

**FINAL LOUISIANA PEARLSHELL
CAPTIVE PRODUCTION, HEAD-STARTING, AND REINTRODUCTION PLAN**



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TABLE OF CONTENTS

Acknowledgements	ii
INTRODUCTION	1
Purpose of Plan	1
Partners	1
SPECIES' INFORMATION	2
Listing and Critical Habitat	2
Range	2
Habitat and Biology	4
Threats	4
Genetics	10
Population Trends	10
CAPTIVE PRODUCING AND HEAD-STARTING JUVENILES	14
Purpose of Program	14
Role in Recovery	14
Louisiana Pearlshells in Controlled Captivity	15
Techniques	16
REINTRODUCING JUVENILES	18
Site Selection	19
Site Plan	20
IMPLEMENTATION GUIDANCE	22
Genetics Management Guidelines	22
Aggregation Restoration Guidelines	23
MONITORING AND MEASURING SUCCESS	23
Survey Methods	23
Verification of Persistence	24
Verification of Reproductive Success	25
RECOVERY MILESTONES	26
ANNUAL REPORT	26
ANNUAL PLAN REVIEW	27
LITERATURE CITED	28

PLAN FOR CAPTIVE PRODUCTION AND REINTRODUCTION OF LOUISIANA PEARLSHELLS

INTRODUCTION

Purpose of Plan

The purpose of this document is to provide guidelines for a program that will positively manipulate the reproductive cycle of gravid Louisiana pearlshells (*Margaritifera hembeli*) in a captive environment to produce and head-start juveniles for release into the wild and to address the role of the program in the conservation and recovery of this federally threatened species. This plan is also intended to facilitate coordination among all parties involved in captive production and population restoration efforts supporting recovery of the Louisiana pearlshell and to ensure those efforts are based on sound science. This document will be reviewed annually and updated periodically as new scientific information becomes available.

Activities of the plan will be implemented to achieve positive conservation benefit while (1) reducing the potential for inbreeding, genetic swamping, and other adverse genetic effects within populations that may result from the enhancement of only a portion of the gene pool; (2) reducing the potential for erosion of genetic differences between populations, and (3) diminishing exposure to novel selection regimes experienced in the controlled environment that may reduce the natural capacity for reintroduced individuals to survive and reproduce in the wild.

Partners

A group of partners is working with the Service in the development and implementation of the captive production, head-starting, and reintroduction program. Our partners are consulted in the development, implementation, and monitoring of the program and may suggest future adaptations to increase the program's efficiency and probability of success. Our partners realize that successful implementation of the program requires long-term commitments. Our main partners include biologists, supervisors, and other experts, technicians, and staff from the U.S. Forest Service (USFS) at the Kisatchie National Forest (KNF); Louisiana Department of Wildlife and Fisheries (LDWF), Wildlife Diversity Program; Alabama Department of Conservation and Natural Resources, Alabama Aquatic Biodiversity Center (AABC); and the Service's Southeast Conservation Genetics Lab. Additionally, current and expected partnerships that contribute to program success include willing private landowners that voluntarily provide long-term habitat conservation and access for Louisiana pearlshell monitoring; the Service's Baton Rouge Fish and Wildlife Conservation Office; and Academia. Private landowners who choose to be involved would participate on a voluntary basis and all personally identifiable information (PII) would be protected from release to outside parties.

Our partners, particularly the USFS and the LDWF, provide assistance in monitoring the range-wide status of Louisiana pearlshell populations; monitoring habitat to identify and address habitat threats; field monitoring of females that were used for captive collection of glochidia (larvae); providing guidance in the development of an efficient and successful captive production program at Natchitoches National Fish Hatchery (NNFH); obtaining or granting access permissions to protected suitable habitat for reintroductions; conducting monitoring efforts at

reintroduction sites to assist the Service in determining the overall success and contribution of the captive production and reintroduction program to Louisiana pearlshell recovery; and continuing research to inform data gaps.

SPECIES' INFORMATION

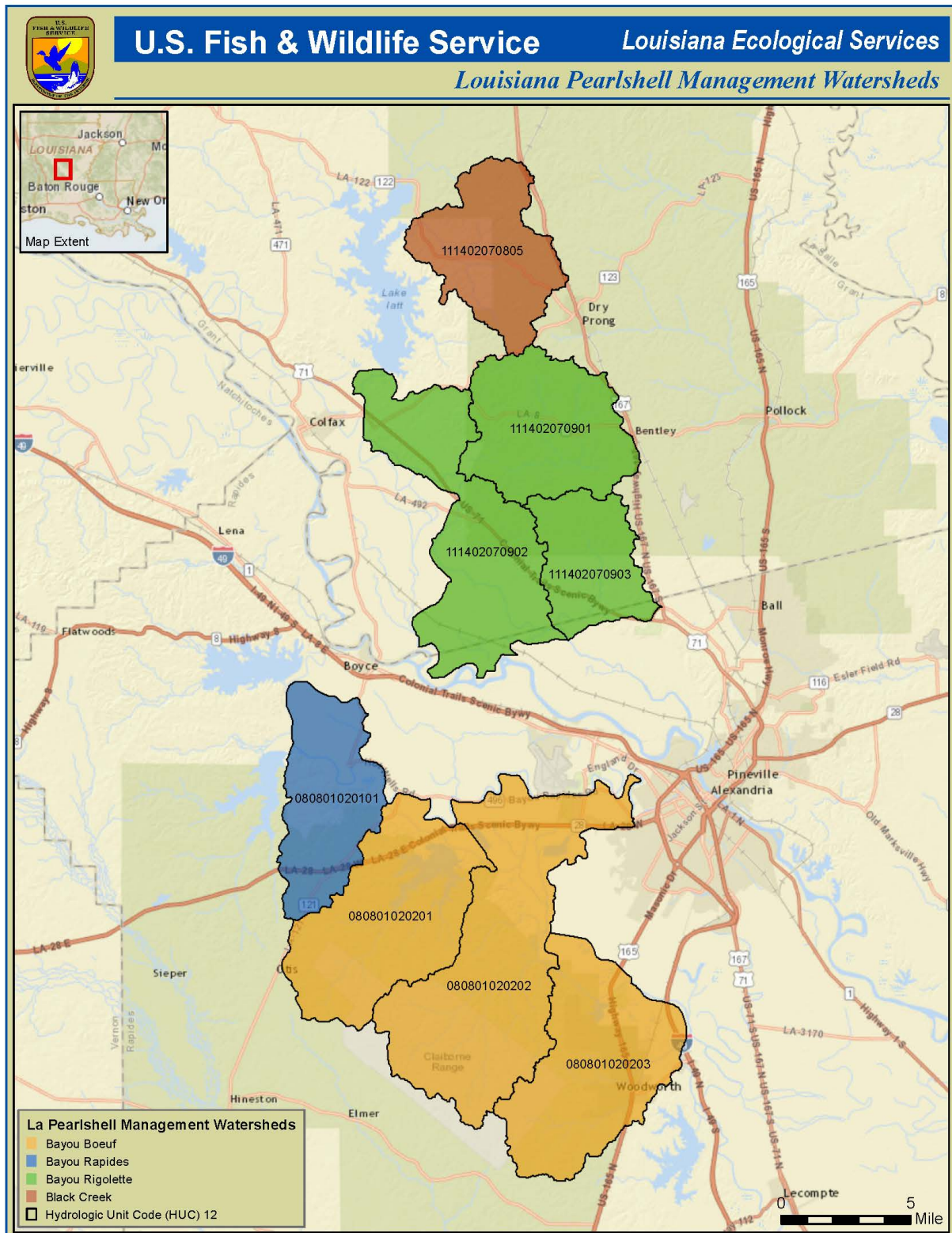
Listing and Critical Habitat

The Louisiana pearlshell is a freshwater mussel that was listed as a federally endangered species in 1988 (53 Federal Register (FR) 3567) and was down-listed to threatened in 1993 (58 FR 49935) after the species was found in new locations. Currently, the Louisiana pearlshell is federally protected as a threatened species under the Endangered Species Act (ESA) of 1973 (87 Stat. 884, as amended; 16 U.S.C. 1531 et seq). No critical habitat has been designated for the species.

Range

The current range of the Louisiana pearlshell is similar to its known historic range, although range-wide local extirpations have occurred. The species is distributed in Louisiana across four management watersheds: Bayou Rapides and Bayou Boeuf south of the Red River in Rapides Parish and Black Creek and Bayou Rigolette north of the Red River in Grant Parish. Management watersheds for the Louisiana pearlshell were delineated by aggregating HUC_12 subwatersheds occupied by Louisiana pearlshells in a way that reflects relatively high connectivity and potential for genetic exchange among subwatersheds within a management watershed, and lower connectivity between management watersheds (Quantitative Ecological Services, Inc. (QES) 2014, pp.2-3). Each management watershed is made up of HUC_12 subwatersheds from a different HUC_10 watershed, but do not include all HUC_12 units within the larger HUC_10 watersheds because they do not all contain Louisiana pearlshells. Figure 1 below shows the combination of the HUC_12 units to delineate management watersheds. Years after we demarcated the four management watersheds, analysis of population genetics data was found to support our delineation of these four management watersheds (Garrison et.al. 2020, entire).

