PLATTE RIVER RECOVERY IMPLEMENTATION PROGRAM (PRRIP -or- Program)

Framing Document – Governance Committee (GC) and U.S. Fish and Wildlife Service (Service) Agreement on Addressing Pallid Sturgeon (PS) as a PRRIP Target Species During the First Increment Extension and Moving Into the Second Increment

At its Virtual Quarterly Meeting on June 9, 2021, the Governance Committee approved the following motion:

GC Motion: *LaBonde moved and Mehling seconded that in accordance with the PRRIP Second Increment Policy Frame, the GC authorizes the EDO to pursue Steps 1-3 as outlined in the Pallid Sturgeon Agreement Framing Document, subject to GC review and approval of all related proposals, budgets, products, and conclusions. The GC and Service agree this fulfills PRRIP pallid sturgeon commitments described in the Program Document. The GC and Service also agree the outlined activities provide the Program ESA compliance for the Milestones during the Extension with the expectation that Program water management activities implemented under this framework will continue to afford defined benefits for the pallid sturgeon during the Second Increment.* Motion approved.

This finalizes the Pallid Sturgeon Agreement Framing Document that follows and memorializes this document as a point of reference for Program pallid sturgeon activities for the remainder of the First Increment Extension and for the purposes of informing Second Increment negotiations.

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Key Understanding

The activities described in this Framing Document will provide defined benefits to pallid sturgeon during the Program's First Increment Extension (2020-2032). They will also be used to identify minimization / avoidance measures to address potential impacts of Program water management during a Second Increment (2033 and beyond). The Service must concur prior to initiation of any of the activities contemplated herein.

As defined in the Second Increment Policy Frame, "regulatory certainty" is based on achieving Milestones that reflect the defined contributions of land, water, and money and continued implementation of the Program (including science activities). This document describes the Program PS science activities to be implemented pursuant to those Milestones that provide regulatory certainty for the Extension.

Background

The endangered pallid sturgeon (PS) is one of four Program target species. Near the end of Program negotiations for the First Increment, the GC agreed to an initial set of PS studies that would inform future species efforts. These included lower Platte River (LPR) water quality monitoring, a PS literature review, and an LPR stage change study (SCS). These initial studies were completed in 2009 with a follow-up peer review of the SCS completed in 2012.

Since that time, the Program struggled to find a mutually acceptable path forward for PS. That is due, in part, to language inconsistencies in the Final Program Document and the Program's Adaptive Management Plan (AMP; Attachment 3 to the Program Document). The PS goal in the Program Document is to test the assumption that managing flow in the central Platte River also improves PS habitat in the LPR.[1] This differs from the PS management objective in the AMP to avoid adverse impacts from Program actions on PS populations.[2] This differing guidance language has increased uncertainty about how to address PS during the Extension.

In a September 2017 PS workshop, the Service stated the PRRIP must not only avoid adverse impacts, but also must provide benefits to PS. The Service also stated they take an expansive approach to defining "benefits" that could include: PRRIP-conducted research, funding others' research, managing physical habitat, or managing flow. This approach was reinforced by the Service in a presentation during the March 2021 GC Virtual Quarterly Meeting.

[1] From the PRRIP Final Program Document, Page 3, **Program goal**: "The Program's long term goal…includes…(3) testing the assumption that managing flow in the central Platte River also improves the pallid sturgeon's lower Platte River habitat."
[2] From the PRRIP Adaptive Management Plan, Page 20, **pallid sturgeon management objective**: "Avoid adverse impacts from Program actions on Pallid Sturgeon populations."

Pallid Sturgeon Impacts/Benefits

Based on this latest guidance from the Service, the EDO developed a progression of PS activities for Program consideration moving forward. The primary goal of these activities is to lay the scientific groundwork for an improved understanding of PS needs for the purposes of:

1) *Addressing the language of the PS Management Objective in the AMP* via implementing management actions to minimize/avoid potential impacts of Program water management[3] on PS during the remainder of the Extension and potentially through the Second Increment of the Program (PS management objective), and

2) *Addressing the PS language of the Program Goal* via quantifying benefits to LPR PS habitat as a result of Central Platte River (CPR) flow management actions.

The activities, structured as sequential steps, are identified below in **Table 1** which is similar to the format provided by the Service in its March 2021 presentation to the GC. In developing these activities, the EDO considered the following assumptions based largely on information presented to the GC by the Service in March 2021[4]:

- The 130,000 acre-feet reduction in target flow shortages is presumed to benefit the pallid sturgeon, but defined benefits are not well understood. The potential for pallid sturgeon impacts from Program water withdrawals is also unclear.

- The Service policy position is that Program water withdrawals will reduce lower Platte River flow and the inability to detect those water withdrawals does not equate to no PS impact.

- PS have been captured upstream of the Elkhorn River confluence.[5] This may expand the potential benefits/impacts of Program water management actions as central Platte River flow represents a higher proportion of total flow upstream of the confluence.

- Careful planning will ensure both long-term benefits from one-time actions such as research and iterative actions such as Program water management and offsets to any PS impacts in perpetuity.

[3] **Program water management** = for the purposes of this document, defined as potential PRRIP water management actions in the CPR including, but not limited to, storage water releases from sources such as the Environmental Account (EA) in Lake McConaughy, excess flow diversions into recharge and/or retiming projects such as the Cottonwood Ranch broad-scale recharge project, and return/recaptured flows from retiming projects.

[4] Agreement by the GC to implement actions to benefit the PS does not reflect GC concurrence with the March 2021 assumptions presented by the Service.

[5] The Program Associated Habitat Reach (AHR) for pallid sturgeon currently only extends from the Platte River confluence with the Missouri River up to the Elkhorn River confluence.

Table 1. Program science actions for the Extension and to develop the foundation for establishing Second Increment actions for PS.

Pallid Sturgeon (PS) Process Steps	Objective	Defines Potential PRRIP Impacts?	Total Cost / Effort	Outcomes	Long-Term Benefits
STEP 1 – Pallid Sturgeon Research					
STEP 1[6] **Habitat & Spawning Research** (UNL / NGPC) + **Genetics Research** (Ed Heist, Southern Illinois University)	Assess PS use, spawning habitat, and spawning success in the LPR as well as the contributions of the LPR to PS population. Assess hybridization, reassess population structure, and estimate effective population size.	Does NOT provide information necessary to define potential impacts. But SHOULD provide information necessary to define LPR spawning habitat and importance of LPR to pallid sturgeon. Also provides population-level information.	**$1,400,000**[7] *Medium*; work completed under Program oversight by contractors; 5 years during Extension.	Contribution to PS knowledge. Assessment of PS habitat and use in LPR that can be used in Phase 2 to define potential PS impacts and minimization / avoidance measures related to Program water management. Genetics research ties learning about habitat and contribution of LPR to recruitment and reproduction to genetically identified PS.	**Medium** Does not itself define effects of Program water management, may not result in conclusive data on PS habitat and use that can be translated into water management guidance in Steps 2 and 3 below.
STEPS 2 & 3 – Minimization / Avoidance of Impacts on PS and Quantification of CPR Water Management Benefits to PS in the LPR					
STEP 2 **PRRIP Water Management Study** (EDO / Contractor)	Identify water operations rules to provide guidance on the benefits of Program water management to and to avoid/minimize impacts on PS in the LPR.	YES.	**$100,000** *Low*; development of operational guidance.	Guidance on the benefits of Program water management to and to avoid/minimize impacts on PS in the LPR.	**High** Identifies operational rules to avoid/minimize impacts due to Program water management on and quantifies the benefits of CPR Program water management for PS in the LPR.
STEP 3 **PRRIP Water Management** (PRRIP / EDO)	Program water management guidance during Extension and Second Increment.	YES.	**Opportunity Costs** *Unknown*; avoidance & minimization during the remainder of the Extension and into the Second Increment.	Avoid/minimize potential Program water management impacts on and quantify benefits of CPR water management to PS in the LPR.	**High** Program water management benefits to and avoidance/minimization of impacts on PS in the LPR.

[6] Step 1 research activities have been designed to occur concurrently, with habitat and spawning research generating genetic samples that will be used in the genetics research.

[7] The EDO has been working with the Missouri River Recovery Program (MRRP) to identify that program's potential contribution to a joint genetics research effort. As of 04_12_21, the total genetics study budget is $366,890 with costs shared between the PRRIP and MRRP as described in Attachment B. The PRRIP cost would be roughly $200,000 for the genetics study.

Step 1 consists of two integrated research projects to fill knowledge gaps about lower Platte River contributions to PS spawning habitat**,** reproduction and recruitment, and population dynamics:

Habitat & Spawning Research

The proposed research is a Platte-specific effort to detect PS use of the lower Platte River and its tributaries. The focus is to identify where and when PS use the lower Platte. The project will utilize both passive and active tracking to assess PS use of the lower Platte and its tributaries, relate seasonal movements to environmental variables, and identify and describe spawning habitat. In conjunction with the genetic research proposed, this project will also evaluate spawning success of PS in the lower Platte and help to assess the contribution of the lower Platte to pallid reproduction and recruitment.

Genetic Research

The proposed research develops a genetic approach with greater resolution to establish new baselines for identifying pallid and shovelnose sturgeon, as well as addressing the issue of hybridization. Utilizing this new approach to species identification ties the learning from habitat and spawning research to pallid sturgeon. It allows us to determine the fraction of young of the year produced on the Platte that are pallid versus hybrid or shovelnose. It facilitates linking pallid sturgeon caught or spawned on the Platte to parental origin to better understand what the Platte contributes to successful reproduction of the Missouri River population. These benefits to Platte River learning depend upon genetic data from pallid sturgeon within the Platte River and the Missouri River to make connections. In return population level contributions include making use of identified pallid broodstock for stocking programs (including at the confluence of the Platte River), a reassessment of population structure to define barriers to reproduction and management units, and an estimate of effective population size for population viability assessments.

If the results of **Step 1** research indicate and quantify LPR habitat and use (e.g., pallid sturgeon presence and successful spawning), then based on the Service's assumptions above (namely, any Program water withdrawal is an "impact" whether or not detectable) the PRRIP would move to **Steps 2 - 3**. These subsequent steps consist of development and implementation of Program water management rules to quantify the benefits of CPR water management actions to avoid future impacts on PS in the LPR as well as to quantify the benefits of CPR water management to PS in the LPR .

