1. Thermal Assessment of Turtle Growth and Habitats at Rehabilitation Sites

Solicitation Topic 3:

Can rehabilitation site design provide adequate thermal heterogeneity for northwestern pond turtle (*Actinemys marmorata*) growth?

Prepared for: Trinity River Restoration Program

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2. Project Description

a. Priority Item

Topic 3: Can rehabilitation site designs provide adequate thermal heterogeneity to promote northwestern pond turtle (*Actinemys marmorata*) growth?

b. Project Intention and Significance

Managed flows since damming of the Mainstem Trinity River have altered physical and thermal habitats downstream, which has ecological implications for fish and wildlife reliant on the river (Asarian et al. 2023). With anadromous fishes restricted from access to cold headwaters upstream of Lewiston and Trinity dams, elevated summer baseflows were implemented to support cold-water fisheries downstream of the dams, with the intention to compensate for the loss of access to upstream habitats. While managing for colder summer water temperatures to benefit adult salmonids and eggs, an unanticipated outcome has been a decrease in growth rate of young of year salmonids, potentially lowering survival of outmigrants when they reach the marine environment (Asarian et al. 2023). Young salmonids benefit from access to warmer waters as they move downstream, which can lead to larger outmigrants with higher survival at sea, and ultimately an increase in returning spawners. Because anadromous fishes experience a wide variety of habitats during their life cycle, it can be challenging to isolate individual components of the environment that contribute to their success.

Native freshwater turtles have high site fidelity, long life spans, and their growth is influenced by water temperature, making them good biological indicators of local aquatic thermal conditions. Studies of the northwestern pond turtle[1] (*Actinemys marmorata*; hereafter, turtle) on the Trinity River have demonstrated demographic and individual impacts to turtles living downstream of the dams. Managed flows since damming, particularly the reduction in magnitude and frequency of high-flow events, resulted in a reduction in turtle rearing habitat and colder water in summer (Reese and Welsh 1998a,b). Following the Record of Decision (USDI 2000), a greater allocation of water retained in the basin has further reduced the growth rate of juvenile turtles (Ashton et al. 2015). Slower growth trajectory of juvenile turtles can increase the duration of predation risk by gape-limited predators and decrease annual survivorship, adult body size, and lifetime reproductive output, leading to negative population impacts for many decades. By evaluating physiological and biological responses of turtles downstream of the dams, we can gain insights to help inform fisheries management.

Channel rehabilitation designs have the potential to increase availability of rearing habitat for young of year salmonids and young turtles and can ameliorate negative thermal impacts imposed by a colder summer thermal regime in the main channel with minimal effects predicted for mainstem temperatures (Zedonis 1994). Thermal heterogeneity in multiple spatial (lateral, vertical, longitudinal) and temporal (diel and seasonal) dimensions allows ectothermic organisms to seek optimal temperatures for metabolism, growth, and survival (Asarian et al. 2023). However, the potential for channel rehabilitation to provide sufficient thermal relief to benefit turtles and other

[1] The northwestern pond turtle (*Actinemys marmorata*; hereafter, turtle) is a California Species of Special Concern (Thomson et al. 2016) and is proposed for federal listing as Threatened under the Endangered Species Act (USFWS 2023). As a federal agency, the Bureau of Reclamation has a responsibility to proactively manage its actions to support conservation of species recognized under the Endangered Species Act.

riverine wildlife and fishes is in need of further evaluation (Lind et al. 1995; Lindke and De Juilio 2018; Cooper-Hertel et al. 2021). Knowing that turtle growth in the main channel has been impaired by colder summer water temperatures from hypolimnetic releases and elevated summer base flows (Ashton et al. 2015; Snover et al. 2015), the intention of the proposed project is to determine whether rehabilitation sites now provide adequate aquatic thermal relief to promote growth of young turtles comparable to that seen in an unregulated river fork in the watershed.(South Fork Trinity River) or other rivers in the region. This study is intended to augment previous studies that assess thermal effects on salmonid growth by using turtles as a biological indicator (see references cited above).

