Wildlife Service 5-year reviews continue to recommend that the Dwarf Wedgemussel remain listed as "Endangered" (USFWS 2007, 2013). In addition, Yellow Lance is listed as endangered in the state of North Carolina.

Habitat and Life History

Habitat use of Dwarf Wedgemussel: Within North Carolina, Dwarf Wedgemussel typically inhabits small to medium streams with moderate flow and stable sand, gravel, and cobble substrates. In addition, the species is sometimes found in clay or under rootwads (Kendig 2014).

Diet of Dwarf Wedgemussel: The Dwarf Wedgemussel is a filter feeder that feeds on a variety of particulate matter suspended in the water column including algae, phytoplankton, zooplankton, bacteria, detritus, and dissolved organic matter (Haag 2012). Juveniles pedal feed by using the cilia on their foot to gather particulate matter from the substrate.

Reproduction of Dwarf Wedgemussel: Similar to most freshwater mussels, Dwarf Wedgemussel has a complex life cycle that requires the use of a fish host to successfully reproduce. Freshwater mussels are dioecious, and sexually mature males release large quantities of sperm into the water column to begin the reproductive life cycle. For fertilization to occur, sperm must pass into the incurrent apertures of sexually mature females. The sperm travel through the aperture while the mussel is filter feeding and fertilize eggs in the suprabranchial chamber. The fertilized eggs are then transferred into the gill chambers, which form a modified brood pouch called the marsupium. While in the marsupium, the fertilized eggs quickly mature into the larval form known as glochidia, and this process usually requires 2-6 weeks for maturation (Haag 2012). Dwarf Wedgemussel is considered to be a long-term brooder (bradytictic) which means that individuals spawn in late summer, females become gravid in September, and release glochidia in April (Michaelson and Neves 1995). Glochidia are released into the water column to attach onto the gills of a suitable fish host, where the glochidia metamorphose from larvae to free-living mussel. Glochidia remain on the host fish for a period of 10-38 days, during this time they receive nutrients from the fish blood and develop its internal organs such as a foot, digestive tract, and gills, as well as forming two adductor muscles (Michaelson and Neves 1995, Haag 2012). Once the glochidia complete their metamorphosis they excyst from the gills of the host fish and settle into the substrate to live as a juvenile freshwater mussel.

Fish Host Trials for Dwarf Wedgemussel: To date, 46 fish species across 11 families have been exposed to Dwarf Wedgemussel glochidia (Michaelson and Neves 1995, St. John White 2007, Levine et al. 2011, St. John White et al. 2017, NCSU unpublished data).

<u>Effective Hosts:</u> *Aphredoderus sayanus* (Pirate Perch), *Cottus bairdii* (Mottled Sculpin), *Cottus cognatus* (Slimy Sculpin), *Etheostoma flabellare* (Fantail Darter), *Etheostoma nigrum* (Johnny Darter), *Etheostoma* olmstedi (Tessellated Darter), *Morone saxatilis* (Striped Bass), *Percina nevisense* (Chainback Darter), *Salmo salar* (Atlantic Salmon)

<u>Poor Hosts:</u> *Etheostoma collis* (Carolina Darter), *Etheostoma vitreum* (Glassy Darter), *Fundulus diaphanous* (Banded Killifish), *Lepomis auritus* (Redbreast Sunfish), *Lepomis cyanellus* (Green Sunfish), *Notropis altipinnis* (Highfin Shiner), *Percina peltata* (Shield Darter), *Salmo trutta* (Brown Trout)

<u>Ineffective Hosts:</u> *Ambloplites rupestris* (Rock Bass), *Anguilla rostrata* (American Eel), *Campostoma anomalum* (Central Stoneroller), *Catostomus commersoni* (White Sucker), *Cyprinella analostana* (Satinfin Shiner), *Cyprinella spiloptera* (Spotfin Shiner), *Etheostoma zonale*

(Banded Darter), *Exoglossum maxillingua* (Cutlips Minnow), *Hypentelium nigricans* (Northern Hog Sucker), *Ictalurus punctatus* (Channel Catfish*), Lepomis gibbosus* (Pumpkinseed), *Lepomis macrochirus* (Bluegill Sunfish), *Luxilus albeolus* (White Shiner), *Luxilus cornutus* (Common Shiner), *Lythrurus matutinus* (Pinewoods Shiner), *Micropterus dolomieu* (Smallmouth Bass), *Micropterus salmoides* (Largemouth Bass), *Nocomis leptocephalus* (Bluehead Chub), *Notemigonus crysoleucas* (Golden Shiner*), Notropis procne* (Swallowtail Shiner), *Noturus insignis* (Margined Madtom), *Oncorhynchus mykiss* (Rainbow Trout), *Perca flavescens* (Yellow Perch), *Percina roanoka* (Roanoke Darter), *Pimephales notatus* (Bluntnose Minnow), *Pomoxis annularis* (White Crappie), *Rhinichthys atratulus* (Blacknose Dace), *Rhinichthys cataractae* (Longnose Dace), *Salvelinus fontinalis* (Brook Trout)

Glochidia of Dwarf Wedgemussel: Dwarf Wedgemussel glochidia are roughly triangular, with hooks, and are relatively large, measuring 325 µm in length and 255 µm in height (Clarke 1981). Glochidia are heavy and typically sink to the bottom of an aquarium. The hooks on the glochidia allow them to attach to the fins of fish and remain there during transformation, which suggests the use of a benthic host fish in the wild.

Conservation Management

<u>Historical Conservation Efforts</u>

North Carolina Wildlife Resources Commission (NCWRC) and US Fish and Wildlife Service (USFWS) biologists conduct 5-10 targeted surveys for Dwarf Wedgemussel on a yearly basis and search for suitable locations for future augmentation efforts. In 2009, North Carolina Department of Transportation, NCWRC, and USFWS partnered with North Carolina State University to identify the host fish and refine captive propagation techniques for Dwarf Wedgemussel. The Marion Conservation Aquaculture Center (MCAC), located at the NCWRC's Marion State Fish Hatchery in McDowell County, NC was established in 2008. The objective of the MCAC is to preclude listing, promote delisting, and prevent the extinction of aquatic species when appropriate by using captive propagation and arking. The MCAC began to "ark" the Neuse River basin Dwarf Wedgemussel population in 2015 and began propagation efforts to augment remaining populations in the future. In 2015, NCWRC initiated beaver management activities on Brinkleyville and Shocco Creek Game Lands so that flowing conditions could be restored to three waterways (Maple Branch, Shocco Creek, and Rocky Swamp) within the Tar-Pamlico River basin. The three focal reaches historically harbored Dwarf Wedgemussel and quality mussel habitat; however, beaver activity severely impacted flow regimes and riparian canopy cover as well as substantially reduced mussel abundance. In addition, the USFWS partnered with species experts to develop a structured decision-making conservation strategy for Dwarf Wedgemussel in 2015. This collaborative effort identified that protecting Tar-Pamlico River basin populations (protect the best) or a hybrid strategy (i.e., protection in the Tar-Pamlico River basin with attempts to expand the distribution in the Neuse River basin) was the optimal conservation strategy for Dwarf Wedgemussel in North Carolina (Smith et al. 2015).

Threats

As with all aquatic species, there are many natural and anthropogenic factors that threaten the long-term viability of Dwarf Wedgemussel (USFWS 1993). Extinction and decline of North American unionid bivalves can be traced to impoundment and inundation of riffle habitat throughout the United States. The loss of obligate hosts, coupled with increased siltation, and various types of industrial and domestic pollution have resulted in the rapid decline of the unionid bivalve fauna in North America (Bogan 1993, NCWRC 2015). Dams, both manmade and natural (created by beavers, see Kemp et al. 2012), are a barrier to dispersal of host fish and attached glochidia. Throughout the Neuse and Tar-Pamlico River basins, beavers have continued to build dams and impound an increasing number of river kilometers. Beaver dams not only inundate and alter riffle/run mussel habitat upstream of the dam but also effect mussel populations downstream of the dam by increasing fluctuations in flow regime, decreasing dissolved oxygen levels, and increasing the variability of food quality and quantity (Hoch 2012, Kemp et al. 2012). Contaminants and water pollution are a significant threat to all aquatic species, especially mussels. Point source discharges from municipal wastewater that contains monochloramine and unionized ammonia compounds are acutely toxic to freshwater mussels and may be responsible for glochidial mortality that results in local extirpation of mussels (Goudreau et al. 1993, Gangloff et al. 2009, NCWRC 2015). Impervious areas in urbanized watersheds contribute to high water levels, even during short rainfall events, which can result in flash flooding. These high or flashy flow events contribute to increased sediment loads, turbidity throughout the water column, and stream bed movements that stress mussel populations (Gangloff et al. 2009, NCWRC 2015). Climate change and development will likely bring additional stressors that need to be evaluated for mussels. Furthermore, specific pollutants that may be introduced into the aquatic environment, the interactions of pollutants and temperature (from climate change), salinity (related to sea level rise), and lower dilution (from altered flows) will need to be considered (NCWRC 2015). In addition, invasive species such as the Asian Clam (*Corbicula fluminea*), the Flathead Catfish (*Pylodictis olivaris*), and Hydrilla (*Hydrilla verticillata*) can create competitive pressures on food resources and habitat availability. These factors can decrease oxygen availability, cause ammonia spikes, alter benthic substrates, impact host fish communities, reduce stream flow, and increase sediment buildup (Belanger et al. 1991, Scheller 1997, NCANSMPC 2015, NCWRC 2015).

Conservation Goal

To prevent the extinction of Dwarf Wedgemussel and promote population viability (i.e., multiple age classes and wild recruitment) within North Carolina for the next 100 years.

Conservation Objectives

The overarching conservation strategy is to promote habitat protection and maintain the best populations of Dwarf Wedgemussel in the Tar-Pamlico River basin and focus efforts within the Neuse River basin on Swift Creek, Little River, and consider options to expand the distribution. Restoration of habitat should be promoted for hydrologic units listed under Objective 1 and should primarily focus on beaver management and protection of riparian habitat and associated uplands.

1) Promote habitat protection and maintain two viable population of Dwarf Wedgemussel in the Neuse River basin and three populations in the Tar-Pamlico River basin (Figure 2). Management Units (MUs) will be defined based on hydrologic units (i.e., HUC10s).
    a. **Neuse River Basin**
        i. Swift Creek MU (0302020110)
        ii. Little River MU (0302020115, 0302020116)
    b. **Tar-Pamlico River Basin**
        i. Fishing creek MU (0302010201, 0302010202, 0302010203, 0302010205)
        ii. Swift Creek MU (0302010107)
        iii. Tar River MU (0302010101, 0302010102, 0302010103, 0302010104)
2) Maintain an ark population of Dwarf Wedgemussel from Neuse and Tar-Pamlico River basin broodstock.
3) Utilize captive propagation and/or translocations to augment or establish subpopulations of Dwarf Wedgemussel where appropriate habitat exists (pending approval from the Habitat, Nongame and Endangered Species Committee). To reduce the potential to minimize the regulatory burden associated with the federal Endangered Species Act, a tool such as Safe Harbor will be established prior to reintroduction into an unoccupied area.
    a. All Neuse and Tar-Pamlico River basin MU hydrologic units listed above.
    b. Additional augmentation areas within the known range of Dwarf Wedgemussel (Figure 2), if propagation efforts exceed MU needs.
        i. Neuse River Basin
            1. Contentnea Creek (0302020301)
            2. Eno River (0302020103)
            3. Middle Creek (0302020109)
            4. Neuse River (0302020107)
        ii. **Tar-Pamlico River Basin**
            1. Stony Creek (0302010105)
    c. Potential reintroduction or introduction of Dwarf Wedgemussel (Figure 2) into areas within the presumed historical range, if propagation efforts exceed MU needs. Ideally located in areas with reduced likelihood of anthropogenic threats.
        i. Neuse River Basin
            1. Black Creek (0302020112)
            2. Contentnea Creek (0302020302, 0302020303, 0302020304, 0302020305, 0302020306, 0302020307)
            3. Falling Creek (0302020114)
            4. Falls Lake (0302020104, 0302020105, 0302020106)
            5. Flat River (0302020101)
            6. Little River (0302020102)
            7. Mill Creek (0302020113)
            8. Neuse River (0302020111, 0302020117, 0302020201, 0302020202, 0302020203)
            9. Swift Creek (0302020204)
        ii. Tar-Pamlico River Basin
            1. Beech Swamp (0302010204)
            2. Conetoe Creek (0302010303)
            3. Fishing Creek (0302010206)

4. Swift Creek (0302010108)
5. Tar River (0302010106, 0302010109, 0302010302, 0302010304, 0302010306)
6. Town Creek (0302010301)
7. Tranters Creek (0302010305)

4) Establish connectivity and gene flow between existing and established populations by either translocating individuals or removal of barriers.
5) Re-establish historical populations of Dwarf Wedgemussel after habitat threats have been reduced.

<u>Research Needs</u>

1) Monitor Dwarf Wedgemussel populations every 2-5 years to assess survival, abundance, population structure, recruitment, and genetic diversity.
2) Develop captive propagation techniques to maximize yield, genetic diversity, and post release survival.
3) Determine locations for establishing Dwarf Wedgemussel populations and monitor the success of population establishment.
4) Determine the genetic diversity and number of genetically distinct populations of Dwarf Wedgemussel throughout its range
5) Develop microsatellite markers or similar genetic tagging techniques to determine age structure, parentage, and hatchery contribution to wild stock.
6) Monitor host fish abundance, population structure, and recruitment.
7) Develop techniques to reduce the abundance of Asian Clam.
8) Determine the known historical range of Dwarf Wedgemussel by verifying the identification of specimens held in museum collections.
9) Determine the impact of Flathead Catfish on Dwarf Wedgemussel host fish populations.