Per GC guidance, the components of **Step 1** have been developed into full research proposals. The UNL/NGPC habitat research proposal is included as **Attachment A** and the SIU genetics research as **Attachment B**. The PRRIP "water operations effects mitigation study" contemplated in **Step 2** has not been scoped but is estimated to cost approximately $100,000. This is substantially less than the First Increment SCS which was conducted, in part, to similarly evaluate the effects of Program water management on LPR stage. The EDO's rationale for reducing effort/cost for a future study: the SCS concluded little to no ability to detect stage change as a result of diversion magnitudes 10 times greater than current Program recharge diversions, except in situations of high central Platte flow and very low LPR flow. As such, we anticipate hydrologic/hydraulic modeling will be of little use in routing and evaluation of much smaller diversions. Instead, we would likely employ tools like scenario planning or other qualitative techniques to identify sensitive periods when diversions should be avoided and when CPR water management would benefit PS habitat in the LPR.

Reporting and Science Review

For **Step 1**, the Habitat & Spawning Research and the Genetics Research will be reported to the PRRIP in the following manner:

1) *Annual reports from each field season* – Mark Pegg and Jon Spurgeon (UNL) and/or their students; Kirk Steffensen (NGPC); and Ed Heist (SIU) and/or his students will attend and present at the annual AMP Reporting Session during each year of the ongoing research projects. These presentations and accompanying update reports will focus on results of data collection from the previous field season, plans for the upcoming field season, cumulative insight from the progression of research, and any challenges encountered that might affect project performance.

2) *Final reports* – The Habitat & Spawning Research leads (Mark Pegg/Jon Spurgeon/Kirk Steffensen) and the Genetics Research lead (Ed Heist) will deliver final reports from the research projects to the PRRIP within one year of the final field season.

3) *Publications* – The research project leads and/or associated students may develop manuscripts for publication at the completion of the research projects. These publications will be presented in draft form to the EDO, TAC, and ISAC for review and comment and may include EDO staff members as co-authors. These publications will address findings from field data collection but will not address Program implications.

For **Step 2**, the PRRIP water management study will be completed in two phases:

1) **Phase 1 of Step 2** – A stand-alone study conducted jointly by the EDO and an independent contractor selected via a competitive selection process. The EDO contribution will be details on Program water management and key science learning from Phase 1 that links PS habitat and use to flow, stage, and other environmental variables in the LPR. The independent contractor will be hired to complete technical aspects of the study. The final product will be a report presented to the TAC, WAC, and ISAC for internal review and will then be peer reviewed using the Program's peer review process.

2) **Phase 2 of Step 2** – This will be a form of synthesis report completed by the EDO that combines science learning from Step 1 relative to pallid sturgeon habitat and use in the lower Platte River and learning from Phase 1 of Step 2 relative to Program water management. The result will be a report that 1) addresses/defines potential PS impacts and provides PS impact avoidance / minimization guidance for the remainder of the Extension and potentially throughout the Second Increment and 2) quantifies the benefits of CPR water management to PS in the LPR. This report will be reviewed internally by the TAC, WAC, and ISAC and will be submitted to the GC for final review and approval.

For **Step 3**, the EDO will develop an operational guidance manual for Program water management during the remainder of the Extension and potentially throughout the Second Increment.

GC/Service Agreement on PS for the Remainder of the Extension and Moving into the Second Increment

- While the GC does not endorse all of the Service's assumptions discussed in this document, the Service and GC agree this work establishes a smart investment on behalf of the Program via contribution to overall PS knowledge and ultimately both quantifying the benefits of CPR water management to PS in the LPR and providing avoidance / minimization of potential Program water management impacts on PS in the LPR.

- Completion of Step 1 satisfies the Program's PS requirements under the Amended Final Program Document and the associated Final Supplemental Biological Opinion. Completion of Step 1 will be a significant step in implementing the Program's Integrated Monitoring and Research Plan (IMRP) consistent with the ESA compliance Milestones through the remainder of the Extension.

- Second Increment Program obligations to PS are not expected to change. Quantification of the benefits of CPR water management to PS in the LPR (to address the "testing the assumption" language in the Program Goal) and implementation of measures to minimize / avid PS impacts as part of Water Action Plan operations (to address the "avoid adverse impacts" language in the AMP PS Management Objective) are expected to provide regulatory certainty under the ESA compliance Milestones for the Program's Second Increment.

- Potential minimization / avoidance measures would be developed during Step 2 and would be weighed alongside other potential Program activities as a part of Second Increment negotiations.

Attachment A

Research Proposal – University of Nebraska-Lincoln (UNL) & Nebraska Game and Parks Commission (NGPC)

Pallid Sturgeon Biology in the Platte River and Its Tributaries

Mark Pegg, School of Natural Resources, University of Nebraska-Lincoln
Jonathan Spurgeon, Nebraska Cooperative Fish and Wildlife Research Unit and School of Natural Resources, University of Nebraska-Lincoln
Kirk Steffensen, Nebraska Game and Parks Commission

Pallid Sturgeon (*Scaphirhynchus albus*) is a long-lived (i.e., > 50 years) species that historically occupied a large proportion of the Mississippi River basin including the mainstem Missouri River and its large tributaries (DeLonay et al. 2016; Hamel et al. 2020). Pallid Sturgeon possesses a life-history strategy that enables individuals to maximize contributions of offspring to future generations through aligning spawning events with environmental conditions that support larval drift, survival, and recruitment to the population. However, Pallid Sturgeon do not spawn until they are many years old (i.e., 10 – 20 years), do not spawn annually when sexually mature, and reach relatively old ages (DeLonay et al. 2016; Hamel et al. 2020).

Pallid Sturgeon have undergone declines in abundance and distribution resulting in listing as a federally endangered species in 1990 (55 FR 36641). These declines are believed to be largely the result of anthropogenic activities including over-harvest and reductions in habitat availability for spawning following fragmentation and alteration of river channels—particularly in the Missouri River basin (DeLonay et al. 2016). Widespread harvest of Pallid Sturgeon is thought to be minimal following protection, but contemporary river conditions remain a suspect in limiting the recovery process. The present distribution of Pallid Sturgeon is truncated due to multiple environmental stressors including lack of connectivity from dams or other flow-control structures that block movements (DeLonay et al. 2016). Furthermore, minimal recruitment of Pallid Sturgeon has occurred within the lower Missouri River (Steffensen et al. 2019) as habitat conditions within the system may limit survival of drifting larvae and limited habitat for post-larval stages (i.e., young-of-year) may exist in altered mainstem channels (e.g., channelized Missouri River). Extensive hatchery supplementation of Pallid Sturgeon has occurred to stabilize population loss and potentially increase the number of reproductively viable adults to a level where natural reproduction can result in recruitment. However, a fundamental understanding of habitat needs of spawning adults across different systems as well as the role of different habitat types in enhancing survival of young is limited.

Studies to inform recovery efforts to date have focused on gaining a better understanding of life-history requirements, population dynamics, habitat use, and propagation in the mainstem Mississippi and Missouri rivers (DeLonay et al. 2016; Steffensen and Mestl 2016; Steffensen et al. 2019; Kroboth et al. 2020). However, limited work has been done to understand the role of tributaries to fill data gaps in the recovery process. Therefore, there is a large need to provide details on Pallid Sturgeon populations and reproduction success in areas where they are not currently being evaluated. For example, the Platte

River is frequently used by Pallid Sturgeon and may be important to species abundance, seasonal distribution, and reproductive ecology.

Past sampling efforts have documented wild- and hatchery-origin Pallid Sturgeon using the lower Platte River to approximately Columbus, NE (rkm 159; Hamel et al. 2014). Additionally, recaptures of previously tagged Pallid Sturgeon have been documented in tributaries to the Platte River (e.g., Elkhorn River and its tributaries; Pegg, unpublished data) as well as anecdotal catches by anglers in other tributaries (e.g., Loup River system). Hamel et al. (2016) did report hydrologic conditions conducive to greater Pallid Sturgeon abundances in the Platte River, but that study did not target specific movements and habitat use. According to information gathered using data storage tags, reproductively ready female Pallid Sturgeon are believed to have spawned in the lower Platte River (DeLonay et al. 2016). Reproductively ready adults have also been captured and used for hatchery propagation from the Platte River (Hamel and Pegg 2018, 2019; Ruskamp, 2021). However, site-specific habitat information as well as documented successful reproduction and recruitment of individuals is lacking.

The Platte River Recovery Implementation Program (PRRIP) is in an ideal position to fill data gaps about the importance of tributaries like the Platte River to the overall biology of Pallid Sturgeon. Specifically, the PRRIP is, among other items, tasked with ensuring management actions related to water and land management in the Platte River Basin have no adverse impact on Pallid Sturgeon in the lower Platte River. The PRRIP uses an adaptive management framework to inform and evaluate management actions in the Platte River. Certainly, knowing more about how Pallid Sturgeon use the Platte River in response to environmental conditions like flow, habitat availability, etc. will be an invaluable contribution to the science that informs both the Platte River and the Missouri River adaptive management programs.

GOAL

Fill knowledge gaps about lower Platte River contributions to Pallid Sturgeon spawning habitat, reproduction, recruitment, and population dynamics.

OBJECTIVES

1. Identify relations among environmental conditions (i.e., river discharge and temperature) with the timing and extent of Pallid Sturgeon movement into and within the lower Platte River.
    a. Quantify seasonal movements of juvenile and adult Pallid Sturgeon into and out of the lower Platte River.
    b. Quantify environmental patterns including—but not exclusive to—components of the flow regime and temperature variation in the lower Platte River.

2. Identify Pallid Sturgeon spawning habitat in the lower Platte River and its tributaries.
    a. Locate probable spawning areas used by gravid Pallid Sturgeon.
    b. Document physical characteristics of the habitat at spawning locations.

3. Verify successful spawning by Pallid Sturgeon in the Platte River and/or its tributaries.
    a. Assess lower Platte River contribution of Pallid Sturgeon offspring to greater Missouri River population.

i. Gather information on free embryo, larva, and exogenous feeding life-stages.

4. Provide Pallid Sturgeon genetic samples for further population and hybridization assessment (in collaboration with Dr. Heist's parallel proposal).
    a. Assess fraction of free embryo/larval/exogenous feeding individuals that are pure pallid, hybrid, and shovelnose sturgeon.
        i. Adult and juvenile Pallid Sturgeon fin clips from telemetry collection.
        ii. Free embryo/larvae/exogenous feeding individuals.

