An Urban Reintroduction of Western Pond Turtles in Southern California

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ABSTRACT

As a cooperative effort between the University of California, Los Angeles (UCLA), the Turtle Conservancy, and the United States Geological Survey (USGS), we have developed an outline for reintroducing western pond turtles (*Emys pallida*) into an urban waterway in the Los Angeles area. First, we present a brief literature review covering the biology and ecology relevant to a reintroduction of western pond turtles. Next, we introduce the research questions central to implementing a reintroduction, followed by the methodology, and results of fieldwork, genetic analysis, and Geographic Information System (GIS) mapping. Finally, a discussion of the work that has been completed so far provides final recommendations for the reintroduction and establishes a framework for the future of the project.

INTRODUCTION

Conservation Biology and Reintroductions

Both the successes and failures of an urban reintroduction represent a learning opportunity for conservation biology. Conservation biology aims to protect threatened and human-impacted environments and endangered species by maintaining self-sufficient, healthy populations of organisms in the wild (McPhee, 2004). A key aspect to this includes species reintroductions, defined as the "intentional movement" of organisms to an area where they were previously or historically located (Fischer & Lindenmayer, 2000; Armstrong & Seddon, 2007). This human intervention has been interchangeably referred to as the reintroduction and translocation of populations. Our goal in this project is to create a self-sustaining population of turtles in a waterway of an urban area in Southern California. Throughout this proposal, reintroduction refers to the release of animals from some captive form of care to the wild and translocation refers to the movement of animals from one location to another within the wild.

WPT as a Candidate for Reintroduction

The western pond turtle (*Emys pallida*, hereafter WPT) is a species native to Southern California, and has been experiencing population declines due to a variety of factors including human development and urbanization. Today, WPT numbers have declined in its native habitat. Contributing factors include a decrease in suitable habitat, alteration of suitable habitat, introduction of exotic flora and fauna, and other anthropogenic forces (Penrod, 2002). WPT is a threatened species residing along the Pacific coast of the United States, from Baja California to the Puget Sound region of Washington (Lovich, 1998). Throughout the range, two distinct WPT species exist (Spinks et al., 2014). This includes a northern species (*Emys marmorata*) with populations extending north along the coast to San Luis Obispo, with inland populations reaching farther south (Spinks et al., 2014). The southern species (*Emys pallida*) occurs along the California coast from San Luis Obispo down to Baja California (Spinks et al., 2014). There is some admixture between the northern and southern species in the central coast mountain range and San Joaquin Valley (Spinks et al., 2014). Because our reintroduction will be carried out in coastal Southern California, we will focus on the southern species of western pond turtle (*E. pallida*).

We have developed a protocol for reintroducing WPT into the Los Angeles Basin region. This protocol is based on surveys of potential reintroduction sites and source populations, and includes strategies for reintroducing WPT based on stakeholders and local jurisdictions. The Los Angeles Basin is located in Southern California. Surrounded by the Los Angeles, San Gabriel, and Santa Ana Rivers, and adjacent to the Pacific Ocean, its presence as a coastal plain has provided habitat for many organisms (Gumprecht, 2001). Mountains also surround the Los Angeles Basin, with the Santa Monica Mountains and San Gabriel Mountains to the north. Its significance as a historical WPT habitat makes it a desirable region for considering reintroductions.

For this project, we have decided to pursue an urban reintroduction that the public can see and interact with. The purpose of this is to measure and record the parameters for success within an urban, and not secluded, reintroduction. Our reasoning for this decision is that while WPT would surely benefit from a secluded, private reintroduction, an urban reintroduction could benefit many species as a whole. If we successfully conduct a reintroduction in urban regions, it can serve as a precedent for other reintroductions in urban regions for other species. As a native organism to the Pacific coast of the United States, their presence in the Los Angeles Basin is an excellent opportunity for educational outreach within a large metropolitan area, bringing into perspective how reintroductions can have a large potential impact in influencing people and attitudes.

WPT Habitat and Ecology

WPT can inhabit fresh or brackish water, and utilize a variety of riparian habitats across the world. They prefer habitats with various vegetation and hydrological gradients that serve different purposes for behaviors such as basking and nesting. They consume insects, annelids, crustaceans, vegetation, and other small organisms (Bury, 1986). WPT appear to be food generalists, exhibiting omnivorous feeding habits. A study conducted in Hayfork Creek in Northern California found that juvenile WPT were more carnivorous than adult WPT, and that this could be due to the higher metabolic demands imposed upon juvenile WPT (Bury, 1986). Overall, WPT tend to prefer a carnivorous diet (this includes small insects and young crustaceans) over an herbivorous diet, but will consume plant tissue when it is more readily available (Bury, 1986). Predators of pond turtles include rodents, larger mammals, other reptiles, fish, and birds (Penrod, 2002). Juvenile pond turtles are especially preyed upon because of their small size and soft shell.

Another important factor that significantly affects habitat suitability is water quality. Without knowing or locating water of suitable quality, we run the risk of introducing turtles to habitat that can harm them in the short and long term (Bury, 1986). Substances with the potential to harm WPTs include chemical toxins and pathogens, with special attention to human induced sources (Holland, 1994). Gauging a waterway for chemical purity requires both testing and qualitative analysis. This means looking for potential threats upstream from the waterway, such as chemical and sewage plants. Monitoring for the presence of pathogens calls for molecular detection assays and PCR amplification. However, we may not need to employ these investigations if we take a comprehensive look at the prospective site. The presence of other organisms in trophic levels above and below that of the WPT is a great place to start, under the assumption that these organisms could not survive in low-grade water.

Relevant ecosystem indicators include the flora and fauna that are involved with the niche of WPT. Annelids, mollusks, insects, other small animals, and various forms of vegetation make up the diet of WPT (Bury, 1986). Predators of the WPT are a necessary component of the prospective site as well. These include opossums, coyotes, birds, skunks, and other medium to larger sized animals (Bury, 1986). The presence of these organisms indicate two things: (1) there is an appropriate niche role for WPT to fill that will not disturb the flow of the ecosystem, and

(2) the ecosystem is healthy enough to support these organisms and therefore healthy enough to support WPT.

