Assessment for the Western Pond Turtle

Final Report

Client: United States Fish and Wildlife Service

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Executive Summary

The western pond turtle (WPT), recently separated into two species, is a candidate for listing under the Endangered Species Act. To assess the current status of both species, we conducted a risk assessment and analysis of historical, current, and future conditions.

Background

This assessment for the WPT compiles the best available literature, scientific information, museum data, and population viability analyses to characterize the biological status of the two species: *Emys (Actinemys) pallida* and *Emys (Actinemys) marmorata*. The goal of this assessment is to inform the listing decision for the two species under the federal Endangered Species Act, and to act as a source of information for future conservation efforts.

Species Biology and Needs

The WPT occurs in a variety of semi-aquatic habitats ranging from lakes, rivers, and streams to man-made channels, agricultural ponds, and sewage treatment ponds. *Emys pallida* can be found from northern Baja California, Mexico to the southern San Francisco Bay area along the coast and inland deserts. It can be found along the Southern Coast Ranges and the western parts of the San Joaquin Valley. *Emys marmorata* can be found from the San Francisco bay area north to Washington state and south along the eastern side of the San Joaquin Valley. The WPT is a medium sized pond turtle that has a maximum life span of about 45 years (Holland 1994, p. 2-11). WPTs may have up to three clutches per year with an average of 7 eggs per clutch, however one clutch is most common (Bury *et al.* 2012, p. 16; Germano 2016, p. 668). The diet of the WPT is generalist and consists of small aquatic invertebrates, vertebrates, vegetation, and carrion (Bury *et al.* 2012, p. 12). Feeding must be carried out in an aquatic environment. Basking and female nesting are the two main activities carried out by the WPT outside of the water.

Adequate population recruitment, which is indicated by individuals of various sizes in health populations, is necessary for the long-term viability of the WPT. As the WPT is a semi-aquatic species, it requires aquatic and dry land habitat of sufficient quality and quantity for survival of the species. In order to withstand catastrophic events that are becoming more common due to climate change, the WPT needs to have resiliency, redundancy, and representation.

Species Separation

Historically, the WPT has been considered one species, *Emys marmorata*. The species has also been referred to as the Pacific pond turtle, *Clemmys marmorata*, and *Actinemys marmorata*, all of which typically refer to one species ranging from Baja California, Mexico to Washington state, USA. However, as a result of genetic research, the WPT is now considered two distinct species: *E. pallida* and *E. marmorata* (we follow Spinks *et al.* 2014 on both species and genetic nomenclature). *Emys pallida* resides in the southern portion of the two species range from Baja California to the San Francisco bay area while *E. marmorata* resides in the northern portion of the two species range from the San Francisco Bay area to Washington state. For the purpose of this assessment, we separate the species in California based on the county they are from and use this as our guide for the rest of the report. In addition to genetic information, the presence of an inguinal scute on the shell of the WPT is typically seen in *E. marmorata* and absent in *E. pallida* (Seeliger 1945, p. 156).

The inguinal, while not infallible, is the most reliable way to morphologically differentiate between *E. marmorata* and *E. pallida.* In order to examine the data gathered on the inguinal from museum specimens, we identified the percentage of *E. marmorata* and *E. pallida* specimens that had inguinal plates present. We found a discrepancy in our results as not all *E. marmorata* specimens had inguinal plates present, and some *E. pallida* specimens did have inguinal plates present. This may be due to our use of county boundaries to delineate the range of the two species, when in fact *E. pallida* and *E. marmorata* may both reside in one county. In order to address this discrepancy we extracted the county data for all the specimens from the museum data and created graphs that examined the frequency of inguinal presence and absence for *E. pallida* and *E. marmorata* respectively in order to identify counties of interest that violated our original hypothesis. These counties may indicate areas where the boundary between *E. pallida* and *E. marmorata* occurs.

Risk Assessment

As a central goal of this assessment, we reviewed, compiled, and analyzed the risk factors affecting the WPT. These risks affect the viability of both *E. pallida* and *E. marmorata* and include drought, predation by non-native species, land conversion, contaminants, flood, fire, disease, invasive species competition, vehicle traffic, natural predation, dams and pumping of water, consumption and pet trade, and climate change. In addition, we took into account the potential effects that artificial ecosystems would have on both species. We ranked the effects of each of these risks for both *E. pallida* and *E. marmorata* by assigning a score based on the severity and prevalence of the risk in available literature. The top five risks for *E. pallida*, in order of decreasing severity, are: drought, predation by non-native species, land conversion, contaminants, and floods. The top five risks for *E. marmorata*, in order of decreasing severity, are: disease, contaminants, land conversion, predation by non-native species, and drought.