METHODS

This study will focus on collecting fish and habitat data throughout the lower Platte River, but will also include areas where Pallid Sturgeon observations have been reported or where detections by the proposed passive telemetry network indicates movements beyond the lower Platte River or into its tributaries (e.g., Elkhorn River, Loup River, Cedar Creek, Salt Creek, etc.). Fish data collection will focus on capturing adult and juvenile individuals for telemetry as well as sampling free embryos, larvae, and exogenous feeding age-0 Pallid Sturgeon.

We propose a 5-year study where the first four years of the project will focus predominantly on intensive field sampling. The field sampling will encompass a single crew from late summer to early spring that will transition to two crews in the field simultaneously during peak Pallid Sturgeon activity (i.e., spawning, or other movement periods) each year. A seasonal, third crew will also be deployed during intensive tracking and early larval fish sampling to maximize data acquisition during this time period. Data summarizations and reporting will be ongoing throughout the project. The fifth year will allow for all genetic samples to be processed in collaboration with Dr. Ed Heist (Southern Illinois University) so results can be verified for any genetically pure Pallid Sturgeon captured. The final year will also be used specifically to compile, synthesize, and analyze all data and complete project summary documents that integrate efforts over the course of the study. Specific methods are detailed below for each objective.

Objective 1: Identify relations among environmental conditions (i.e., river discharge and temperature) and timing and extent of Pallid Sturgeon movement into and within the lower Platte River.

This objective uses active and passive telemetry as the centerpiece means of data collection to document Pallid Sturgeon movement into and within the Platte River and its tributaries. Pallid Sturgeon movements will be coupled with habitat and water quality data to evaluate movement triggers both into and within the Platte River and its tributaries. Objective 2 will specifically address telemetry data associated with identifying spawning site location(s) by narrowing in on reproductively ready fish to gather habitat data.

Telemetry

Approach: The main goals of these telemetry efforts are to track the movement of adult, reproductive Pallid Sturgeon; document spawning to determine spawning-site habitat characteristics; and document movements and habitat use by non-reproductive individuals using the Platte River. We will accomplish these goals using two sources of Pallid Sturgeon implanted with telemetry transmitters – fish caught in the Platte River and its tributaries (within this project) and fish being studied by a concurrent project on the Missouri River. First, we will establish passive and active telemetry networks for Pallid Sturgeon captured and tagged in the Platte River and its tributaries. Second, we will work in collaboration with Nebraska Game and Parks Commission's (NGPC) Pallid Sturgeon Population Assessment crew to use the lower Missouri River telemetry portfolio of fish to track Pallid Sturgeon when they enter the Platte River system from the Missouri River. All telemetry equipment (i.e., transmitters, passive listening stations, active tracking receivers) will be compatible with concurrent telemetry efforts in the Missouri River as listed in Table 1. Generally, we will monitor Pallid Sturgeon movement and habitat use in the Platte River and its tributaries following the basic guidelines outlined in Welker et al. (2020).

Table 1. Summary of telemetry tools used to track movements of Pallid Sturgeon > 800 g in the Platte River and its tributaries. All items listed are from InnovaSea Systems Inc. (formerly VEMCO) to be compatible with concurrent Pallid Sturgeon telemetry projects on the Missouri River (coded, 69 kHz transmitters and receivers). The minimum number column represents our targeted minimum sample size for transmitters with optimum numbers indicated in parentheses. Actual sample size will be dictated by catch.

Item	Model #	Use	Minimum number (ideal number)
Receiver	VR2Tx	Passive tracking	21 (30)
Receiver	VR-100	Active tracking	4
Transmitter	V-16TP*	All tracking efforts	20 (40)
Transmitter	V-13TP*	All tracking efforts	20 (40)

*Each acoustic transmitter is uniquely coded to provide individual fish identification. Transmitters are registered by the manufacturer to each buyer so "unknown" tag detections can be linked back to the tagging agency/university. Each receiver is able to detect any InnovaSea Systems Inc. tag in the 69 kHz range (i.e., no manual programming needed to detect tags as with radio receivers).

Pallid Sturgeon Collection for Telemetry: Our intent is to implant transmitters in as many Pallid Sturgeon as we can possibly capture, under environmental conditions that federal regulations allow sampling (water temperature < 15°C) and use the lower stations of the passive telemetry to inform movements into the lower Platte River from the mainstem Missouri River. Unfortunately, there is substantial uncertainty in the actual number of fish we will be able to capture. Data from previous studies suggest captures of reproductively ready adults could be relatively low; historical catches ranged from 1 to 5 per year but reproductively ready fish moving into the lower Platte River from the mainstem Missouri River

is likely. Sub-adult captures were somewhat greater with catches ranging from 1 to dozens per year (Hamel and Pegg 2019). However, those studies used a single crew and a random site selection process to meet different objectives than proposed herein. We will build on these previous works to improve Pallid Sturgeon captures for the telemetry objectives in three ways: 1) longer duration sampling window (i.e., months vs. weeks), 2) two to three crews to increase sampling effort, and 3) non-random selection of sample locations to optimize captures. As such, we anticipate implanting a ***minimum*** sample size of 40 individuals (Table 1) during the first three years of the field season but will maintain capacity to put additional transmitters into Pallid Sturgeon should the opportunity arise. We will put an emphasis on implanting transmitters earlier in the project to facilitate as much data collection as possible.

Collection of Pallid Sturgeon suitable for telemetry will primarily occur during March – May each of the first three years. Sample efforts will be focused from the Elkhorn River confluence to the Missouri River. The lower Platte River reach has historically been a reach with the greatest abundance of Pallid Sturgeon in the Platte River system (Hamel et al. 2016). For instance, the reach of river 8-30 km upstream from the confluence with the Missouri River provided the greatest catch rates for collecting Pallid Sturgeon compared to areas outside of that reach (Hamel et al. 2014). At least initially, we will concentrate fish collection efforts there. We will use information gained from the ongoing telemetry observations to identify additional potential high probability capture locations when such data are available.

Fish collection methods will follow established collection techniques used in the Platte River and within the Pallid Sturgeon Handling Protocols. Specifically, we will target Pallid Sturgeon for transmitter implantation using baited trotlines (Peters and Parham 2008; Hamel and Pegg 2019), which has proved to be the most productive capture method. Each crew will set 10 - 20 trotlines per day in locations ideal for sturgeon captures (e.g., channels, behind sandbars, riprap bank lines, etc.). Trotlines will be 32 m long and made of 6 mm diameter nylon main line with a lead core. Hooks will be fished using a 30 cm line attached to the main line at 1.5 m intervals (N = 20 hooks/line). We will use O'Shaughnessy (size = 3/0) hooks baited with nightcrawlers *Lumbricus terrestris* for all trotline sets. The sets will typically be allowed to fish overnight with a maximum set time of 24 hours but will follow the requirements within the federal handling protocols. Start and stop times will be recorded in addition to georeferenced location (e.g., latitude and longitude coordinates) for each trotline set. Habitat measures (described below) will be taken at all trotline sample sites.

Collected fish will be temporarily held in a holding tank for processing. All fish *except* sturgeon species will be identified, measured for total length (mm) and mass (g), then released near the capture location. All sturgeon will be identified, measured for fork length (mm) and mass (g). Shovelnose sturgeon will be released near their capture location. Putative Pallid Sturgeon will be further processed for inclusion in the telemetry portion of this study when appropriate (Objectives 1 & 2) and genetic assessment (Objective 4). Pallid Sturgeon will be checked for presence of a Passive Integrated Transponder (PIT; 125.0 or 134.2 kHz; Biomark unencrypted) tag that would have been implanted during previous studies. We will implant a new PIT tag if one is not present. The PIT tagging effort is an ongoing, collaborative component of the Pallid Sturgeon recovery plan across the Missouri River Basin to assess movements

and develop population parameters. A fin clip will also be collected and preserved in 70% ethanol for genetic identification from all fish identified in the field as Pallid Sturgeon or suspected hybrids (Objective 4). Genetic samples will be shipped to Dr. Ed Heist for species identification and origin determination (wild or hatchery-origin).

Gender identification and reproductive evaluations for adults will be assessed using an ultrasound, oocyte biopsy, and/or visual examination during surgical implantation as outlined in Wildhaber et al. (2007). Gender identification will allow determination of the sex ratio of Pallid Sturgeon using the lower Platte River for comparison to the mainstem, while evaluations on reproductive status will also provide valuable information into the population characteristics (i.e., reproductive readiness ratio, age/length-of-maturity, etc.) of the lower Platte River. Also, knowing the gender and reproductive stage of individual fish will allow us to target likely reproductive fish in future years for intensive telemetry tracking efforts.

Implanting transmitters into Pallid Sturgeon: We will use two sizes of uniquely coded transmitters for tracking Pallid Sturgeon. We will use a V-16TP (16 mm X 71 mm; 26 g) for fish > 870 g and V-13TP (13 mm X 39 mm; 11 g) for fish 370 – 870 g. Each tag has a unique acoustic signal so that individual fish can be specifically identified. Both tag sizes provide battery life sufficient to track individuals for 3+ years and do not exceed the 3% of total body weight threshold often used in telemetry studies to reduce potential impacts of the transmitter on fish buoyancy (Cooke et al. 2012). The transmitters will also be equipped with temperature and pressure (depth) sensors that will provide additional insight into depth and temperature conditions used by these individuals through time. Changes in depth and temperature are particularly useful for identifying when Pallid Sturgeon move between the Platte and Missouri river systems (Haas et al. 2019).

As required under our Federal Endangered Species Collectors Permit and the revised Pallid Sturgeon Handling Protocols, all personnel responsible for surgery will undergo training for proper surgical methods and oocyte extraction by certified staff (training provided by USGS science center in Columbia, Missouri). Surgery procedures will follow guidelines developed for proper care and handling of Pallid Sturgeon (USFWS 2012, Kroboth et al. 2020). Transmitters will be inserted into the body cavity of the Pallid Sturgeon via an incision cut in the abdomen near, but not along, the ventral line. The incision will be closed using non-absorbable, monofilament suture material with independent sutures to ensure proper closure. All procedures will follow field sterilization procedures for instruments, transmitters, and suture materials and not occur when air temperatures are below 2^{o}C. Surgical implantations will also not occur in late-fall to prevent delayed incision heeling before the sutures dissolve due to cold water temperatures. A post-operative injection of antibiotics (Liquamycin Vaccination at 0.045 mg/kg) will also be administered to reduce the probability of infection when the procedure is complete. Individuals will be released near the point of capture once they are able to hold themselves upright and transmitter signal is verified as turned on and functional.