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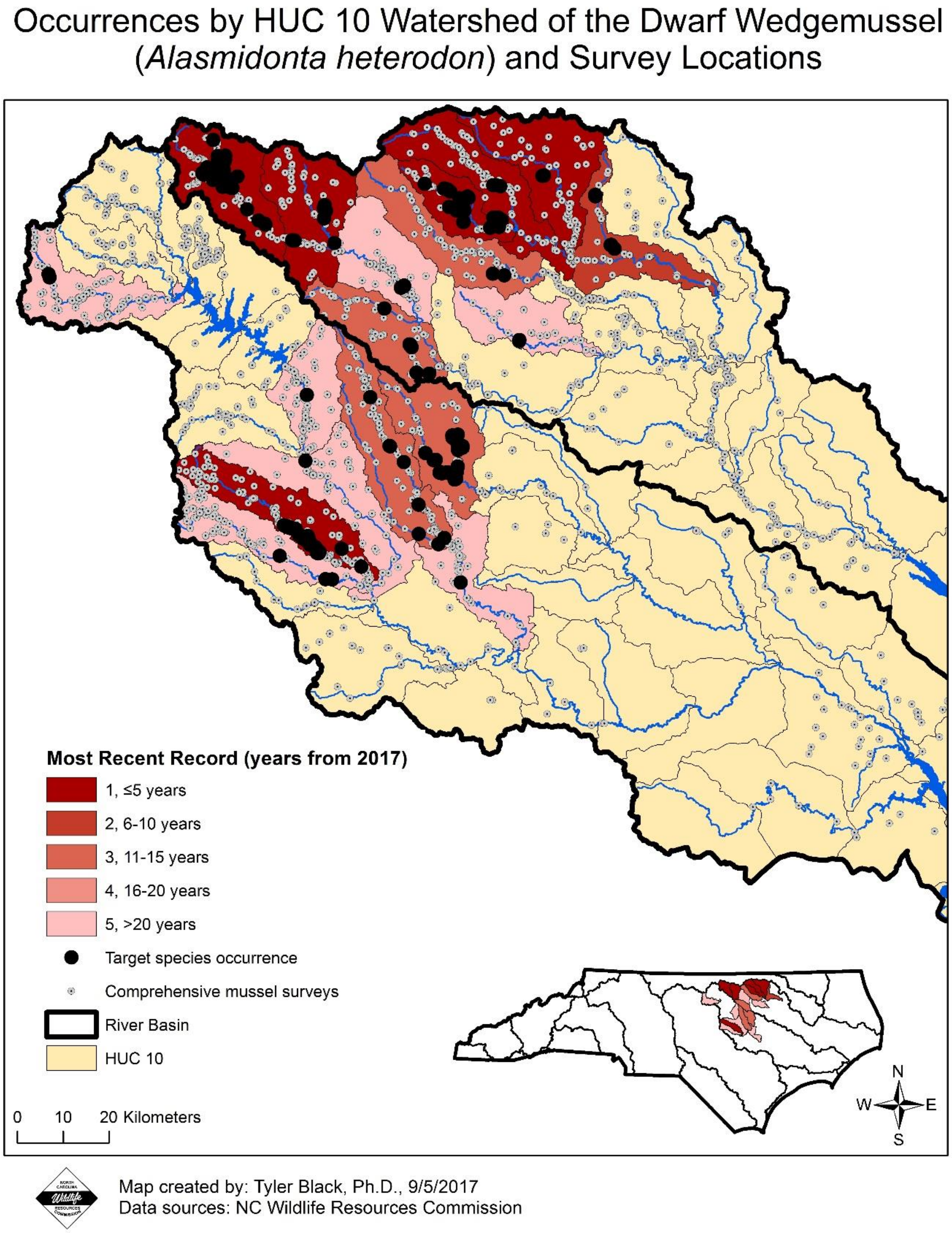

Figure 1. Distribution map of Dwarf Wedgemussel (*Alasmidonta heterodon*) within the Neuse and Tar-Pamlico River basins depicting 10-digit hydrologic units (colored and categorized based on year of observation), collection locations (black dots), and survey locations (gray dots).

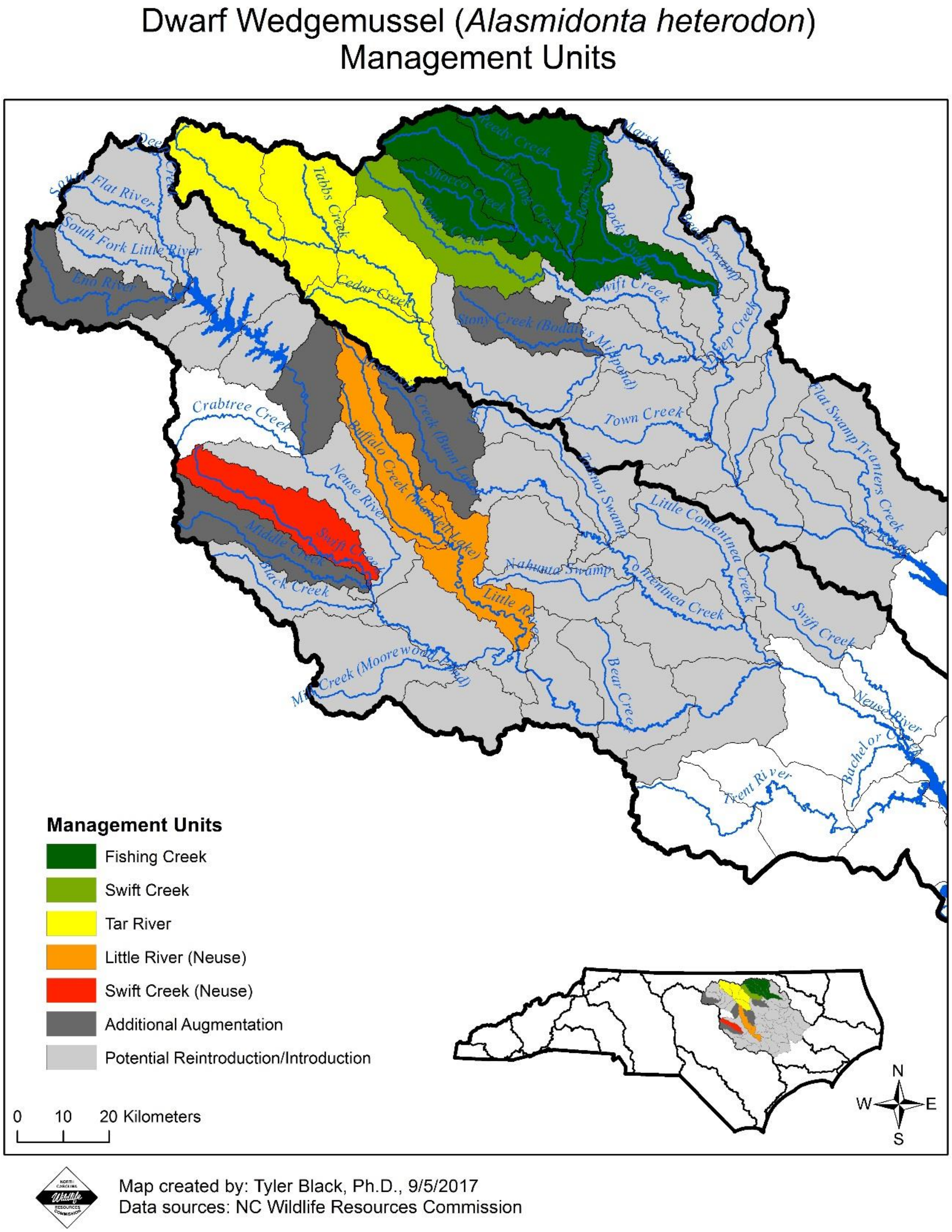

Figure 2. Management units of Dwarf Wedgemussel (*Alasmidonta heterodon*) within the Neuse and Tar-Pamlico River basins depicting 10-digit hydrologic units (colored based management units and future management scenarios).

Yellow Lance (*Elliptio lanceolata*)

Biological Information

Description and Taxonomic Classification

The Yellow Lance (*Elliptio lanceolata* (Lea 1828)) is a state endangered and federally threatened freshwater mussel that is restricted to the Neuse and Tar-Pamlico River basins in North Carolina. It has a bright yellow elongate shell that is over twice as long as it is tall and usually not more than 86 mm in length (Bogan 2017). Its periostracum has a smooth and waxy appearance with brownish growth rests, and it rarely ever has rays (Alderman 2003). The posterior ridge is distinctly rounded and curves dorsally toward the posterior end (Lea 1828, Bogan 2017). The lateral teeth are long and thin, with two in the left valve and one in the right valve; each valve has two pseudocardinal teeth with the posterior one on the left valve and the anterior one on the right valve being vestigial (Lea 1828, Kendig 2014). The Yellow Lance was originally described as *Unio lanceolatus* in 1828 by Isaac Lea. For many years, the Yellow Lance was recognized as part of the "lanceolate *Elliptio*" species-complex that incorporated 25 species (Johnson 1970). However, in 2009, Bogan et al. identified *Elliptio lanceolata* as described by Lea to be a distinct species, but its placement in the genus *Elliptio* remains questionable.

Taxonomic Hierarchy (Integrated Taxonomic Information System 2017):

Kingdom:	Animalia
Phylum:	Mollusca
Class:	Bivalvia
Order:	Unionoida
Family:	Unionidae
Genus:	*Elliptio*
Species:	*Elliptio lanceolata*

Distribution and Population Status

Yellow Lance has a historical range of the Patuxent River basin in Maryland; possibly the Potomac River basin in Maryland and Virginia; the Rappahannock, York, James, and Cowan River basins in Virginia; and the Tar-Pamlico and Neuse River basins in North Carolina (Figure 3; USFWS 2018). A range wide Species Status Assessment Report was recently completed by the U.S. Fish and Wildlife Service and provides a comprehensive review of the species (USFWS 2018). Historically, the distribution of Yellow Lance in North Carolina appeared widespread within the two basins. In the Neuse River basin, it historically occurred in Swift Creek, Mill Creek, Middle Creek, and the Little River. In the Tar-Pamlico River basin, occurrence records exist in Swift Creek, Richneck Creek, Fishing Creek, Sandy Creek, Tabbs Creek, Shocco Creek, Crooked Creek, Fox Creek, and the Tar River proper. Given the distribution of Yellow Lance it is presumed that it historically occurred within the Roanoke and Chowan River basins in North Carolina; however, there are no verified records.

To date, Yellow Lance have been collected within 17 watersheds (i.e., 10-digit hydrologic units) in North Carolina (Figure 3). Within the past decade (2008 – 2017), Yellow Lance have been collected from 2 of 5 watersheds (40%) and 7 of 12 watersheds (58%) within the Neuse and Tar-Pamlico River basins, respectively. The range and number of sites that Yellow Lance has been found in recent years has been decreasing. However, this species seems to be locally abundant in a few locations, as NCWRC biologists found 53 Yellow Lance in 10 person-hours at a new site in Swift Creek (Tar-Pamlico River basin) in 2016. The Tar-Pamlico River basin holds the best known remaining populations of Yellow Lance, with the Swift Creek sub-basin being the primary stronghold of the species. During recent surveys, two locations in the Tar River proper were documented to harbor Yellow Lance; however, given the cryptic nature of this species, its proclivity for burying deep into the substrate, and the large size and depth of the mainstem Tar River, it is possible that other locations and populations in the Tar River have yet to be discovered. Yellow Lance has been found at only two sites in Fishing Creek in the past 10 years, and it appears that the habitat at one of the sites has degraded in recent years and may no longer be suitable for this mussel to persist. Thus, only one remaining known site is left in Fishing Creek that can serve as a broodstock collection location. With no healthy populations from which to collect broodstock, the Yellow Lance populations in the Neuse River basin are in far worse shape than the populations in the Tar-Pamlico River basin. While there have been several observations in Swift Creek within the past 10 years and as recently as 2015, every observation found only one or two individuals during the survey. There have been recent (2014-2016) intensive surveys in the Swift Creek watershed, and only one Yellow Lance has been observed. Available habitat in Swift Creek has been observed to continually decline over the past 10 years, and with the impending construction of the I-540 Outer Loop Southeast Extension and continued development and urbanization within the Swift Creek sub-basin, the persistence of Yellow Lance within Swift Creek appears bleak. There appears to be more available habitat in the Little River sub-basin; however, there has not been a Yellow Lance observation in this sub-basin since 2009.

Yellow Lance is listed as endangered (soon to be changed to threatened) in the state of North Carolina and on May 3, 2018, the U.S. Fish and Wildlife Service made a final ruling that the Yellow Lance be listed as a threatened species with protection provided by the Endangered Species Act of 1973.

Habitat and Life History

Habitat use of Yellow Lance: Yellow Lance is often found in stable, clean, coarse- to medium-sized sandy substrate, although it has also been found in gravel substrates and migrating with shifty sands (Alderman 2003). This species is highly mobile and has been shown to migrate up to 15 m upstream in sandy substrates (NCWRC unpublished data). Due to its high mobility, Yellow Lance will often be found within a few inches of exposed substrate, migrating towards the thalweg when the water level drops. This mussel can often be found on the downstream end of stable sand and gravel bars, sometimes buried up to six inches in the substrate. Clean flowing water with high dissolved oxygen and minimal nutrient loading is important for the survival of Yellow Lance (USFWS 2018).

Diet of Yellow Lance: Yellow Lance is a filter feeder that feeds on a variety of particulate matter suspended in the water column including algae, phytoplankton, zooplankton, bacteria, detritus, and dissolved organic matter (Haag 2012). Juveniles pedal feed by using the cilia on their foot to gather particulate matter from the substrate. It has been shown that the addition of the probiotic bacteria *Bacillus subtilis* enhances early juvenile growth and survival (Eads and Levine 2012).

Reproduction of Yellow Lance: Similar to most freshwater mussels, Yellow Lance has a complex life cycle that requires the use of a fish host to successfully reproduce. Freshwater mussels are dioecious with sexually mature males releasing large quantities of sperm into the water column to begin the reproductive life cycle. For fertilization to occur, sperm must pass into the incurrent apertures of sexually mature females. The sperm travel through the aperture while the mussel is filter feeding and fertilize eggs in the suprabranchial chamber. The fertilized eggs are then transferred into the gill chambers, which form a modified brood pouch called the marsupium. While in the marsupium, the fertilized eggs quickly mature into the larval form known as glochidia, and this process usually requires 2-6 weeks for maturation (Haag 2012). Yellow Lance is a short-term brooder (tachytictic) which means that when the eggs develop into mature glochidia they are released shortly thereafter into the water column to attach onto the gills of an appropriate fish host where the glochidia metamorphose from larvae to free-living mussel occurs. In a hatchery setting, female Yellow Lance have been observed to become gravid multiple times in one spawning season and release between 2-3 broods from April-July in North Carolina (Eads and Levine 2009). Glochidia remain on the host fish for a period of 7-17 days, during this time they receive nutrients from the fish blood and develop its internal organs such as a foot, digestive tract, and gills, as well as forming two adductor muscles (Haag 2012). Once the glochidia complete their metamorphosis they excyst from the gills of the host fish and settle into the substrate to live as a juvenile freshwater mussel.

Fish Host Trials for Yellow Lance: To date, 26 fish species across 8 families have been exposed to Yellow Lance glochidia (Eads and Levine 2009).

<u>Effective Hosts:</u> *Luxilus albeolus* (White Shiner), *Lythrurus matutinus* (Pinewoods Shiner)

<u>Poor Hosts:</u> *Anguilla rostrata* (American Eel), *Catostomus commersonii* (White Sucker), *Etheostoma vitreum* (Glassy Darter), *Fundulus rathbuni* (Speckled Killifish). *Lepomis cyanellus* (Green Sunfish), *Lepomis macrochirus* (Bluegill), *Micropterus salmoides* (Largemouth Bass), *Nocomis leptocephalus* (Bluehead Chub), *Notropis procne* (Swallowtail Shiner), *Noturus insignis* (Margined Madtom), *Percina roanoka* (Roanoke Darter), *Semotilus atromaculatus* (Creek Chub)

<u>Ineffective Hosts:</u> *Ambloplites cavifrons* (Roanoke Bass), *Ameiurus platycephalus* (Flat Bullhead), *Aphredoderus sayanus* (Pirate Perch), *Cyprinella analostana* (Satinfin Shiner), *Enneacanthus gloriosus* (Bluespotted Sunfish), *Erimyzon oblongus* (Creek Chubsucker), *Etheostoma nigrum* (Johnny Darter), *Hypentelium nigricans* (Northern Hogsucker), *Lepomis auritus* (Redbreast Sunfish), *Notropis hudsonius* (Spottail Shiner), *Noturus furiosus* (Carolina Madtom), *Percina nevisense* (Chainback Darter)

Glochidia of Yellow Lance: Yellow Lance glochidia are small, rounded, and hookless, and they measure approximately 200 µm in length and 190 µm in height (Eads and Levine 2009). Broods are released as clumps of mucus and glochidia that stick to each other and ball up at the bottom of an aquarium in a laboratory setting. However, it is possible that in the wild, the glochidia release is more string-like and floats in the water column, resulting in it being targeted as food by minnows (USFWS 2018, C. Eads personal communication). Fecundity for wild Yellow Lance is typically 4,000-15,000 glochidia; however, when held in a hatchery setting, fecundity is increased to 20,000-56,000 glochidia.