Aside from trophic level dynamics, there are other factors that contribute to the suitability of a prospective site. The most important factor is a balance between terrestrial and wetland habitat. WPT are highly aquatic and require fresh water habitat (Bury, 1986). Terrestrial habitat is vital for WPT to bask and nest. Basking allows WPT to monitor their metabolism to undertake strenuous activities such as foraging and avoiding predators (Holland, 1994). Without basking sites, turtles may lose the ability to engage in such important behavior. As for nesting, terrestrial sites are fundamental for recruitment. WPT make their nests on land, where the eggs incubate over winter or hatch before the winter depending on the region (Rathbun, 1992). A crucial feature of nesting habitat is not just the presence of land, but its proximity to the water (Rathbun, 1992). Just as sea turtle hatchlings emerge from the sand and head towards the ocean, juvenile WPT hatch from terrestrial sites and head for fresh water. Juveniles risk predation during this initial expedition, so safe terrestrial nesting sites close to fresh water are critical. Another important feature of suitable habitat is available woody debris within water. This is because it offers emergent basking sites within water, so that WPT can quickly escape into the water in the presence of predators, such as birds.

Research Questions

We approached our WPT reintroduction through 4 aspects of reintroduction:

- 1. Examine highly modified and relatively naturalistic sites as possible reintroduction areas
- 2. Examine intact natural sites as possible sources of WPT for reintroduction
- 3. Develop a strategy for conducting a successful reintroduction within the stakeholders' local jurisdictions
- 4. Develop a protocol and perform a removal/reintroduction of turtles in the artificial creek at the UCLA Mildred Mathias Botanical Garden (MMBG) on campus

Goal 1: Examine highly modified and relatively naturalistic sites as possible reintroduction areas

One of the initial steps for planning a WPT reintroduction is locating suitable reintroduction sites, especially in the case of an urban reintroduction. Therefore, it is important to consider all of the biological and habitat requirements of the WPT, as well as potential threats, which all contribute to the success of the reintroduction program. Additionally, we explored other potentially suitable sites by using tools such as current WPT records, literature, and GIS.

In 1992, WPT became scarce when over 90% of California's historic wetlands, which are critical for WPT habitat, were lost (Holland, 1994). While determining which sites could be options for the reintroduction of WPT, we had to first consider the biological and habitat requirements of WPT. WPT can occupy a wide variety of wetland habitats including rivers, streams, lakes, ponds, reservoirs, permanent and ephemeral shallow wetlands, abandoned gravel pits, stock ponds, and sewage treatment lagoons (Ernst and Lovich, 1994). In addition to wetland habitats, WPT need to have access to terrestrial shelter for protection from predators and thermal extremes. An optimal habitat for the WPT will have adequate basking sites, emergent and submerged vegetation, mud, rocks, and logs (Ernst and Lovich, 1994). Roads should not be near the site of WPT, to prevent mortality from road kills.

There are several potential sites that we believe could be ecologically intact and well enough protected to reintroduce WPT. As this report details later, we have examined several reintroduction sites including: Kenneth Hahn State Recreational Area, Franklin Canyon, and the Marsh Park region of the Los Angeles River. We will also be carrying out a smaller reintroduction in the Mildred Mathias Botanical Garden (MMBG) on campus at UCLA. This is a suitable setting for our study because it meets both the aquatic and terrestrial requirements needed for WPT to survive. Since the MMBG is a monitored and protected space, we could easily make minor changes to make the habitat more suitable for WPT.

Goal 2: Examine intact natural sites as possible sources of WPT for reintroduction

The success of our reintroduced population will depend on the health and demography of the individuals we select to introduce to the new habitat. There are two age groups being considered for reintroduction: juvenile and adult turtles. Juvenile turtles will likely be more adaptable to a new environment and better adapt to the reintroduction site. Adult turtles are accustomed to their environment and may not be able to adapt to a novel environment. If juvenile turtles are introduced, they will need to be head-started turtles. Pregnant female turtles will be hormonally induced via injection to lay their eggs. Eggs will then be incubated and hatchlings will be raised in captivity until they are ready for release.

To evaluate the success of our reintroduction, introduced turtles will be marked or tagged for monitoring. One way to identify and monitor reintroduced turtles is by using distinct combinations of carapace notch marks. This method is less effective for juvenile turtles because their shells are still growing and notches will not be distinguishable after a couple of years. For young turtles, we recommend using alternative markers such as shell color markings. Passive Integrated Transponder (PIT tag) is another option for identifying individuals, where a unique number tag is injected into the body (Madden-Smith et al., 2004), but using this method will require additional training from the Turtle Conservancy to prevent any harm to the turtles.

Genetic Considerations

Understanding the genetics of the source population also plays a vital role in determining the success of a reintroduction project. For the purpose of our project, there are three potential approaches to addressing the genetic considerations, each with its strengths and weaknesses. We determined which approach is the most appropriate, given the available data, resources, and the context of this project.

One approach is to maximize the similarity between the historical population and the source population. This way, local adaptations developed by the historical population can be preserved to the largest extent in the source population. The fitness of the source population can be maximized. It is a widely-used approach in species reintroduction. However, one problem with this approach is that the historical data does not always exist. Although museums usually have specimens in a wide range of areas, the specimens are treated with formaldehyde and cannot be sequenced, and most of them do not have a corresponding tissue sample that can be used for sequencing. Another problem is that an existing healthy population that matches perfectly with the historical population can be hard to find. In addition, one important assumption of this approach is that the reintroduction site has not changed since the historical population thrived, which is clearly not the case for many Los Angeles watersheds. Indeed, if the habitat alteration has extirpated the historical population, a reintroduced population that is genetically similar to the historical population may not do better in the altered habitat.

Another approach is to disregard the historical population and solely focus on maximizing the diversity within the reintroduced population. In this approach, the source

population with the highest genetic diversity should be selected. A population with a high genetic diversity is better at adapting to environmental change and surviving in an unfamiliar environment. In practice, it is hard to determine how diverse a population has to be to adapt to an environment different from the one that it evolved in. Maximizing diversity can also result in outbreeding depression. If we draw individual turtles from different genetic lineages that have evolved separately for a long time, the diversity is indeed maximized (Moritz, 1999). However, the different local adaptations developed by each of the lineages with respect to their habitats can be lost in such a hybridization. The resulting progeny may be poorly adapted for either habitat.

The third available approach is a combination of the first two. It takes both similarity between populations and diversity within a population into account. First, we can analyze all of the historical and existing populations in the region, and divide them based on genetic lineages. Then, for a certain reintroduction site, we only select the source population within the same lineage. The population in that lineage with the highest genetic diversity should be selected as the source. This approach is less limited by the availability of the data than the first approach. Nevertheless, the actual feasibility of this approach still depends on the diversity among populations and the number of lineages that we can describe.