Figure 1. Delineation of Management Watersheds using HUC_12 Units.



Habitat and Biology

Louisiana pearlshells are endemic to narrow streams in upland forests of the Lower West Gulf Coastal Plain. The riparian forest adjacent to these streams is comprised of typical upland riparian vegetation, such as southern magnolia (*Magnolia grandiflora*); American beech (*Fagus grandifolia*); laurel, water, and cherrybark oaks (*Quercus laurifolia*, *Q. nigra*, and *Q. pagoda*, respectively); sweetgum (*Liquidambar styraciflua*); bluebeech (*Carpinus caroliniana*); longleaf and loblolly pine (*Pinus palustris* and *P. taeda*, respectively); baldcypress (*Taxodium distichum*); etc. (Johnson and Brown 2000, p.274; Holcomb et al. 2015, pp.159-164). These riparian forests are generally densely vegetated and have 51 to 75 percent canopy closure (LNHP 1998, p.2).

Louisiana pearlshells are found in spring-fed, headwater streams that are relatively narrow (less than 4.9 meters in width), clear, and moderately swift-flowing. These streams have stable mineral substrate, such as a sandy bottom with rocky outcroppings, and an approximate riffle-to-pool ratio of 3:1 (Johnson 1995, pp.10, 19). Within these streams, Louisiana pearlshells usually occur in shallow water (12 to 24 inches deep) in headwater riffles where the substrate is dominated by loose, fine, or very fine sand with infrequent patches of larger gravel substrate (Johnson 1995, pp.10, 19). Occurrence and abundance data indicate a preference for slightly acidic, oligotrophic systems with low sediment organics, low suspended particulates, low specific conductivity, and harder water with the species' distribution skewed toward areas of the stream having a gravel-cobble substrate (Johnson and Brown 2000, p.273).

Louisiana pearlshells have a complex life cycle similar to other freshwater mussel species. Fertilization occurs after females siphon sperm that males release into the water during the spawning period. Research and field observations conducted by biologists from the NNFH beginning in 2010 to current indicate that spawning occurs once annually and glochidial development occurs between February and March. Recent genetic research indicates multiple paternity (Garrison et.al. 2020, pp.19, 23-24). Females brood developing embryos until releasing fully developed glochidia (larvae) into the water (Smith 1988, p.161). Glochidia must infest a suitable fish host to transform from larval stage to juvenile stage. The length of metamorphosis from glochidia into juveniles for the Louisiana pearlshell is 35-51 days in a captive environment, with the largest number of juveniles dropping off at day 41 (Service 2018, p.4).

Threats

The following is a summary of threats potentially facing the Louisiana pearlshell based on analysis of the five factors as outlined under section 4(a)(1) of the ESA:

Factor 1: Habitat

The main threat to the Louisiana pearlshell is habitat loss and degradation resulting from a variety of land uses. Influences on the viability of the species that result in the destruction or modification of Louisiana pearlshell habitat include impoundments, beaver activity, water quality degradation, forestry practices, gravel pits and mining, cattle grazing, and construction activities. Consequently, some populations from historically occupied sites are extirpated and others have suffered declines in abundance. The life history traits and habitat requirements of the Louisiana pearlshell, and other freshwater mussels in general, make them extremely susceptible to decreased water quality and environmental change. Unlike other aquatic organisms (e.g., aquatic insects and fish) mussels are limited in mobility to escape or seek refuge

from habitat disturbance (e.g., droughts, sedimentation, chemical contaminants). Additionally, because the species is a narrow endemic of specialized habitat, localized impacts to habitat have the potential to influence the species at a population or watershed level.

Impoundments

All man-made impoundments that potentially impact the Louisiana pearlshell pre-date listing of the species. Reservoirs, lakes, and other impoundments fragment the spatial distribution of Louisiana pearlshell habitat on the landscape, as they have done from the time of initial listing. Kincaid Reservoir impounds the uppermost headwaters of Bayou Boeuf. At the time of listing, Mack Branch and Valentine Creek were the only streams above Kincaid Lake that supported the species. Since then, Louisiana pearlshells in Mack Branch have become extirpated; however, the extent to which the Kincaid Reservoir impoundment contributed to this local extirpation event is not known. Habitat degradation was likely the primary cause of Louisiana pearlshell extirpation in Mack Branch; consequently, the number of Louisiana pearlshells in the stream continuously declined in response to water quality decreases that rendered the stream unsuitable for the species (Darden 1988, pp.20, 23). The largest Louisiana pearlshell populations within the Bayou Boeuf watershed occur in unimpounded tributaries to Bayou Boeuf in Loving Creek, Long Branch, and Bayou Clear drainages. Other impoundments of the Bayou Boeuf system are Indian Creek Reservoir, Oden Lake, and Cotile Lake. Lake Iatt impounds the headwater region of Bayou Rigolette although some of the largest extant Louisiana pearlshell populations are found there. The presence of these permanent impoundments cannot be addressed by recovery actions; however, population level impacts resulting from limitations to host fish movement and genetic interchange could be addressed by reintroducing captive reared Louisiana pearlshells into suitable habitat.

Beaver Activity

Beaver (*Castor canadensis*) activity has been documented as a source of disruption throughout the species' range since the beginning of monitoring and survey efforts. Beaver dams create small impoundments within Louisiana pearlshell watersheds that have the potential to alter hydrology and affect the spatial distribution of the Louisiana pearlshell throughout its range. Louisiana pearlshell mortality can occur upstream, downstream, or at beaver dam locations for various reasons. For instance, beaver dams can lead to indirect negative impacts and mortality when water flow is inhibited by the dam causing upstream habitat to become deeper and more stagnant, whereas downstream habitat becomes shallower or completely dry. Inundation of Louisiana pearlshells upstream of dams could lead to suffocation in a stagnant hypoxic environment (Ice and Sugden 2003, p.95), and downstream drying could lead to stranding of mussels on dewatered areas of the stream. The downstream impacts of beaver dams are likely exacerbated during drought and by the influences of low precipitation and increased rates of evaporation during warmer time periods. Lastly, the location of beaver dam construction can lead to direct mortality of Louisiana pearlshells when dams are built on top of mussels. Thus, beaver control and dam removal is an important conservation tool in areas where beavers pose a local threat to resident Louisiana pearlshells.

Land Use Impacts to Water Quality

Multiple land use practices are impacting Louisiana pearlshell habitat through reductions in water quality. Water quality is a limiting factor on the local abundance of the Louisiana

pearlshell among different streams, and decline in water quality contributes to species' declines (Johnson 1995, p.37).

➤ *Forestry*

Forestry practices that involve the harvesting of trees up to the streambank can decrease bank stability, cause direct soil erosion and increase runoff into the stream, with resultant increases in water turbidity and scouring of the streambed, all of which can create unsuitable or unstable habitat for mussels (58 FR 49936). Streams that lose vegetated riparian buffers suffer a loss in the natural ability to filter sediment, debris, and pollutants.

In accordance with their Revised Land and Resources Management Plan (RLRMP), multiple restrictions have been implemented by the USFS that protect the Louisiana pearlshell and its habitat on the KNF (USFS 1999). For example, the USFS minimizes potential impacts to riparian areas through the effective use of streamside management zones (SMZ) that provide protection of riparian habitat within 50 to 100 feet along the banks of perennial and intermittent streams. These SMZs are maintained for the benefit of water quality and wildlife habitat. Any timber harvest permitted within SMZs is restricted to selective cutting of individual trees for the purpose of wildlife habitat improvement. However, SMZs do not necessarily extend to private lands. Many industrial timber owners in Louisiana implement SMZs to meet Sustainable Forestry Initiative (SFI) requirements. The Louisiana Forestry Association (LFA) published a manual on Forestry Best Management Practices (BMPs) for Louisiana which covers SMZs (1997, pp.14-15); however, not all private landowners follow these guidelines because participation in the SFI and implementation of the BMPs is a voluntary decision. Therefore, Louisiana pearlshells found adjacent to and downstream of private lands where detrimental forestry practices occur are likely to be negatively impacted.

➤ *Gravel Pits*

There are two known gravel pit operations in the species' current range. One is currently inactive and is located in Grant Parish on Beaver Creek with seemingly no current impacts to Louisiana pearlshells or their habitat. The other one is a large operation in Rapides Parish on Hospital Bayou, which empties into Bayou Clear, which is an occupied stream. In the area of the active gravel pit, there are very few Louisiana pearlshells; however, it is unclear if this is a result of the gravel pit operation. It is also unclear if there are impacts to Louisiana pearlshells further downstream.