Additional source of currently transmitter-implanted Pallid Sturgeon: Individual Pallid Sturgeon, already implanted with transmitters and of known reproductive status, have previously entered the Platte River from the Missouri River (Haas et al. 2019). Missouri River telemetry crews are not authorized to follow these fish into the Platte River. Valuable information is lost when fish move out of the Missouri River

and into the Platte River or its tributaries. Our study would provide a means for the Missouri River telemetry crews to hand off the fish to the Platte River crews to the benefit of all involved. In essence, this transfer of information would maintain continuity in tracking individuals across wider movement patterns and provide an opportunity to possibly increase our sample size of adult, reproductive-ready Pallid Sturgeon using the Platte River and its tributaries. Likewise, we will also coordinate details with NGPC should Pallid Sturgeon implanted with transmitters in the Platte River system move to the Missouri River.

Habitat measures at Pallid Sturgeon capture locations: Habitat will be assessed at multiple spatial scales for **Objectives 1-3**. Micro-scale and meso-scale habitat information will be collected at each gear deployment location. Micro-scale habitat variables will be summarized across the area where gears were deployed and will include water velocity, water depth, dominant substrate type(s), water temperature, turbidity, conductivity, and presence of wood structure. Mesoscale habitat variables used to describe the area surrounding the location of capture will include—but not be limited to—categories such as main-channel, side-channel, overflowing bar, emerged-bar with main-channel border, or back-water. Reach-scale habitat variables will include measures of channel width and complexity as well as distances to tributary inflows. A river reach is defined—for the purposes of this study—as the 1km above and below the location where a Pallid Sturgeon is recaptured. Channel complexity will be analyzed using remote sensing methods and approaches similar to O'Neill and Thorp (2011), where the River Channel Complexity Ratio is measured using length of individual channels within a river reach compared to the total length of bank along the outside boundary. These data are consistent with previous habitat measures from the Platte River (Peters and Parham 2008; Hammen et al. 2018; Hamel and Pegg 2019; Platte River Recovery Stage-Change Study 2009). We will also assess hydrologic patterns by linking Pallid Sturgeon locations with the nearest USGS hydrologic gage. Flow metrics will be calculated on daily and sub-daily timescales (Spurgeon et al. 2016) and will summarize the magnitude, duration, frequency, rate-of-change, and timing of flow events. Additional flow metrics estimated at the yearly time scale also will be assessed. The combination of physical habitat measurements with hydrologic patterns may provide insight into the dynamic nature of habitat availability and Pallid Sturgeon use along the Platte River.

Passive Tracking: Passive tracking allows continuous monitoring of fish movements across a greater temporal and/or spatial scale than is typically logistically feasible solely using active tracking techniques (Kraus et al. 2018). The basic premise is that a network of receivers can be deployed across a study area to document when exactly a fish implanted with a transmitter has encountered the receiver at a given location. This network approach has been used in numerous studies across a range of aquatic environments and provides a coarse view of where fish are located within the system as well as documents large-scale movement (e.g., Enders et al. 2019). We intend to use the passive tracking portion of the telemetry study to assist with documenting large scale movements as in other studies, but to also assist in determining if and when Pallid Sturgeon have moved to new areas within the Platte River or into one of its major tributaries. Specifically, we will deploy passive telemetry stations (VR2Tx receivers) in the Platte River at strategic locations to document specific fish movements (Figure 1).

These receivers operate by detecting signals within a “line of sight”, meaning there needs to be an unobstructed path between the transmitter and the receiver through the water. Therefore, we will create the passive tracking network by placing receivers in locations (e.g., narrowing channel with potential complete line of sight, known sturgeon congregation areas, etc.) starting just upstream from the confluence with the Missouri River, continuing to a location past the Loup River confluence with the Platte River. Some locations will likely require more than one receiver to ensure full line of sight coverage across the river. Furthermore, we will use the passive tracking network to monitor Pallid Sturgeon movements into or out of major tributaries to the Platte River where they have been reported in the past. Each tributary will be monitored by placing a receiver just upstream from its confluence, yet far enough into the tributary to be sure the fish is actually in the tributary. We will initially place 21 listening stations (Table 2; Figure 1) prior to implanting any transmitters in fish during the first year of this study. We will place additional receivers (maximum of 9 additional receivers) to target specific data collection based on movements of Pallid Sturgeon over the course of the project or to replace damaged or lost receivers in the original network design. Additional receivers may also be needed in areas with braided channels to minimize missing individuals when multiple channels are present.

The passive tracking network will be an integral component of the entire telemetry portfolio to document the extent of Pallid Sturgeon movements in the Platte River, its tributaries, and emigration into the mainstem Missouri River. The passive tracking network provides continuous listening abilities (i.e., always listening for transmitters upon being deployed) that active tracking cannot always deliver. The passive tracking network will also be able to monitor movement information for the entire telemetry portfolio when crews are intensively tracking reproductive fish. Receivers document exactly when (i.e., uniquely coded individual fish detections with a date and time stamp) any individual passes a respective receiver. We anticipate coupling the data gathered by the passive network and the active tracking to test the relation between Pallid Sturgeon movements and potential environmental cues like discharge, temperature, etc.

Table 2. *Approximate* location and estimated number of VR2Tx receivers to be initially placed in the Platte River and its tributaries to establish a passive telemetry network. Location numbers correspond to mapped locations in Figure 1. An additional nine transmitters are included as additional passive tracking sites emerge or as replacements if deployed receivers are damaged or lost.

Station	Map location (from Figure 1)	Longitude	Latitude	Estimated number of receivers
Platte Confluence	1	-95.88220	41.053346	2
Cedar Creek	2	-96.11301	41.036487	2
Suspected Spawning Site	3	-96.12966	41.029207	2
Highway 50	4	-96.15746	41.012596	2
Salt Creek	5	-96.33799	41.051616	2
Highway 6	6	-96.32663	41.061275	2
Elkhorn River	7	-96.29643	41.204528	2
Above Elkhorn Confluence	8	-96.31282	41.121036	2
Fremont	9	-96.50269	41.403659	1
Rogers	10	-96.92405	41.456900	1
Platte River-Loup Power Canal	11	-97.28230	41.398238	1
Loup River	12	-97.40016	41.431202	1
Columbus Bridge	13	-97.36772	41.397570	1
Additional receivers (for spawning locations or replacements)				9
			Total	**30**

The network will also serve as a "safety net" of sorts to help us locate fish that are not found during active tracking. We expect the receivers will remain in place year-round, or nearly so, once positioned. However, receivers placed in the small tributaries (i.e., Cedar Creek, Salt Creek) may be removed prior to potential ice-flow events in the winter to protect the receivers from damage. Similar activities may also occur on the mainstem Platte River if the receivers are at risk of ice scour but will largely depend on exact location of receiver placement (e.g., bridge abutments, anchored in the channel, tethered to shore, etc.). As long as the receiver remains submerged and fish "line-of-sight" is clear of physical obstructions, they will be actively collecting data. Gathering data from each receiver in the network will require physically retrieving each receiver to attain data since the previous download event. Retrieving the data from these receivers will likely be done in accordance with the fish movements we have been observing to balance optimal data collection under both the passive and active segments of this project. At the very least, we anticipate downloading data from each receiver twice annually. Actual downloads will likely be more frequent depending on last known locations of fish within the system. For example, if an individual has been in the Platte River-Elkhorn River confluence area and active tracking fails to find the fish, we will download data from receivers in that general area to assess the suspected direction of movement. We believe targeting specific receivers for data downloads in this strategic manner will be especially crucial if/when individuals move into one of the tributaries or move into the Missouri River as

it will provide valuable direction on where a more extensive search should be initiated or if the fish will be handed off to NGPC.

Active Tracking: Active tracking will include *extensive* tracking to locate all fish and *intensive* tracking to follow reproductively ready adults during the spawning season. Details regarding the intensive tracking events are detailed in Objective 2. However, both tracking events will use InnovaSea VR100 deck boxes equipped with a multidirectional hydrophone and a directional hydrophone to locate fish (Table 1). Each receiver is capable of detecting any transmitters it encounters with an InnovaSea transmitter in the 69 kHz range.

Extensive tracking will be primarily conducted by one field crew for much of the year and will attempt to gather monthly locations for all fish with transmitters within the Platte River and its tributaries outside of the spawning season and when water conditions allow (i.e., January – March and July – December each year). The search event will generally follow a systematic approach where the field crew will search for fish in the Platte River and then radiate into the tributaries as needed or as the passive tracking network indicates. We anticipate most fish will initially remain in the Platte River near where we focus our fish collection efforts and then disperse upstream or downstream accordingly. It seems reasonable to start extensive searches in this area until we observe dispersal events. We will develop the most efficient search approach based on fish behaviors observed from our tracking data collection efforts. We will use the passive listening network to bolster our ability to locate fish and determine whether or not they remain in our study area as described in the passive telemetry section. This is especially true for fish whose last known location was near one of the tributaries or the upstream and downstream extents of our network.

We will typically use the omnidirectional hydrophone to locate fish during the monthly tracking events. Once a fish is located, we will switch to the directional hydrophone to get as specific to the location of the fish as possible without disturbing the fish. Location accuracy should be < 5 m and will provide direct insight into the type of microhabitat in which the fish is located. A habitat assessment will then be conducted near the fish's location.

Habitat measures at Pallid Sturgeon relocation sites: Habitat for relocated Pallid Sturgeon will be assessed at multiple spatial scales. Micro-scale, meso-scale, and reach-scale habitat information as described above will also be collected when Pallid Sturgeon can be relocated. As described above, flow patterns according to the nearest USGS hydrologic gage will be linked to Pallid Sturgeon relocation sites. Additionally, if environmental conditions permit (i.e., water depth and hydrologic conditions), we will attempt to provide side-scanned images of the location where individual Pallid Sturgeon are located for further analyses.

Objective 2: Identify Pallid Sturgeon spawning habitat in the lower Platte River and its tributaries.