Conservation Management

Historical Conservation Efforts

Prior to 2009, NCWRC biologists conducted general mussel surveys in the Neuse and Tar-Pamlico River basins in North Carolina to document the distribution of Yellow Lance throughout its range. In 2009, the NCWRC partnered with North Carolina State University (NCSU) to conduct targeted surveys, perform fish host trials, and develop captive propagation techniques for Yellow Lance. Refinement of captive propagation techniques continued in subsequent years, including the development of *in vitro* propagation methods to successfully transform Yellow Lance without using a fish host. The Marion Conservation Aquaculture Center (MCAC), located at the NCWRC's Marion State Fish Hatchery in McDowell County, NC was established in 2008. The objective of the MCAC is to preclude listing, promote delisting, and prevent the extinction of aquatic species when appropriate by using captive propagation and arking. In 2015, NCWRC biologists conducted an experimental release of 270 propagated Yellow Lance split between two sites in Sandy Creek, a tributary of the Tar River. Biologists were evaluating habitat suitability, detection, growth, and survival of the released mussels in an effort to gain information that will guide future augmentation efforts throughout its range. While exhibiting good growth and survival, annual monitoring surveys of the released mussels have also demonstrated that the propagated mussels will become gravid in the wild. NCWRC again partnered with NCSU in 2015 to collect additional broodstock and propagate Yellow Lance from the Tar-Pamlico River basin, identify future augmentation areas, and evaluate the suitability of several ponds to serve as grow-out locations for Yellow Lance. From 2016-2017, NCWRC biologists conducted targeted surveys for Yellow Lance, resurveying the locations from 2009 and adding several more survey locations throughout its range to update the current species distribution.

Threats

As with all aquatic species, there are many natural and anthropogenic factors that threaten the long-term viability of Yellow Lance. Extinction and decline of North American unionid bivalves can be traced to impoundment and inundation of riffle habitat throughout the United States. The loss of obligate hosts, coupled with increased siltation, and various types of industrial and domestic pollution have resulted in the rapid decline of the unionid bivalve fauna in North America (Bogan 1993, NCWRC 2015). Dams, both manmade and natural (created by beavers, see Kemp et al. 2012), are a barrier to dispersal of host fish and attached glochidia. Throughout the Neuse and Tar-Pamlico River basins, beavers have continued to build dams and impound an increasing number of river kilometers. Beaver dams not only inundate and alter riffle/run mussel habitat upstream of the dam but also effect mussel populations downstream of the dam by increasing fluctuations in flow regime, decreasing dissolved oxygen levels, and increasing the variability of food quality and quantity (Hoch 2012, Kemp et al. 2012). Contaminants and water pollution are a significant threat to all aquatic species, especially mussels. Point source discharges from municipal wastewater that contains monochloramine and unionized ammonia compounds are acutely toxic to freshwater mussels and may be responsible for glochidial mortality that results in local extirpation of mussels (Goudreau et al. 1993, Gangloff et al. 2009, NCWRC 2015). Impervious areas in urbanized watersheds contribute to high water levels, even during short rainfall events, which can result in flash flooding. These high or flashy flow events contribute to increased sediment loads, turbidity throughout the water column, and stream bed movements that stress mussel populations (Gangloff et al. 2009, NCWRC 2015). Climate change and development will likely bring additional stressors that need to be evaluated for mussels. Furthermore, specific pollutants that may be introduced into the aquatic environment, the interactions of pollutants and temperature (from climate change), salinity (related to sea level rise), and lower dilution (from altered flows) will need to be considered (NCWRC 2015). In addition, invasive species such as the Asian Clam (*Corbicula fluminea*), the Flathead Catfish (*Pylodictis olivaris*), and Hydrilla (*Hydrilla verticillata*) can create competitive pressures on food resources and habitat availability. These factors can decrease oxygen availability, cause ammonia spikes, alter benthic substrates, impact host fish communities, reduce stream flow, and increase sediment buildup (Belanger et al. 1991, Scheller 1997, NCANSMPC 2015, NCWRC 2015).

<u>Conservation Goal</u>

To prevent the extinction of Yellow Lance and promote population viability (i.e., multiple age classes and wild recruitment) within North Carolina for the next 100 years.

<u>Conservation Objectives</u>

The overarching conservation strategy is to promote habitat protection and maintain the best populations of Yellow Lance in the Tar-Pamlico River basin and focus efforts within the Neuse River basin on Swift Creek and Little River. Restoration of habitat should be promoted for hydrologic units listed under Objective 1 and should primarily focus on the protection of riparian habitat and associated uplands.

1) Promote habitat protection and maintain for two populations of Yellow Lance in the Neuse River basin and three populations in the Tar-Pamlico River basin (Figure 4). Management Units (MUs) are defined based on hydrologic units (i.e., HUC10s).
    a. **Neuse River Basin**
        i. Little River MU (0302020115, 0302020116)
        ii. Swift Creek MU (0302020110)
    b. **Tar-Pamlico River Basin**
        i. Fishing Creek MU (0302010201, 0302010203, 0302010205, 0302010206)
        ii. Swift Creek MU (0302010107, 0302010108)
        iii. Tar River MU (0302010102, 0302010103, 0302010104, 0302010106, 0302010109, 0302010302)
2) Maintain an ark population of Yellow Lance from Neuse and Tar-Pamlico River basin broodstock.
3) Utilize captive propagation and/or translocations to augment or establish subpopulations of Yellow Lance where appropriate habitat exists (pending approval from the Habitat, Nongame and Endangered Species Committee). To reduce the

potential to minimize the regulatory burden associated with the federal Endangered Species Act, a tool such as Safe Harbor will be established prior to reintroduction into an unoccupied area.

   a. All Neuse and Tar-Pamlico River basin MU hydrologic units listed above.
   b. Additional augmentation areas within the known range of Yellow Lance (Figure 4), if propagation efforts exceed MU needs.
      i. **Neuse River Basin**
         1. Middle Creek (0302020109)
         2. Mill Creek (0302020113)
      ii. **Tar-Pamlico River Basin**
         1. Stony Creek (0302010105)
         2. Tar River (0302010101)
   c. Potential reintroduction or introduction of Yellow Lance (Figure 4) into areas within the presumed historical range, if propagation efforts exceed MU needs. Ideally located in areas with reduced likelihood of anthropogenic threats.
      i. Neuse River basin
         1. Black Creek (0302020112)
         2. Contentnea Creek (0302020301, 0302020304, 0302020307)
         3. Eno River (0302020103)
         4. Flat River (0302020101)
         5. Little River (0302020102)
         6. Neuse River (0302020107, 0302020111, 0302020117, 0302020201, 0302020202, 03020203)
      ii. Tar-Pamlico River basin
         1. Little Fishing Creek (0302010202)
         2. Tar River (0302010304, 0302010306)
         3. Town Creek (0302010301)

4) Establish connectivity and gene flow between existing and established populations by either translocating individuals or removal of barriers.
5) Reestablish historical populations of Yellow Lance after habitat threats have been reduced.

Research Needs

1) Monitor Yellow Lance populations every 2-5 years to assess survival, abundance, population structure, recruitment, and genetic diversity.
2) Conduct Yellow Lance focused surveys within the Roanoke and Chowan River basins to assess presence or absence of the species.
3) Develop captive propagation techniques to maximize yield, genetic diversity, and post release survival.
4) Determine locations for establishing Yellow Lance populations and monitor the success of population establishment.
5) Determine the genetic diversity and number of genetically distinct populations of Yellow Lance throughout its range.
6) Develop microsatellite markers or similar genetic tagging techniques to determine age structure, parentage, and hatchery contribution to wild stock.
7) Monitor host fish abundance, population structure, and recruitment.

8) Develop techniques to reduce the abundance of Asian Clam.
9) Determine the known historical range of Yellow Lance by verifying the identification of specimens held in museum collections.
10) Determine the impact of Flathead Catfish on Yellow Lance host fish populations.

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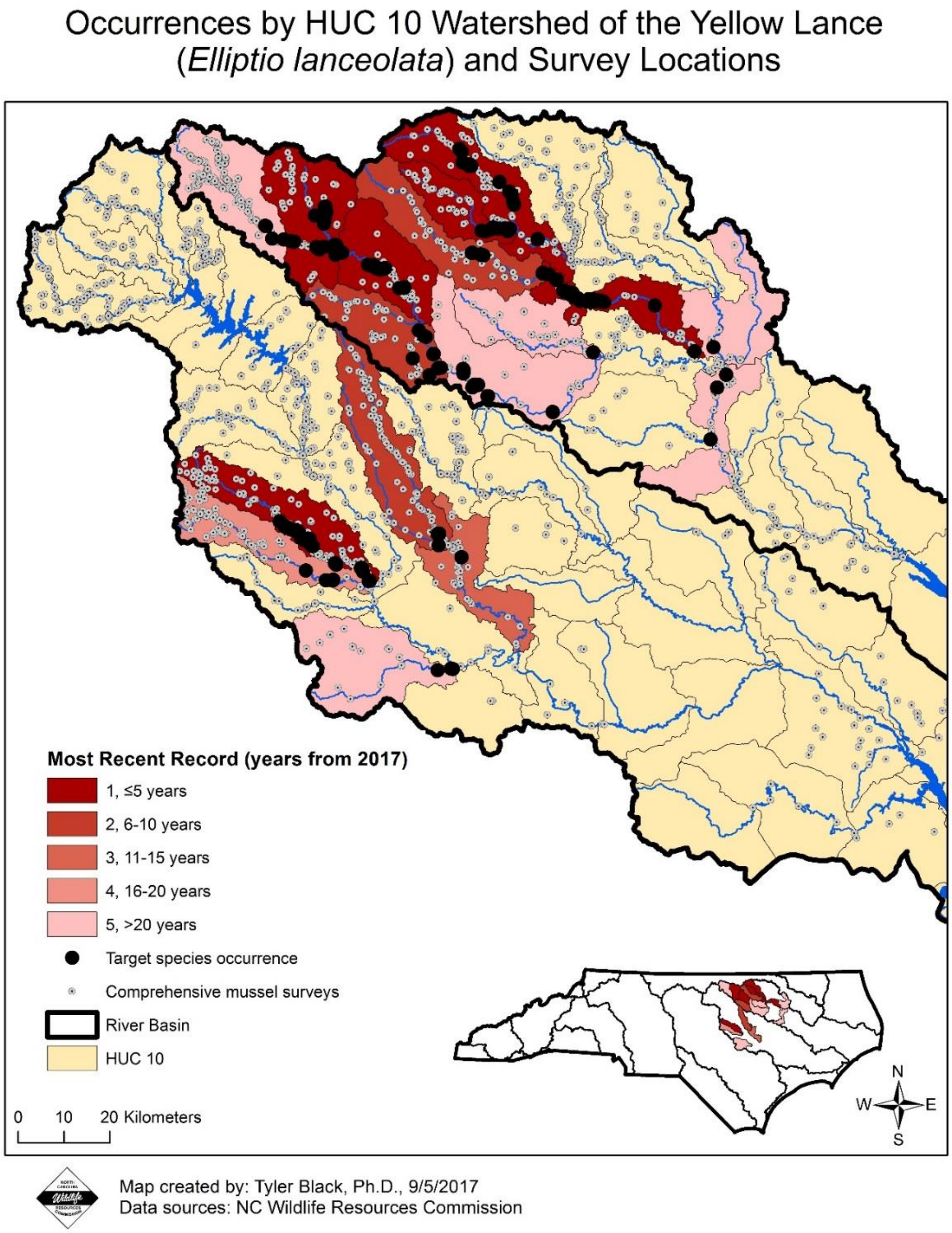

Figure 3. Distribution map of the Yellow Lance (*Elliptio lanceolata*) within the Neuse and Tar-Pamlico River basins depicting 10-digit hydrologic units (colored and categorized based on year of observation), collection locations (black dots), and survey locations (gray dots).

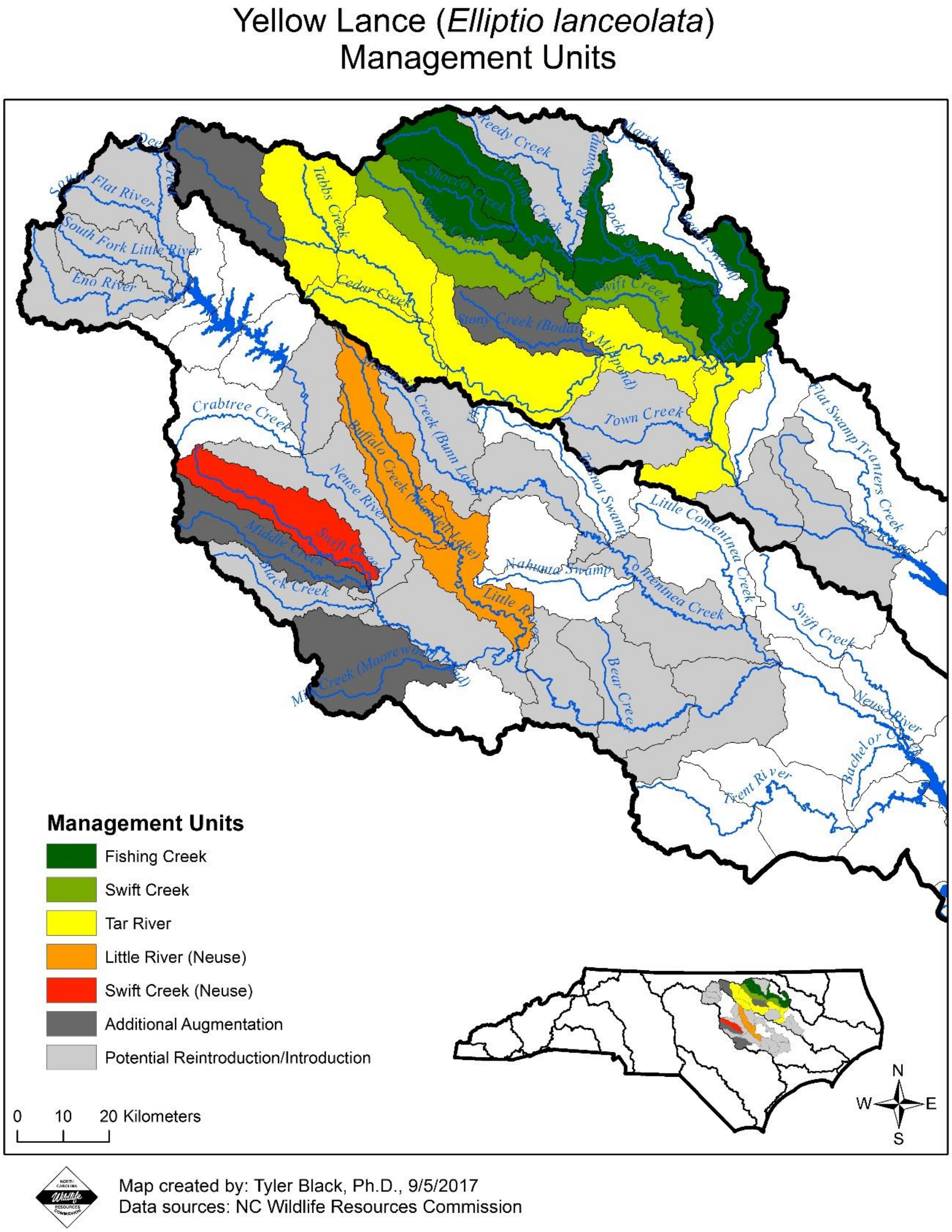

Figure 4. Management units of the Yellow Lance (*Elliptio lanceolata*) within the Neuse and Tar-Pamlico River basins depicting 10-digit hydrologic units (colored based management units and future management scenarios).