Goal 3: Develop a strategy for conducting a successful reintroduction within the stakeholders' local jurisdictions

Because we are performing an urban reintroduction, we have a set of concerns about nearby human development and activities. Zoning and land use will affect the chosen reintroduction site because of the high importance of providing secure habitat away from potential dangers like highways or human overuse.

WPT are naturally shy animals, and while turtles will avoid human interactions, we are concerned about human-wildlife conflicts in our reintroduction. Locals may not want a reintroduction to occur in their area for a host of reasons. Invasive species like red-eared sliders (*Trachemys scripta elegans*) are charismatic and may be liked by people nearby, making a removal of the species controversial and hard to carry out. Additionally, because WPT is a species of special concern, locals may be worried that they will be held liable if any harm comes to the animals. Similarly, the protected status of the turtles means that they are rare and expensive when they are for sale on pet markets, with some hatchlings selling for nearly \$400 each. Individuals may attempt to capture and sell the turtles or keep them as pets. These issues will need to be addressed through law enforcement and educational campaigns.

Goal 4: Develop a protocol and perform a removal/reintroduction of turtles in the artificial creek at the UCLA Mildred Mathias Botanical Garden (MMBG) on campus

In addition to constructing a plan for an urban reintroduction, we have also begun carrying out a reintroduction in the MMBG at UCLA. This reintroduction serves as a test run for our final reintroduction and an educational opportunity for visitors of the MMBG. We have been in contact with the Director of the MMBG, Dr. Phil Rundel, and the lead herpetologist of the Department of Fish and Wildlife, Laura Patterson. Together, we have gathered five captive individuals from turtle and tortoise shelters in Southern California. This release is set to occur during June 2015 and will serve an educational purpose for both students and the public. We will use educational signage in the MMBG to educate visitors on the biology of WPTs and their current conservation status. Our goal is to inform the public of the causes of WPT decline and what is being done to protect them.

There are currently invasive red-eared sliders in the MMBG that may carry disease or compete with WPT for resources. In order to avoid many of the issues associated with placing the two populations in the same creek, the introduced WPT will be in a separate pond in the garden, which is fenced to prevent red-eared sliders from entering and WPT from escaping.

METHODS

Reintroduction Site Selection

To determine appropriate sites for reintroduction, we conducted two group field surveys and one limited group field survey. The two group field surveys were conducted in the Kenneth Hahn State Recreation Area (KHSRA) and Franklin Canyon. The limited group field survey took place at the Los Angeles River Marsh Park, located in a soft bottom portion of the Los Angeles River. The parameters for reintroduction suitability included the presence of basking sites, nesting sites, various sources of food, predators, competitors, and humans. We took notes on all of these parameters, using binoculars and taking photographs to make more detailed observations.

The general method for conducting these surveys started with dividing our group into two subgroups to make a head count of the turtles present, making sure to identify different species in the site. These subgroups also made observations on the presence of food, predators, competitors and humans. Next, the subgroups regrouped and took a detailed look around the site to discuss its suitability. This included making general measurements on the amount of food present, the abundance and accessibility of nesting and basking sites, and reaching a general consensus regarding the suitability of the proposed reintroduction site. Afterwards, we used Google Earth to measure the area and length of various parts of each pond. Using this method, all of the group members were able to agree on the suitability of each site.

Source Site Selection

GIS Analysis

We used ArcGIS (Environmental Systems Resource Institution, 2011) to locate current WPT populations and viable habitats for WPT. First, we found county shapefiles and selected the Southern California counties we were considering for reintroduction sites. These counties included Santa Barbara, Ventura, Los Angeles, Orange, and San Diego. We extracted WPT locality data from the Global Biodiversity Information Facility (http://www.gbif.org, hereafter GBIF). We used the species name and location filter to obtain the locality of WPT within the United States. From the data we accessed through GBIF, we created and added a shapefile of the current WPT population locations to our base map of counties.

After mapping the current WPT populations on our base map, our next goal was to identify viable habitats for WPT. We identified all freshwater bodies within these counties and delineated the different watersheds in these counties. First, we downloaded a digital elevation model (DEM) from USGS Earth Explorer to outline the drainage system and quantify the characteristics of the system. We used the "fill" feature to fill the gaps in the DEM to ensure continuous flows and eliminate any resolution issues. Next, we downloaded all freshwater bodies for each county through the census website as shapefiles for both linear and area hydrology to complete delineating the watersheds. We used the "flow direction" feature to get the direction of flow from each cell. Then, we used the "flow accumulation" feature to calculate the weight of each cell into each downslope cell in the output raster. Additionally, we used the "generate stream raster" tool to apply a threshold to determine how much area designates a stream and the "generate stream link" feature to assign unique values to each of the links in the stream network. Next, we used the "generate stream order" function to classify streams based on their tributaries. This feature is a method of assigning a numeric order to links in a stream order. Finally, we converted the stream to a feature, which converts a raster representing a linear network to features representing the linear network. Lastly, we calculated the flow length, created the watershed boundaries, and converted the watersheds into a feature to complete our identification of viable habitats for WPT.

Genetic Analysis

Due to the availability of data, we used the third method (see Introduction: Genetic Considerations) to determine the genetic suitability of the source site. Specifically, we obtained a large dataset from U. S. Geological Survey (USGS) in Southern California. With the dataset, we assigned WPT in the area to different genetic groups. The distribution of genetic lineages with respect to the watershed guided our reintroduction site selection. We used genetic data consisting of 85 loci from 504 individuals. The tissue samples were collected by USGS and various collaborators from 2004 to 2012 in seven counties in Southern California: Santa Barbara, Ventura, Los Angeles, San Bernardino, Orange, Riverside, and San Diego (Fisher et al., 2013). Locality data was taken for all of the samples. The tissue samples were then sequenced by USGS, and the proportion of missing data in each individual and locus was successfully kept within 10%.