Surgically implanting transmitters provides an opportunity to assess adults and their stage of reproductive readiness while the fish is in hand. Likewise, the NGPC PSPAP crews will also be tracking reproductively ready adults and will notify our crew(s) when a fish moves near or enters the lower Platte River from the Missouri River to make sure we follow that individual appropriately. We will use this information to intensively follow fish we identify as being reproductively ready during their spawning period and attempt to document spawning behaviors and location. Two telemetry crews will try to collect individual locations daily when spawning timing approaches (~April) and water temperatures are less than 16^{o}C. As water temperatures near 16-18^{o}C, intensive active tracking efforts will begin locating reproductive fish approximately every 15 to 60 minutes during daylight hours. Overnight tracking on the major river systems is difficult and dangerous so will likely not occur. However, crews will continue active tracking the following morning based on observed swimming speed and potentially information retrieved from passive stations to anticipate relocation areas if the fish is not immediately found. Behaviors that typically suggest a spawning event include an individual halting its upstream movement, remaining in one localized area for a short period (1-5 days) with consistent patterned movement of approximately 100-m along spawning locations, then moving back downstream (DeLonay et al. 2014). After continued downstream movement is detected, crews will attempt to recapture the fish to assess spawning success.

Intensive tracking requires a lot of time and effort to locate individual fish daily and the number of fish tracked annually depends upon fish availability, distance between reproductive fish, and available crews. To maximize information return, we will use two crews during spawning and immediately after spawning to maximize the number of fish that can be simultaneously, yet intensively tracked. However, this will limit the information acquired for juveniles and non-reproductive adults for Objective 1 to incidental detections during the spawning season window.

Habitat measures at Pallid Sturgeon spawning locations: Habitat information from the spawn site(s) will be recorded to characterize spawning sites following the spawning season to ensure we do not disturb or interrupt spawning behaviors. Habitat at spawning locations will be estimated at multiple spatial scales. Micro-scale and meso-scale habitat information will be collected when spawning Pallid Sturgeon sites are documented. Reach-scale habitat variables such as channel width and complexity as well as distances to tributary inflows will also be measured. If environmental conditions permit (i.e., water depth and hydrologic conditions), we will attempt to provide side-scanned images of the location where individual Pallid Sturgeon are/were located. Hydrologic patterns will be assessed by linking Pallid Sturgeon locations with the flow metrics described above as obtained from the nearest USGS hydrologic gage. These measurements will document the physical and hydrological conditions of Pallid Sturgeon spawning habitat on the Platte River. After the reproductive fish concludes spawning behavior and begins downstream movement, we will attempt recapture to determine if that individual successfully spawned as well as attempt to capture free embryos in the general spawning area (Objective 3).

Objective 3: Verify successful spawning by Pallid Sturgeon in the Platte River and/or its tributaries.

Young-of-year Pallid Sturgeon Collection

Approach: We will use two sampling approaches to document Pallid Sturgeon spawning success in the Platte River and its tributaries. These approaches will facilitate gathering information on the contribution of the lower Platte River and its tributaries to Pallid Sturgeon population dynamics on the lower Missouri River and document locations where Pallid Sturgeon are spawning successfully within the Platte River system.

First, we will document the contribution of Platte River collected Pallid Sturgeon embryos, larvae, and exogenous feeding individuals to the lower Missouri River system through a systematic sampling process. We will use ichthyoplankton nets (gear deployment described below) to target early life-stage Pallid Sturgeon at the confluence of the Platte River with the Missouri River as well as above and below the confluence in the Missouri River (Figure 1). Sampling at the confluence area of the Platte River with the Missouri River will document the Pallid Sturgeon being produced in the Platte River and "exported" to the Missouri River when such an instance occurs. In essence, a cumulative perspective of the Platte River's contribution to the greater Pallid Sturgeon population. Furthermore, sampling in the Missouri River above and below the confluence will give insight into the relative contribution of Pallid Sturgeon from the Platte River compared to the mainstem Missouri River above the confluence. Weekly sampling will commence when water temperatures exceed 15^{o}C or May 1, whichever occurs first, through June 30 each year of field data collection. We will sample the Platte River along a transect (perpendicular to flow) approximately 1-km upstream from the confluence. Habitat diversity is greater in the Platte River compared to available sample locations on the Missouri River. Therefore, we will sample three locations along a transect wherein we deploy gear about 50-m away from each bank and a mid-river location. Exact sample locations will likely be dictated by water conditions and presence of sandbars, but we will target the deepest water available. Conversely, DeLonay et al. (2016) reported the majority of Pallid Sturgeon were collected in outside bend habitats on the Missouri River. Hence, we will target outside bend habitats in the Missouri River about 1-km upstream and 1-km downstream of the Platte River confluence and all samples will be collected on the riverbed. Each Missouri River sampling location will be sampled in triplicate for parity with the Platte River sampling. We will use a third crew to sample the confluence area during this time given the logistical demands on the two telemetry crews also operating at this time. These logistical plans will be dictated by how many reproductively active individuals are currently in the Platte River and its tributaries each year during spawning.

Second, we will initiate an intensive sampling effort at site(s) where behavior by individuals implanted with transmitters suggest spawning is occurring or has occurred (per Objective 2). Here, one or both of the telemetry crews that are following reproductively active adults will deploy ichthyoplankton nets downstream of the spawning location to target collection of free embryos and/or larval Pallid Sturgeon to document that successful spawning took place. The amount of water sampled by the ichthyoplankton nets will be dependent on debris load. Sampling sites will be selected based on channel configuration to maximize capture probability. Sampling will begin 3 days post-spawning and continue

for 5 to 7 days depending on the number of Acipenseriformes larvae being collected. Field samples will be immediately processed or "picked" in the field and preserved in alcohol. All individuals from Acipenseriformes in each sample will be submitted to Dr. Ed Heist for species identification (Objective 4).

Sample Collection: Ichthyoplankton nets will be used to collect free embryos, larvae, and early life-stages of exogenous feeding individuals. These 750-micron mesh nets are conical in shape, with 0.75-m diameter openings that extend 4-m to a cod end equipped with a jar to hold captured material. These nets will be set from an anchored boat to ensure stationary deployment and consistent gear operation. We will deploy nets in tandem (i.e., one net starboard/one net port) at each sample location. Both nets will be placed at or near the bottom of the water column at the water-substrate interface to target Acipenseriformes (sturgeon and paddlefish) species (DeLonay et al. 2016). Drift net deployment times will likely vary depending on quantities of organic flotsam in the water column, but we will target 10-minute sets in all locations. Sampling times will likely vary, but sampling effort will be standardized according to the amount of water sampled in each net. We will collect three replicate samples at each of the Platte River confluence and Missouri River sites. Intensive spawning site sample collection will likely vary but will be at least five (5) samples per day when verifying spawning success at a particular site.

We will determine relative density of fish captured in the nets as number of fish per unit volume of water sampled (e.g., number of individuals per cubic meter; #/m^3). Volume of water sampled (V) will be calculated as:

$$V = \pi * R^2 * H$$

where R is the net radius, and H is the distance of water sampled. We will determine H using a General Oceanics (Miami, FL) flow meter positioned at the mouth of the drift net during deployment. This flow meter counts propellor revolutions in the instrument that can then be multiplied by a manufacturer-provided constant to convert flow into distance of water sampled.

Processing Suspected Sturgeon Species: All samples will be initially screened immediately following net retrieval for individuals from the Order Acipenseriformes (i.e., sturgeon and paddlefish). Species-specific identification of individuals from Acipenseriformes is not possible from morphometric features when assessed in the field at the free embryo and early larval stages. Therefore, individuals identified as being from Acipenseriformes will be immediately preserved in 70% ethanol. Remaining fish in the sample will be preserved in either a 10% buffered formalin or 70% ethanol solution to be processed at a later date. Preserved specimens will be identified to the lowest possible taxonomic level (e.g., Family, Genus, Species). All suspected *Scaphirhynchus* spp. will be submitted for genetic identification per Objective 4 to verify successful spawning by Pallid Sturgeon in the Platte River and assess the Platte's contribution of Pallid Sturgeon offspring to the greater Missouri River population.

Habitat measures taken at suspected spawning sites: Habitat information from the spawn site(s) will be recorded to characterize spawning sites following the spawning season as described above for Objective 2.

Objective 4: Annual sampling of fish and free embryo/larvae/young-of-year from the Lower Platte to provide samples for Dr. Ed Heist's genetic study.

Tissue samples from all Pallid Sturgeon captured as part of this project will be provided to Dr. Ed Heist's laboratory at Southern Illinois University for genetic analyses to assess species identification, demographics, and the potential degree of hybridization with Shovelnose Sturgeon (See companion proposal from Dr. Heist for specific details). Juvenile and adult Pallid Sturgeon (and potential hybrids that are phenotypically identified in the field) collected under **Objective 1** will provide insights into Pallid Sturgeon population dynamics (i.e., ratio of wild to hatchery-origin, natural recruitment via juvenile wild-origin fish collected, occurrence/rate of hybridization). Age-0 (embryos, larval, and exogenous feeding) individuals collected under **Objectives 2 and 3** will provide insights into reproductive success in the lower Platte River, purity of these reproductive events, and contribution of the Platte River to the lower Missouri River sturgeon populations.

All samples (e.g., fin clips or whole specimens depending on life stage) will be preserved, documented, and shipped to the laboratory for further processing following protocols provided by Dr. Heist. Results derived from this objective will be provided in reporting documents as described in the companion proposal to this document. Results from genetic analyses will be incorporated into the current project to verify field identification of adults and juveniles as pure Pallid Sturgeon and make Pallid-specific assessments of Platte River habitat use, successful spawning, and contributions to the Missouri River population(s).

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Timeline

We are proposing a 5-year study to gather information on pallid sturgeon use and spawning in the Platte River system with field work beginning during spring 2022. This project will include four years of intensive field data collection efforts. Telemetry data verification, genetic lab-sample processing, and data analyses are anticipated to be time consuming and continue into a fifth year. Therefore, we will follow the field-data collection years with one additional year to allow time for final data analyses, results synthesis, completion of genetic results, and composing the final report.

Included in the study is support for one Ph.D. student and two M.S. students (in consecutive sequence). Specific graduate project descriptions will be developed with input from Platte River Recovery Implementation Program (PRRIP) Executive Director's Office (EDO) staff where appropriate and we will investigate the potential for Dr. Malinda Henry to serve on the graduate committee for students. Timelines for data collection, analyses, and summarization (Table 3) and graduate student timelines (Table 4) are below. Years are divided into quarters to highlight specific activities at approximate times throughout the project timeline (Table 3; Table 4).