Tar River Spinymussel (*Parvaspina steinstansana*)

Biological Information

Description and Taxonomic Classification

The Tar River Spinymussel (*Parvaspina steinstansana* Johnson and Clarke 1983) is a state and federally endangered freshwater mussel that is restricted to the Neuse and Tar-Pamlico River basins of North Carolina. It is a small to medium sized mussel with adults typically ranging between 30-50 mm in length; however, individuals reaching up to 60 mm have been documented. The Tar River Spinymussel is one of three freshwater mussel species in North America that are characterized by the presence of spines. Short spines (up to 5 mm in length) are found on most young specimens (Bogan 2017). As many as 12 spines have been found on juveniles; however, adults tend to lose some or all their spines as they mature (Bogan 2017). On the nacre, fine iridescent lines radiate from where the spines originate, helping to identify shells that have lost their spines (Kendig 2014). The left valve contains two triangular pseudocardinal teeth. The right valve has two parallel pseudocardinals, one triangular and serrate (posterior) and one low and vestigial (anterior) (Johnson and Clarke 1983). The umbo is slightly elevated above the hinge line and more centrally located than that of *Elliptio* species, which sometimes exhibit a similar shell shape (Kendig 2014). The periostracum is smooth orange-brown and can be covered with greenish rays when young, becoming darker or blackish brown, and the rays can become inconspicuous in adult mussels (Johnson and Clarke 1983). These mussels appear to have extensive wear and erosion around the umbo because they are older than their small size would suggest (Kendig 2014).

This species has been informally cited as "spiny naiad" by Shelly (1972), "*Canthyria sp.*" by Fuller (1977) and the "Tar River spiny mussel (*Canthyria sp.*)" by Biggins (1982). It was first formally described by Johnson and Clarke (1983) as *Elliptio* (*Canthyria*) *steinstansana*. The reasons for placement in the genus *Elliptio*, with *Canthyria* as a subgenus, are described by Clarke (1983; Section 3.4). A recent study examining the molecular systematics of the North American spinymussels concludes that *Elliptio steinstansana* and *Pleurobema collina* (James Spinymussel) form a monophyletic clade that is distinct from both *Elliptio* and *Pleurobema,* and a new genus (*Parvaspina* gen. nov.) is described to reflect this relationship (Perkins et al. 2017). **Etymology:** *steinstansana*, referring to the honorary naming of the Tar River Spinymussel after Dr. Carol B. Stein and Dr. David H. Stansbery, who discovered the species in the Ohio State Museum of Natural History in 1964 and ownership of a specimen that was used in Shelly (1972) figures, respectively (Johnson and Clarke 1983).

Taxonomic Hierarchy (Integrated Taxonomic Information System 2017; Perkins et al. 2017):

Kingdom:	Animalia
Phylum:	Mollusca
Class:	Bivalvia
Order:	Unionoida
Family:	Unionidae
Genus:	*Parvaspina* (Elliptio)
Species:	*Parvaspina* (Elliptio) *steinstansana*

Distribution and Population Status

The Tar River Spinymussel has a historical range that is restricted to the Neuse and Tar-Pamlico River basins in North Carolina. To date, Tar River Spinymussel have been collected within 14 watersheds (i.e., 10-digit hydrologic units) in North Carolina (Figure 5). Within the past decade (2008 – 2017), Tar River Spinymussel have been collected from 2 of 3 watersheds (67%) and 3 of 11 watersheds (27%) within the Neuse and Tar-Pamlico River basins, respectively. It is probable that the Tar River Spinymussel may have once occurred throughout much of the Tar-Pamlico River basin prior to settlement of the area during the 1700s (USFWS 1992). In the Tar-Pamlico River basin, occurrence records exist in Chicod Creek, Fishing Creek, Little Fishing Creek, Sandy Creek, Swift Creek, Shocco Creek, and the Tar River. In the Neuse River basin, it has been collected in the Little and Neuse rivers; however, historically it likely inhabited many waterways throughout the basin. Monitoring and other surveys for Tar River Spinymussel have documented a continued decline in nearly all the surviving populations of the species. For example, a robust population of Tar River Spinymussel in Swift Creek (Tar-Pamlico River basin) experienced a substantial mussel kill due to a chemical spill in 1990 (Fleming et al 1995). Although limited levels of reproduction and recruitment may be occurring within the Little Fishing Creek/Fishing Creek and Little River populations, the amount of recruitment occurring does not appear to be at levels high enough to maintain these populations (USFWS 2014). All surviving populations are small to extremely small in number and restricted in range, and based on the most recent survey data within each river system, each of the surviving populations appears to be isolated from the other populations in the same river system by impoundments and/or extensive unoccupied stream reaches (USFWS 2014).

The Tar River Spinymussel is listed as endangered in the state of North Carolina, and on July 29, 1985, the U.S. Fish and Wildlife Service made a final ruling that the Tar River Spinymussel be listed as an endangered species with protection provided by the Endangered Species Act of 1973.

Habitat and Life History

Habitat use of Tar River Spinymussel*:* Tar River Spinymussel is often found in relatively fast-flowing, well-oxygenated waters with a circumneutral pH. The substrate is usually comprised of silt free, clean, stable, gravel/coarse sand substrate (Alderman 1988). Many individuals have been found in a small, stable seam of habitat where the substrate transitions from cobble/pebble to sand/gravel.

Diet of Tar River Spinymussel*:* The Tar River Spinymussel is a filter feeder that feeds on a variety of particulate matter suspended in the water column, including algae, phytoplankton, zooplankton, bacteria, detritus, and dissolved organic matter (Haag 2012). Juveniles pedal feed by using the cilia on their foot to gather particulate matter from the substrate.

Reproduction of Tar River Spinymussel*:* Similar to most freshwater mussels, the Tar River Spinymussel has a complex life cycle that requires the use of a fish host to successfully reproduce. Freshwater mussels are dioecious, and sexually mature males release large quantities of sperm into the water column to begin the reproductive life cycle. For fertilization to occur, sperm must pass into the incurrent apertures of sexually mature females. The sperm travel through the aperture while the mussel is filter feeding and fertilize eggs in the suprabranchial chamber. The fertilized eggs are then transferred into the gill chambers, which form a modified brood pouch called the marsupium. While in the marsupium, the fertilized eggs quickly mature into the larval form known as glochidia, and this process usually requires 2-6 weeks for maturation (Haag 2012). The Tar River Spinymussel is a short-term brooder (tachytictic) which means that when the eggs develop into mature glochidia they are released shortly thereafter into the water column to attach onto the gills of an appropriate fish host where the glochidia metamorphose from larvae to free-living mussel. In a hatchery setting, female Tar River Spinymussel have been observed to become gravid multiple times in one spawning season and are known to release up to 5 broods between late-March and early-August (Eads and Levine 2009, R. Hoch personal communication). Glochidia remain on the host fish for a period of 27-39 days, during this time they receive nutrients from the fish blood and develop its internal organs such as a foot, digestive tract, and gills, as well as forming two adductor muscles (Eads and Levine 2008, Haag 2012). Once the glochidia complete their metamorphosis they excyst from the gills of the host fish and settle into the substrate to live as a juvenile freshwater mussel.

Fish Host Trials for Tar River Spinymussel: To date, 18 fish species across 7 families have been exposed to Tar River Spinymussel glochidia (Eads and Levine 2008, Eads and Levine 2009, Levine et al. 2011, Eads and Levine 2015).

<u>Effective Hosts:</u> *Luxilus albeolus* (White Shiner), *Lythrurus matutinus* (Pinewoods Shiner), *Nocomis leptocephalus* (Bluehead Chub)

<u>Poor Host:</u> *Cyprinella analostana* (Satinfin Shiner), *Notemigonus crysoleucas* (Golden Shiner), *Notropis procne* (Swallowtail Shiner), *Pimephales promelas* (Fathead Minnow), *Semotilus atromaculatus* (Creek Chub)

<u>Ineffective Hosts:</u> *Anguilla rostrata* (American Eel), *Enneacanthus gloriosus* (Bluespotted Sunfish), *Erimyzon oblongus* (Creek Chubsucker), *Esox americanus* (Chain Pickerel), *Etheostoma olmstedi* (Tessellated Darter), *Etheostoma vitreum* (Glassy Darter), *Lepomis auritus* (Redbreast Sunfish), *Moxostoma cervinum* (Blacktip Jumprock), *Noturus furiosus* (Carolina Madtom), *Percina roanoka* (Roanoke Darter)

Glochidia of Tar River Spinymussel: Tar River Spinymussel glochidia are very small (170 µm wide), hookless, and relatively spherical, which causes them to naturally lay with their hinge down (Eads and Levine 2008). The glochidia are packaged in a single row along the margin of a ribbon-like, flat conglutinate that is 5-7 mm long (Eads and Levine 2008). The only gravid females found in the wild had a very low percentage of the brood fertilized, less than 8%. However, when held in a hatchery setting, the percent of brood fertilized can regularly exceed 90%, with a typical fecundity of 3,000-10,000 glochidia (Eads and Levine 2014).

Conservation Management

Historical Conservation Efforts

The first targeted surveys for Tar River Spinymussel were conducted in 1983 when Arthur Clarke surveyed throughout the Neuse, Tar, and Roanoke River basins (Clarke 1983). Since the late 1980s, NCWRC and USFWS biologists conducted both targeted surveys for Tar River Spinymussel and general mussel surveys throughout its range. From 2007 – present, NCWRC and USFWS partnered with North Carolina State University to conduct a series of experiments investigating the life history of Tar River Spinymussel. Some of the research objectives completed were finding gravid females in the wild, collecting individuals for broodstock to begin arking a population at a NCWRC fish hatchery, identifying effective fish hosts, investigating life history characteristics and spawning periods, refining captive propagation and culture techniques, evaluating creeks for future augmentation through *in situ* monitoring of caged juveniles, and identifying appropriate habitats for future augmentations (Eads and Levine 2008, Eads and Levine 2009, Levine et al. 2011, Eads and Levine 2014, Eads and Levine 2015). The Marion Conservation Aquaculture Center (MCAC), located at the NCWRC's Marion State Fish Hatchery in McDowell County, NC was established in 2008. The objective of the MCAC is to preclude listing, promote delisting, and prevent the extinction of aquatic species when appropriate by using captive propagation and arking. Between December 2014 and September 2016, NCWRC, in partnership with the USFWS and others released over 9,500 propagated Tar River Spinymussel at four locations in Fishing Creek and Little Fishing Creek (Tar-Pamlico River basin). To evaluate the success of the initial augmentations, 1,310 Tar River Spinymussel, were individually tagged, measured, and released into an experimental reach of Little Fishing Creek from December 2014 to October of 2015. In August 2015 and August 2016, a two-pass snorkel survey was conducted in the experimental stocking reach and 35% (2015) and 20% (2016) of the released mussels were recaptured as live individuals. Mean growth of recaptured individuals was 1.04 mm (SD=0.7 mm). Preliminary results suggest that stocking propagated individuals of Tar River Spinymussel into the best available habitat has the potential to bolster dwindling populations and assist in the recovery of this species.

Threats

As with all aquatic species, there are many natural and anthropogenic factors that threaten the long-term viability of Tar River Spinymussel. Extinction and decline of North American unionid bivalves can be traced to impoundment and inundation of riffle habitat throughout the United States. The loss of obligate hosts, coupled with increased siltation, and various types of industrial and domestic pollution have resulted in the rapid decline of the unionid bivalve fauna in North America (Bogan 1993, NCWRC 2015). Dams, both manmade and natural (created by beavers, see Kemp et al. 2012), are a barrier to dispersal of host fish and attached glochidia. Throughout the

Neuse and Tar-Pamlico River basins, beavers have continued to build dams and impound an increasing number of river kilometers. Beaver dams not only inundate and alter riffle/run mussel habitat upstream of the dam but also effect mussel populations downstream of the dam by increasing fluctuations in flow regime, decreasing dissolved oxygen levels, and increasing the variability of food quality and quantity (Hoch 2012, Kemp et al. 2012). Contaminants and water pollution are a significant threat to all aquatic species, especially mussels. Point source discharges from municipal wastewater that contains monochloramine and unionized ammonia compounds are acutely toxic to freshwater mussels and may be responsible for glochidial mortality that results in local extirpation of mussels (Goudreau et al. 1993, Gangloff et al. 2009, NCWRC 2015). Impervious areas in urbanized watersheds contribute to high water levels, even during short rainfall events, which can result in flash flooding. These high or flashy flow events contribute to increased sediment loads, turbidity throughout the water column, and stream bed movements that stress mussel populations (Gangloff et al. 2009, NCWRC 2015). Climate change and development will likely bring additional stressors that need to be evaluated for mussels. Furthermore, specific pollutants that may be introduced into the aquatic environment, the interactions of pollutants and temperature (from climate change), salinity (related to sea level rise), and lower dilution (from altered flows) will need to be considered (NCWRC 2015). In addition, invasive species such as the Asian Clam (*Corbicula fluminea*), the Flathead Catfish (*Pylodictis olivaris*), and Hydrilla (*Hydrilla verticillata*) can create competitive pressures on food resources and habitat availability. These factors can decrease oxygen availability, cause ammonia spikes, alter benthic substrates, impact host fish communities, reduce stream flow, and increase sediment buildup (Belanger et al. 1991, Scheller 1997, NCANSMPC 2015, NCWRC 2015).

Conservation Goal

To prevent the extinction of Tar River Spinymussel and promote population viability (i.e., multiple age classes and wild recruitment) within North Carolina for the next 100 years.

Conservation Objectives

The overarching conservation strategy is to promote habitat protection and maintain the best populations of Tar River Spinymussel in the Tar-Pamlico River basin and focus all efforts within the Neuse River basin on the Little River. Restoration of habitat should be promoted for hydrologic units listed under Objective 1 and should primarily focus on the protection of riparian habitat and associated uplands.

1) Promote habitat protection and maintain for one population of Tar River Spinymussel in the Neuse River basin and three populations in the Tar-Pamlico River basin (Figure 6). Management Units (MUs) will be defined based on hydrologic units (i.e., HUC10s).
    a. **Neuse River Basin**
        i. Little River MU (0302020115, 0302020116)
    b. **Tar-Pamlico River Basin**
        i. Fishing creek MU (0302010201, 0302010202, 0302010203, 0302010205, 0302010206)
        ii. Swift creek MU (0302010107, 0302010108)
        iii. Tar River MU (0302010103, 0302010104, 0302010106, 0302010109, 0302010302)

2) Maintain an ark population of Tar River Spinymussel from the Neuse and Tar-Pamlico River basin broodstock.
3) Utilize captive propagation and/or translocations to augment or establish subpopulations of Tar River Spinymussel where appropriate habitat exists (pending approval from the Habitat, Nongame and Endangered Species Committee). To reduce the potential to minimize the regulatory burden associated with the federal Endangered Species Act, a tool such as Safe Harbor will be established prior to reintroduction into an unoccupied area.

    a. All Neuse and Tar-Pamlico River basin MU hydrologic units listed above.
    b. Additional augmentation areas within the known range of Tar River Spinymussel (Figure 6), if propagation efforts exceed MU needs.
        i. **Neuse River Basin**
            1. Neuse River (0302020117)
        ii. **Tar-Pamlico River Basin**
            1. Chicod Creek (0302010306)
            2. Tar River (0302010304)
    c. Potential reintroduction or introduction of Tar River Spinymussel (Figure 6) into areas within the presumed historical range, if propagation efforts exceed MU needs. Ideally located in areas with reduced likelihood of anthropogenic threats.
        i. **Neuse River Basin**
            1. Black Creek (0302020112)
            2. Contentnea Creek (0302020301, 0302020302, 0302020304, 0302020307)
            3. Eno River (0302020103)
            4. Flat River (0302020101)
            5. Little River (0302020102)
            6. Middle Creek (0302020109)
            7. Mill Creek (0302020113
            8. Neuse River (0302020107, 0302020111, 0302020201, 0302020202, 03020203)
            9. Swift Creek (0302020110)
        ii. **Tar-Pamlico River Basin**
            1. Stony Creek (0302010105)
            2. Tar River (0302010101, 0302010102)
            3. Town Creek (0302010301)
4) Establish connectivity and gene flow between existing and established populations by either translocating individuals or removing barriers.
5) Reestablish historical populations of Tar River Spinymussel after habitat threats have been reduced.