In this study, we propose to test these two hypotheses:

1) Channel rehabilitation sites can provide aquatic microhabitats that exceed 21℃ in mid-summer (July), or approximately 4℃ above adjacent main channel temperatures, in the Douglas City to Junction City stretch of the river under the current flow regime with summer baseflows at 450 cfs.
2) Assuming warmer aquatic microhabitats are provided by rehabilitation sites; these warmer waters improve annual growth of young turtles as determined by annuli measurements of younger turtles (< 10 years old).

c. Project Goals, Objectives, and Adaptive Management

The Trinity River Flow Evaluation Study (TRFE; USFWS & HVT 1999) proposed a fishery resource restoration strategy recommending management actions expected to create a functioning river ecosystem that could provide and maintain the variety and abundance of habitats necessary to restore the anadromous salmonid and other riverine dependent fish and wildlife populations of the Trinity River. The Record of Decision (ROD; USDI 2000), signed by the Secretary of the Interior, adopted the TRFE recommendations, plus a watershed restoration component, for restoring the mainstem fishery resources of the Trinity River. The TRFE and ROD provide a restoration strategy, including management actions and associated targets. However, these documents do not provide detailed methods for assessing the effectiveness of the management actions in achieving Program goals or management targets.

This science study plan proposes to increase our understanding of a key component identified in the recently proposed RAEWG Target, Objective RAE-2 (modified from the original IAP target 6.5.2) specifying the importance of maintaining suitable water temperatures for growth of juvenile northwestern pond turtles. The proposed target currently reads as follows,

"Increase thermal gradients to provide warmer waters exceeding 21℃ *mean daily maximum in July in peripheral habitats and around structural features used by various age classes of northwestern pond turtles."*

The goals of this proposal are to assess the growth of younger turtles at channel rehabilitation sites and measure the range of thermal heterogeneity at selected rehabilitation sites, prioritizing rehabilitation sites being used by turtles. In a sense, we're using turtles to help pre-select the sites for assessment. Within the rehabilitation sites, younger turtles will preferentially seek microhabitats that provide the best available conditions for growth and survival including backwaters, alcoves, and off-channel wetted areas.

We propose to address two objectives with this research (details provided in Methods):

- Measure annuli (growth rings) of young turtles (< 10 years old) to assess juvenile growth rates within and outside of constructed rehabilitation sites. This objective is intended to evaluate annual growth of young turtles in recent years to assess annual growth relative to thermal regimes at micro-habitats available to young turtles. Annuli measurements will also be compared to data previously collected on the mainstem and south fork Trinity rivers in the 2000s, as well as comparable data from other localities in Northern California and Southern Oregon across a range of thermal regimes.

- Determine the range of aquatic thermal heterogeneity within selected rehabilitation sites to determine the degree to which channel rehabilitation can influence water temperature in peripheral habitats during mid-summer. This includes measuring temperatures where turtles are seen as well as across the range of microhabitats observed throughout the site and in the mainstem adjacent to the selected sites. In still water areas, where limited mixing is perceived, we will also measure water temperatures throughout the water column; these measurements will focus on areas most suitable for rearing of turtles – areas of calm, warmer water with aquatic vegetation or other cover.

If the proposed study demonstrates that desired thermal heterogeneity (i.e., adequate warming in some peripheral habitats within rehabilitation sites to promote growth of young turtles) is not achieved by the topographic configuration of rehabilitation sites, then recommendations for increased structural complexity or adaptive management approaches for flow releases to achieve the desired magnitude of thermal heterogeneity will be provided to the Program for consideration.

d. Methods

i. Site selection

We will conduct a field reconnaissance tour for one week in June, after spring hydrograph peak flows recede, to select sites for water temperature monitoring and turtle growth assessments. During this visit, we will select three rehabilitation sites for evaluation. We will prioritize site selection based on turtle occupancy, particularly where younger turtles are most likely to be encountered. This includes sites where turtles have previously been seen, or seen during the reconnaissance visit, sites with areas of calm waters with emergent or submerged aquatic vegetation (often used as cover by young turtles), and/or sites adjacent to known nesting locations or suspected nesting habitat. Our site selection will initially focus on sites along the mainstem between Douglas City and Junction City, where past surveys have documented higher turtle abundance relative to other sections of the mainstem (Snover and Adams 2016; Don Ashton pers. obs.). Some of the rehabilitation sites being considered include Douglas City, Lorenz Gulch, Steiner Flat, Dutch Creek, Deep Gulch and Sheridan, Chapman Ranch, Oregon Gulch, and Junction City. Sites in the Lewiston area were excluded from this study because turtles are uncommon in the colder reaches closer to the dam and the few turtle observations have been in off-channel habitats isolated from hypolimnetic releases. While these upstream sites may have a greater difference in temperature between off-channel and main channel waters, sample size per effort is expected to be quite low based on previous surveys, with the exception of the Lowden Ranch ponds which are isolated from the river (Sloan 2012).