Table 3. Proposed project timeline. All field-related activities indicate an ***approximate period*** of the indicated activity and will actually be conducted when specific timing is appropriate and as dictated by water conditions and fish availability.

Task	2022	2023	2024	2025	2026
Hire personnel					
Acquire telemetry and sample equipment					
Deploy/replace listening stations					
Fish collection and transmitter implantation					
Active Tracking (all fish)					
Active Tracking (reproductively ready adults)					
Passive Tracking Listening station downloads					
Free embryo and exogenous feeding sampling					
Data processing (QA/QC)					
Data analyses (interim and preliminary)					
Annual summary/report					
Student dissertation/theses completion					
Final Report analyses and preparation					

572 **Table 4.** Tentative student timeline

Student	2022	2023	2024	2025	2026
Ph.D.					
MS					
MS					

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574 We recognize this study has substantial complexity related to timing and allocation of effort. Logistical
575 constraints tied to boat availability, safe sampling conditions, fish availability, etc. can alter plans as we
576 have presented them here. However, we have collectively accrued over 60 years of river sampling
577 experience and over 20 years of experience coordinating and conducting telemetry projects in riverine
578 conditions across North America. Additionally, the NGPC Missouri River Program's staff has extensive
579 telemetry experience in the mainstem Missouri River and will be available for training and logistical
580 knowledge/support consultation at all times. This expertise has given us a solid foundation and insight
581 into how to successfully adapt to real-time conditions to meet project objectives. While we cannot
582 anticipate every situation that may require a contingency plan, we do have a prioritization process in
583 place. The below highlights that prioritization in terms of data collection priorities. This prioritization is
584 most poignant during the spawning season and embryo collection phases of the project when all crews
585 will be actively conducting field work.

586 Spawning/embryo sampling time period prioritization

587 Intensive telemetry of reproductively active adults will be our highest priority when we have such within
588 the system. Females will be ranked higher than males and will take top priority. Correspondingly,
589 attempts to collect embryos immediately following an indication of spawning will be coupled with the
590 intensive tracking as a logical next step and top priority. Intensive tracking is very time consuming,
591 requiring non-traditional working hours and days. Monthly searches for all juveniles or non-
592 reproductively active adults will be deferred to the passive tracking network during this time if both
593 crews are dedicated to intensive tracking. If reproductive fish are limited or not available in a given
594 year, focus on the juvenile and non-reproductive fish movements and habitat use will be the main
595 priority.

596 **Communications and Coordination with PRRIP**

597 We envision regular formal and informal communications and coordination with PRRIP EDO staff
598 and other facets of the PRRIP throughout this study. Examples of these efforts could include, but are
599 not limited to, monthly updates/discussions on field sampling and progress toward meeting objectives,
600 updates on data analyses and their results, and presentation(s) of interim study progress and results at
601 PRRIP-led meetings. We also anticipate technical and public presentations of our results at scientific
602 conferences, public media outlets (as requested), and lay presentations to the general public (as
603 requested) by the students and PIs.

Deliverables

In addition to items delivered as part of our regular communications listed above, we anticipate providing the following written documents: 1) Annual reports (by December of each year starting 2022), 2) 2 M.S. Theses, 3) 1 Ph.D. Dissertation, and a final report to supplement documents developed during the interim upon project completion. We will also publish results of our findings in peer-reviewed, scientific journals.

Budget

Draft Budget

(totals are subject to change following formal review by UNL Sponsored Programs Office)

UNL Basic Request Budget

	Person Months					Year 1	Year 2	Year 3	Year 4	Year 5	Total
Senior Personnel	Yr1	Yr2	Yr3	Yr4	Yr5						
					Total Senior Personnel	-	-	-	-	-	-
Other Personnel	# of Ppl										
Post Docs	0	0	0	0	0	-	-	-	-	-	-
Other Professionals	1	1	1	1	0	7,000	7,210	7,426	7,649	-	29,285
Graduate Students	2.00	2.00	2.00	2.00	2.00	55,000	56,650	58,350	60,100	61,903	292,003
Undergraduate Students	3	3	3	3	1	22,560	23,236	23,934	24,652	14,046	108,428
Secretarial	0	0	0	0	0	-	-	-	-	-	-
Other	0	0	0	0	0	-	-	-	-	-	-
					Total Other Personnel	***84,560***	***87,096***	***89,710***	***92,401***	***75,949***	***429,716***
Fringe Benefits						28,816	30,384	32,068	33,878	32,676	157,822
					Total Salaries and Benefits	**113,376**	**117,480**	**121,778**	**126,279**	**108,625**	**587,538**
Equipment						74,000	-	-	-	-	74,000
Travel						23,740	24,452	25,186	25,941	9,432	108,751
Supplies						15,000	10,000	5,000	5,000	5,000	40,000
Subawards						-	-	-	-	-	-
Other						104,500	46,500	25,000	5,000	1,500	182,500
					Total Other Direct Costs	**217,240**	**80,952**	**55,186**	**35,941**	**15,932**	**405,251**
					Total Direct Costs	**330,616**	**198,432**	**176,964**	**162,220**	**124,557**	**992,789**
F&A Base	MTDC					234,156	175,154	152,834	137,206	100,621	799,971
F&A	26.0%					60,881	45,540	39,737	35,674	26,161	**207,993**
					Total Request	**391,497**	**243,972**	**216,701**	**197,894**	**150,718**	**1,200,782**

MTDC Exclusions	*Year 1*	*Year 2*	*Year 3*	*Year 4*	*Year 5*	*Total*
Equipment	*74,000*	-	-	-	-	*74,000*
Tuition Remission	*20,460*	*21,278*	*22,130*	*23,014*	*23,936*	*110,818*
Subawards in excess of $25K	-	-	-	-	-	-
Participant Support Costs	-	-	-	-	-	-
Rent	*2,000*	*2,000*	*2,000*	*2,000*	-	*8,000*
Alterations and Renovations	-	-	-	-	-	-
Total Exclusions	***96,460***	***23,278***	***24,130***	***25,014***	***23,936***	***192,818***

Budget Justification/Comments

The total project cost is $1,200,782 broken down to $992,789 in direct cost and $207,993 in indirect cost. Requested direct costs include personnel (3% cost of living increase per year), travel, equipment, and supplies, all of which are broken down further below. Facilities & Administrative (F&A) costs, sometimes referred to as indirect costs, are costs incurred for common or joint objectives and therefore are not readily identifiable with a particular sponsored project, an instructional activity, or any other institutional activity. Because these are actual (real) costs of doing business, we seek their recovery from grant awards in the form of F&A costs. Facilities costs can include, but are not limited to, utilities, custodial services, plant operations such as heating and cooling services, and maintenance expenses.

Administrative costs can include, but are not limited to, payroll and accounting services, office supplies, departmental administration, procurement services, library services and sponsored projects administration.

Personnel ($587,538)

- Project supports 1 PhD student ($30,000/yr.) and 2 consecutive M.S. students ($25,000/yr.) plus tuition remission and benefits for each.

- Two additional field technicians to assist the graduate students will be hired seasonally. All seasonal technicians will be hired at a starting hourly rate of $12/hr. The budget lists these technicians as "undergraduate technicians" in the line item, though they may or may not be currently enrolled as a student. Duration of employment for each season technician will be:
    - Technician 1 at 1,040 hours ($12,480) annually during years 1-4 to facilitate field planning, preparation, data collection, and sample processing). This technician will be used to assist with project completion (e.g., data entry, final lab sample processing, etc.) in year 5.
    - Technician 2 at 520 hours ($6,240) annually during years 1-4 will be used to complete field work as part of the two-crew telemetry effort.

- The short-term, third crew will be used for larval sampling at the Platte River confluence only. This crew will consist of one research associate and one seasonal technician.
    - The research associate is currently on staff at UNL and time can be allocated to larval sampling before field work on other projects begins each year. We will buy 2 months ($42,000 annual salary) of the associate's time to lead this effort during years 1-4. This equates to about $10,000 per year including benefits (calculated at 40% of salary).
    - Technician 3 at 320 hours ($3,840) during years 1-4 will be used to complete field sampling and processing larval fish data collections.

Travel ($108,751)

- Current budget puts 1 crew in the field during winter and early spring (January - March) and late summer - winter (August through December) as conditions allow. We will use 2-3 field crews on the river during spring and summer (late March - July). Crew 1 would initially include the PhD student and 1 M.S. student when the hourly technicians are not available. The graduate students will then lead their independent crews during May through July. Travel expenses largely cover vehicle rental, mileage, and fuel per the UNL currently published rates for ¾-ton trucks that can pull airboats (e.g., current rate is $500/month plus $0.29 per mile above 1,110 miles per month and actual fuel costs) and travel to meetings with PRIPP EDO as needed.
    - Crew 1 would focus on passive telemetry setup, fish collection, transmitter implantation, active tracking to document spawning and support free embryo sampling and habitat associations,

- Crew 2 would focus on active tracking to document spawning and support free embryo sampling and habitat associations,
- Crew 3 will be used to sample embryos and larvae at the confluence of the Platte River with the Missouri River when Crews 1 & 2 are engaged in intensive, active tracking during and following spawning events.

Equipment ($74,000)

The University of Nebraska-Lincoln's policy is that any single item over $5,000 is inventoried equipment. The following identifies equipment needs for this project.

- We request four (4) VR-100 receivers ($10,000 each X 4 = $40,000), including omnidirectional and directional hydrophones, to support active tracking for both field crews. Two receivers per crew are needed to simultaneously operate the omnidirectional and directional hydrophones and is consistent with Missouri River Pallid Sturgeon telemetry protocols that are currently in use.

- We request two (2) multi-probe sensors ($9,000 each X 2 = $18,000) to assess water quality parameters (e.g., temperature, dissolved Oxygen, Conductivity, etc.) to support active tracking for both field crews.

- We request two (2) flow meters ($8,000 each X 2 = $16,000) to measure water velocity at fish locations in support active tracking for both field crews.

Supplies + Other ($222,500)

The University of Nebraska-Lincoln lists individual items under $5,000 as supplies or other items. Specific breakdowns of those items are:

- Supplies for field work and sample collection ($40,000) include fuel for 2-3 boats, rope, ichthyoplankton nets, surgical supplies, etc.