Historical & Current Conditions

Historically, the WPT was widely distributed across the west coast. However, it has experienced drastic reductions in its overall range and population size. Today, the WPT exists in highly urbanized and agricultural areas due to the loss of its natural habitat. While some populations have seemed to adapt to new urban environments, as is the case with populations in agricultural ponds and sewage treatment plants, most are in decline due to a variety of threats.

Using museum data, we evaluated the historical conditions of the WPT by mapping the locations of where specimens were collected, quantifying changes in sex ratios over time, and analyzing trends in body size to understand the age composition of the species over time. Data were collected from the Los Angeles Museum of Natural History, the California Academy of Sciences, and the Museum of Vertebrate Zoology on both *E. pallida* and *E. marmorata* from 1892-2005. For both species, we found a male-biased sex ratio over time along with an increase in mean carapace length, implying aging populations. In addition to the museum data, we looked for sex ratio and carapace length trends using trapping survey data from the United States Geological Survey (USGS) from 2006-2018. However, this data was only available for *E. pallida* and was combined with our museum data for *E. pallida*. With the addition of the USGS data, we observed a significant increase in mean carapace length over time and are confident that sex ratios remain male-biased today.

Additionally, we identified all of the WPT population sites we could find in primary literature and from the USGS data. From the literature, we included the most current sighting/trapping data for 44 sites across Mexico, California, Nevada, Oregon, and Washington. From the USGS data, we included the number of turtles trapped at 61 different sites across the range of *E. pallida*. We then projected the data onto a map that shows the number of turtles sighted and captured at each location from the literature and the USGS data. This provides us with a rough estimate of the WPT's current range and how many we would expect to see at each location.

Future Conditions (PVAs)

In order to explore the likelihood of population persistence of the WPT, we conducted population viability analyses (PVAs). Using the modeling program Vortex, we carried out several different PVAs for several different populations of WPTs. For *E. marmorata* we utilized the Goose Lake and Russian River populations with parameters from the authors Bury, Cook, Germano, and Holland. For *E. pallida* we utilized the Coyote Creek and Pine Valley Creek with parameters from the authors Belli, Bury, Germano, and Holland. We found that the populations were extremely sensitive to hatchling survival rates as no populations were viable using the 91.25% mortality rate for individuals aged 0-1 from Holland (Holland 1994, p. 2-11). Every population that used this mortality rate for hatchlings had a 100% chance of extinction within 100 years. Following this, we carried out a sensitivity analysis to find the mortality rate for individuals aged 0-1 at which the population for Goose Lake would persist. Although this mortality rate was still high, it was not as high as the rate proposed by Holland. The only population that persisted was Goose Lake with a 49% hatchling mortality rate from Germano (Germano 2016, p. 670). Overall, the PVA's we conducted suggest that the majority of populations of WPTs tested are not sustainable for the future when using the best available population demographic estimates.

Species Risk Conclusions

Based on our research and evidence, *E. pallida* and *E. marmorata* have declined significantly and are susceptible to further decline. However, *E. pallida* populations appear to be more at risk of extirpation than *E. marmorata*.

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ChE - Cholinesterase Enzymes
CMI - T-Cell Mediated Immune
CUPs **-** Current-Use Pesticides
CWCP - Chico Water Control Plant
ESA - Endangered Species Act
EV - Environmental Variation
H/L - Heterophil / Lymphocyte
HUPs **-** Historic-Use Pesticides
PAHs - Polycyclic Aromatic Hydrocarbons
PCB - Polychlorinated Biphenyl
PVA - Population Viability Analysis
SD - Standard Deviation
SE - Standard Error
SVOC - Semi-Volatile Organic Compounds
T3 - Triiodothyronine
T4- Thyroxine
USFWS - United States Fish and Wildlife Service
USGS - United States Geological Survey
WPT - Western Pond Turtle

Section 1: Background

The WPT is California's only extant native freshwater turtle. Its regional decline makes it a potential candidate for listing under the Federal Endangered Species Act (ESA). Given its range-wide decline, the United States Fish and Wildlife Service (USFWS) is performing a pre-listing assessment to evaluate the current status, population health, and threats to the species.