At the end of the reconnaissance tour in June, temperature data loggers will be deployed at the selected sites to record water temperature during the duration of the study. Three data loggers will be placed at each selected rehabilitation site with an additional data logger in the main channel adjacent to the site. Data logger model and exact placement have yet to be determined but our goal is to document the range of thermal heterogeneity at each site by placing data loggers in locations where warmer water, and presumedly younger turtles, are expected along with a corresponding main channel data logger. We will aim to suspend data loggers in the water column 10 cm below the water surface, but we will make a final determination of placement based on onsite observations and consideration of risk of vandalism or theft. Data loggers will remain in place until the end of the field component of the study (approximately 2 months). We will use data logger temperatures to supplement the manually measured temperatures collected during on-site field data collection.

ii. Data collection

We will return to the selected rehabilitation sites in mid-summer to conduct field measurements. Site visits will target mid-summer (mid-July to mid-August) when maximum warming of peripheral waters is expected, and young turtles will have experienced growth within the year. Site visits will include the selected rehabilitation sites and nearby habitats in main channel used by turtles. We plan for at least two separate data collection site visits approximately one week apart at each study site to increase the sample size of turtles and allow for repeated temperature measurements. Additional visits to some sites may be needed to increase the likelihood of encountering young turtles for annuli measurements.

We propose to evaluate turtle growth to assess whether increased thermal heterogeneity provides a biologically relevant growth advantage. Each week, at each site, we will capture young turtles (< 10 yrs old) and measure growth annuli to evaluate growth rate. Annuli preserve a record of annual growth rate for about the first decade of life. Measurements of annuli have been correlated to annual environmental conditions in desert tortoises (Germano 1988, 1992; Medica et al. 1975, 2012), but use of this method to assess growth of freshwater turtles in response to environmental conditions has rarely been employed (e.g., Rickard et al. 1989). Although factors beyond aquatic thermal regime influence turtle growth, a previous study of turtles in the Trinity River suggests water temperature is a key factor in this system (Ashton et al. 2015). Individual annuli will be measured in the field using digital calipers, starting from zero (the natal annulus) and continuing to the current year. The plastron will also be photographed with a ruler for scale to preserve a record of the annuli. Growth annuli from previous years will allow retrospective assessments of annual growth in recent years (similar to using tree rings to estimate variation in annual climate). Growth annuli for turtles at sites with increased thermal will be compared to growth at locations with lower thermal heterogeneity to assess whether increased thermal heterogeneity provides a biologically relevant growth advantage. Growth slows, or essentially ceases, in adult turtles (a determinate growth pattern) so new growth annuli are not discernable on adults. Also, annuli tend to wear down over time, so the record of juvenile growth is no longer retained on the plastron of most adult turtles, therefore, we will not be measuring annuli on adult turtles (generally ≥ 10 years of age or ≥ 125 cm carapace length). We will document the occurrence and location of adult turtles in the rehabilitation sites to assess turtle use and occupancy at constructed sites, but adults will not contribute to our assessment of annual growth rate in relation to thermal regime.

Because use of annuli measurements to evaluate effects of aquatic thermal regime is a novel approach, and an adequate sample size of young turtles is not assured, we will also assess the thermal heterogeneity provided by rehabilitation sites, both for comparison to turtle growth and as an independent assessment. In addition to temperature data loggers, we will measure water temperature where turtles (young turtles and adults) were first observed, and two randomly generated locations nearby, one location within the rehabilitation site and one location nearby in the main channel near-shore (within 4 meters of shore; same day and similar to turtle observation time). Random locations within the rehabilitation site will be generated by selecting a random number from 10 to 60 for distance in meters, and a random number from 1 to 16 to provide a direction. If the randomly generated location is not wetted, the opposite direction will be used. If the opposite direction does not lead to a wetted location, a new set of random numbers for distance and direction will be generated until a nearby, wetted, random location is found. Corresponding locations in the nearby main channel will largely be determined by access and feasibility and will be augmented by data logger readings. We will also measure water temperature at a variety of depths in the water column to get a sense of thermal stratification in calm waters favored by turtles. This may also be important for growth of young salmonids (e.g., Cooper-Hertel et al. 2022). We plan to manually measure temperature from the water surface down through the water column to the substrate at 10 cm increments. These will not be continuous measurements (i.e., not data loggers), but we will document the time and location of water column temperature measurements. Additionally, we will measure water temperature at additional locations that are noticeably warmer or colder. These water temperature data will be used to assess the range of thermal heterogeneity within the rehabilitation site and to compare to main channel temperatures.

