

### Example Recovery Permit Application Proposal for Specialized Research Activities:

Our research aims to understand the genetic diversity, disease-carrying prevalence, and population dynamics of the California tiger salamander (CTS) using various methodologies. This document outlines: I. specific methodologies, II. the rationale for site selection, and III. potential challenges in genetic analysis.

#### I. Proposed Research Methods

We will conduct genetic collections at each site, focusing on tail and toe clips, as well as swabbing for chytrid fungus (Bd and Bsal). We will sample 20 – 50 CTS from each of the below sites (refer to Rational for Site Selection section below) across Sacramento, Yolo, Solano, San Joaquin, and Stanislaus Counties. Collections will occur over a 1–3-year period, and collections will only occur once at each site.

At each site, we will:

- Conduct visual encounter surveys.
- Capture CTS with clean gloves, rinse with DI water, and place in clean Whirlpak bags.
- Measure SVL and weight in the bags, to prevent pathogen transmission.
- Keep bags cool during processing.
- Swab each CTS with clean swabs, storing samples in ethanol and RNALater.
- Clip 2mm of the fourth toe, storing samples for genetic sequencing and Bd and Bsal isolation. Toe clips will only be taken from adults  $\geq$  to a specific length or snout-vent length.

We will sterilize scissors between animals and monitor CTS health, releasing any distressed individuals immediately. This procedure ensures the health and survival of CTS populations while collecting necessary genetic data.

#### **Genetic Analysis Methods:**

- **DNA Extraction and Amplification:** We will extract DNA from tail and toe clips using standard protocols. The extracted DNA will be amplified using polymerase chain reaction (PCR) to target specific genetic markers.
- **Microsatellite Analysis:** Microsatellites, or short tandem repeats, will be used to assess genetic diversity and population structure. These markers are highly polymorphic and provide detailed insights into genetic variation.
- **Mitochondrial DNA Sequencing:** Sequencing of mitochondrial DNA (mtDNA) will help trace maternal lineages and understand the evolutionary history of CTS populations.

- **Next-Generation Sequencing (NGS):** NGS technologies will be employed to obtain comprehensive genomic data, allowing for the identification of adaptive genetic variations and potential resistance to diseases like chytrid fungus.
- **Quantitative PCR (qPCR):** qPCR will be used to quantify Bd infection loads in collected samples, providing data on pathogen prevalence and virulence.

## II. Rationale for Site Selection

The selected sites represent a range of habitats and environmental conditions within the California Central Valley. This diversity allows us to study the genetic variation and adaptability of CTS across different landscapes. By including both natural and man-made habitats, we can assess the impact of habitat modifications on CTS populations. Additionally, the presence of other amphibian species at these sites provides an opportunity to study interspecies interactions and their influence on CTS genetic diversity.

**Willow Creek Pond, Sacramento County:** Chosen for its diverse amphibian population.

**Sunset Mitigation Marsh, Sacramento County:** Man-made site isolated in a large area of residential development.

**Blue Heron Pond, Yolo County:** Known for its stable CTS population and accessibility for long-term monitoring.

**Golden Meadow Mitigation Wetland, Yolo County:** Features a mix of natural and man-made habitats, providing a comparative study environment.

**Eagle Nest Pond Mitigation Reserve, Solano County:** Offers a controlled environment to study the impact of habitat modifications on CTS.

**Silver Lake Marsh, Solano County:** Selected for its high biodiversity and presence of both CTS and Western Toad.

**Cedar Grove Biological Site, San Joaquin County:** Chosen for its historical data on CTS and Western Toad populations.

**Maple Leaf Wetland, San Joaquin County:** Provides a diverse habitat with a mix of native and invasive species.

**Oak Ridge Cow Pond, Stanislaus County:** Known for its consistent CTS sightings and varied aquatic habitats in human-made cow watering holes.

**Pine Valley Creek Vernal Wetlands, Stanislaus County:** Selected for its pristine condition and minimal human disturbance.

### Conservation Implications:

1. **Genetic Diversity Preservation:** Understanding the genetic diversity within and between CTS populations is crucial for maintaining their long-term viability. High

genetic diversity enhances the species' ability to adapt to environmental changes and resist diseases.

2. **Informed Management Strategies:** Genetic data can inform conservation strategies, such as identifying critical habitats for protection and designing effective breeding programs to prevent inbreeding and genetic bottlenecks.
3. **Disease Resistance:** By identifying genetic markers associated with disease resistance, conservationists can develop targeted interventions to mitigate the impact of pathogens like Bd on CTS populations.
4. **Habitat Restoration:** Insights into the genetic structure of CTS populations can guide habitat restoration efforts, ensuring that restored habitats support genetically diverse and resilient populations.

### III. Potential Challenges in Genetic Analysis

1. **Large Genome Size:** Amphibians, including CTS, often have large and complex genomes, which can be challenging to sequence and analyze due to their size and repetitive elements.
2. **Sample Degradation:** Genetic material can degrade quickly if not properly preserved, especially in field conditions. Ensuring the integrity of samples from collection to analysis is critical.
3. **Technical Limitations:** Despite advances in sequencing technologies, technical limitations such as sequencing errors and incomplete genome assemblies can hinder the accuracy of genetic analyses.
4. **Environmental Contamination:** Contamination from environmental DNA (eDNA) can complicate the analysis, requiring stringent protocols to ensure sample purity and accuracy.

This research will contribute valuable insights into the conservation and management of CTS, ensuring the long-term survival of this species in its native habitat. By integrating genetic analysis with field surveys, we aim to develop a comprehensive understanding of CTS population dynamics and inform effective conservation practices.

### Citations:

- [1] Johnson, Sarah. 2021. Genetic Analysis Techniques for Amphibian Conservation. Animal Conservation Society. Volume 5. pp. 34-56.
- [2] Harper et al. 2022. Advancing Genetic Research. The North American Amphibian Genomics Consortium. Volume 3. pp. 78-102.