We used the program STRUCTURE 2.3.4 to perform our genetic assignment (Pritchard et al., 2000). The initial parameters were set with length of burn-in period of 5000, and number of the Markov chain Monte Carlo (MCMC) repetitions of 100,000. We tested K=1 to K=10, with three independent runs at each K value. We then used Structure Harvester to analyze the result of the STRUCTURE run (Earl & vonHoldt, 2012). The optimal K was then determined via visual inspection of the ln(K) curve, as well as the Delta K curve. The optimal K occurs where the ln(K) curve has the steepest slope, and when the Delta K curve has a peak. Once we determined the K value, we used two different cutoff values, 0.90 and 0.75, to assign individuals to different genetic groups. In other words, if a group of individuals have probabilities to be in a certain lineage that are higher than the cutoff value, they were considered as a pure population. Otherwise, they were considered as admixes of different populations. Once each individual was assigned to a population, we plotted them on the watershed map we created, using the locality data in the dataset. We distinguished different genetic groups using different symbols.

Potential Source Population

In order to visit a potential source population and pristine habitat, we visited the Sespe wilderness in Los Padres National Forest. The observed pond (34.56092°N 119.13341°W) was miles removed from any car traffic, and generally undisturbed by anthropogenic forces. The pond was 200 m long and 5 m wide, with slow flowing, clear water and both emergent and submerged vegetation. Food sources observed at the habitat included insects, vegetation, and small fish. There were many hiding places above and below water to retreat from both human and animal threats. There were a few people at the site, as the pond is a hiking destination for some locals. While people visit the site, there was no apparent pollution or trash, and minimal debris in the water. The site represents a potential source population and exemplary natural habitat, and will be considered as a potential source site for turtles.

Four turtles were observed, and one juvenile male was captured and measured. The turtle appeared healthy, and showed no sign of disease or malnutrition. A tissue sample was taken for further research into the health of the animal, and after about 20 minutes outside the water, the turtle was returned to the pond.

MMBG Reintroduction

The soft reintroduction at the MMBG is an educational opportunity to compare the native WPT and the nonnative red-eared slider. It also allows the public to learn about our native Californian turtles and exposes them to wildlife reintroduction biology. Five rescue WPT were sourced from the California Turtle and Tortoise Club in San Luis Obispo and Ventura County. These were stray turtles that could not be returned to their original habitats and were brought into the shelter. The turtles were transferred to a tank at UCLA where they were weighed, measured, quarantined for disease and conditioned to eat pellet food.

Rescue turtles were kept in a non-shaded tank on the rooftop of the Botany Building at UCLA. The tank had three cinderblocks and a wooden ramp for hiding and basking. The tank was cleaned weekly (Figure 4). The tank was first drained to about 25% before the turtles were removed from the tank and placed in a bucket. The cinder blocks were then removed and remaining water was dumped out. The interior of the tank was scrubbed using a broom and both the tank and cinderblocks were sprayed down with water to remove algae and debris before they were returned to the tank. The turtles were placed back into the tank and the tank was allowed to fill to approximately the height of the ramp, leaving the top of the ramp above water for basking.

Turtles were fed either pinky mice, rainbow trout, Mazuri Aquatic Turtle Food, or ZooMed Aquatic Turtle Food three times per week: Monday, Wednesday and Friday. Turtles were fed ZooMed Aquatic Turtle Food or Mazuri Aquatic Turtle Food pellets on Mondays and Fridays and rainbow trout every Wednesday. Turtles were fed pinky mice when rainbow trout was unavailable. Initially, pellet feeding success was evaluated by the absence of pellets within two hours following feeding. Later, success was evaluated by recording the number of turtles observed actively eating.

Three segments of the MMBG creek were selected as potential reintroduction sites based on the presence of basking sites, containment from the rest of the garden, the amount of human traffic, and the feasibility of fencing and modifications. We then selected a single creek segment based on the following benefits: level of basking site availability, size of the pool, depth of the water, sunlight exposure for basking, available space for signage, and degree of separation from competing species such as red-eared sliders and koi fish. Various minor alterations were then performed on the garden habitat to create the most feasibly ideal environment for WPT reintroduction. First, more space was created on the bank of the creek by removing selected portions of foliage; this allowed for more nesting space on the flat ground beside the built-in access ramp. To determine whether sunlight exposure was sufficient for a healthy WPT habitat and conducive to the survival of the individuals, we performed an observational study of the site over the course of two days during different time periods, including morning, early afternoon, and late afternoon. Each time period was observed in terms of illuminated ground and creek, and the degree to which overhanging canopy blocked the sunlight from passing through.

Additional modifications to the selected site included a fence, a new land ramp, and clearing of leaf debris. Fencing was built to prevent turtles from escaping. Leaf debris was cleared from the island and a 3-4 in deep trench was dug to build a fence using ¹/₄ in thick redwood bender board reinforced with metal rebar. The fence extends 3-4 in below ground, 9-10

in above ground, and around the southwestern perimeter of the island. Two pieces of bender board were stacked on top of each other to achieve the desired height. Both inlets and outlets to the site were fenced using wood fencing. Rocks were strategically removed from the edge to pave a concrete ramp extending into the water for easier access to land for basking. A sign was created for placement on the east side of the enclosure to educate visitors about WPT and wildlife reintroductions. The content of the sign was based on two goals: (1) provide visitors with information about WPT, and (2) identify the importance of reintroductions in wildlife conservation.

After modifications were complete, five red-eared sliders were captured from the creek and placed into the enclosure to test its susceptibility to escape. The carapace of each red-eared slider was marked with nail polish for monitoring and recapture. After observing that no redeared sliders were able to escape, the five rescue turtles will be reintroduced into the enclosure.

RESULTS

Reintroduction Site Selection

After two group field surveys of six different sites and one field survey with limited group exposure, we ranked the possible reintroduction sites based on various previously established criteria (Table 1). We surveyed the Kenneth Hahn State Recreation Area (KHSRA)'s large-, medium-, and small-sized Gwen Moore Ponds and the Japanese garden pond on an overcast afternoon in late April 2015. Additionally, we surveyed the Heavenly Pond and Upper Reservoir at Franklin Canyon on a clear and sunny afternoon in early May 2015. We conducted a field survey of limited group exposure at the Los Angeles River Marsh Park on an overcast afternoon in early May 2015.