<u>Research Needs</u>

1) Monitor Tar River Spinymussel populations every 2-5 years to assess survival, abundance, population structure, recruitment, and genetic diversity.
2) Develop captive propagation techniques to maximize yield, genetic diversity, and post release survival.

3) Determine locations for establishing Tar River Spinymussel populations and monitor the success of population establishment.
4) Determine the genetic diversity and number of genetically distinct populations of Tar River Spinymussel throughout its range
5) Develop microsatellite markers or similar genetic tagging techniques to determine age structure, parentage, and hatchery contribution to wild stock.
6) Monitor host fish abundance, population structure, and recruitment.
7) Develop techniques to reduce the abundance of Asian Clam.
8) Determine the known historical range of Tar River Spinymussel by verifying the identification of specimens held in museum collections.
9) Determine the impact of Flathead Catfish on Tar River Spinymussel host fish populations.

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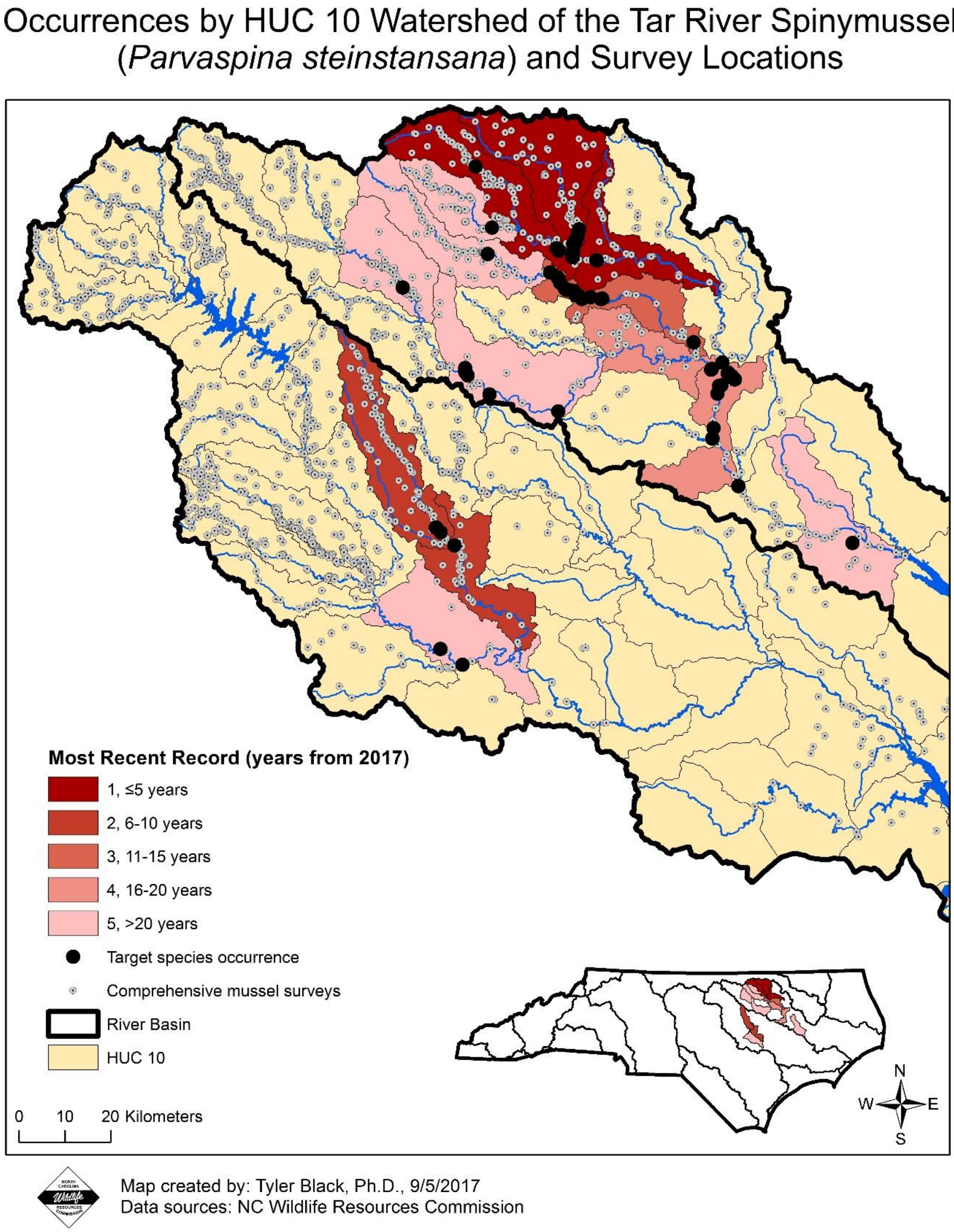

Figure 5. Distribution map of the Tar River Spinymussel (*Parvaspina steinstansana*) within the Neuse and Tar-Pamlico River basins depicting 10-digit hydrologic units (colored and categorized based on year of observation), collection locations (black dots), and survey locations (gray dots).

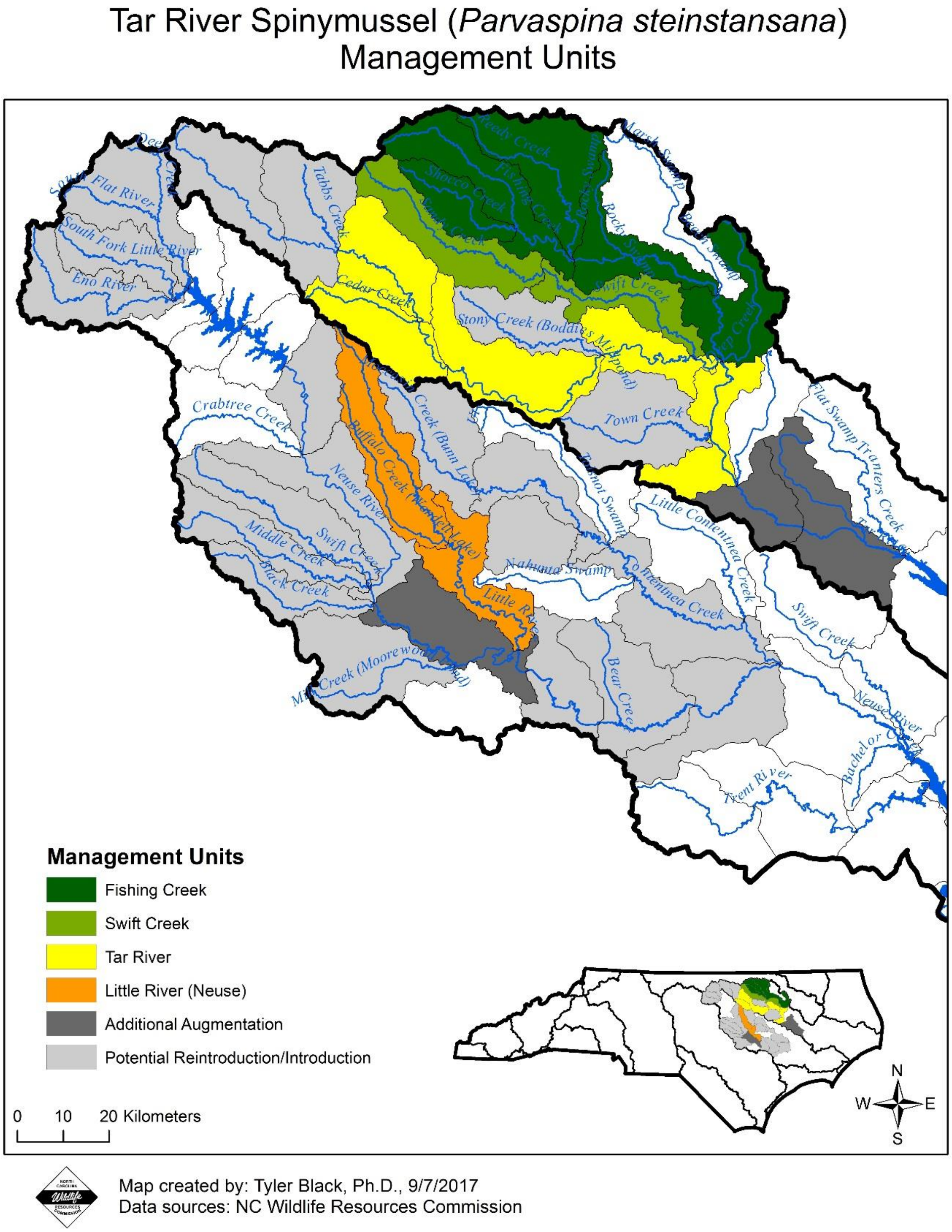

Figure 6. Management units the Tar River Spinymussel (*Parvaspina steinstansana*) within the Neuse and Tar-Pamlico River basins depicting 10-digit hydrologic units (colored based management units and future management scenarios).

Carolina Madtom (*Noturus furiosus*)

Biological Information

<u>Description and Taxonomic Classification</u>

The Carolina Madtom, *Noturus furiosus* (Jordan and Meek 1889) is a small, rare catfish restricted to the Neuse and Tar-Pamlico River basins in North Carolina. Catfishes within the genus *Noturus* are often referred to as "madtoms" and are easily distinguished from other catfishes by an adipose fin that is fused to the body along the entire length. The Carolina Madtom is a member of the subgenus *Rabida*, which includes 15 species that often exhibit boldly marked black and yellow dorsal saddles and curved pectoral spines equipped with prominent, curved serrae. Furthermore, the Carolina Madtom is easily distinguished from other madtom species within the Neuse and Tar-Pamlico River basins because it is the only species to exhibit distinct black saddles (3-4) and curved pectoral spines with large serrae. Adults often range from 36 to 84 mm in length (Burr 1997). **Etymology:** f*uriosus* = "mad" or "raging", referring to the strongly serrate pectoral spines that are armed with a virulent venom (Jordan 1889).

Taxonomic Hierarchy (Integrated Taxonomic Information System 2017):

Kingdom:	Animalia
Phylum:	Chordata
Class:	Actinopterygii
Order:	Siluriformes
Family:	Ictaluridae
Genus:	*Noturus*
Species:	*Noturus furiosus*

<u>Distribution and Population Status</u>

The Carolina Madtom is endemic to the Piedmont and Coastal Plain of the Neuse and Tar-Pamlico River basins in North Carolina (Figure 7). The historical range of the Carolina Madtom included all major and many minor tributaries to the Neuse and Tar-Pamlico River basins (Burr et al. 1989). Within the Neuse River basin, the Trent River sub-basin represents a disjunct population because it is isolated from the Neuse River by brackish water.

Surveys for Carolina Madtom occurred in the 1960s (Bayless and Smith 1962; Smith and Bayless 1964), the 1980s (Burr et al. 1989), and 2007 (Wood and Nichols 2011). Specifically, the North Carolina Wildlife Resources Commission (NCWRC) conducted basin-wide rotenone surveys for fishes in the 1960s and collected Carolina Madtom at 26 of 281 sampling stations. In the 1980s, Burr et al. (1989) surveyed 31 localities within the Neuse and Tar-Pamlico River basins, collected Carolina Madtom at 17 localities, and described the species abundance as rare or uncommon. Wood and Nichols (2011) surveys at 30 sites throughout the range of the Carolina Madtom detected the species at 11 sites.

In 1977, the status of Carolina Madtom was listed as “special concern” by Bailey, although no rationale for this status was given. In 1987, Menhinick evaluated the Carolina Madtom and determined that it warranted no special conservation status because Carolina Madtom were found at 38 sites from 23 different streams. However, Burr (1997) identified the Carolina Madtom *as* “special concern”. Due to limited distribution and presumed declines, Carolina Madtom was up-listed from Special Concern to State Threatened in 2006. Wood and Nichols (2011) found strong evidence for a decrease in the occupied range of Carolina Madtom by examining data from the 1960s, 1980s, and 2007 surveys. They noted a decrease in the frequency of occurrence (FOO; no. of sites Carolina Madtom detected/no. of sites surveyed) from 0.70 in the 1960s to 0.37 in 2007. However, this decrease was exclusively due to declines in the Neuse River basin, where FOO dropped from 0.80 in the 1960s to 0.13 in 2007. FOO in the Tar-Pamlico River drainage remained virtually unchanged (Figure 7; Wood and Nichols 2011). A subset of the sites surveyed in all three studies of the Neuse River basin (Bayless and Smith 1962; Burr et al. 1989; Wood and Nichols 2011) noted the same pattern. Burr et al. (1989) found Carolina Madtom at only 60% of the sites where they had been found in the Neuse River basin by Bayless and Smith (1962). The 2007 surveys revealed that Carolina Madtom were found at only 13% of the sites in the Neuse River basin where they were found by Bayless and Smith (Wood and Nichols 2011). Within the Neuse River basin, the only remaining populations inhabit Contentnea Creek and Little River (Woods and Nichols 2011). The Tar-Pamlico River basin still contains good populations of Carolina Madtom in Fishing Creek, Swift Creek, and the main stem of the Tar River. As previously noted, there was no change in the Tar-Pamlico River basin populations of Carolina Madtom from the 1960s to 2007, indicating stability in this drainage (Wood and Nichols 2011). The North Carolina Wildlife Resources Commission currently classifies Carolina Madtom as threatened. The NC Natural Heritage Program categorizes Carolina Madtom as S2, G2 – Imperiled. The Center for Biological Diversity has filed a petition with the US Fish and Wildlife Service (USFWS) to designate Carolina Madtom as either threatened or endangered (CBD 2010). This resulted in a positive 90-day finding. A range wide Species Status Assessment (SSA) Report was recently completed by the U.S. Fish and Wildlife Service and provides a comprehensive review of the Carolina Madtom (USFWS 2017). The USFWS is now conducting a 12-month finding for this species to determine if it merits listing under the Endangered Species Act of 1973.

Habitat and Life History

Habitat use of Carolina Madtom: Carolina Madtom typically inhabit medium to large streams with moderate flow and sand, gravel, cobble and detritus substrates (Burr et al. 1989; Burr 1997; Midway et al. 2010). Specifically, Midway et al. (2010) found that Carolina Madtom use water depths of 0.1 to 0.19 m, water velocities of 0.10 – 0.24 m/s, and substrates of sand, gravel, and cobble. Cover objects occupied by Carolina Madtom often include cobble, boulder, woody debris, leaf packs, mussel shells, and beverage cans or bottles (Burr et al. 1989; Midway et al. 2010; Wood and Nichols 2011).

Diet of Carolina Madtom: Adult and young Carolina Madtom are nocturnal, benthic insectivores that feed primarily on immature aquatic insects (Burr et al. 1989). Comparisons between spring and summer diets indicate that Carolina Madtom forage on elmid larvae (riffle beetles) in the spring and shift to simulid larvae (black flies), ephemeropteran nymphs (mayflies) and trichopteran larvae (caddisflies) in the summer (Burr et al. 1989). In addition, Burr et al. (1989)

observed that the presence of chironomid larvae (midges) and odonate nymphs (dragonflies and damselflies) did not change between seasons.

Reproduction of Carolina Madtom: The sex ratio for Carolina Madtom is 1:1, and reproduction has been observed to occur between mid-May and late-July when water temperatures range from 18-25 $^{\circ}$C (Burr et al. 1989; Wood and Nichols 2011; NCWRC unpublished data). Nesting occurs within or under cover objects (e.g., cobble or boulder, mussel shells, beverage cans or bottles) that are located within runs upstream of riffles or pools with moderate flow (Burr et al. 1989). Parental care of the eggs and young is likely provided by the male. Females reach sexual maturity within two years and can produce clutch sizes of approximately 80 to 300 eggs (Burr et al. 1989). The age at which males reach sexual maturity is unknown; however, males guarding nesting sites were 2 to 4 years old (Burr et al. 1989).