1.1 Taxonomy

Recent research has identified two species of WPT: *E. marmorata* from Washington through the San Joaquin Valley and *E. pallida* from the central coast range to Baja California (Spinks *et al.* 2014, p. 2238) [Figure 1]. This most recent genomic-scale analysis of the WPT helped clarify the confusing taxonomy that potentially limited WPT conservation efforts. The authors of that study highlighted that the unique evolutionary lineage of *E. pallida* potentially faces a more severe decline than *E. marmorata*. Recognition that the WPT consists of two species means that the status of each species must be assessed separately; each may be experiencing different threats and have differences in viability.

For the purposes of this report, the species of WPT for each county in California is identified (Table 1). We based the geographical breakdown by county off of the molecular evidence of the Spinks *et al* paper from 2014. The Spinks *et al* paper used Single Nucleotide Polymorphism analysis to find different population structure across the range of the WPT and further delineate the WPT into two species. Breaking down counties by species in this way is necessary for many of the analyses done in this report as all past literature and data identifies the WPT as one species. The breakdown of the two species guides the remainder of this report.

Figure 1. Range of *E. marmorata* and *E. pallida* (USFWS).

***Table 1.* WPT Species by California Counties based on Spinks *et al.* 2014.**

County	Species
Alpine	*E. marmorata*
Amador	*E. marmorata*
Butte	*E. marmorata*
Calaveras	*E. marmorata*
Colusa	*E. marmorata*
Del Norte	*E. marmorata*
El Dorado	*E. marmorata*
Fresno	*E. marmorata*
Glenn	*E. marmorata*
Humboldt	*E. marmorata*
Inyo	*E. marmorata*
Kern	*E. marmorata*
Kings	*E. marmorata*
Lake	*E. marmorata*
Lassen	*E. marmorata*
Madera	*E. marmorata*
Marin	*E. marmorata*
Mariposa	*E. marmorata*
Mendocino	*E. marmorata*
Merced	*E. marmorata*
Modoc	*E. marmorata*
Mono	*E. marmorata*
Napa	*E. marmorata*
Nevada	*E. marmorata*
Placer	*E. marmorata*
Plumas	*E. marmorata*
Sacramento	*E. marmorata*
San Joaquin	*E. marmorata*
Shasta	*E. marmorata*
Sierra	*E. marmorata*
Siskiyou	*E. marmorata*
Solano	*E. marmorata*
Sonoma	*E. marmorata*
Stanislaus	*E. marmorata*
Sutter	*E. marmorata*
Tehama	*E. marmorata*
Trinity	*E. marmorata*
Tulare	*E. marmorata*
Tuolumne	*E. marmorata*

Yolo	*E. marmorata*
Yuba	*E. marmorata*
Alameda	*E. pallida*
Contra Costa	*E. pallida*
Imperial	*E. pallida*
Los Angeles	*E. pallida*
Monterey	*E. pallida*
Orange	*E. pallida*
Riverside	*E. pallida*
San Benito	*E. pallida*
San Bernardino	*E. pallida*
San Diego	*E. pallida*
San Francisco	*E. pallida*
San Luis Obispo	*E. pallida*
San Mateo	*E. pallida*
Santa Barbara	*E. pallida*
Santa Clara	*E. pallida*
Santa Cruz	*E. pallida*
Ventura	*E. pallida*

1.2 Species Concern

Information regarding the current status of the WPT is needed to determine whether listing under the ESA is warranted. To aid in this decision, we formulated several research questions: What threats are putting populations at risk and to what degree? Do numbers seem to be stable, increasing, or decreasing? Is there recruitment? What is the status and trend of WPT populations across their range? How does the WPT's size, sex, and distribution change over space and time? To answer these our team has analyzed published and unpublished data, performed risk assessments, and collected new data on size, sex-ratios, and body condition from three museum collections. Additionally we conducted population viability analysis (PVA) models for both species using literature information for relevant life history parameters.