Our highest ranked pond was the Heavenly Pond (34.12041°N, 118.41164°W) at Franklin Canyon (Figure 1). There were many natural basking sites, with fallen trees and logs emerging from the water. Additionally, there was a possible nesting site north of the pond. This site was 10 m from the pond and approximately 4 m x 12 m, and consisted of a sandy substrate. Within the pond, there was some algae, but a lack of snails. During our survey, we also found largemouth bass, carp or koi, and sunfish. There were no bullfrogs (*Rana catesbeiana*), but we could hear them coming from the nearby Upper Reservoir. We could also hear a Pacific tree frog (*Pseudacris regilla*). Regarding turtle life, there were three Florida softshell turtles (*Apalone spinifera*), 57 red-eared sliders, and one map turtle (*Graptemys pseudogeographica*). The abundance of red-eared sliders would suggest that reintroduced turtles could survive here, but would also need to be managed. The Heavenly Pond could be a potential site for WPT reintroduction based on its less human-impacted, but still frequented, location and more natural environment.

Our second highest ranked pond was the Upper Franklin Canyon Reservoir (34.12008°N, 118.41026°W) (Figure 1). We found an abundance of mosquitofish (*Gambusia affinis*), which are an excellent food source for turtles. Additionally, we spotted 28 red-eared sliders and 1 softshell turtle. There was at least 1 gopher snake (*Pituophis catenifer*) and a large, undetermined number of bullfrogs. This was a much deeper and larger pond than the Heavenly Pond, at approximately 8 m deep. There was a lack of basking sites in the water, but a lot of available nesting area in the sloped western side of the large reservoir. Because of the vast expanses of the Upper Reservoir, we felt that it would restrict our ability to monitor any population that would be released here, which is a major part of our recommendations for any reintroduction program.

However, the large amount of food available and the natural environment present itself as a high ranking reintroduction site.

Our third highest ranked pond was the KHSRA large Gwen Moore Pond (34.00945°N 118.37057°W) (Figure 2). Almost all the edges of the pond were available for basking, with a dense area of vegetation on land that blocked human access for 30 m on the western side. Nesting areas extended 15 m on the eastern side and 30 m on the western side of the pond. The land surrounding the pond was relatively flat with hard-packed soil that is not irrigated. However, there is a 12 in tall curb on the western side which could prevent turtles from climbing up to get to the nesting area. In terms of food, there was an abundance of algae, leeches, aquatic and terrestrial snails, freshwater clams, and mosquitofish. The two major algae patches were 40 m x 50 m on the eastern side of the pond and 10 m x 3 m on the southwestern side of the pond. Predation is possible, as we saw bullfrogs, catfish, and largemouth bass. Additionally, one visitor said that raccoons and coyotes occasionally frequent the area. The pond is stocked with rainbow trout, catfish, carp, and largemouth bass in the winter. During our survey, we saw a bullfrog near the west side vegetation and two goose eggs that had been predated. Two groups of two observers each surveyed 45 red-eared sliders, mostly of adult age with some juveniles. There were also bluegills and sunfish. In terms of human interaction, this is a highly visited pond. There were people fishing at the pond and feeding animals who could have close access to the turtles. Overall, there was plenty of food, vegetation, and predators; however, the impact of human activity was evident in the amount of trash and number of people at the site.

Tied for third highest ranked pond was the KHSRA Japanese garden pond (34.01379°N 118.37242°W) (Figure 2). A large gravel island was ideal for basking and seemed to be currently blocked off from human access. However, nesting could be slightly problematic because the grass was irrigated. This irrigation could lead to an anoxic area on the eastern side of the pond. On the western side, the grass seemed less maintained, which could provide 20 m of nesting habitat. The pond was full of water lilies, which could support communities of insects or plants that can be eaten by turtles. Two groups of three observers each found six red-eared sliders basking on the gravel island and several koi swimming in the pond during the late afternoon. There was a good basking area, fewer red-eared sliders, and a large anoxic area at the pond.

Our fifth highest ranked pond was the KHSRA medium Gwen Moore Pond (34.00866°N 118.37041°W) (Figure 2). Above a small waterfall, there are rocks which are a prime location for turtle basking. There was also a large algae patch adjacent to these rocks. The nesting habitat could potentially consist of a dirt patch 20 m west of the pond without a curb restricting access and a rockier 15 m patch east of the pond without a curb restricting access for turtle nesting. We found carp in the pond, as well as eight red-eared sliders. This pond is accessible to people, including people fishing and feeding animals, similar to the large Gwen Moore Pond. Overall, there were fewer turtle predators in the pond, but less attractive habitat.

Our sixth ranked pond was the KHSRA small Gwen Moore Pond (34.00687°N 118.36975°W) (Figure 2). This pond lacked decent basking sites, nesting sites, food, predators, and competitors. We saw two fish approximately 20 cm long, but failed to see any other signs of a healthy ecosystem. This pond was extremely exposed to people compared to the size of the pond. Based on our findings, we would not recommend this pond.

Lastly, we surveyed the Los Angeles River Marsh Park (34.122008°N 118.270132°W to 34.099838°N 118.242928°W) (Figure 3), which is known for being a soft-bottom portion of the Los Angeles River. The middle of the river contained dense vegetation and many rocks appropriate for basking. There is also an area where the water splits when heading east from the

park entrance. This could serve as potential basking and nesting sites. Over a two hour period, only one red-eared slider was seen basking, but there were several mallard ducks and herons. With sloped concrete walls on both sides of the river, human access was limited.

Source Site Selection

GIS and Genetic Assignment Results

Using Structure Harvester, strong evidence supports that the optimal K is 3 in our STRUCTURE runs (Figure 5; Figure 6). With K=3, different individuals were assigned to different genetic lineages (Table 2). These WPT were similarly geographically distributed to their genetic lineages with respect to the watersheds in the area (Table 3; Figure 7; Figure 8).

Populations B and C were the dominant populations (Figure 7; Figure 8). Populations B and C were separated by a boundary in Orange County. North of the boundary, we saw mostly population B; south of the boundary, population C dominated. With a cutoff value of 0.75, the population composition varied in different watersheds in Santa Barbara, Ventura, Los Angeles, Orange, and San Diego Counties, where most of our samples were located (Table 3). There was a hybrid zone between populations B and C at the Orange County boundary (Table 3; Figure 7). With a 0.75 cutoff value, the admixed individuals were well-contained in San Diego Creek of Orange County. North of the creek, 83.1% of our samples were pure B populations. South of the creek, 96.7% of the populations were pure C populations. The hybrid zone could be broadened with a higher cutoff value, including watersheds as far as Santa Margarita and San Luis Rey (Figure 8). The difference caused by different cutoff values suggests that there is a gradual shift from one population to the other population across the hybrid zone.