➤ *Cattle Grazing*

Open-range cattle grazing was once considered a range-wide threat to the Louisiana pearlshell because cattle created stream crossings to access water, which resulted in increased sedimentation and organic input into the stream and direct mortality to mussels from being crushed (Service 1990, p.5). Open-range cattle grazing has been discontinued on the KNF; however, the extent to which cattle grazing affects Louisiana pearlshells on private property is uncertain.

➤ *Minerals Mining*

Minerals mining is not thought to be a threat to the species because it is regulated. The RLRMP (USFS 1999, pp.1-10, 1-11, D-4, D-9) restricts the zones of mineral development within the

Louisiana pearlshell range on the KNF to protect the water quality of Louisiana pearlshell streams. There are no known restrictions on private lands; however, such details are addressed under Section 7 consultation when there is a federal nexus for the proposed action when planned within the species' range.

➤ *Construction*

Construction and other soil-disturbing activities (e.g., bridge replacement, culvert installation, road maintenance, utility rights-of-way, etc.) with inadequate erosion control measures within Louisiana pearlshell watersheds can cause the following: (1) a direct loss of habitat; (2) reduced capability of host fish passage; (3) reduced water quality as a result of increased erosion, run-off, sediment loading, reduced flow, and decreased levels of dissolved oxygen; and (4) changes in stream geomorphology through processes such as head-cutting, bank sloughing, and perched water tables.

Improper stream crossing construction, placement, or bank span width can cause fragmentation of habitat by altering upstream and downstream hydrology and inhibit host fish passage during Louisiana pearlshell critical reproductive periods. Potential impacts of insufficient erosion control can result from project-related soil disturbance during excavation, vegetation removal, etc., as well as from erosion occurring through failure to implement and maintain long-term erosion control measures, including but not limited to, restoring herbaceous groundcover on disturbed soil and armoring the stream bank to protect from scouring. As filter feeders, mussels can close their shells for short periods to protect internal tissue when sediment loads in the water temporarily increase (e.g., sedimentation due to a storm event); however, chronic sedimentation, even at a relatively smaller load, can affect mussels because at some point they must open their shell and resume siphoning water across the gills. Excessive siltation degrades water quality and substrate, clogs gills, reduces feeding efficiency and growth, and can eventually smother mussels (Service 2000, entire).

Factor 2: Overutilization

Overutilization is not a threat to the species. The Louisiana pearlshell is not harvested for consumption. It is not a commercially valuable species, and the small streams it inhabits are not subject to harvesting for commercial mussel species. Commercial or private harvest for reasons other than research is not a legal action, and neither the U.S. Fish and Wildlife Service (Service) nor the LDWF could legally issue a permit for commercial or private harvest. There is some need for collection of the species for research purposes, which is regulated through ESA, Section 10 permitting and Section 7 consultation and results in an overall beneficial impact to the species.

Factor 3: Disease and Predation

Disease

There is no evidence to support the instance of disease in any wild or captive population of the Louisiana pearlshell. Disease is not known to be prevalent in freshwater mussels; however, the potential for disease to occur in species of freshwater mussels has received little attention in published literature. There is currently no tool to screen Louisiana pearlshells for disease.

Predation

The shallow stream habitat of this species does make it vulnerable to predation by river otters (*Lontra canadensis*), raccoons (*Procyon lotor*), muskrats (*Ondatra zibethicus*), and possibly feral hogs (*Sus scrofa*). Evidence of suspected otter predation on Louisiana pearlshells at the individual level has been frequently observed (Gregory 2010, pers. comm; Shively 2010, pers. comm.; Smith 2010, pers. comm.; Kaller 2018, pers. comm.). The USFS (2009a, p.5) reports depredation of Louisiana pearlshells in Gray Creek, Grant Parish, to be a significant contributing factor to the decline of that local population. At that site, predation could be from otters or raccoons or both. It is possible that there is increased incidence of predation in times of low water, especially by raccoons, due to easier access to the mussels. This is suspected to have occurred in 2012 during a large predation event during a time of low water in Gray Creek on the KNF (USFS 2012, pp.3, 8). To date, this is the only documented instance of depredation on an aggregation level although it is unclear if the Louisiana pearlshells were living, injured, or recently dead at the time depredation started. However, wildlife cameras placed on site after the majority of the aggregation had been decimated showed raccoons foraging on remaining living Louisiana pearlshells.

Factor 4: Inadequacy of Existing Regulations

The existing regulations are adequate for protection of the species. The Louisiana pearlshell is federally protected as a threatened species under ESA and also under state statute (Louisiana Revised Statutes 56:1901 and 1904).

Factor 5: Other Natural or Man-made Factors

All-Terrain Vehicles

Recreational use of all-terrain vehicles (ATVs) in Louisiana pearlshell watersheds can decrease bank stability and lead to gully formation and heavy silt loading into streams, reducing instream water quality (LNHP 2009, Figure 7). An additional threat exists from direct mortality by crushing if vehicles cross streams where Louisiana pearlshell aggregations occur. In response to the impacts caused by the use of ATVs in Louisiana pearlshell habitat on the KNF, the USFS has enacted regulations that limit the use of ATVs to established trail systems (USFS 2007b, entire). However, according to recommendations given by the USFS in the 2009 Louisiana Pearlshell Mussel Survey for Grant Parish, there remains a need to establish effective enforcement of the ATV regulations. This indicates that there is still some level of associated threat, as evidenced by reported impacts from the use of ATVs on several Louisiana pearlshell streams on the KNF (USFS 2007a, p.8; 2009a; 2010, p.8). Even so, the establishment of the ATV regulation provides substantial protection of Louisiana pearlshell habitat from recreational activity on the KNF. There are no such regulations for the use of ATVs in Louisiana pearlshell habitat on private lands, which supports the vast majority of known Louisiana pearlshells.

Nuisance and Invasive Species

The Asian clam (*Corbicula fluminea*) is a freshwater bivalve that has been introduced into North America. Its prolific reproductive capability has allowed it to quickly spread its range across the continent, and the species is now almost ubiquitous throughout the range of the Louisiana pearlshell. The species is believed to compete with native mussels for resources such as food, nutrients, and space (Kraemer 1979, pp.1092, 1094). High densities of Asian clams have been

found to negatively affect the survival and growth of native mussels by disturbing and displacing young juveniles. Findings also indicate they may ingest glochidia and newly metamorphosed individuals (Strayer 1999, p.82; Yeager et al. 2000, p.255). However, typical Louisiana pearlshell streams are spring-fed with flowing water, which could lead to dispersal of Asiatic clams and prevent large congregations of this species at any particular location, likely reducing potential impacts of this invasive species on Louisiana pearlshells. Future studies may be needed to determine the potential level of threat this species has on Louisiana pearlshells. However, this invasive species has been present in Louisiana pearlshell streams since the time of monitoring, and there is no documented evidence that the presence of this species currently poses a threat to Louisiana pearlshells.

Feral hogs have been implicated in declines of other freshwater mussels through water quality degradation from hog fecal coliforms (Kaller et al. 2007, p.174), although this has not been documented in Louisiana pearlshell streams. Louisiana pearlshell streams are flowing and spring-fed in nature; thus, there is a possibility that fecal coliforms introduced by animal defecation would be quickly diluted and dispersed downstream to the Red River. Additionally, rutting by feral hogs could lead to bank instability, accelerated bank erosion, and silt loading in Louisiana pearlshell streams. Incidence of this happening in areas of Louisiana pearlshell streams is currently not well known.

Drought and Climate Change

Extreme drought or drought-like conditions can cause the drying of Louisiana pearlshell streams. In August 2011, a drought led to stream drying and local extirpation of Louisiana pearlshells in Moccasin Branch and Cress Creek (which had already lost a large number of mussels from beaver activity). Warmer temperatures and decreased precipitation are predicted in the region where Louisiana pearlshells occur as the climate changes, which would lead to increases in water temperatures; changes in runoff regimes; and increases in the frequency, duration, and intensity of droughts in the southeastern United States (Poff et al. 2002, pp. ii, 7, 10,). Droughts cause decreases in water flow and dissolved oxygen levels and increases in temperature in stream systems. Although the impacts of climate change on the Louisiana pearlshell and its habitat are not certain, exceptionally hot and dry summers could lead to drying of small streams, similar to that observed in Moccasin Branch and Cress Creek in 2011.