With these data we will attempt to answer the questions, “Are turtles selecting warmer portions of the rehabilitation site?” and “Are rehabilitation sites used by turtles warmer than near-shore habitats in the main channel?”

While there are other worthy questions about turtles to investigate, the purpose of this proposed study is to assess the effectiveness of rehabilitation site designs to increase thermal heterogeneity to improve growth of ectothermic organisms in the river by using turtles as a biological indicator. If the Program requests information on turtle population trends and other demographic metrics in a future solicitation for scientific investigations, a proposal for a mark-recapture study replicating the methods used for comparisons of the mainstem and south fork Trinity River in the 1990s and 2000s (Reese and Welsh 1998a; Ashton et al. 2015) can be prepared.

iii. Data processing and analysis

We will compare measurements of turtle annuli for locations with different thermal profiles. Success of this component relies on the sufficient sample size of young turtles captured in zones with different thermal regimes. If there is little temperature difference between locations where turtles were captured, we will use previously collected data on young turtles from the mainstem (cold water) and the south fork (warm water) of the Trinity River for comparisons (Ashton et al. 2015) and other rivers in the region (D. Ashton, unpublished data).

After field data collection, Yurok Tribal Fisheries collaborators will run the SRH-2D temperature model (independent of this study) for one or more locations (channel rehabilitation sites) where we collected field measurements (turtle and temperature measurements), with the modeled date(s) corresponding to the field data collection date(s) to account for the meteorological patterns experienced. We will compare the range of thermal heterogeneity recorded in the field to the range of thermal heterogeneity predicted by the SRH-2D temperature model for the selected channel rehabilitation sites. This comparison is dependent on the capacity of Yurok Tribal Fisheries collaborators to run the model and process the results in a time frame compatible with the proposed deliverables timeline for this project. Otherwise, the data from the project will be made available for future comparisons to the SRH-2D model predictions.

We will evaluate the temperature range from the warmest locations found during the field visits, locations where turtles are found, and mainstem temperatures adjacent to the site. To the extent feasible, we will coordinate with Hoopa Valley and Yurok Tribal Fisheries, as well as Trinity River Restoration Program staff, to maximize the compatibility of temperature data across disciplines.

iv. Reporting

We will generate a written report explaining the results of the proposed study with comments on the thermal heterogeneity at channel rehabilitation sites and recommendations for habitat enhancements to benefit turtles. A data package with temperature data by location, turtle measurements and photos, and turtle marking codes will be included with the final report. We will submit the final report and data package to TRRP for peer-review by December 2026. We plan to respond to reviewer comments in a timely manner. Following a review with the Riparian and Aquatic Ecosystem Working Group (RAEWG), we will present a summary of the study results to the Interdisciplinary Team (IDT) and Trinity Management Council (TMC). Presentations may need to occur after December 2026, depending on RAEWG, IDT and TMC meeting schedules.

e. Project Timeline

TASK	DESCRIPTION	TIMELINE
Task 1	Project planning and coordination	Upon release of funds
Task 2	Field reconnaissance, site selection	June 2026
Task 3	Field work and data collection	July – August 2026
Task 4	Data processing and analysis	September – October 2026
Task 5	Reporting and presentations*	November – December 2026

3. Budget

Budget summary table, detailed budget provided as a spreadsheet.

TASK	DESCRIPTION	ARS TOTAL COST ESTIMATE FOR TASK	GRAND TOTAL COST ESTIMATE FOR TASK
TASK 1	Project Management/Coordination	$12,928	$15,627
TASK 2	Field Reconnaissance, site selection	$16,921	$20,589
TASK 3	Filed Work and Data Collection	$28,699	$36,034
TASK 4	Data Processing and Analysis	$21,768	$21,768
TASK 5	Reporting and Presentation to TMC	$30,796	$32,596
	TOTAL:	**$111,112**	**$126,614**

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