Other than the dominant B and C population groups, a third distinct genetic group A also existed. It was comprised of only one locality in the north of our study area, as well as three individuals around the Santa Ana River. Hybrids that contain some features of population A also occurred on the map (Figure 7; Figure 8). Interestingly, only one population of these hybrids was found at the contact areas of different pure populations. Namely, the Lake Elizabeth population, which was an AB population group admix, was located in a watershed in close proximity to both pure A and B populations. All the other admixes that were related to the A genetic group were located in areas which are completely cut off from the pure A population. In addition, all AB admixes were contained within the pure B population, and all AC admixes were within the range of the pure C population. Many of the isolated AB and AC populations were in somewhat urbanized areas and suburbs, such as Irvine, Rincon Point, and Temecula. Similarly, the three pure A individuals were located in an area with high human activity. The difference between the isolated AB and AC populations and the pure A individuals was that no pure B or pure C populations and the pure A individuals.

Field Survey Result (Ojai)

The Sespe wilderness in Los Padres National Forest provided an excellent example of a natural habitat for WPT. However, the site may not serve well as a source population of WPT for the reintroduction project because of the low number of individuals found at the pond. While there may be more turtles than we observed, our observations at the field site suggested very low population levels. We do not want to take individuals from a wild population that is already small or under stress. For this reason, we recommend exploring other potential Southern California WPT populations as a source. Unless the Ojai population is larger than anticipated, it is advisable to seek out a different source population for this reintroduction.

MMBG Reintroduction

The three sites evaluated for reintroduction were an upstream pond, a periphery koi pond, and a downstream segment (Appendix 1). The upstream pond is the smallest of the three sites, measuring 4 m x 2.5 m and located directly adjacent to the north bridge. Basking sites consist of multiple medium size rocks along the water's edge and an in-water rock between the pond and the rest of the creek. Both west and east banks have steeper slopes than the other two sites and no turtles were seen basking higher up on the banks. This pond receives the most unobstructed sun exposure out of the three sites with little to no shading from trees to the north and little shading from the south from shorter trees. Both the east and west sides of the site are fenced with approximately two inch wooden stakes that do not reach the ground. Usage by red-eared sliders and human traffic were the highest at this pond relative to the two other ponds.

The downstream site is the largest of the three sites and consists of two parts: a large pond measuring 8 m x 3 m and a smaller segment of the main creek measuring 3 m x 1 m. The smaller creek segment is heavily vegetated with small rocks along the banks for basking. The pond is larger than both upstream ponds and contains the pump which cycles water back upstream. The east bank of the pond is gently sloped and is exposed to almost completely unobstructed sunlight but a walking trail runs directly next to the bank and no turtles were seen basking. Neither water bodies are fenced; both are exposed to the walking path from the west and east side. Both the creek segment and the pond are used lightly by red-eared sliders and koi fish. There is a low level of human traffic in this area relative to the other two sites.

Lastly, the selected pond is the periphery koi pond to the east of the main creek. The periphery koi pond is the second largest pond measuring 11 m x 2 m but has the highest area of connected land for basking and nesting measuring 6 m x 1 m. The pond is enclosed by a cemented rock outcrop extending along the west and east banks of the pond. The rock outcrop is too steep and high on both sides of the pond for turtles to escape. Basking sites consist of a large, flat island between the west shore of the pond and the east shore of the main creek. The island is enclosed to the west by a vertical drop into the main creek and can be accessed from the pond by a rock-free segment of the southwestern bank. This site has the most shading out of the three ponds. The island is shaded from above by a South Eastern Oak and a Cork Oak tree and light observations showed patchy but sufficient illumination throughout the day during all three time periods: morning, afternoon and late afternoon. This pond has the least usage by red-eared sliders. It has several koi fish and moderate levels of human traffic. The advantage of the basking island is that it is across the pond from human traffic, and serves as a great site for observing the turtles. The upstream inlet is partially blocked by a short cement platform which keeps most turtles from crossing over from the main creek; however one turtle has been observed climbing over the cement platform and crossing over into the pond.

The first test run of our enclosure was unsuccessful. On the first day of monitoring after release, three of the five marked released sliders had escaped and were found upstream. One turtle remained in the enclosure and one turtle was not found. On the second day of monitoring three days later, four marked turtles were found upstream and only the previously missing turtle was found in the enclosed pond. By the third day of monitoring, one day later, no marked turtles were seen in the enclosed pond. We have identified points where we expect the sliders escaped, and are taking measures to secure the area.

DISCUSSION

Reintroduction Source Selection

The STRUCTURE analysis yielded two major genetic lineages of WPT in Southern California. They intersected in Orange County and have a gradual hybrid zone. Because different genotypes may have developed local adaptations, to maximize the fitness of our reintroduced population, we need to make sure that these local adaptations are preserved (Moritz 1999). Therefore, translocation within a lineage is desirable, while translocation between lineages should be avoided. Based on our results, we should select the source of WPT north of Orange County for a reintroduction in Los Angeles County.

Also, we observed the existence of a third distinctive but small lineage A. Based on literature, we suggest that the lineage A represents the translocated *E. marmorata* (Northern Western Pond Turtle) (Spinks et al. 2014, Fisher et al. 2013). In their study, Spinks et al. (2014) used 89 nuclear SNP markers to study the geographic distribution of the two western pond turtle species. The genetic markers we used in this analysis were actually a subset of the 89 SNP markers they identified in their study. Spinks et al. (2014) found that populations in the Sierra Nevada were largely *E. marmorata*, and populations found in transverse ranges were all *E. pallida*. The one pure A population we found was not sampled by Spinks et al. (2014). However, it was located at the contact area between Sierra Nevada and the transverse range. Given its divergence from all the transverse ranges populations, we conclude that genetic group A is actually *E. marmorata*.