Climate change also has the potential to increase vulnerability of the Louisiana pearlshell to random catastrophic events or alter habitat suitability within the species' range. The climate in the southeastern United States has warmed about 1°C (about 2 °F) from a cool period in the 1960s and 1970s, and is expected to continue to rise (Carter et al. 2014, pp.398-399). Inter-annual variability in precipitation has been increasing over the last several decades, with this region exhibiting either exceptionally wet or exceptionally dry summers (Kunkel et al. 2013, p.28). Various emissions scenarios suggest that, by the end of the 21st century, average global temperatures are expected to increase 0.3 °C to 4.8 °C (0.5 °F to 8.6 °F), relative to the period 1986–2005 (Intergovernmental Panel on Climate Change (IPCC) 2014, p.10). By the end of 2100, it is virtually certain that there will be more frequent hot and fewer cold temperature extremes over most land areas on daily and seasonal timescales, and it is very likely that heat waves and extreme precipitation events will occur with a higher frequency and intensity (IPCC 2014, pp.15-16). Projections for future precipitation trends in the Southeast suggest that overall

annual precipitation will decrease, and that tropical storms will occur less frequently, but with more force (more category 4 and 5 hurricanes) than historical averages (Carter et al. 2014, p.399).

Genetics

Research indicates that some Louisiana pearlshell streams on each side of the Red River and upstream of Lake Iatt are isolated from each other (Roe 2009, p.11; Garrison et.al 2020, pp.21-22). Barriers to host fish movement have led to limited or no occurrence of genetic interchange among some streams. Genetic studies have found evidence of genetic structuring, inbreeding depression, and bottlenecks in Louisiana pearlshell populations (Roe 2009, p.11; Garrison et.al. 2020, pp.15, 18-19).

Recent research assessed genetic diversity and structure among four wild populations from across the species' range, along with the genetic diversity of a captive reared cohort (Garrison et.al 2020, pp.2, 8). All of the study sites had similarly low levels of heterogeneity and other measures of genetic diversity. Analysis of a wild fertilized but captive reared cohort displayed higher levels of genetic diversity than expected, which likely represents a case of multiple paternity (Garrison et.al 2020, pp.2, 18-19) that occurred during the natural spawning period at the native maternal site. Like earlier research, this analysis indicated genetic differentiation between Louisiana pearlshells north and south of the Red River, with the Red River being a barrier to host fish movement (Roe 2009, p.11; Garrison et al. 2020, pp.18-19). However, analysis indicated that genetic interchange among separated populations on the same side of the Red River happens on occasion when glochidia infested host fish swim between those populations during high water events (Garrison et.al 2020, pp.18-19).

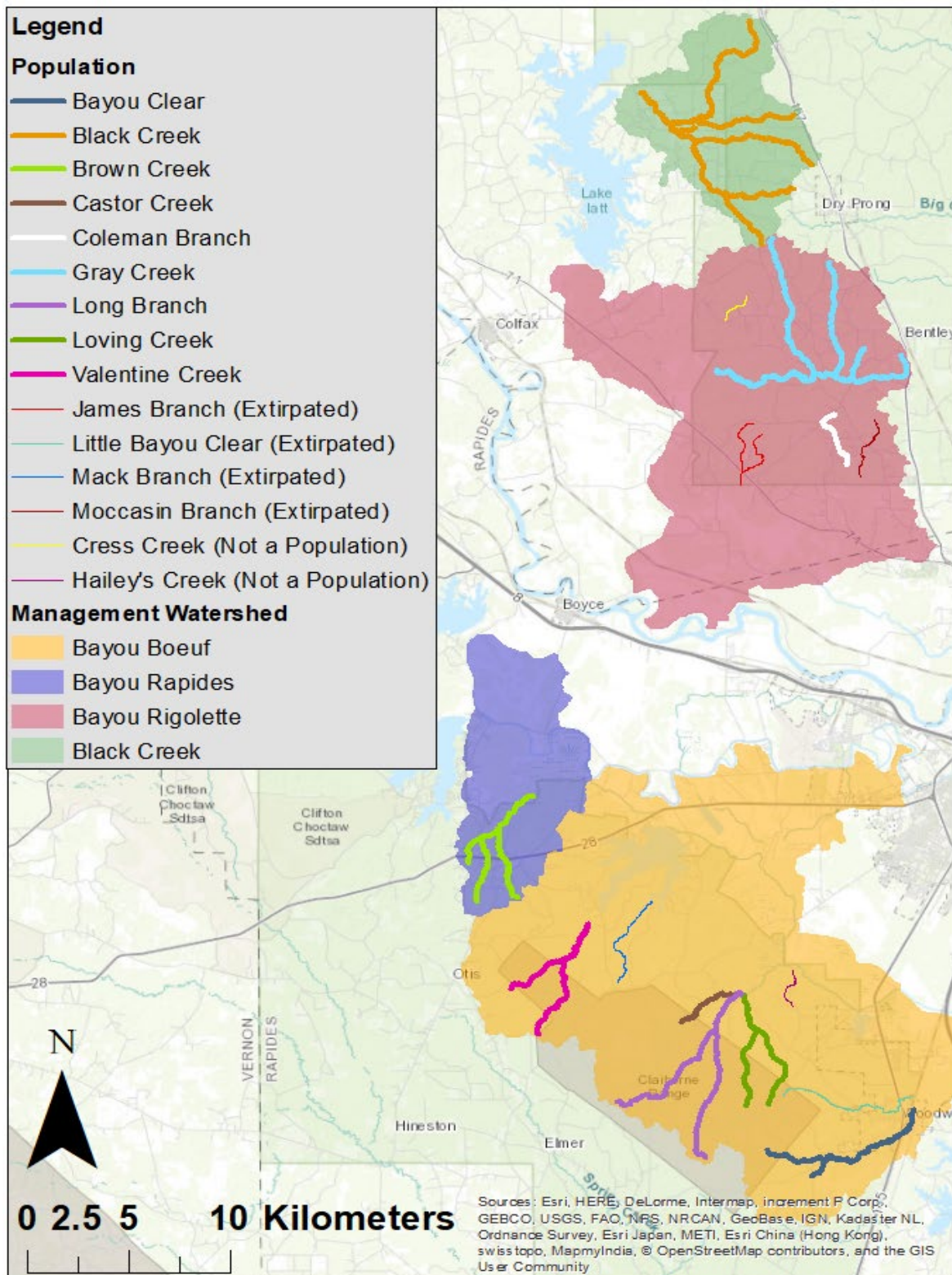
Population Trends

In total, there are 27 historically occupied streams. Currently, 19 of those remain occupied by the species. Since the time of listing and initiation of species' monitoring, eight streams across the entire range have become extirpated or functionally extirpated of Louisiana pearlshells.

The Louisiana Pearlshell Species Status Assessment (SSA, Service 2019a) analyzed range-wide monitoring data and degrees of stream connectivity to delineate populations and establish metrics to score population condition. Range-wide, the SSA delineated nine extant Louisiana pearlshell populations and four extirpated or functionally extirpated populations across the species' natural range. Populations were delineated in the SSA by grouping together mussel streams that shared the highest level of connectivity. All populations delineated as extant were required to support one or more aggregations of at least 100 mussels. Figure 2 below, taken from the SSA (Service 2019a, Figure 9, p.34), depicts current populations using the thicker lines. Thinner lines represent (1) populations that have been extirpated or functionally extirpated (both labeled as "extirpated") and (2) currently or formerly occupied locations that never contained aggregations of Louisiana pearlshells since monitoring began (labelled as "not a population"). The population naming convention in the SSA reflects the name of the stream considered to be most essential to the population. As shown in Figure 2 below, the nine extant populations were named the following: Bayou Clear, Black Creek, Brown Creek, Castor Creek, Coleman Branch, Gray Creek, Long Branch, Loving Creek, and Valentine Creek. Of these, Black Creek, Coleman Creek, and Gray Creek populations are in Grant Parish (north of the Red River); and Bayou

Clear, Brown Creek, Castor Creek, Long Branch, Loving Creek, and Valentine Creek populations are in Rapides Parish (south of the Red River). Additionally, Figure 2 shows the four delineated populations that are extirpated or functionally extirpated, which are James Branch, Little Bayou Clear, Mack Branch, and Moccasin Branch. James Branch and Moccasin Branch are in Grant Parish, and Little Bayou Clear and Mack Branch are in Rapides Parish. Lastly, Cress Creek and Hailey's Creek are shown as locations that have supported Louisiana pearlshells but never at an aggregation level; thus, neither are considered an extant or an extirpated population.

Figure 2. Louisiana Pearlshell Populations.



Between the two management watersheds south of the Red River in Rapides Parish, there are 13 historic Louisiana pearlshell streams, of which 10 are currently occupied. The 13 Louisiana pearlshell streams in Rapides Parish are Patterson Branch, Brown Creek, and Burney Branch of the Bayou Rapides management watershed; of which Burney Branch has been extirpated of mussels (see Appendix A, Figure A1 in Service 2019a for map); and Little Loving Creek, Loving Creek, Little Brushy Creek, Long Branch, Castor Creek, Hailey's Creek, Little Bayou Clear, Bayou Clear, Mack Branch, and Valentine Creek in the Bayou Boeuf management watershed; of which Little Bayou Clear has been functionally extirpated and Mack Branch has been extirpated of mussels (see Appendix A, Figure A2 in Service 2019a for map).