- Other ($182,500)

  - Listening Stations ($75,000) – VR-X2Tx receivers (30 X $2,500 each and includes supplies needed to deploy the receiver like anchors, weighted base, bridge bolts, etc.)
  - Transmitters ($80,000) – V16TP and V13TP transmitters with Temperature and Pressure (Depth) sensors ($1000 each X up to 80 transmitters).
  - Computers and software ($10,500) - Field rugged laptops (e.g., Toughbooks; $4,500 each x 2 = $9,000) and software (e.g., ArcView, InnnovaSea dedicated telemetry data, etc.; $1,500) for data entry and receiver download data collection.

- Temperature loggers ($6,000) – 30 data loggers will be purchased ($200 each) to monitor temperature conditions in specific habitats during spawning and other key time periods.
- Publication/printing ($3,000) – Costs incurred for printing and publication fees for reports, publications, etc.
- Rent ($8,000) – The University of Nebraska-Lincoln does not currently have sufficient space to store large boats and sampling equipment. We currently rent commercial storage space with annual rent currently at about $40,000. The university has partially subsidized commercial storage space ($15,000 per year), but a consortium of four researchers (Pegg and Spurgeon are 2 of the 4) pay the remainder plus utilities. The $2000 per year is requested in support of airboat/gear storage for this project in a secured location and is prorated to reflect current use and space allocations relevant to this project.

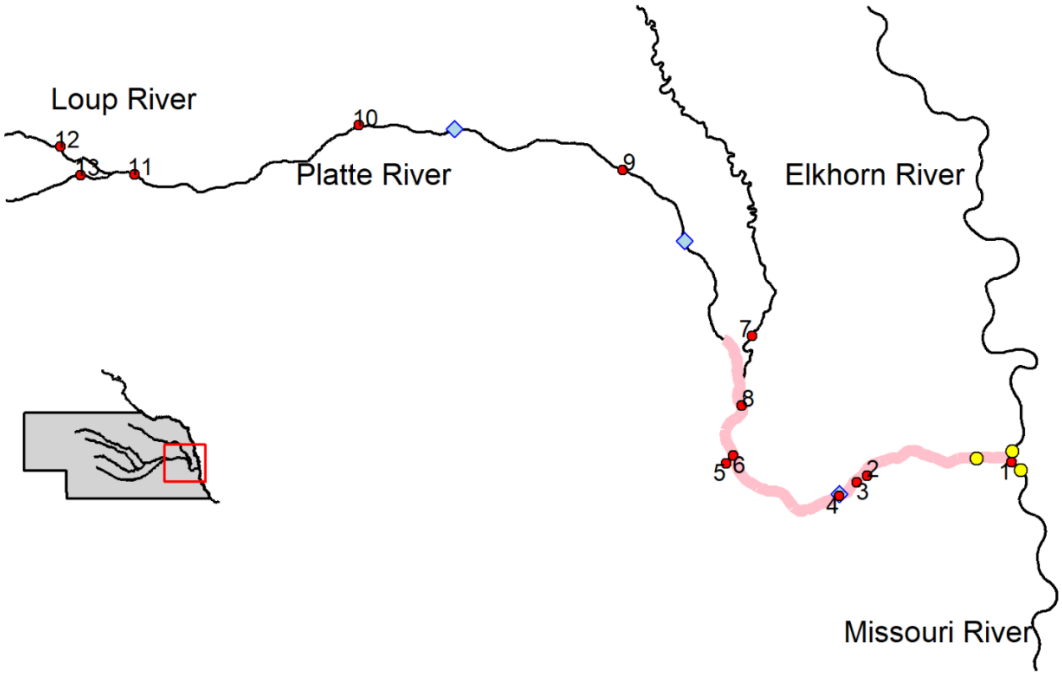

Figure 1. Rivers where Pallid Sturgeon will be tracked during the course of the proposed study. Potential listening station locations in lower Platte River are represented by red dots. The locations of USGS gaging stations are represented by blue triangles. The section of the lower Platte River where sampling for Pallid Sturgeon will occur initially is represented by a solid pink line. The locations where free embryo/larval drift samples are initially proposed are indicated by yellow circles. Some listening station areas will likely require >1 receiver to detect fish across width of river due to limited "line of sight" detection from channel braiding (refer to Table 2).

Attachment B

Research Proposal – Ed Heist/Southern Illinois University Genetics Study (Lower Platte River + Missouri River)

Resolving Pallid Sturgeon Species Identification, Demographics and Hybridization using GT-Seq
Edward J. Heist
Southern Illinois University Carbondale

Introduction – Pallid sturgeon (*Scaphirhynchus albus*) is an endangered species endemic to the Missouri and Mississippi river basins where it is far less common than shovelnose sturgeon (*S. platorhynchus*). Pallid and shovelnose sturgeon are genetically very similar, more similar than are intraspecific populations of many other species (Allendorf et al. 2001; Campton et al. 2000). The species hybridize and produce fertile offspring (Schrey et al. 2011), meaning that an individual fish may have inherited a variable fraction of its genes from both species. Hybridization is listed as one potential threat to the survival of pallid sturgeon (Dryer and Sandvol 1993), as hybridization between a rare and common species may result in the rare species going extinct as its genome is subsumed into that of the common species (Rhymer and Simberloff 1996). Use of genetically pure pallid sturgeon as broodstock for the conservation stocking program is critical because stocking of hybrid and backcross fish can further the deterioration of species boundaries and thus accelerate extinction of pallid sturgeon. Field-research programs including the Habitat Assessment Monitoring Program (HAMP, Jacobson et al. 2015) which monitors the response of several fish species to stream modifications and the Pallid Sturgeon Population Assessment Program (PSPAP, Colvin et al. 2018) which monitors trends in juvenile sturgeon population abundance, rely on accurate species ID to manage and conserve pallid sturgeon.

Currently pallid and shovelnose sturgeon are identified using a panel of 19 microsatellite loci but recently Jordan et al. (2019) demonstrated that these markers are insufficient for identifying the species and their hybrids. Preliminary results from our ongoing project to develop hundreds of Single Nucleotide Polymorphism (SNP) markers indicate that these new markers provide much greater resolution than the current microsatellites (Figure 1). The markers were developed using a technique called ddRAD (Peterson et al. 2012) in which I sequenced small portions of the genomes of 57 putatively pure pallid and 52 putatively pure shovelnose sturgeon. The ddRAD approach is too inefficient for scoring large numbers of individuals. However, a new approach called Genotyping in Thousands by Sequencing (GT-Seq, Campbell et al. 2015) will allow us to use our ddRAD sequences to design primers and probes for efficiently genotyping larger numbers of individuals for the most powerful markers. In year one we will focus on the development of these GT-seq markers for pallid genotyping.

Species ID and hybridization – Over the past 20 years my laboratory has collected several thousand sturgeon genetic samples. Initial identification was made based on a combination of genetic and morphological traits. In years two and three of this project we will determine GT-Seq genotypes from a minimum of 2000 adult and sub-adult individuals including putative pallid, shovelnose and hybrid sturgeon distributed across the entire range of pallid sturgeon. A variety of analytical approaches will be

used to provide better resolution of the species status of each individual and identify more reliable criteria for identifying pure pallid sturgeon. Discriminant Analysis using Principal Components (DAPC, Jombart et al. 2010, Figure 1) will be used to sort genetic data into "natural" groups composed of pure species with hybrids spanning the space between the clusters. STRUCTURE (Pritchard et al. 2000) will assign the fraction of each fish's genome comprised of pallid and shovelnose sturgeon genes. Results from both analyses will be used to construct NewHybrids (Anderson and Thompson 2002) baselines for identifying pure pallid and shovelnose sturgeon and the reliability of these baselines will be evaluated using modeling as was done in Jordan et al. (2019).

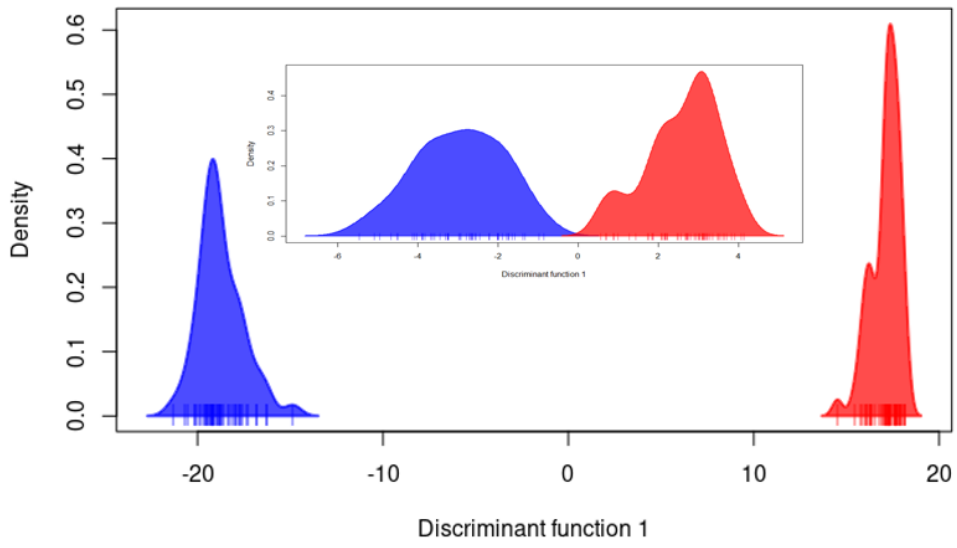

Figure 1. Discriminant analysis using principal components (DAPC, Jombart et al. 2010) resolution between putatively pure pallid (blue) and shovelnose (red) sturgeon. Main graph is for the new SNP markers, inset is for microsatellites. SNP resolution was 100% consistent with putative species identification; microsatellites falsely assigned one of the shovelnose sturgeon to the pallid sturgeon cluster.

Population Structure – Currently pallid sturgeon has four recognized management units. The GPMU is found in the upper Missouri River, the CLMU in the lower Missouri River to the mouth of the Grand River, 250 miles upstream of the confluence with the Mississippi River, the IHMU from the Grand River downstream to the confluence of the Mississippi and Ohio rivers, and the CPMU in the Mississippi from the Ohio to the Gulf of Mexico. Analyses based on microsatellites indicate that hybridization is rare in the GPMU, uncommon in the upper reaches of the CLMU, and then increasingly common in the IHMU, and pervasive in the CPMU (Jordan et al. 2019). Microsatellite data indicate that the GPMU and CLMU are genetically distinct but the CLMU and IHMU are not. However, genetic structure within species may be compromised by the presence of undetected hybrids among the purported pure pallid sturgeon, and no conclusions can currently be drawn about stock structing involving the CPMU because of the difficulty of identifying pure pallid sturgeon there. Once we have identified which sturgeon from each of

the management units are pure pallid sturgeon we will re-analyze genetic stock structure as an aid to better define management units and perhaps the designation of distinct population segments under the US Endangered Species Act.