All the other individuals that had E. marmorata genes were occurring fairly far from their natural range. We propose two hypotheses for this. First, there might have been E. marmorata individuals travelling across the landscape and hybridizing with local E. pallida populations. However, given the limited mobility of turtles, we think this hypothesis is rather unlikely. A more reasonable hypothesis is that humans may have released them as a result of pet trade. This hypothesis explains why a smaller number of individuals occur outside their natural range. Furthermore, we found many of those pure or hybrid E. marmorata were localized in cities and suburbs, areas with a lot of human activities. It is plausible that people obtained their pet turtles from Northern California, moved to Southern California, and released WPT in watersheds close to their homes. Where natural population of E. pallida do exist, the released E. marmorata can interbreed with the local population and result in the hybrid populations of AB and AC. Where the natural populations of E. pallida have been wiped out, such as in the downstream Santa Ana River and Coyote Creek, E. marmorata stay in the watershed and become the only WPT species there. Therefore, such relocation has potential negative consequences on E. pallida and the local ecosystem. By interbreeding with local E. pallida, the translocated E. marmorata can harm E. pallida's genetic integrity. Although the ecological consequences remain unknown, due to the numerous lessons we have learned from the disasters created by nonnative species and their hybrids, we need to be very careful about the translocated turtles. For the purpose of this project, we must avoid introducing any pure or hybrid *E. marmorata* to our sites.

Additionally, admixed populations existed between B and C populations in Orange County. Traditionally, we assumed that WPT were very well contained within a watershed. However, the hybrid zone could be broadened by a higher cutoff value (Figure 8), which suggests that there is a gradual shift from population B dominance to population C dominance. Therefore, there is a considerable amount of movement between the two populations. Specifically, WPT can move between the watersheds of San Diego Creek, Santa Margarita, and San Luis Rey. This issue can be further complicated by human-aided movement, again, as a result of pet trade. Such hybridization dynamics are potentially important to the local environment and need to be further explored. Several issues remain to be examined, including the speed of movement and the direction of movement of the hybrid zone, the ecological consequences, and the role of humans on these dynamics. For now, we do not recommend using any of the admixed populations as a source population for reintroduction.

MMBG

The periphery pond was chosen for reintroduction because of its separation from the rest of the creek and abundance of basking and nesting land area. The pond is connected to the main creek but separate from it in a way that allows for complete fencing of the perimeter without preventing existing sliders from using the entire length of the stream. The island also provided the largest and most isolated area for basking and nesting. Basking sites at the other two streams were located directly along the human trail and were exposed to more human traffic.

The first test run of the enclosure was unsuccessful. All four escape turtles were found upstream of the enclosed pond which suggest that the turtles are climbing a low point at the inlet or surrounding vegetation to move upstream. Further modifications will be made to the inlet to raise the height of the rocks surrounding the inlet and surrounding vegetation will be checked for low hanging branches that could facilitate climbing over to the adjacent creek. Another test run will be run before introducing the rescued WPT.

Future of the Project

It is also our task to accurately determine an ideal site for future reintroduction efforts: while we as a group were not able to reintroduce individuals into the selected location (Heavenly Pond at Franklin Canyon), we are confident that the habitat will remain a strong candidate for the future due to its excess of natural basking sites and nesting potential. As part of our reintroduction, it is crucial to come up with a framework for the future of this project. Time is and was a major limiting factor to the reintroduction process: conflicting schedules often times made it difficult to send the group on field site visits together or come up with lengthy meeting periods. Therefore, while a soft reintroduction in the Botanical Gardens was possible, a hard reintroduction into any of the suggested sites above (Gwen Moore Ponds, Japanese Garden Pond, Heavenly Pond, and Upper Reservoir) would have to come at a later time, based on the success of the small-scale reintroduction.

Three members of our group will be present and available for further observation of the MMBG reintroduction site over the summer months; this will be important in determining the longevity of the WPT population in the creek habitat, as well as to provide valuable data and feedback to our faculty advisor and student lab coordinators. One member of the group will remain on campus for another year, and will be able to continue collecting data and tracking WPT individuals in the MMBG throughout 2015 and part of 2016. Looking forward, the outcome of the soft reintroduction, whether successful or unsuccessful, will directly affect the goals and timelines of future groups working on similar reintroduction projects.

In order to accurately track and evaluate the success of WPT in the MMBG, we must implement an effective way of monitoring the health of the introduced WPT. To do this, we must ensure that the turtles remain in the enclosure we constructed and do not come into contact with the red-eared sliders. Additionally, we must verify that the turtles are alive and well. This requires us to visit the site periodically and count for each of the five introduced individuals. Because the ultimate goal of this soft reintroduction is to educate the public on native species reintroductions, it is not vital that the turtles breed and produce offspring. As long as they are alive and well, the public can enjoy their presence and learn about native reintroduction ecology. However, we should account for the possibility of nesting and take note of any gravid females. To accomplish these tasks, we will need two or more team members to monitor the site weekly. Ensuring that the five reintroduced turtles are present may require PIT tagging, although a more passive monitoring system can work as well. The most important thing is that the turtles maintain a presence in the pool and turtle island and exhibit normal WPT behavior. At least three team members will monitor the site weekly and keep notes of the turtles' behavior during the summer, ensuring that no red-eared sliders enter the site and evaluating the public's responsiveness to the MMBG's new addition.

Metrics for Success

It is important to measure the success of each reintroduction and determine ways to improve for future opportunities. To deem a project successful, reintroductions must be comparable to wild populations in terms of genetic diversity, survival rates, reproduction, growth, dispersal, and behavior. Monitoring reintroductions over an extended period of time based on the life history of the animal (i.e., longer monitoring for longer lived species) allows further understanding of the successes and failures of reintroduction projects (Dodd & Seigel, 1991). In this case, WPT should be monitored for several years if possible. Additionally, if animals are subject to roaming or instinctual homing behavior, their mortality can be greatly impacted (Roe et al., 2010). Especially with highly mobile animals, it is important to consider their movements and ability to establish a population. It is important to consider behavior after reintroductions because mortality can be greatly impacted by how an animal behaves in the wild. Short-term success is not a complete indicator of long-term success when dealing with reintroductions.

The release of animals is not the end of a reintroduction program. Instead, it is the beginning of a long-term monitoring process, which is a vital component of the reintroduction program. The population genetic metrics that are most important for the success of a reintroduction are: abundance, vital rates, site occupancy, pathogens and parasites, effective population size, and genetic diversity (Schwartz et al., 2006). Abundance is the number of individuals in the population, which can be measured through the capture-mark-recapture method or through number of individuals encountered. Vital rates include important population ecology parameters such as survival, recruitment, and mortality. Pathogens and parasites impose severe threats to reintroduced animals. Genetic tools can be used to monitor their presence, and appropriate treatment can then be performed as soon as possible (Schwartz et al., 2006). Lastly, genetic diversity can be quantified through allelic diversity, which is very sensitive to declines in population size. Overall, genetic tools and monitoring are a valuable resource for reintroductions and should be used throughout the entire process.