Between the two management watersheds in Grant Parish, there are 14 historic Louisiana pearlshell streams, of which nine are currently occupied. The 14 Louisiana pearlshell streams in Grant Parish are Cypress Creek, Swafford Creek, Beaver Creek, Gladly Hollow, Black Creek, and Clear Branch in the Black Creek management watershed; of which Clear Branch has been extirpated (see Appendix A, Figure A3 in Service 2019a for map); and Coleman Branch, Chandler Creek, Jordan Creek, Gray Creek, James Branch, Moccasin Branch, Cress Creek, and Hudson Creek in the Bayou Rigolette management watershed; of which James Branch, Moccasin Branch, Cress Creek, and Hudson Creek have been extirpated (see Appendix A, Figure A4 in Service 2019a for map).

Using an analysis detailed in Table 12, Figure 13, and Appendix C of the SSA; the current resiliency values of populations were scored, and depending on the score, the current resiliency of populations was categorized as "good, moderate, or poor". The current resiliency of five populations was categorized as "good", the current resiliency of three populations was categorized as "moderate", and the current resiliency of one population was categorized as "poor". The SSA indicated that Bayou Clear, Long Branch, and Loving Creek populations in the Bayou Boeuf management watershed; Gray Creek population in the Bayou Rigolette management watershed; and Black Creek population in the Black Creek management watershed have good resiliency values. Castor Creek and Valentine Creek populations in the Bayou Boeuf management watershed and Brown Creek population in the Bayou Rapides management watershed have moderate resiliency values; and Coleman Branch population of the Bayou Rigolette management watershed has a poor resiliency value. Information regarding these resiliency values is presented in tabular form in Appendix C of the SSA. To be noted, the SSA indicated that Coleman Branch was the only population where no evidence of recruitment has been documented. Population condition and evidence of recruitment could prove useful in locating release sites that would most benefit recovery.

CAPTIVE PRODUCING AND HEAD-STARTING JUVENILES

Purpose of Program

The primary purposes of Louisiana pearlshell captive production program are to produce individuals (1) for reintroduction to conserve the species' genetic diversity, which has already been affected by processes such as population bottlenecks and inbreeding depression; (2) to repopulate areas of local extirpations; and (3) to increase range-wide species' distribution to alleviate population and species' level risks from threats. Captive production and head-starting juveniles for release into unoccupied, suitable areas in the species' natural range is intended to offset local extirpations and population declines, to prevent the Louisiana pearlshell from becoming endangered, and to help achieve recovery of the species.

Role in Recovery

The Louisiana pearlshell is listed as a threatened species under the ESA, which means that it is "likely to become an endangered species within the foreseeable future throughout all or a significant portion of its range". Louisiana pearlshell reintroduction efforts are intended to reestablish and create new aggregations that become breeding units, provide genetic maintenance or enhancement for populations, and reduce the potential of endangerment by spreading out the species across its natural range to decrease potential impacts of localized events. As shown through preliminary analysis of monitoring data and observed documentation of changes in populations; barriers to species' expansion, high levels of genetic inbreeding, range-wide local extirpations, and population declines are impacting the species. No reversal of these impacts or future positive trajectory are expected without intervention. In light of current and potential threats and species' level impacts; habitat restoration and protection, in combination with captive production and release of head-started juveniles, are needed to reverse species' and genetic declines and increase species' redundancy and resiliency across the landscape. Successful reintroductions are intended to help achieve recovery criteria described in the Revised Recovery Plan (Service 2019b, pp.5-6) and move the Louisiana pearlshell closer to a recovery threshold and potential delisting. The Revised Recovery Plan lists the following three recovery criteria, which specify at what point species' recovery should be assessed:

- 1) At least six populations exhibit a stable or increasing trend, as evidenced by natural recruitment and multiple age classes;
- 2) At least one population occurs in each of the following management watersheds: Bayou Boeuf, Bayou Rapides, Bayou Rigolette, and Black Bayou; and
- 3) Threats have been addressed and/or managed to the extent that the species will remain viable into the foreseeable future.

The first two criteria in the recovery plan speak to the status of populations at the time of species' recovery. These criteria specify the number of resilient populations and what proportion of the range must be occupied for species' recovery. The third criterion addresses minimizing or eliminating threats and increasing habitat quality, distribution, and availability. Providing an adequate amount of quality habitat is vital to achieve species' recovery and is an important first step before reintroducing the species into a site.

Populations have degrees of isolation, mostly due to anthropogenic influences. The recovery

criteria assume genetic variation exists between populations within management watersheds where there is adequate connectivity for host fish movement between populations (Service 2019b, pp.5-6). To retain or improve genetic integrity of populations, gene flow must occur, either naturally or with assistance from humans through reintroduction efforts. Additionally, reintroduction will increase the species' ability to repopulate and establish new Louisiana pearlshell aggregations in key areas, which is important for increasing population connectivity and maintaining and expanding the species' distribution to increase probability of range-wide persistence.

The primary goal of Louisiana pearlshell conservation actions is to ameliorate threats to such a level that the species' no longer needs ESA protection. However, application of current conservation methods have not resulted in corresponding population increases. Rather, since monitoring began in the 1980's; population, species', and genetic declines have been detected. In fact, Louisiana pearlshells have been completely extirpated from eight former streams encompassing four former populations, and species' literature documents processes such as population bottlenecks (Roe 2009, p.11; Garrison et.al. 2020, pp.15, 18-19). We believe these declines and losses are likely to continue to impact the species at higher levels into the future without intervention. Like with many other endemic species that occupy a narrow, specialized niche; the restricted distribution of the Louisiana pearlshell increases species' vulnerability to catastrophes and the potential for aggregation, stream, and population level impacts; diminishes the ability for natural population expansion and recolonization of formerly occupied areas; increases genetic risk; and exacerbates other inherent limitations and anthropogenic impacts posed by the species being a habitat specialist within a narrow range. We believe population and species' vulnerability will be alleviated by implementation of a successful reintroduction program that establishes new aggregations in key areas.

An additional benefit of the captive production, head-starting, and reintroduction program is the opportunity for research that will increase our knowledge of aspects important to Louisiana pearlshell conservation. The following are examples of additional research opportunities that could provide needed information:

- Continue host fish trials to determine if there are other natural host fish species and monitor status of host fish accordingly;
- Document the number of Louisiana pearlshells, average density, and demography found in effective reintroduced aggregations to better define what constitutes a mussel bed;
- Document the age of sexual maturity;
- Document habitat characteristics that are present in areas where reintroductions are most successful; and
- Determine general success and utility of the reintroduction program to inform future recovery actions.

Louisiana Pearlshells in Controlled Captivity

The NNFH began reproductive studies in 2010 and host fish trials in 2011, using several different fish species that were collocated in streams with Louisiana pearlshells (Service 2010, entire; Service 2011, entire). For this research, gravid Louisiana pearlshells were taken from the wild, and their released glochidia were placed into a controlled aquatic environment with

potential host fish. Once the maternal Louisiana pearlshell released glochidia, she was tagged and returned to her natal location.

In April 2016, the NNFH successfully infested and transformed Louisiana pearlshell glochidia from a wild, gravid females using grass pickerel (*Esox americanus vermiculatus*) as the host, resulting in production of approximately 9,000 juvenile stage animals from a hatchery infested fish (Service 2016, p.3). The NNFH studies were conducted to inform unknown aspects of Louisiana pearlshell biology and to facilitate development of methodology to produce and rear captive juveniles to develop a captive production and reintroduction release program for the species. However, at the time the first juveniles were produced in 2016, the NNFH was unequipped to rear the animals, especially given the large number of juveniles produced from one infested fish. To facilitate the chances of survival, over 5,000 transformed juveniles were moved to the AABC, which was better equipped for rearing mussels (Service 2016, p.3). The AABC reports 94 remaining Louisiana pearlshells produced as a product of the NNFH's 2016 research (Fobian et al. 2019, p.5). The remaining survivors are candidates for reintroduction into the species' range in Louisiana.

Once host fish trials found the grass pickerel to be a suitable host in captivity, the NNFH continued research to develop and improve captive production and develop methods to successfully rear juvenile Louisiana pearlshells at their facility. Since the first successful transformation of Louisiana pearlshells in 2016, the NNFH has continued research to improve in-house methods and capabilities, which has led to successful production and rearing of juvenile Louisiana pearlshells (Service 2016, entire; Service 2018a, entire).