Demographics -- Effective population size (N_e) can be thought of as the number of successful breeders and is inversely proportional to the rate at which populations are losing genetic variation and becoming inbred. N_e is generally smaller than census population size (N_c) and studies from many species indicate that N_e is, on average, approximately 10% of N_c (Frankham 1995). Franklin (1980) recommended that healthy populations have and N_e of at least 500, which was the basis for the current Pallid Sturgeon Recovery Plan's (USFWS 2014) recommendation that recover goals for each management unit will be met when "a self-sustaining genetically diverse population of 5,000 adult Pallid Sturgeon is realized and maintained within each management unit for 2 generations (20-30 years)" (USFWS 2014). Thus, reliable estimates of N_e are critical for evaluating current status and progress towards recovery goals. N_e can be estimated using genetic data, provided data can be obtained from large numbers of unlinked genetic markers (i.e., the SNP markers currently being developed).

We will determine N_e from GT-Seq data from genetically pure wild pallid sturgeon from the GPMU and CLMU management units using the NEEstimator program of Do et al. (2014). The wild GPMU pallid sturgeon represent the last remaining adults that were spawned prior to the recruitment collapse. Understanding N_e of this population will tell us N_e of a pre-collapse population, which may not have been as large as 500. The CLMU wild pallid sturgeon represent what are currently the only naturally-recruiting pallid sturgeon (Steffensen et al. 2019). We have tissue samples from more than 100 GPMU and several hundred wild CLMU pallid sturgeon that, when confirmed as pure pallid sturgeon, will make for robust estimates of N_e. Note that N_e estimates are based on comparisons between all pairs of sampled individuals and you do not need to sample 500 individuals to get an estimate greater than 500.

Population composition based on larval identification – Currently we know little about the actual composition of *Scaphirhynchus* sturgeons that are spawned throughout the lower Missouri River. Most sturgeon captured are morphological shovelnose sturgeon, while those that are submitted for genetic analyses exhibit some pallid-like morphologies. The least biased sampling of *Scaphirhynchus* are the larval and free embryo collections done by HAMP and PSPAP. In a typical year, several thousand wild-caught sturgeon free embryos and larvae are screened for 2 SNPs following the protocols of Eichelberger et al. (2014). This technique efficiently identifies most free embryos and larvae as pallid sturgeon or "other" with the "other" category including shovelnose sturgeon and hybrids. In 2014 we identified 7 pallid sturgeon larvae including 4 that were caught near the mouth of the Platte River (Heist et al. 2015). In years 2 – 5 of this project we will use GT-Seq with the new species-ID protocols to identify 2000 larval/free embryo sturgeon from the waters of Nebraska and Missouri to characterize the species composition of sturgeon that are spawned in the wild. Sampling will include reanalysis of individuals collected in 2014, which may have been a relatively successful year for pallid sturgeon spawning in the lower Missouri. This study will provide the first examination of the numbers of pallid;

shovelnose and hybrid sturgeon spawned and will provide benchmarks for determining the extent, and in future years the trajectory, of hybridization and successful pallid sturgeon reproduction.

Contributions to Platte River Learning – In addition to the contributions this project makes to our understanding of pallid sturgeon along their range as a whole, the project fills important gaps in our knowledge of the importance of the Platte River for pallid reproduction and recruitment. With SNP technology pallid free larvae that are collected at the Missouri River confluence with the Platte River can be traced back to known parents and telemetry fish. Previously this required genetic information from both parents, but with multiple SNP markers at multiple loci, genetic information from a single parent is enough for a match. Coupling this technology with the expansion of free larvae sampling into the Platte River and its tributaries will help us link parental origin to free larvae capture locations helping us understand how important the Platte is for pallid reproduction and recruitment.

The proposed research will allow us to determine the composition of Platte River pallid free larvae. GT-seq combined with SNP technology allows us to determine the fraction of free larvae at the confluence or in the Platte River itself that are pure pallid, hybrid, and shovelnose sturgeon. Without SNP technology, we currently are unable to distinguish between hybrids and shovelnose sturgeon.

Current stocking efforts have focused on the confluence of the Platte River with the Missouri River. The proposed research project contributes to pallid hatcheries and stocking by identifying pure pallids, making as much use of them as possible, while avoiding the use of hybrids and backcross fish as broodstock which further propagates their genes into future generations of pallid sturgeon.

The genetic research proposed here will be supplied genetic samples from captured putative pallid sturgeon and larva/free embryos collected during a 5-year study by UNL/NGPC that focuses on the Platte River. Previous studies on the Platte River varied in effort, sampling period, and spatial distribution of sampling. We know little about what we may be able to catch there in terms of larva/free embryo/exogenous feeding young of the year, making it difficult to get a reliable estimate of the number of samples that may be collected from the Platte. We are estimating a total number of genetic samples (adults, juveniles, larva/free embryo) collected annually from the Platte to be 25 – 40 samples.

With the more rigorous sampling effort, accompanied by tracking of telemetered fish, focused strictly on the Platte as proposed by the UNL/NGPC study, we may obtain a greater number of samples. Genetic research combined with a focused effort to capture, take genetic samples, tag and track pure pallid sturgeon within the Platte River and its tributaries fills an information gap that will help us determine how the Platte River contributes to pallid reproduction and its potential role in species recovery.

Deliverables – We will provide annual reports for each year of the project and a final report describing results, accomplishments, and interpretations. Findings will be presented at national and regional meetings including Pallid Sturgeon Recovery Team meetings, the three regional pallid sturgeon meetings, the pallid sturgeon fall science meeting and the national meeting of the American Fisheries Society. We will also publish peer-reviewed journal articles describing the findings. All papers and

presentations will acknowledge the support of the Platte River Recovery Implementation Program and the Army Corps of Engineers.

BUDGET

Year 1 (July 1, 2021 – June 30, 2022)
Illumina MiSeq DNA sequencer (with 5-year service plan) $143,810
Labor $20,457
GT-Seq Primers $5750
Consulting (GTseek) $3000
Indirect (47.5% all but equipment) $13,873

Total year 1 = $186,890

Total Year 1 Costs paid by PRRIP for equipment purchase and GT-seq consulting.

Year 2 (July 1, 2022– June 30, 2023)
1000 samples run w/ GTseq
$45 per sample*
1000 samples = $45,000

Year 3 (July 1, 2023– June 30, 2024)
1000 samples run w/ GTseq
$45 per sample
1000 samples = $45,000

Year 4 (July 1, 2024– June 30, 2025)
1000 samples run w/ GTseq
$45 per sample
1000 samples = $45,000

Year 5 (July 1, 2025– June 30, 2026)
1000 samples run w/ GTseq
$45 per sample
1000 samples = $45,000

Total Years 2-5 = $180,000

Year 2-5 Costs are shared by PRRIP and ACOE based upon the number of samples analyzed from each source (see budget justification below).

ACOE estimated contribution based upon 960 samples/year at $45/sample over 4 years	$172,800
PRRIP estimated contribution based upon Total Year 1 cost for GT-seq equipment and development 40 samples/year at $45/sample over 4 years	$194,090
Total 5-year project budget	**$366,890**

Budget Justification

Student Personnel:

Funding is requested to support a Ph.D. student in years 1 through 5. The graduate student will perform the GT-Seq and bioinformatics under the supervision of the PI.

Equipment:

In year 1 I am requesting $143,810 for the purchase of an Illumina MiSeq desktop genome sequencer. This instrument is necessary to run the GT-Seq genotyping. This model was chosen because of the flexibility in the amount of data that can be collected in a single run and because the instrument cost and cost per run are appropriate for a project of this size. The quote includes five years of service contract to ensure that the instrument will be operational for the duration of the project.

Commodities:

Appendix 1 in Campbell et al. (2015) provided a detailed list of the commodities and costs associated with GT-Seq genotyping including microtubes, pipet tips, enzymes etc. and determined a cost of $3.98 per sample based on the use of an Illumina Hi-Seq instrument (Campbell Appendix 1 attached). The Hi-Seq costs less per megabase of data, but the instrument costs ≈ 10 times as much as the MiSeq and would require far more throughput of samples than is practical for this project. Substituting the MiSeq instrument into Campbell's analysis changes the cost of item #8 from $42.26 per 96 samples to $197.80 per 96 samples for a total cost of $6.47 per individual. The price of $197.80 per 96 well plate is based on the $989 per Illumina v3 150 cycle kit (see instrument quote) which provides enough sequence data for five plates of 96 samples each. The calculations provided by Campbell (2015) are based on the pricing at the time and do not include shipping, inflation, and the inevitable samples that fail on the first run and need to be run again. To compensate for these, I am multiplying the $6.47 per sample by 1.25 to get a current realistic commodities cost estimate of $8.09 per sample. $8.09 per sample times 1000 samples per year = $8090 in commodities each in years 2-5. There will also be a one-time expense of $5750 to purchase 250 pairs of primers at $23 per primer pair in year 1.

Other Direct Costs:

Nate Campbell, who invented the GT-Seq and now has his own company called "GTseekLLC" will serve as a consultant on the project. He will be paid a flat fee of $2000 for primer development and will be paid $100/hr for up to 10 hours of consultation during the genotyping (quote attached).

Indirect Costs:

Southern Illinois University Carbondale's (SIUC) predetermined rate of 47.5%, effective July 1, 2016 was used. SIUC's Facilities and Administrative (F&A) rates are approved by the Department of Health and Human Services. The distribution base for the F&A rate is modified total direct costs (MTDC).

Costs Per Sample:

In each of years 2-5 of the project, 1000 individuals will be analyzed using GT-Seq. Estimated costs for 1000 samples in year 2 includes $8090 in commodities, $21,060 for the graduate student stipend, and $13,846 indirect cost = $42,996. Graduate student stipends are expected to rise 3% each year and commodity costs will likely rise as well. I estimate the average cost per sample for 1000 samples over each year of the project to be $45 per sample.

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