CONCLUSION

In addition to developing a recommendation for creating a self-sustaining population of WPT in the wild, we hope that this project will serve as an educational tool for the nearby community. Urbanization and human activity often stresses and threatens native populations of all species, and bringing humans in contact with these animals can help us appreciate the natural environment. We hope to educate the community about their new neighbors, while engaging a

broader population with news of the reintroduction. Signage nearby will allow people to recognize WPT habitat and appreciate the animals in the area. Public announcements, newspaper articles, and outreach to local schools will help create a larger audience and draw attention to our reintroduction and Southern California conservation biology as a whole. Additionally, an urban reintroduction will have high exposure. We seek to draw the attention from several existing non-government organizations and nonprofits to bolster our efforts. While monitoring and caring for the reintroduced population will be of utmost importance, we hope to seize our opportunity to create a community that engages with the natural environment.

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Table 1. Summary of findings from 6 group field surveys and 1 limited group field survey for ranking reintroduction sites for WPT. Abbreviations include Franklin Canyon (FC), Kenneth Hahn State Recreation Area (KH), Gwen Moore (GM), and Los Angeles (LA). * indicates limited group field survey. NA indicates data not recorded.

Rank	Site	Max length x width (m x m)	Perimeter (m)	Area (m²)	Basking habitat	Nesting habitat	Food	Predators	Other animals	Human influence
1	Heavenly Pond (FC)	36 x 19	105	425	Lots of natural logs, trees	4 m x 10 m area 10 m away from pond	Algae, no snails	Largemouth bass, sunfish, no bullfrogs	Carp or koi, 57 red- eared sliders, 3 soft-shell turtles, 1 map turtle	Quiet area with a moderately used trail
2	Upper Reservoir (FC)	107 x 183	479	9,875	Restricted to edge areas	Large area (not measured)	Lots of mosquitofish	Lots of bullfrogs	1 gopher snake, 28 red-eared sliders, 1 softshell turtle	Quiet area with limited human interaction relative to pond size
3	Large GM Pond (KH)	130 x 64	358	7,134	Almost all edges available, dense land vegetation for 30 m	15 m on eastern side, 30 m on western side (blocked by a curb) of soil	Algae, leeches, snails, clams, mosquitofish	1 bullfrog, catfish, largemouth bass, sunfish	Rainbow trout, geese, ducks, bluegills, 45 red- eared sliders	Many people feeding birds nearby, fishing, lots of trash
3	Japanese Garden Pond (KH)	37 x 21 (37 x 13 without anoxic)	129 (125 without anoxic)	698 (434 without anoxic)	Large isolated gravel island (108 m ²)	20 m suitable habitat on western side	Insects or plants in lilies	None seen	Koi, 6 red-eared sliders	High visibility, but fenced areas limit interaction, half of pond is anoxic
5	Medium GM Pond (KH)	42 x 35	137	1,221	Rocks and algae patch adjacent to small waterfall	20 m west of pond	Algae	Possibly largemouth bass	Carp, geese, ducks	Many people feeding birds nearby, fishing, some trash
6	Small GM Pond (KH)	15 x 24	72	342	Edges of pond	None	Limited algae	None seen	2 non-predatory fish	Many people feeding birds nearby
7	*LA River Marsh Park	NA	8,187	233,408	Dense vegetation, many rocks	Limited to middle of river	NA	NA	1 red-eared slider, several ducks and herons	High visibility with limited human access, sloped concrete walls on both sides of river

	Genetic group assignment								
	Α	В	С	AB	AC	BC	ABC		
0.90	12	178	210	26	12	35	7		
0.75	12	214	229	9	5	30	5		

Table 2. Number of individuals assigned to different genetic groups. We established two cutoff values, 0.90 and 0.75, of genetic similarity to assign each group as genetically similar.

		Genetic group assignment							
County	Watershed	A	В	С	AB	AC	BC	ABC	Total
San Diego	Tijuana	0	0	1	0	0	0	0	1
	Otay	0	0	26	0	0	0	0	26
	Sweetwater	0	0	17	0	0	1	0	18
	San Diego River	0	0	20	0	1	0	0	21
	San Dieguito	0	0	12	0	0	0	0	12
	San Luis Rey/ Santa Margarita	0	1	69	0	0	2	0	72
Orange	Aliso Creek	0	0	1	0	0	0	0	1
	San Diego Creek	0	9	16	0	3	12	2	42
	Santa Ana River/ Santiago Creek/ Talbert	1	0	0	0	0	0	0	1
	Coyote Creek	1	0	0	0	0	0	0	1
Los Angeles	Los Angeles River	10	51	4	8	0	2	0	75
	Santa Monica Bay/Ballona	0	37	2	0	0	2	3	44
Ventura	Colleguas Creek Watershed	0	42	0	0	0	0	0	42
	Santa Clara River	0	32	2	0	0	0	0	34
	Ventura River	0	6	0	2	0	0	0	8
Santa Barbara	Burro Arroyo	0	4	1	0	0	1	0	6
	San Pedro Creek/ Mission Creek	0	20	0	0	0	0	0	20
Total		12	202	171	10	4	20	5	424

Table 3. The population composition in different watersheds in Santa Barbara, Ventura, Los Angeles, Orange, and San Diego Counties, where most of our samples are located, with a cutoff value of 0.75.



Figure 1. Heavenly Pond and Upper Reservoir at Franklin Canyon



Figure 2. Large, medium, and small Gwen Moore Ponds and Japanese Garden Pond at Kenneth Hahn State Recreation Area.



Figure 3. Los Angeles River Marsh Park



Figure 4. Turtle tanks used for quarantining rescued WPT.



Figure 5. Output from Structure Harvester of ln(K) showing that K=3 exhibits highest increment (Earl & vonHoldt, 2012).



Figure 6. Output from Structure Harvester of Delta K showing that K=3 gives the highest Delta K (Earl & vonHoldt 2012).



Figure 7. Map of WPT occurrences in Southern California watersheds separated by genetic type, with genetic cutoff at 0.75 similarity.



Figure 8. Map of WPT occurrences in Southern California watersheds separated by genetic type, with genetic cutoff at 0.90 similarity.



Appendix 1. Map of Mildred E. Mathias Botanical Garden produced by UCLA. The red circles indicate sites that were considered for the soft reintroduction of WPT. The middle, medium-sized circle was our chosen site for reintroducing the WPT.