Techniques

Captive Production and Head-Starting

The NNFH staff first developed methods for captive production of the Louisiana pearlshell in 2016, which have been improving through research (Service 2016, entire; 2018a, entire). Beginning in February, NNFH staff visited potential source aggregations to examine the gills of Louisiana pearlshell for signs of gravidity using a non-damaging, widely-accepted method for examining the internal structure of mussels. Gravid females are temporarily removed from the native location and brought into controlled captivity at the NNFH to supply glochidia for captive production. After glochidia are naturally released by females, the glochidia are put into controlled containment with grass pickerel (which serves as a host fish in captivity), where they encapsulate in the host fish and metamorphose from larval to juvenile stage. Production of juveniles involves fertilization in the wild; however, all other aspects of the reproductive cycle leading to the production of juvenile mussels are manipulated in a controlled, captive environment (e.g., glochidial release, number of glochida and amount of water per fish, introduction and infestation of a suitable host fish, and transformation from glochidia to juvenile mussels). Under this situation, one female is capable of producing thousands of juveniles in controlled captivity from one reproductive event, which is much higher than what would be produced naturally. Methods of infestation help prevent glochidiosis, which happens in captive environments by over-infestation of one fish and usually leads to fish death and corresponding loss of encysted glochidia. Glochidiosis was noted in the 2016 Louisiana pearlshell host fish studies before methods to reduce glochidia to fish ratio were implemented at NNFH. Natural

factors allow for fertilization of the female brood stock; however, natural glochidial infestation on the host fish, survival of the encysted fish throughout the phase of glochidial metamorphosis into juvenile, and juvenile drop off from the fish in habitat conducive for survival must all be met for a female to have reproductive success in the wild in any given year.

As quickly as feasible following glochidial release, the maternal mussels are marked and transported back to their capture location and repositioned naturally into the substrate near the periphery of the mussel aggregation to limit disturbance to the other mussels. Wild stock, hatchery born Louisiana pearlshell juveniles are successfully produced and reared in high numbers in controlled captivity. Captive production and rearing of juveniles for two to three years (head-starting) decrease the chances of mortality associated with the two most vulnerable life stages that experience poor survival in the wild (i.e., the glochidial stage and the early juvenile stage). Some captive inoculations of host fish may require more than one female to reach the number of glochidia needed, in which case progeny would not be directly traced back to a single female. However, when glochidia from multiple females are used for inoculation, the females are taken from the same source aggregation.

Captive production efforts should be based on sound scientific principles to conserve genetic variation and species' integrity. The genetic identity of source females used in all captive production efforts will be recorded. Unique identifiers on the females allows post-release monitoring and precludes the repeated collection of a single specimen to prevent additional genetic contribution from a single female. Likewise, efforts will be made to verify and record the aggregation-level parentage of captive produced Louisiana pearlshells. Prior to release, juveniles will be uniquely marked for individual identification and to identify the lineage. Marking and identification methods are decided at the discretion of the NNFH staff, in coordination with the Louisiana Ecological Services Office (LESO), and the methods used will be included in the Site Plan (see section below). The identifiers must be useful to researchers, but inconspicuous to ensure that mussels can maintain their natural camouflage in the wild. Other records will be maintained, as practicable, to document and track additional pertinent information important to program objectives and management. The NNFH will subject juvenile mussels to an acclimation period before release to help ensure that juveniles adjust to the wild. As determined by the NNFH staff; methods for captive production, head-starting, and release may change over time to allow for improvements in feasibility and success. Proposed changes will be discussed with the species' lead at the LESO before changes are implemented.

Disposition of Excess Progeny

Captive production efforts may result in Louisiana pearlshells in excess of needs for conducting reintroduction activities. This may occur due to the following instances: (1) there are individuals found to be unfit for release into the wild, (2) there are surplus individuals such as when captive production produces more than required for program needs or the program is concluded but individuals remain in captivity; (3) there are no reintroduction sites suitable at a given time due to insufficient habitat or genetic considerations; or (4) there are individuals in surplus of what is needed to meet the recovery objectives of the program.

In the above instances, the NNFH may coordinate with the Service's Louisiana pearlshell recovery lead at the LESO to determine the best course of action for the disposition of excess

Louisiana pearlshells. Some options may include (1) using unfit individuals for outreach and public education; (2) transferring individuals to other Service-approved facilities or individuals for display, research, or other approved purposes; (3) holding surplus individuals in captivity for observation of morphological and behavioral information; (4) using surplus mussels for emergency repatriation following a catastrophic loss; and (5) euthanizing individuals to further species' research or when there may be a chance of disease, parasites, or other suspected abnormality.

Precautions

The NNFH staff will take all known precautions to prohibit the potential introduction or spread of diseases and parasites into controlled environments or suitable habitat. At this time, there is no established screening protocol for disease in Louisiana pearlshells. However, some of the measures necessary to guard against the potential of disease and parasite transmission are proper containment in clean vessels during holding at the facility and during transport; proper upkeep of equipment; and assuring water quality conditions at the facility are appropriate and free of known pathogens, invasive species, and parasites. Additionally, NNFH will not use unfit Louisiana pearlshells in reintroduction efforts whose growth patterns, morphology, or other observable or testable condition is not similar to that of other captive cohorts, which could be an indicator of health. If a reliable disease screening process is developed, it will be implemented prior to reintroductions.

Additionally, NNFH staff will take all known precautions to prevent the escape or accidental introduction of individuals outside their natural range. However, this does not present a realistic concern for the species because Louisiana pearlshells will be reintroduced within areas of the species' natural range. Limited mobility in freshwater mussels precludes movement of juvenile and adult mussels outside of the species' range. If host fish infested with next-generation glochidia (i.e., those from generations subsequent to reintroduction efforts but from reintroduction lineages) swim outside of the Louisiana pearlshell range, encysted glochida would not survive juvenile stage because the habitat would be unsuitable for species' survival.

REINTRODUCING JUVENILES

The terms "reintroduction" and "release" are used herein to refer to the human introduction of captive produced and head-started Louisiana pearlshell juveniles that are transported and placed into the wild within unoccupied, suitable areas of the species' natural range. The NNFH, in cooperation with applicable partners, will reintroduce Louisiana pearlshells into unoccupied sites that were historically inhabited by Louisiana pearlshells, as well as those sites with no prior records of species' occurrence. Whether target areas are former Louisiana pearlshell sites or sites that have no records of species occurrence, releases will be conducted to do the following:

- Maintain or increase current levels of genetic diversity by appropriate selection of donor populations and brood stock in accordance with genetic research for the Louisiana pearlshell;
- Increase the number of released individuals from different cohorts through repeated reintroductions into target areas to maximize the overall chances of survival and reproductive success; and

- Establish an effective aggregation size and demographic structure to facilitate successful reproduction and recruitment of viable offspring.

Decisions for reintroduction efforts will be made at the discretion of the Service, the LDWF, and applicable land management agency or landowner in consideration of the (1) need, based on meeting recovery goals; (2) the available amount and the quality of the habitat into which individuals are being placed; (3) risk of disease transmission; (4) genetic concerns; and (5) success of prior reintroductions.

Site Selection

Because the threats of habitat loss, alteration, and fragmentation are thought to be the most consequential factors in the Louisiana pearlshell's decline, it is essential that any releases of captive animals for reintroduction purposes occur only where habitat quality and amount is sufficient to sustain those mussel releases, and a long-term commitment has been made by the landowners to provide ongoing management activities to maintain habitat and participate in Louisiana pearlshell conservation. Sites for reintroduction activities will be selected based on criteria including the historical and current distribution of the species; habitat conditions; and past, present, or future threats. Primary habitat considerations include land use, presence of suitable riparian area, and adequate water quality at the site and upstream of the site. Priority will be given to release sites with quality habitats where there is room for nearby expansion, and threats facing the species have been eliminated or ameliorated to below thresholds of significant impact; a commitment for long-term access and habitat protection is expected; where the location of the site provides a benefit to the species' distribution and recovery; and where the source stock and the release site best match the highest priority genetic considerations. Additional considerations include selection of sites where there is minimal probability of considerable pollution, manmade disaster; or exposure to exotic or introduced predators, competitors, or diseases (AZA 1992).

In line with the recovery goals for this species, locations unable to potentially support 100 or more mussels should not be considered as candidate release sites. Release sites will be selected based on consideration of genetic management and location specific recommendations (Garrison et al. 2020, entire; Frater et al. 2019, p.6), including (1) analysis of historic, current, and desired future distribution of the species within the natural range; (2) status of current on-site habitat conditions; (3) analysis of known or expected current or future threats; and (4) the ability for long-term, on-site habitat protection, habitat management, and access to the site for release and monitoring activities. All reintroductions within a particular drainage will be focused on limited sites until conditions best suited for survival are verified (Frater et al. 2019, p.6) to reduce potential for future reintroductions into inadequate release sites and to provide valuable information for future site selection. Also, reintroducing juveniles over several years at a given location will mimic a more natural demographic situation by allowing for various age classes and more genetic diversity, while increasing number of Louisiana pearlshells at the site to an adequate number to exhibit natural reproductive behavior and produce viable offspring.

Areas where local extirpations have occurred will be considered candidate reintroduction sites only when the source of the impact has been identified and ameliorated through habitat management or other threat management actions. Additionally, sites within apparently suitable,

protected habitat within the natural range will be considered candidate release sites even in the absence of historic records, but only when adequate habitat and threat management mechanisms are in place. Both types of site selection will create opportunity for population expansion and increased species' resiliency.