Conservation Management

Historical Conservation Efforts

To date, conservation efforts for Carolina Madtom have focused on monitoring surveys and acquisition of conservation lands or conservation easements. NCWRC biologists conducted targeted surveys for Carolina Madtom throughout its range in 2007 to update its current distribution and status. NCWRC also partnered with North Carolina State University (NCSU) in the same year to examine habitat suitability for Carolina Madtom across its range. NCWRC again partnered with NCSU in 2016 to repeat the surveys conducted in 2007, and complete a genetic evaluation of the different Carolina Madtom populations in order to guide future broodstock collection and augmentation efforts.

Threats

As with all aquatic species, there are many natural and anthropogenic factors that threaten the long-term viability of Carolina Madtom (USFWS 2017). The primary threats to Carolina Madtom include an apparent decline related to invasive species and habitat degradation. It is suspected that Flathead Catfish (*Pylodictis olivaris)* were introduced into the Neuse and Tar-Pamlico River basins in 1980s or 1990s. Since introduction, Flathead Catfish have expanded throughout the Neuse and Tar-Pamlico River basins and currently inhabit a substantial portion of the historical range of Carolina Madtom (Figure 8). Diet analysis and feeding chronology of Flathead Catfish in North Carolina indicate that the species is an opportunistic generalist that exhibits an ontogenetic dietary shift (300 mm TL) to larger prey items, such as centrarchids, clupeids, and ictalurids (Pine et al. 2005; Baumann and Kwak 2011). Furthermore, Flathead Catfish are known to directly restructure or suppress native fish communities through predation and cause rapid and substantial declines in native catfish populations (Guier et al. 1981; Pine et al. 2005; Dobbins et al. 2012). Currently, there are two known sympatric populations of Carolina Madtom and Flathead Catfish; however, few Carolina Madtom have been observed in these areas, potentially indicating rapid extirpation of Carolina Madtom once Flathead Catfish invades. Suspected mechanisms for Carolina Madtom extirpation related to Flathead Catfish introductions include direct predation, competition for prey, and competition for cover habitat. In addition, invasive species such as the Asian Clam (*Corbicula fluminea*) and Hydrilla (*Hydrilla verticillata*) can create competitive pressures on food resources and habitat availability. These factors can decrease oxygen availability, alter benthic substrates, impact fish communities, reduce stream flow, and increase sediment buildup (Belanger et al. 1991, NCANSMPC 2015, NCWRC 2015). Dams, both manmade and natural (created by beavers, see Kemp et al. 2012) are robust barriers to fish dispersal and alter natural temperature and flow regimes. Contaminants and water pollution are a significant threat to all aquatic species and impervious areas in urbanized watersheds contribute to high water levels, even during short rainfall events, which can result in flash flooding. These high or flashy flow events contribute to increased sediment loads, turbidity throughout the water column, and stream bed movements (NCWRC 2015). Climate change and development will likely bring additional stressors that need to be evaluated for fish. Furthermore, specific pollutants that may be introduced into the aquatic environment, the interactions of pollutants and temperature (from climate change), salinity (related to sea level rise), and lower dilution (from altered flows) will need to be considered (NCWRC 2015).

<u>Conservation Goal</u>

To prevent the extinction of Carolina Madtom and promote population viability (i.e., multiple age classes and wild recruitment) within North Carolina for the next 100 years.

<u>Conservation Objectives</u>

The overarching conservation strategy is to promote habitat protection and maintain the best populations of Carolina Madtom in the Tar-Pamlico River basin and focus efforts within the Neuse River basin on Contentnea Creek and Little River. Restoration of habitat should focus on areas that have not been invaded by Flathead Catfish and should primarily focus on the protection of riparian habitat and associated uplands.

1) Promote habitat protection and maintain for two populations of Carolina Madtom in the Neuse River basin and three populations in the Tar-Pamlico River basin (Figure 9). Management Units (MUs) will be defined based on hydrologic units (i.e., HUC10s).
    a. **Neuse River Basin**
        i. Contentnea Creek MU (0302020304)
        ii. Little River MU (0302020115, 0302020116)
    b. **Tar-Pamlico River Basin**
        i. Fishing Creek MU (0302010202, 0302010203, 0302010205)
        ii. Swift Creek MU (0302010107, 0302010108)
        iii. Tar River MU (0302010102, 0302010103, 0302010104)
2) Establish and maintain a ark population of Carolina Madtom from Neuse and Tar-Pamlico River basin broodstock.
3) Utilize captive propagation and/or translocations to augment or establish populations of Carolina Madtom where appropriate habitat exists (pending approval from the Habitat, Nongame and Endangered Species Committee). To reduce the potential to minimize the regulatory burden associated with the federal Endangered Species Act, a tool such as Safe Harbor will be established prior to reintroduction into an unoccupied area.

a. All Neuse and Tar-Pamlico River basin MU hydrologic units listed above.
b. Additional augmentation areas within the known range of Carolina Madtom (Figure 9), if propagation efforts exceed MU needs, and threat of Flathead Catfish invasion is low or threats related to Flathead Catfish populations have been reduced.
   i. **Neuse River Basin**
      1. Eno River (0302020103)
      2. Contentnea Creek (0302020306, 0302020307)
      3. Middle Creek (0302020109)
      4. Mill Creek (0302020113)
      5. Neuse River (0302020107, 0302020111, 0302020117, 0302020201, 0302020202, 0302020203, 0302020206)
      6. Swift Creek (0302020110)
      7. Trent River (0302020401, 0302020402)
   ii. **Tar-Pamlico River Basin**
      1. Beech Swamp (0302010204)
      2. Fishing Creek (0302010206)
      3. Tar River (0302010106, 0302010109, 0302010302)
      4. Town Creek (0302010301)
c. Potential reintroduction or introduction of Carolina Madtom (Figure 9) into areas within the presumed historical range, if propagation efforts exceed MU needs. Ideally located in areas with reduced likelihood of anthropogenic threats and invasion by Flathead Catfish.
   i. **Neuse River Basin**
      1. Contentnea Creek (0302020301, 0302020303)
      2. Black Creek (0302020112)
      3. Falls Lake (0302020104, 0302020105, 0302020106)
      4. Flat River (0302020101)
      5. Little River (0302020102)
   ii. **Tar-Pamlico River Basin**
      1. Shocco Creek (0302010201)
      2. Stony Creek (0302010105)
      3. Tar River (0302010101, 0302010304, 0302010306)

4) Establish connectivity and gene flow between existing and established populations by either translocating individuals or removal of barriers.
5) Reestablish historical populations of Carolina Madtom after invasive species or habitat threats have been reduced.

<u>Research Needs:</u>

1) Monitor Carolina Madtom populations every 2-5 years with surveys replicating the methods of Wood and Nichols (2011).
2) Develop captive propagation techniques to maximize yield, genetic diversity, and post release survival.
3) Delineate the distribution of Flathead Catfish and monitor the invasion rate.
4) Develop techniques to reduce the rate of Flathead Catfish invasion and population size.

5) Determine locations for establishing Carolina Madtom populations, and monitor the success of population establishment.
6) Determine the genetic diversity and number of genetically distinct populations of Carolina Madtom throughout its range.
7) Develop microsatellite markers or similar genetic tagging techniques to determine age structure, parentage, and hatchery contribution to wild stock.
8) Monitor the need for additional population or genetic augmentations.

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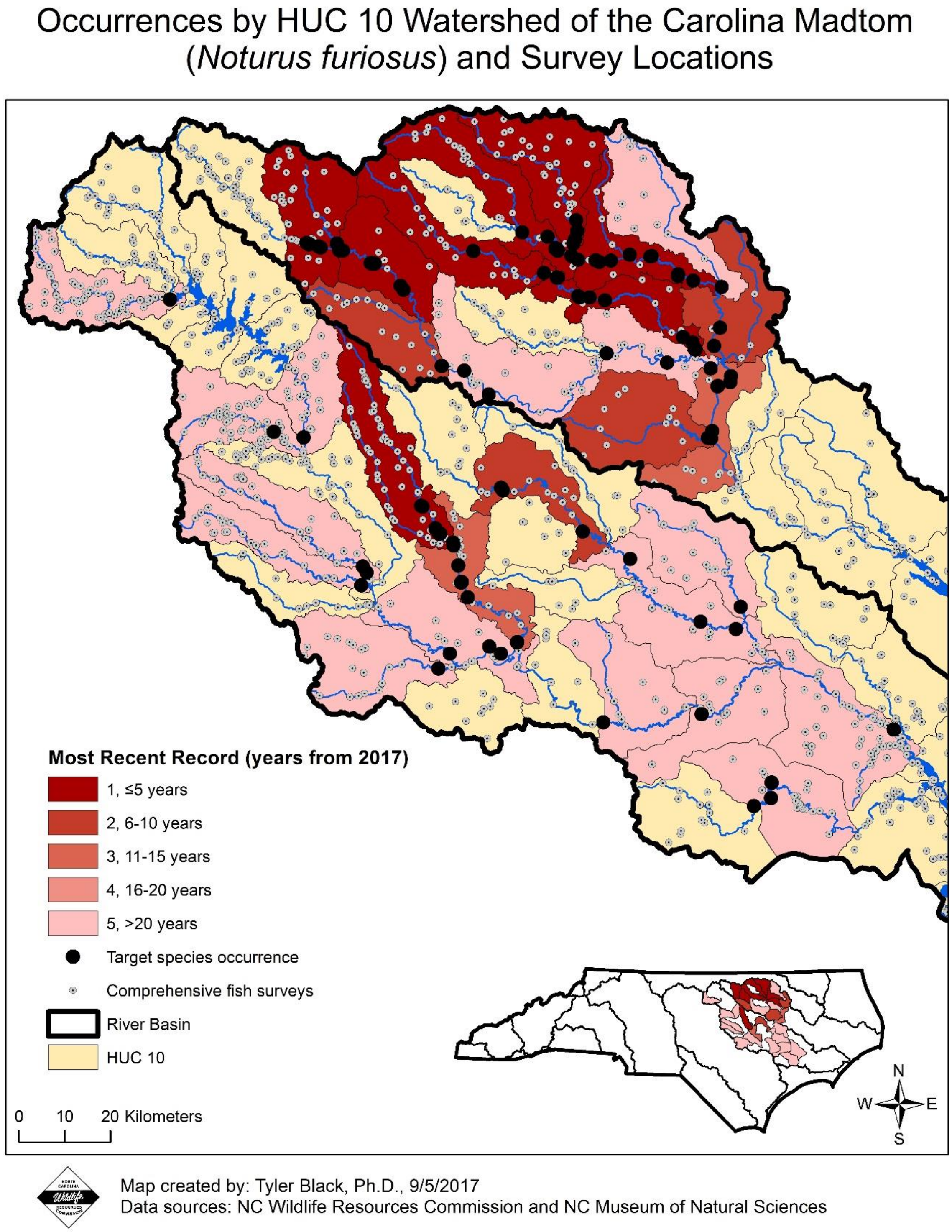

Figure 7. Distribution map of Carolina Madtom (*Noturus furiosus*) within the Neuse and Tar-Pamlico River basins depicting 10-digit hydrologic units (colored and categorized based on year of observation), collection locations (black dots), and survey locations (gray dots).

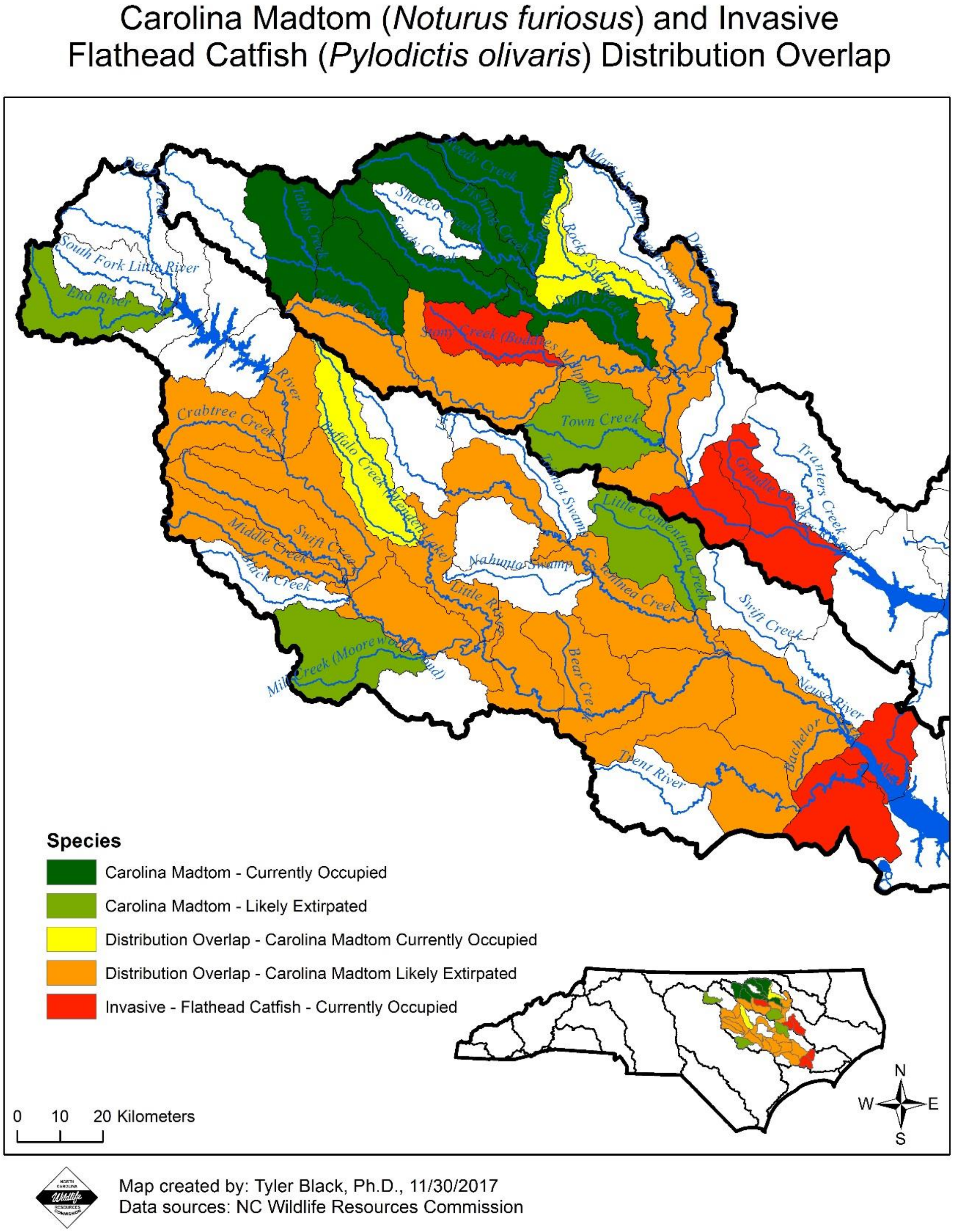

Figure 8. Distribution map of Carolina Madtom (*Noturus furiosus*) and invasive Flathead Catfish (*Pylodictis olivaris*) within the Neuse and Tar-Pamlico River basins depicting 10-digit hydrologic units (colored based species occurrence or distribution overlap).