In California, *E. pallida's* distribution and abundance, particularly in the southern part of its range classifies it as a Priority 1 Species of Special Concern (Thomson *et al.* 2016). In the north, *E. marmorata* populations are also declining, although not as severely. As a result, Thomson *et al* (2016) considered California *E. marmorata* a Priority 3 Species of Special Concern. In Washington, *E. marmorata* has been state listed as endangered since 1993 (Hallock *et al.* 2017, p. 6) and in Oregon it is considered a "sensitive species"- a species facing one or more threats to its population or habitat (Rosenburg *et al.*, 2009, p. 9). However, not much is known or available on the WPT's status in Baja California. Despite the progress that has been made in recovering *E. marmorata* populations in Washington, their recovery plan goals for downlisting to threatened have yet to be realized. The statewide population is still reliant on supplementation with head-started individuals because of low hatching success and predation on hatchlings.

1.3 Previous Listing Attempt

It is important to note there was a previous listing attempt for the WPT. On January 29, 1992 the USFWS was petitioned to list the WPT in its entirety (that is, both *E. marmorata* and *E. pallida*) under the ESA. However, the USFWS concluded that the listing was not warranted (USFWS 1992, p. 45761-45762). At the time of its submission, the WPT had been completely extirpated or ecologically extirpated (reduced to such low abundance that it no longer interacted significantly with other species) from many areas, especially throughout southern California and the Great Central Valley (USFWS 1992, p. 45761-45762). The petition recognized the WPT as one species (*Clemmys marmorata)* and cited the following threats across its range: loss and degradation of wetland terrestrial habitat, predation by introduced species, overexploitation, habitat fragmentation, drought, and various other factors (USFWS 1993, p. 42717-42718).

However, a one-year petition finding in 1993 concluded that the WPT remained in the vast majority of its historical range and did not meet the definition of an endangered or threatened species (USFWS 1993, p. 42717-42718). The USFWS stated that most of the available information regarding these threats was anecdotal and consistent information on the long-term effects of these activities was lacking on a range-wide basis. For example, the alteration of wetlands as a threat was not consistent with the occurrence of WPTs in altered habitats such as sewage treatment ponds, irrigation canals, reservoirs, and stock ponds (USFWS 1993, p. 42717-42718). Additional threats such as contaminant spills, grazing, and off-road vehicle use were considered localized and thus did not threaten the species throughout most of its range (USFWS 1993, p. 42717-42718).

Section 2: Species Biology and Needs

The general biology and historical conditions of the WPT are important for understanding the risks and current conditions of the species. Life history knowledge is critical for properly parameterizing PVAs.

2.1 Species Description & Taxonomy

The WPT is a semi-aquatic turtle native to the west coast of North America. While coloration varies by geography and sex, WPTs are generally dark brown dorsally and yellow ventrally. Markings also vary, but generally the species has spots or streaks dorsally on their carapace and dark blotches ventrally on their plastron (Bury *et al.* 2012, pp. 3-4). The WPT is the most common name for the turtle although Pacific pond turtle is also used in literature.

Taxonomy of the WPT has been in a great deal of flux due to new genetic information. Originally, the WPT was regarded as one species. Then, two separate populations were found based on morphological analysis: a northern population and a southern population with some mixture between the two in the San Joaquin Valley (Seeliger 1945, entire). These two populations were proposed as to be recognized as distinct subspecies of *E. marmorata* (*E. m. marmorata* and *E. m. pallida*). Later genetic studies differed in how many evolutionary lineages existed within the nominate species until the most recent genetic separation concluded that there are two distinct evolutionary lineages (Spinks *et al.* 2014, entire). As previously stated, there are now two recognized species of WPT— *E. pallida and E. marmorata* — based on this most recent separation.

Both species are grouped in the family Emydidae, but many scientists disagree on the genus the species should be placed under (Fritz *et al.* 2011, entire). Although the WPT has historically been placed in different genera, the most recent work has proposed it belongs in *Emys* (Spinks *et al.* 2016, entire) which is the taxonomy we will use in this document.