Genetic impacts to the free-ranging populations must be considered when selecting sites for reintroduction. Because fertilization between wild, brood stock females and males occurs in the natural habitat before captive production, the genetic makeup of captive produced individuals for reintroduction is a match to the donor aggregation and free-ranging population from where the gravid female was captured. In keeping with the species' specific genetic management recommendations, all reintroductions will occur in suitable habitat areas where Louisiana pearlshells are absent but as close as possible to the maternal stock location. Augmentation of existing aggregations may be detrimental to the integrity of the species' genetics (Garrison et al., p.24) and is not a method that will be used for the Louisiana pearlshell. Additionally, for the same reason, reintroductions will not occur on the opposite side of the Red River from the donor population.

Site Plan

Reintroductions will occur after a Site Plan specific to the release location is finalized. The Site Plan will provide details about location of the brood stock relative to release location of head-started juveniles, using the principles of genetic management and goals for aggregation restoration in site selection.

A draft site-specific plan for reintroduction and release will be developed by the NNFH in coordination with federal and state agencies and the appropriate land management agency or landowner. If habitat managers are willing private landowners, part of another agency, or non-governmental organizations; they will also be included in the planning process and provided with the approved Site Plan prior to relocating or releasing animals into the wild. All recovery partners, and any other potentially affected private or public entities identified by the partners, will be notified of planned reintroduction activities. Under provisions of NEPA, the USFS has concluded a programmatic Environmental Assessment for the reintroductions on their lands, thereby notifying the public and addressing public comment regarding reintroduction of the Louisiana pearlshell on the KNF in Louisiana pearlshell management watersheds.

Site Plans should be designed to ensure adequate survival rates, to avoid genetic bottlenecks or swamping, and to maximize species' distribution within the range. In addition, they should address site-specific habitat management and conservation actions needed to support potential reintroduction activities. The final draft of the Site Plan will be distributed to the collaborating agencies for review at least 20 days prior to reintroductions (Frater et al. 2019, p.5), barring extenuating circumstances that may lead to the NNFH requesting a shortened review time. Final Site Plans must be approved by the LESO, in collaboration with LDWF, prior to any reintroduction effort that involves the release of captive produced Louisiana pearlshells into the wild. Given the understanding that collection of gravid females, successful production and number of juveniles, and availability of suitable reintroduction sites in a given year are difficult to predict, Site Plans will include as much information as possible (Frater et al., p.6). The following lists the type of information that should be included in the site plan, when feasible:

- Cooperating and responsible partners and the respective roles in planned activities and monitoring;
- Location of the maternal stock (e.g., stream name, management watershed, GPS coordinates, and map), including landowner name and location information; such as property address and directions (any non-publicly available information will be privately secured and maintained by the LDWF);
- Number of females removed from site and protocols used; such as checking for gravidity, method of production, tagging or use of other identifier; and how mussels will be transported;
- Location of the release sites (e.g., stream name, management watershed, GPS coordinates, and map), including landowner name and location information; such as property address and directions (any non-publicly available information will be privately secured and maintained by the LDWF);
- Spatial and population level relationship of the reintroduction site to extant locations, in keeping with the guidelines for genetic management;
- How reintroduction at the planned location benefits species' conservation, in keeping with the recovery plan;
- Status of the Louisiana pearlshell at the reintroduction site; for example, extirpated, never known to be present, and number of individuals present from previous reintroductions;
- Number of and cohort of juveniles for the planned reintroduction event;
- Method of health and disease screening of mussels for release;
- Release protocols, such as methods for pre-release conditioning, type and method of tagging or other identifier, and how mussels will be transported;
- Current habitat conditions and site-specific habitat management actions;
- Potentially limiting factors at the reintroduction site; including current or future threats; and how limiting factors and threats, if any, will be addressed;
- Beaver control and predator management actions, if necessary;
- Other pertinent information as identified by participants and partners;
- An alternatives analysis, in keeping with the internal control requirements of the federal fish hatchery system;
- All legal and appropriate documents including necessary State and Federal permits; ESA consultations; certification, based on reasonable certainty, that captive produced progeny are pathogen-free at the time of release into the wild; written landowner permission for Louisiana pearlshell releases and access to property for post-release monitoring (may be in LDWF files for private landowners); and landowner agreement to on-site species' and habitat conservation. Additional documents not mentioned here may be required, as deemed necessary. The Site Plan does not have to include copies of these documents but rather acknowledge these documents are present and current.
- By reference, site plans will incorporate the monitoring protocols of this document and identify the means, roles, and responsibilities for which monitoring will be implemented. Site Plans may use information from previous monitoring activities to inform new or modified actions, as necessary

IMPLEMENTATION GUIDANCE

Initial reintroductions of Louisiana pearlshells will be limited to no more than five streams on each side of the Red River for at least three years. Fewer sites will address potential limitations on the availability of good habitat, will increase numbers of individuals at a particular location over time, and will allow for focused monitoring to determine species' persistence at release sites. After the initial reintroduction of Louisiana pearlshells at a site, post-release monitoring will be conducted to evaluate persistence of reintroduced mussels and determine if releases at a particular site will continue in following years, as specified above.

Genetics Management Guidelines

Genetic management is informed by genetic findings and recommendations stemming from research and the best available science (Roe 2009, entire; Garrison et al. 2020, entire). The following guidelines are based on specific genetic management recommendations that produce genetically diverse Louisiana pearlshell juveniles from a small number of wild caught females, which is enhanced by evidence of multiple paternity (Garrison et al. 2002, pp.25-26).

- Only wild caught, gravid females will be used in captive production efforts.
- Individual females will be used to propagate juveniles only once to guard against inbreeding effects.
- Reintroduction of juveniles from multiple females per release site per year is encouraged.
- Wild caught maternal females should come from the area closest to the reintroduction area.
- Head-started juveniles resulting from wild gravid females native to one side of the Red River will be reintroduced into an area on the same side of the Red River to guard against outbreeding effects.
- As based on geographic proximity of reintroduction locations to the maternal capture location, the following guidelines apply in order of preference:
 - 1) In an unoccupied area of the same population, closest to the maternal capture aggregation;
 - 2) In an unoccupied area of the same management watershed with the greatest degree of connectivity to the donor population, closest to the maternal capture aggregation; and
 - 3) In an unoccupied area of a different management watershed on the same side of the Red River (i.e., same Parish), closest to the maternal capture aggregation.
- In addition, the following restrictions apply:
 - Do not develop a captive brood stock or breed Louisiana pearlshells in captivity for any purpose.
 - Do not use an individual maternal female for more than one captive production event.
 - Do not reintroduce captive progeny onto the opposite side of the Red River from the maternal capture aggregation (i.e., do not cross Parishes in reintroduction efforts).
 - Do not conduct captive production for the purpose of augmentation of an existing aggregation.

Aggregation Restoration Guidelines

The following guidelines are consistent with the genetic management guidelines above and the recovery strategy for the species in accordance with the Revised Recovery Plan (Service 2019b, entire).

- Aggregation restoration efforts will only take place within the Louisiana pearlshell's known natural range and at release sites where the species is currently absent.
- The habitat at the candidate release site must be suitable and under long-term protection. Human activities that may lead to negative impacts to the reintroduction must be identified and discontinued.
- Produced Louisiana pearlshells will only be released into the wild as long as these efforts will not reduce the survivability of existing wild Louisiana pearlshell aggregations.
- There will be minimal risk of disease from population restoration efforts. Louisiana pearlshells are not known to carry or transmit disease. However, if the presence of disease is identified in this or similar freshwater mussel species, or if there is reason to suspect disease; the reintroduction efforts will cease, and the NNFH and other partners will discuss with the species' lead at the LESO whether future reintroductions are advisable. If it is determined that captive production and reintroduction should continue, measures will be developed for rigorous health screening by qualified veterinarians or other experts in animal health and disease transmission and implemented before reinitiating the program.
- There will be minimal disturbance at the relocation site from reintroduction and monitoring activities, especially in the presence of formerly reintroduced Louisiana pearlshells. A limited number of trained, qualified people authorized for the activity will be involved to minimize disturbances to the species and its habitat during release and monitoring activities.
- Aggregation restoration efforts will focus on establishing aggregations of size, demographic, and genetic structure necessary for natural reproduction and recruitment.
- Aggregation restoration efforts will focus on no more than five of the best sites on each side of the Red River to reduce risk from local threats and attempt to establish aggregations of at least 100 mussels. Introduced aggregations of 100 or mussels showing persistence as established herein will count toward the number of potential reproductive units and be included in the data to be analyzed for species' recovery.
- Habitat and survey protocols established herein will be followed to identify and address location specific threats and determine persistence and occurrence of reproduction.
- All planning, reporting, and other specific requirements will be met in accordance with this plan and provisions of the ESA.