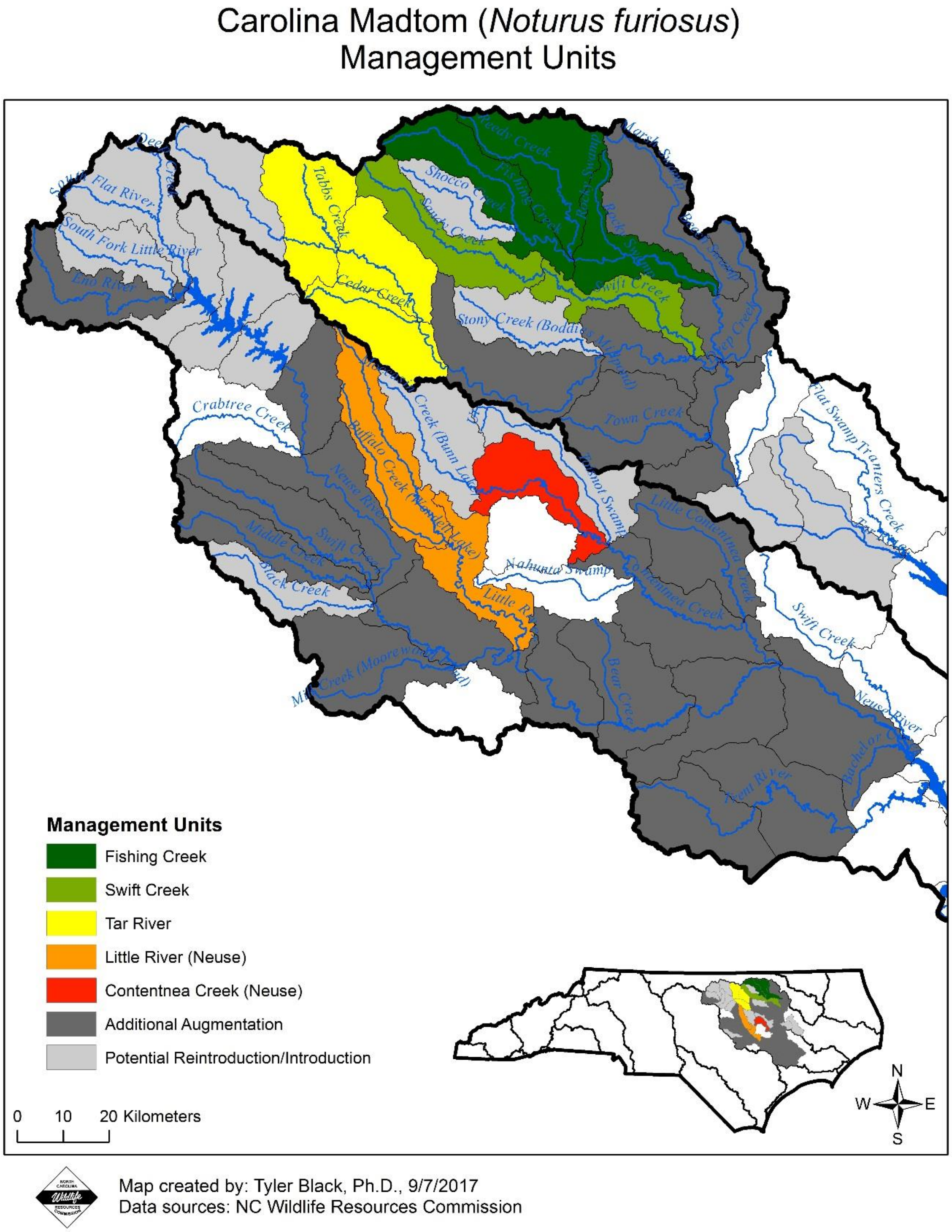

Figure 9. Management units of Carolina Madtom (*Noturus furiosus*) within the Neuse and Tar-Pamlico River basins depicting 10-digit hydrologic units (colored based management units and future management scenarios).

Neuse River Waterdog (*Necturus lewisi*)

Biological Information

Description and Taxonomic Classification

Neuse River Waterdogs are from an ancient lineage of permanently aquatic salamanders in the genus *Necturus*. Adult Neuse River Waterdogs have been described by Bishop (1943), Brimley (1924), Cahn and Shumway (1926), Viosca (1937), and Hecht (1958), while the first accurate descriptions and illustrations of hatchlings and larvae were documented by Ashton and Braswell (1979).

Hatchlings are light brown in color with dark lines from each nostril through the eye to the gills, with a white patch behind the eye and above the line (Ashton and Braswell 1979). Their heads are round compared to the square, elongated heads of the adults. Hatchlings have melanophores scattered on the gills, upper surfaces of the legs, lower jaw, and parts of the head, with concentrations highest on the tail, making the tail darker than the head and trunk (Ashton and Braswell 1979). Hatchlings have developed forelimbs, with three complete toes and the fourth, inner toe is only a bud and the hindlimbs are pressed close to the lower tail fin and not fully developed (Ashton and Braswell 1979).

Adults lose the striped pattern, and the side melanophores decrease in intensity while the dorsal melanophores increase in intensity and definition, on top of a reddish-brown skin (Ashton and Braswell 1979). The underside is brown/grey and has dark spots but smaller than those on the back. Adults have a set of external bushy dark red gills. Their tail is laterally compressed, and each foot has four toes. Adults can be up to 9 inches long.

Taxonomic Hierarchy (Integrated Taxonomic Information System 2017):

Kingdom:	Animalia
Phylum:	Chordata
Class:	Amphibia
Order:	Caudata
Family:	Proteidae
Genus:	*Necturus*
Species:	*Necturus lewisi*

Distribution and Population Status

The Neuse River Waterdog is endemic to the Neuse and Tar-Pamlico River basins in North Carolina. Its historical distribution includes two physiographic provinces (Piedmont and Coastal Plain) comprising all major tributary systems of the Neuse and Tar-Pamlico, including the Trent River sub-basin (Braswell and Ashton 1985). Because of saltwater influence, the habitats in the Trent River system are isolated from the Neuse River and its tributaries; therefore, we consider the Trent River system as a separate basin (i.e., population), even though it is technically part of the larger Neuse River basin.

A concerted effort to survey the range of Neuse River Waterdog was first conducted from 1978-81 (Braswell and Ashton 1985). Over 300 sites throughout the possible range of the species were trapped and results are shown in Figure 9. A subset of those exact sites were trapped again from 2011-15 by NCWRC staff and other partners, with 81 individuals captured. Comparing the same 170 sites from historical versus recent surveys, 56% (95 of 170 sites) were positive during historical surveys compared to 37% (63 of 170 sites) during recent surveys. Trends in population "loss" or "gain" varied among sub-basins and trends are shown in Figure 10. Current conditions of the status of the Neuse River Waterdog and possible future scenarios are shown in Figure 11.

Habitat and Life History

Habitat use of Neuse River Waterdog: The Neuse River Waterdog is endemic to the Neuse and Tar-Pamlico River basins of North Carolina. They are distributed from larger headwater streams in the Piedmont to coastal streams up to the point of saltwater intrusion, and none have been found in lakes or ponds (Braswell and Ashton 1985). Braswell and Ashton (1985) noted that waterdogs are usually found in streams wider than 15m and deeper than 1m, and with a main channel flow rate greater than 0.1m/sec. Further, these stream salamanders need clean, flowing water characterized by high dissolved oxygen concentrations (Brimley 1924, Braswell and Ashton 1985, Ashton 1985). The preferred habitats vary with the season, temperature, dissolved oxygen content, flow rate and precipitation (Ashton 1985), however the waterdogs do maintain home retreat areas under rocks, in burrows, or under substantial cover in backwater or eddy areas.

Diet of Neuse River Waterdog: Neuse River Waterdogs use both olfactory and visual cues to detect prey (Ashton 1985). Both adults and larvae are opportunistic feeders (Braswell and Ashton 1985), and most commonly waterdogs lie in wait for a small organism to swim or float by (Ashton 1985). However, Neuse River Waterdogs also use other feeding techniques when they are active at night, often leaving their retreats to actively search of food. Larvae eat a variety of small aquatic arthropods (primarily ostracods and copepods), and adults eat larger aquatic arthropods and also any aquatic and terrestrial invertebrates (including hellgrammites, mayflies, caddisflies, crayfish, beetles, caterpillars, snails, spiders, earthworms, centipedes, millipedes, slugs) and some vertebrates (including small fish like darters and pirate perch) (Bury 1980, Braswell and Ashton 1985). All prey are ingested whole, and larger items are sometimes regurgitated and then re-swallowed.

Reproduction of Neuse River Waterdog: Neuse River Waterdogs reach sexual maturity at around 5.5-6.5 years, or at a length of 102 mm SVL (snout-vent length) for males and 100 mm SVL for females (Fedak, 1971). The sexes are similar in appearance and can be distinguished only by the shape and structure of the cloacal area. Neuse River Waterdogs breed once per year, with mating in the fall/winter and spawning in the spring (Pudney et al. 1985). After courtship, the male will deposit a packet of sperm which the female places into her vent, thus fertilization occurs internally (Pudney et al. 1985). During the spring (May-

June), females will lay a clutch of ~25-90 eggs in a rudimentary nest, under large rocks in moderate currents (Braswell and Ashton 1985). Ashton (1985) noted that nest sites were often found under large bedrock outcrops or large boulders with sand and gravel beneath them, often placed there by the waterdogs. Females guard the nest (Braswell 2005).

Conservation Management

Historical Conservation Efforts

Conservation efforts to date have mainly consisted of conducting surveys for the Neuse River Waterdog throughout its range, and to monitor populations through repeated surveys. Initial survey efforts for the species were conducted throughout the species' possible range in the late 1970s and early 1980s (Braswell and Ashton 1985). Subsequent surveys were completed by NCWRC staff and partners at a subset of historically-surveyed sites from 2011-15. No other direct conservation actions for Neuse River Waterdogs have occurred, except for collecting tissue samples for ongoing genetic analysis.

Threats

As with all aquatic species, there are many natural and anthropogenic factors that threaten the long-term viability of Neuse River Waterdog. The primary threats to Neuse River Waterdog include a myriad of issues that affect water quality, habitat quality, connectivity of populations, and possibly adverse effects from invasive species. The U.S. FWS (2017) Draft Species Status Assessment identifies the following general threats to the viability of Neuse River Waterdog Populations:

1. Development and pollution
2. Improper agricultural practices
    a. Nutrient and chemical pollution
    b. Pumping for irrigation
    c. Confined animal feeding operations
3. Improper forestry practices
4. Invasive species
5. Dams and other barriers
6. Energy production and mining
7. Climate change

Conservation Goal

To prevent the extinction of the Neuse River Waterdog and promote population viability (i.e., multiple age classes and wild recruitment) within North Carolina for the next 100 years.

Conservation Objectives

The overarching conservation strategy is to promote habitat protection and maintain the best populations of *N. lewisi* throughout the Neuse and Ta-Pamlico River basins, as well as the Trent River sub-basin. The Neuse River Waterdog appears to have maintained better populations in the Tar-Pamlico River basin compared to the Neuse River basin, comparing historical to more contemporary survey efforts.

More research is needed to determine why the species appears to have declined drastically in specific watersheds compared to others (e.g., compare land use, water quality, etc. in watersheds with seemingly different levels of population loss). Since the Trent River sub-basin is isolated from the rest of the species' range, concerted effort should be made to maintain that population. Augmentation and/or re-introduction of the species may prove useful in increasing populations, however, reasons for the decline of the species need to be determined and habitat assessments need to be me made before these actions are implemented. To reduce the potential to minimize the regulatory burden associated with the federal Endangered Species Act, a tool such as Safe Harbor will be established prior to reintroduction into an unoccupied area. Specific objectives include:

1) Work collaboratively with landowners adjacent the species' habitat to protect riparian buffers and limit sediment runoff.
2) Work to remove barriers that limit interactions between Neuse River Waterdog populations.
3) Target point-source pollution issues and work to reduce issues related to water quality downstream of these sources.
4) Continue surveys and studies to increase knowledge about abundance, demography, and life history of Neuse River Waterdogs in order to better manage specific populations (e.g., the "best" remaining populations).

<u>Research Needs</u>

1) Improve our knowledge of population density, demographics, and land use effects on populations of waterdogs.
2) Conduct genetic analysis of waterdog tissue samples to determine the effects of population declines on the species, and to determine whether distinct genetic populations exist.
3) Determine the effect various pollutants on waterdog populations.
4) Monitor the need for additional population or genetic augmentation and possible re-introductions.

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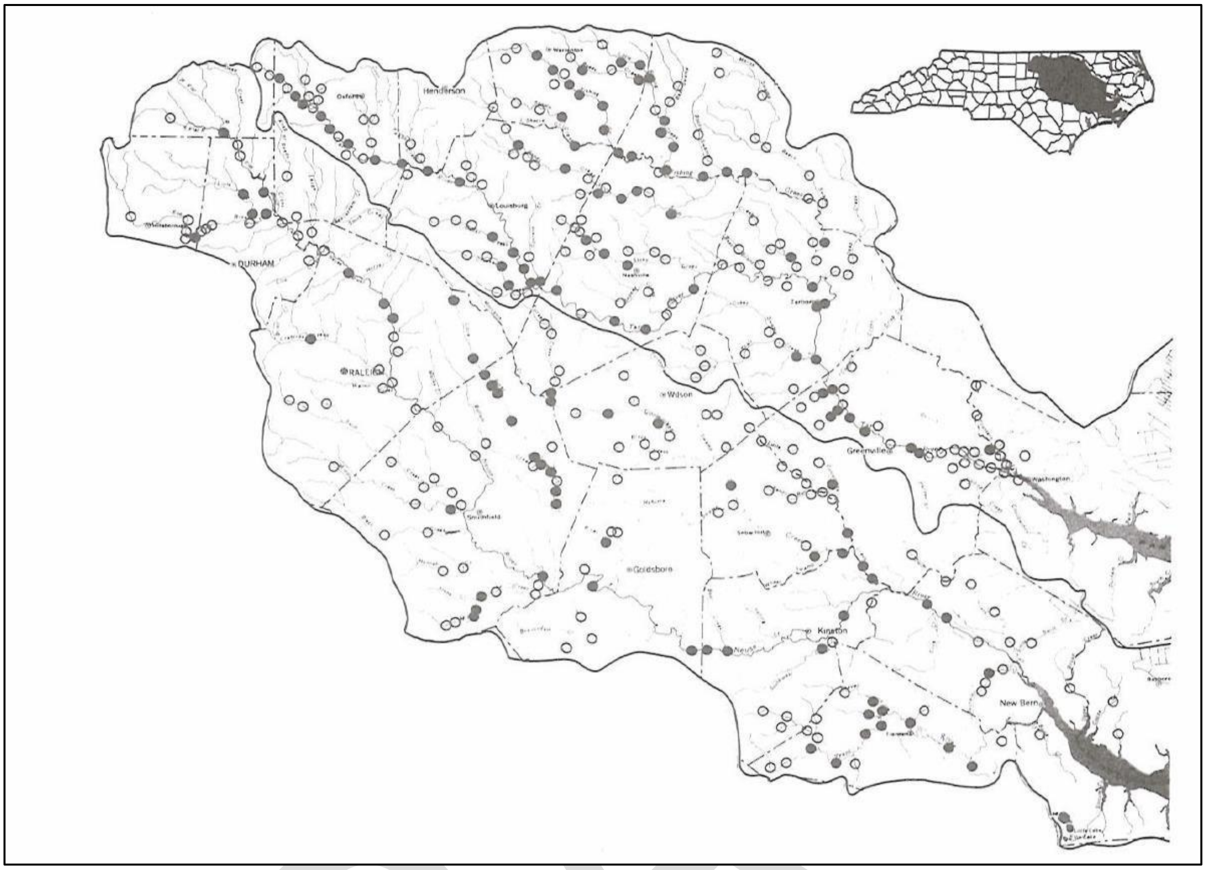

Figure 9. Historical surveys for Neuse River Waterdog (*Necturus* lewisi) from Braswell and Ashton (1985). Closed circles indicate species presence and open circles indicate species absence.