MONITORING AND MEASURING SUCCESS

Survey Methods

There will be a collaboration with the USFS, NNFH, LDWF, and all landowners for post-reintroduction monitoring efforts. Routine monitoring of the reintroductions is critical to determine survival rates and to look for and address any new habitat or other threats that may be present in the area.

Methods for Louisiana pearlshell monitoring and population assessment use quantitative sampling techniques, as described in the *Report Determining Population Trends for the Louisiana Pearlshell Mussel and Development of a Long- Term Monitoring Protocol* (QES 2014). This QES report was coordinated and funded by LDWF; in cooperation with the NNFH, USFS, and LESO; to establish a sound, range-wide standard monitoring protocol. It was founded on the general principles described in the *Guide to Sampling Freshwater Mussels* (Strayer and Smith 2003, entire), which is a practitioner's guide that uses knowledge of statistical principles and mussel biology to assist in the development of methods for assessing freshwater mussel populations. The standard monitoring protocol is currently used range-wide for Louisiana pearlshell surveys on the KNF and private land to collect sufficient data to conduct population and species' level analyses for estimating trends and informing future recovery decisions.

The minimum number of Louisiana pearlshells in an aggregation that are biologically required for successful reproduction is poorly understood. Currently, monitoring data show that 90 percent of Louisiana pearlshells occur in aggregations of 100 or more individuals (QES 2014, Appendix G). Thus, for purposes of species' management and standardization of range-wide data for sound comparison, only aggregations of 100 or more mussels are counted toward a self-sustaining population, as per the standard monitoring protocol, although the presence of non-aggregating mussels are also documented.

Release efforts will focus on the best sites as much as practicable to establish aggregations of at least 100 mussels. Persistence of introduced aggregations of 100 or more mussels may be representative of a potential breeding unit and will be included in the data analysis for population trends. Monitoring of release sites will follow the same standard protocol as those developed for aggregations across the species' range but at an increased frequency initially to determine annual persistence. This methodology is explained in greater detail below.

Verification of Persistence

By the time of release, head-started juveniles should be visible at release. Monitoring for persistence will generally not require handling of individuals. However, removal of living mussels by authorized individuals is acceptable when required to gather additional information, as needed. Dead study specimens will be documented and identified, when possible, and may be collected by authorized individuals and subject to the disposition methods explained in this plan.

After initial release, there will be site monitoring at mid-year and again at twelve months post-release. After verification of persistence at the 12-month post-release monitoring effort, different female brooders will be used for captive production of juveniles for multiple year releases at the same release site. Only when the site remains suitable and monitoring is positive for on-site persistence at the 12-month post-release monitoring event, annual reintroductions and post-release monitoring should continue at the same release site for at least two additional years, totaling at least three consecutive years of reintroductions per site (i.e., the initial release year and at least two subsequent release years), whenever feasible. During the site specific monitoring efforts following the three annual releases, presence of reintroduced mussels must be documented at the 12-month, 24-month, and 36-month effort for a particular site to be a candidate for a fourth consecutive year of reintroduction, if applicable. After following these

guidelines to verify persistence, monitoring of release sites can coincide with routine population monitoring efforts already in place for USFS and LDWF surveys. Reintroduction at a particular release site will discontinue if the finding of post-release monitoring is negative for Louisiana pearlshell or if future survival is reduced or precluded by new threats or habitat conditions. If an alternate site is available, the Site Plan will be modified accordingly. Also, if persistence of reintroduced Louisiana pearlshells is negative at all locations after five years of implementation, consideration should be given to discontinuation of the program. New data obtained through the successes and failures will be considered annually during the annual review process and will be used to assist in our five-year evaluation to decide whether to continue or discontinue the program and to allow for other appropriate modifications to program objectives, on a frequency as needed to increase probability of success.

After three years of reintroductions and three subsequent years of verified persistence at a given release site, future reintroductions will be conducted as determined by the availability of protected suitable habitat, the availability of juveniles from appropriate maternal brood stock, and the potential contribution toward recovery, as follows:

- Reintroduction may continue at the same location,
- Reintroduction may expand longitudinally upstream and downstream of the original release location; and/or
- Reintroduction may occur in new locations.

This type of focused release effort and intense monitoring of site-specific persistence is intended to measure overall success of the program and reveal which locations are better suited for implementing a wider, sustained effort to facilitate establishment of new aggregations of 100 or more mussels.

Verification of Reproductive Success

The reproductive age of Louisiana pearlshells has not been the focus of research or well documented in the literature. A study of the shells of naturally dead Louisiana pearlshell specimens used annuli to estimate growth rates and predict a correlation between growth rate and age of maturity, which was estimated to be five years (Daniel and Brown 2012, p.26). The reproductive age of other closely related species are reported to take up to 12 years to reach sexual maturity (McMahon 1991, pp.342, 345). Some species' experts expect that the likely age of reproduction for the Louisiana pearlshell may be in the range of 10-15 years (Johnson 2020, pers. comm). Based on consideration of these data and expert opinion, it is possible that female gravidity may be observed at release sites as early as five years post-release (i.e., at seven to eight years old).

Because Louisiana pearlshells are long-lived animals, there is not sufficient time to estimate reproductive age or generate data needed to establish an accurate metric for expected reproductive success prior to our reintroduction efforts. As is the case for many other long-lived species that exhibit delayed reproduction; empirical data on this aspect of the reproductive phase will not be available until years after implementation of the captive production, reintroduction, and long-term monitoring efforts. Field observation of wild born progeny may be possible in a time no shorter than 24 months following verification of gravidity. However, female gravidity is

the most accurate measure of reproductive success because the life cycle and specific biological and habitat related-needs for successful production of juvenile mussels are complex and confounded by many external factors (such as infection rate of host fish and the movement of host fish from the point where they became infected by glochidia to the point where glochidia drop off, for example). Factors such as these will likely have similar influence on breeding and juvenile recruitment in reintroduced aggregations to that of comparable aggregations that are naturally formed.

RECOVERY MILESTONES

Aggregations of 100 or more mussels will be more resistant to stochastic stressors than smaller ones, and the chances of reproductive success will increase as the aggregation size increases. All of these factors will contribute to increased species' resiliency and will be considered in context to contributions to Louisiana pearlshell recovery. The ultimate measure of success is establishment of one or more self-sustaining, reproductive aggregations in populations suffering high degrees of isolation to protect genetic integrity and to increase species' distribution within a population and across its natural range. Reintroduced aggregations of 100 or more mussels that have persisted for three or more years will be the success parameter used when determining to include the monitoring data from reintroduced aggregations with the range-wide species' monitoring data. Inclusion of these monitoring data will show the benefit of successful reintroduction effort to population status by generating a more stable population trend estimate during analysis. Additionally, documentation of gravidity in reintroduced aggregations will be the parameter used to show an overall increased reproductive capacity of the recipient population. Both of these milestones will directly contribute toward the achievement of recovery criteria (Service 2019b, pp.5-6) to assist in species' recovery.

ANNUAL REPORT

The NNFH will provide an annual report on the status of program activities to the Louisiana pearlshell lead at the LESO by September 30th. Depending on the applicability and the availability of information, examples of the information that should be included in the annual report include the following:

- Itemized expenditures for the past fiscal year associated with the program, including those of partnering agencies (e.g., equipment, estimated staff time, costs of travel, etc. separated out by agency, when possible);
- List of cooperators and respective roles;
- Description of methodology for captive production and reintroduction at the sites;
- Information on the fulfillment or deviations from the Site Plan and explanations for deviations;
- Information on donor populations and the recipient locations; including things such as the following, when applicable:
 - Why the locations were chosen and how the maternal capture location relates to the reintroduction area;
 - Recipient site habitat description;

- Description of the former and current status of Louisiana pearlshell occupation at the recipient site (i.e., extirpated, never present, prior reintroduced); including the status of prior on-site reintroductions;
 - Reasons Louisiana pearlshells were formerly extirpated from the planned reintroduction site and how the threat has been addressed, and
 - Description of additional improvements in habitat and other measures implemented or needed;
- Methods and results of post-release monitoring;
 - Information on disease, predation, parasitism, etc.;
 - Information on disposition of surplus individuals;
 - Information on mortality and disposition of research specimens;
 - Prospects for and obstacles to achieving research, captive production, or reintroduction objectives; and
 - Additional information not included here that is later identified as a reporting requirement.

ANNUAL PLAN REVIEW

This plan is a working document that is subject to modification based on results of current and future research, monitoring, and recovery activities involving Louisiana pearlshell captive production, head-starting, and reintroduction. Recovery partners are encouraged to communicate with one another about the utility of the plan and to provide comments and suggestions to the NNFH staff and the species' recovery lead at the LESO, as needed, throughout the life of the program. The Service will conduct an annual review of this plan and a full evaluation of the plan and program every five years. We will incorporate new information, protocols, and other pertinent information no later than the five-year evaluation.

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