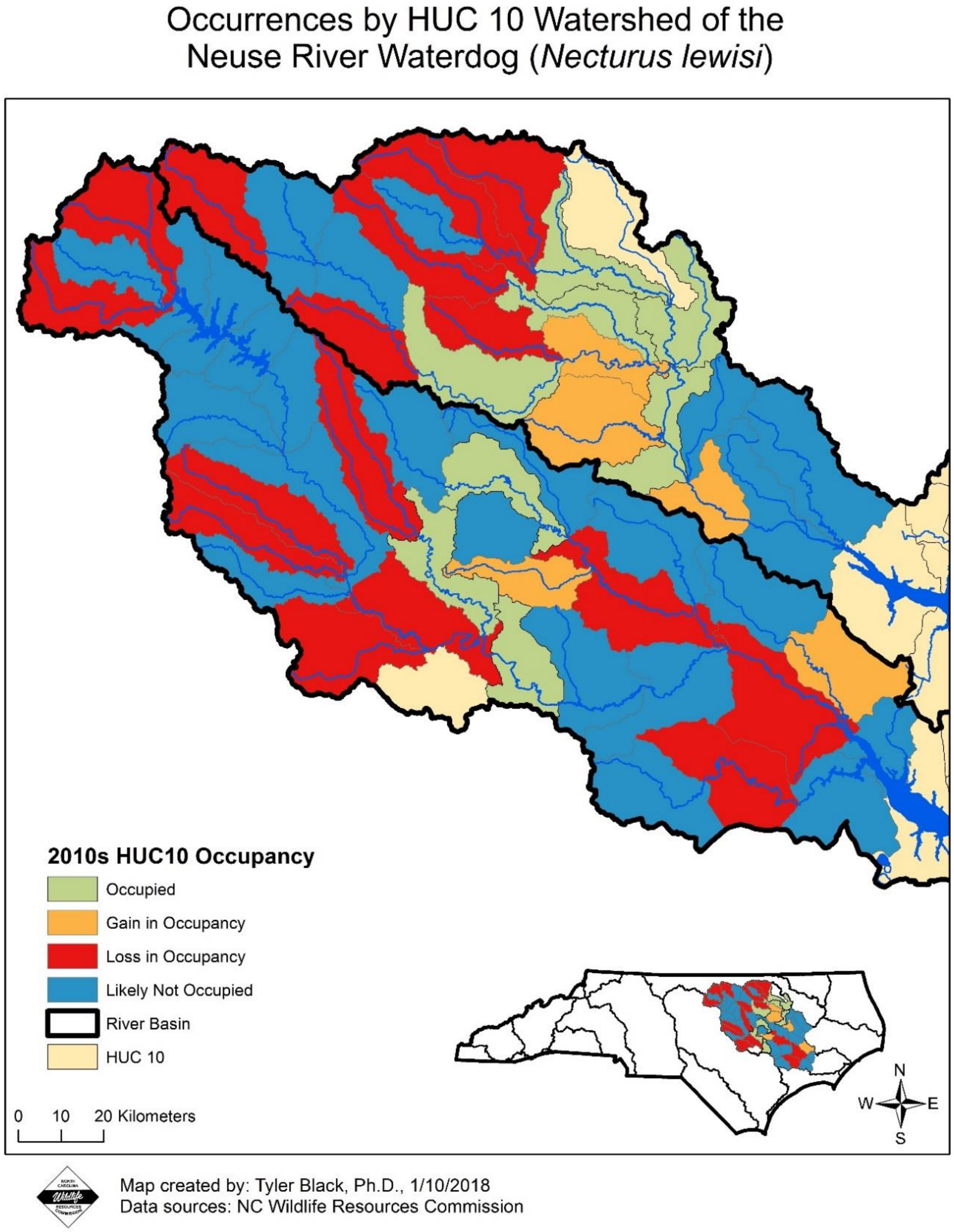

Figure 10. Occupancy observations for Neuse River Waterdog (*Necturus* lewisi) within the within the Neuse and Tar-Pamlico River basins depicting 10-digit hydrologic units.

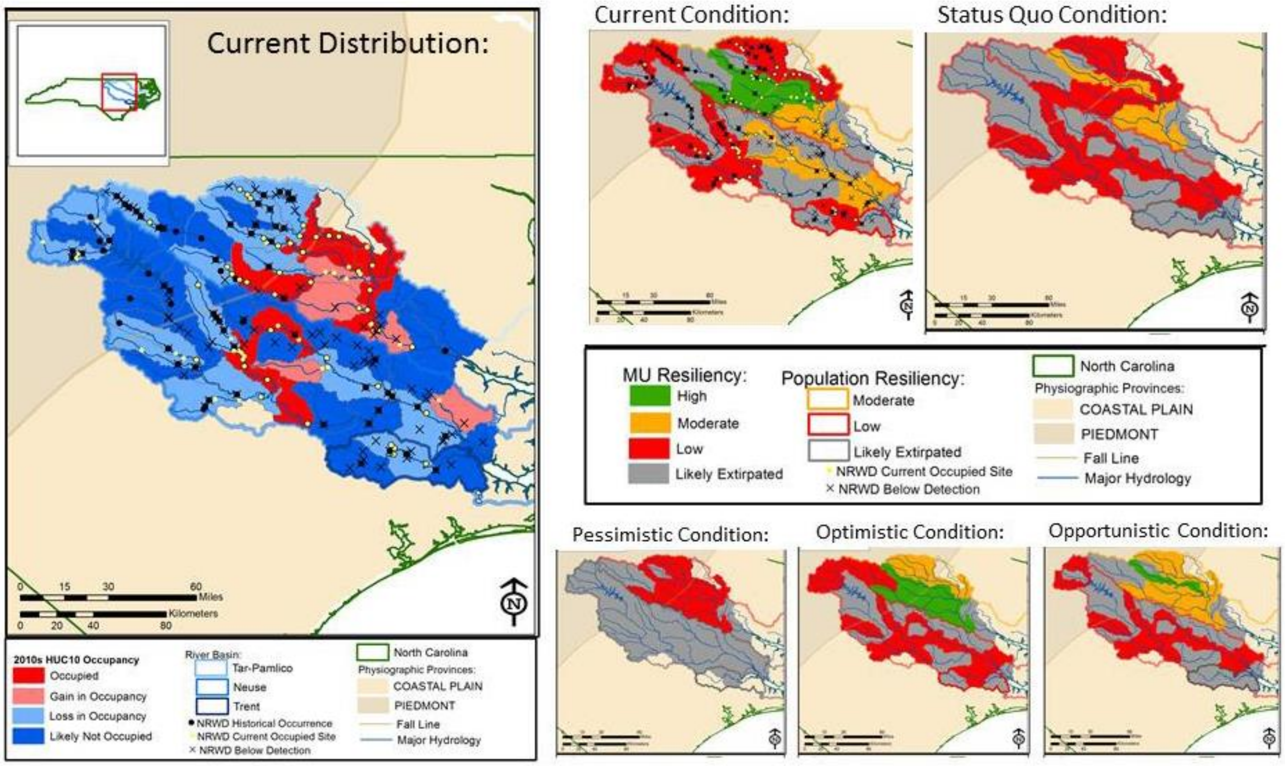

Figure 11. Current distribution and possible future scenarios concerning the status of the Neuse River Waterdog (U.S. FWS 2017).

CONSERVATION ACTIONS

This section outlines conservation actions intended to guide activities needed to achieve conservations objectives. These conservation actions focus on protection and management of habitats, law enforcement, educational outreach, and fostering conservation partnerships.

Habitat Protection and Habitat Management

Federal, state, local, and private organizations own and protect significant habitats within the Neuse and Tar-Pamlico River basin. Publicly owned lands (game lands, national wildlife refuges, national forests, and state parks) include over 274,000 acres. These lands help to promote the viability of Carolina Madtom, Dwarf Wedgemussel, Neuse River Waterdog, Tar River Spinymussel, and Yellow Lance populations by protecting high-quality water resources and associated riparian habitats. However, long-term maintenance of viable populations will require additional habitat protection efforts within the species management units and high priority areas (i.e., 12-digit HUCs and riparian buffers) highlighted within the North Carolina Wildlife Action Plan. Land acquisition will require support from a combination of federal, state, local, and private organizations and lands management strategies should follow “best management practices” that maintain or improve water-quality and natural flow regime. In addition, support will be needed to control beaver populations and exotic invasive species such as Asian Clam, Flathead Catfish, Hydrilla, and Mystery Snails.

Permitting

State and federal biologist will review permit applications for projects that might impact waterways within the range by Carolina Madtom, Dwarf Wedgemussel, Neuse River Waterdog, Tar River Spinymussel, and Yellow Lance.

Protective Laws

<u>Federal</u>

The Tar River Spinymussel (*Parvaspina steinstansana*) and Dwarf Wedgemussel (*Alasmidonta heterodon*) are listed as Endangered by the U.S. Fish and Wildlife Service (USFWS) while the Yellow Lance (*Elliptio lanceolata*) is proposed to be listed as Threatened. These species are protected by regulations listed in the Code of Federal Regulations (CFR) which implement the Endangered Species Act of 1973, 87 Stat. 884, 16 U.S.C. 1531-1543. The USFWS regulates the import/export, take, possession, sale, and captive breeding of threatened and endangered wildlife under 50 CFR 17.21 and 50 CFR 17.31. Section 404 of the Clean Water Act (CWA) regulates the discharge of dredged or fill material into the waters of the United States, regulating such activities as fill for development, water resource projects (such as dams and levees), infrastructure development (such as highways and airports) and mining projects. Section 404 requires a permit that is reviewed by the U.S. Army Corps of Engineers be administered before any of these activities commence. Under Section 401 of the CWA, an applicant for a federal license or permit to conduct any activity that may result in a discharge to water of the United States must provide the federal agency with a Section 401 certification which is issued by the North Carolina Division of Water Resources (DWR). The CWA also prohibits anybody from discharging pollutants through a point source into waters of the United States unless they have a NPDES permit. The NPDES permit is issues by the DWR and contains limits on what can be discharged, monitoring and reporting requirements, and other provisions to ensure that the discharge does not hurt water quality, wildlife, or people's health. The Fish and Wildlife Coordination Act requires federal agencies that construct, license, or permit water resource development projects to first consult with FWS and state fish and wildlife agencies regarding the impacts on fish and wildlife resources and measures to mitigate these impacts.

<u>State</u>

The species in this conservation plan are listed on the protected wild animal list at endangered, threatened, or special concern. It is unlawful to take, possess, transport, sell, barter, trade, exchange, or export any animal on the protected wild animal list without a valid permit and is currently prohibited under NC law and administrative code (15A NCAC 10I .0102) and is considered a Class 1 misdemeanor (§ 113-337b).

Conservation Incentives

Several conservation incentive programs focus on restoring water quality by preventing runoff and siltation. Each of the following incentive programs, except for the North Carolina Wildlife Conservation Land Program, come from the Farm Bill.

The Conservation Reserve Program is administered by the Farm Services agency and pays a yearly rental payment in exchange for farmers removing environmentally sensitive lands from agriculture and planting species that will improve environmental quality. The Conservation Reserve Enhancement Program provides rental payments to landowners with high priority conservation issues in exchange for removal of these lands from farm production.

The Farmable Wetlands Program is designed to restore wetlands and wetland buffer zones that are farmed. It also provides annual rental payments to farmers willing to restore wetlands and establish planted buffers.

The Grassland Reserve Program works to prevent grazing and pasture land from being converted into cropland or used for development. In return, landowners receive an annual rental payment.

The Environmental Quality Incentives Program (EQIP) is a Farm Bill program that provides financial and technical assistance to farmers who plan and implement conservation practices that improve soil, water, plant, animal, air and related natural resources on agricultural land and on-industrial private forestland.

The North Carolina Wildlife Conservation Land Program provides tax incentives to landowners willing to manage priority habitats such as wetlands, or protected state listed species. This program is administered by NCWRC, and allows landowners a reduced assessment for taxation purposes. Although this program has not been used much in eastern North Carolina, it has significant potential to improve habitat.

The North Carolina Division of Mitigation Services (DMS) works with willing landowners who are interested in conservation efforts to improve and protect water resources. All projects that receive funding from DMS must offer perpetual conservation protection through the voluntary use of a conservation easement.

The North Carolina Forest Service administers cost-sharing assistance through the Forest Development Program (FDP) to support prompt reforestation after timber harvesting and afforestation of fallow ag fields. Given the apparent linkage between the abundance of many candidate aquatic species populations, and their relative close proximity to existing forested watersheds, it should be recommended to support the FDP and other programs that encourage the sustainable management of forests.

Education and Outreach

Education and outreach are important components of managing imperiled aquatic species. Citizens who are well informed regarding the merits of an imperiled species, and the habitat that supports such species, can make better decisions and support sound conservation measures to secure those species' continued survival. A concerted effort needs to be made to educate anglers about the perils of moving fish between bodies of water and the ecological damage that invasive species, such as the flathead catfish, can cause. The benefit of freshwater mussels from the ecological services standpoint of filtering river water and serving as an important sentinel species needs to be highlighted to the public.

Conservation Partnerships

Establishing and maintaining working relationships between governing bodies (federal, state, and local), universities, private landowners, private companies, and conservation organizations will be critical to the long-term persistence of Carolina Madtom, Dwarf Wedgemussel, Neuse River Waterdog, Tar River Spinymussel, and Yellow Lance. Some potential partners within the Neuse and Tar-Pamlico River basins include the following:

Duke Energy
North Carolina Department of Agriculture
North Carolina Department of Environmental Quality
North Carolina Division of Parks and Recreation
North Carolina Coastal Land Trust
North Carolina Natural Heritage Program
North Carolina State University
North Carolina Museum of Natural Sciences
North Carolina Cooperative Fish and Wildlife Research Unit
Tar River Land Conservancy
United States Fish and Wildlife Service
Various forestry associations

ECONOMIC IMPACTS

Potentially Affected Parties

Implementation of this conservation plan will predominately affect the North Carolina Wildlife Resources Commission. The NCWRC will be responsible for virtually all the population management, habitat management, monitoring, and research.

To a lesser extent, parties applying for development permits may also be affected.

Agency Costs

Costs for implementing the conservation actions outlined in this plan are estimated to be approximately $3,513,000 over a 10-year period. There is no way to estimate how many projects NCWRC staff will review where these species may be affected, but permit review requires approximately two hours of staff time per project and would cost an estimated $74 per project.

Costs to Others

Developers may be required by the NC DEQ or US COE to assess projects for any potential impacts to listed species as part of the permit application process for development. All currently available species data is available free of charge on the Natural Heritage website and applicants can request free assistance in interpreting the data at any time. However, if data do not exist on a species, a survey may need to be completed, at the developer's expense, before the project begins. A site survey for a species is nominal to the developer compared to the total expense of a project. The costs associated with the survey are typically absorbed into other scoping, survey or environmental fees that developers plan for as part of the site development.

Efforts to Minimize Costs and Adverse Economic Impacts

The NCWRC will utilize two main strategies for minimizing the economic impacts of implementing this plan. The first strategy is that the NCWRC will utilize federal grant funding to carry out most of the actions called for in this plan. These activities are eligible for funding through the State Wildlife Grants (SWG) Program or Endangered Species (Section 6) grants. SWG will cover 65% and Section 6 will cover 75% of the costs of virtually all the actions called for in this plan. Secondly, the NCWRC will not stock federally listed species or species likely to become federally listed without some sort of mechanism in place such as a Safe Harbor Agreement or Candidate Conservation Agreement with Assurances to reduce the potential regulatory burden associated with the Endangered Species Act.