2.2 Historical & Current Distribution

According to historical records, the WPT once had a maximum range from northern Baja California to southern Canada along the west coast of North America. A majority of recorded WPT populations are in California (56% of total records) and on non-federal land (71% of total records) (Barela and Olson 2014, p. 4). Current evidence indicates that the distribution of the WPT is shrinking. For example, a 1993 survey in Oregon only found WPTs at 83 of 313 (26.5%) previously occupied sites (Barela and Olson 2014, p. 6). In addition, estimations indicate that declines may be occurring in more than 80% of the overall range. The most severe declines for *E. marmorata* have been in Washington, while *E. pallida* appears to be suffering losses throughout most of its range (Bury *et al.* 2012, p. 6). However, the severity of distribution loss may have been either underestimated or overestimated as sampling may not have been comprehensive throughout all historically occupied sites (Barela and Olson 2014, pp. 5-11). Despite population declines, WPTs seem to thrive in some new habitats that have been created by humans including artificial bodies of water such as water treatment plants and stock ponds (Bury *et al.* 2012, p. 6). Comprehensive sampling is necessary to identify the true current range of the WPT, particularly *E. pallida* which may be facing more severe distribution shrinkage than *E. marmorata*.

2.3 Life History and Ecology

2.3.1 Life History

Hatchlings are a critical life stage in turtle populations. On average, hatchlings enter aquatic habitats 49 days after hatching and make several stops during their movement from terrestrial habitat to aquatic habitat (Rosenberg and Swift 2013, p. 111). In terms of growth, hatchlings experience the most rapid growth rates of a life stage and typically must reach a carapace length of around 110 mm to reach sexual maturity (Bury *et al.* 2012, p. 16). Once reaching adulthood, WPTs are long-lived with some reaching over 55 years of age (Bury *et al*. 2012, pp. 17-19). The long life span of WPTs has made approximations of mortality rates at the adult stage difficult. Approximations of survivorship for earlier stages is difficult as well due to low detection of juvenile turtles.

As a semi-aquatic turtle, WPTs have both a terrestrial and aquatic life history. The amount of time spent on land changes depending on location and aquatic habitat type. Ultimately, intermittent bodies of water result in turtles spending longer amounts of time on land than in perennial bodies of water (Bondi and Marks 2013, entire). A study of a seasonal pond found that the turtles spent an average of 235 days of the year out of water with 95% of their terrestrial sites within 187 meters of a pond (Zaragoza *et al.* 2015, p. 439).

Temperature-dependent sex determination is a common characteristic of turtles including WPTs, which have a higher likelihood of being female if they spend 30% or more of the sex-determining period of incubation above 29 degrees Celsius (Christie and Geist 2017, p. 47). With temperatures predicted to increase due to climate change, WPTs may see a higher proportion of hatchlings born as females due to their temperature-dependent sex determination.

Historically, the sex ratio for WPTs was 1:1 (Bury *et al.* 2012, p. 15). However, terrestrial life history can be highly impacted by motorized vehicles resulting in the death of turtles, particularly females who must spend more time on land each year for nesting (Nyhof and Trulio 2015, entire). While the age WPTs reach sexual maturity varies between sites, most gravid (egg-carrying) females are over 6 years old (Bury *et al*. 2012, p. 15). Eggs are typically deposited May through July, although populations of *E. marmorata* deposit eggs later in the season than *E. pallida* (Bury *et al*. 2012, p. 15). The mean clutch size for WPTs varies from 4.5 to 8.5 eggs. The number of clutches varies from 1 to 3 per year by location. Based on evidence from southern California and coastal central California populations, *E. pallida* consistently has 2 clutches/year. However, *E. marmorata* has much more variation in clutch size with 3 clutches recorded in the San Joaquin Valley, 2 clutches recorded in Oregon's Willamette Valley, and 1 clutch in western Oregon populations.

The life history of WPTs can change in response to environmental conditions, such as temperature: colder water results in slower growth and fewer gravid females in the breeding season because individual turtles divert resources to storage instead of growth (Ashton *et al*. 2015, entire). Warmer water has the opposite effect with faster growth and more gravid females in the breeding season because they do not have to divert resources to storage.

2.3.2 Habitat

The habitat of the WPT can be very diverse as they are found in most freshwater bodies including rivers, lakes, and ponds. Large populations are typically not found 1500 meters above sea level (Bury *et al*. 2012, p. 12). Due to the geography of their range, great distances can exist between WPT populations (Bury *et al.* 2012, pp. 12-13). Suitable habitats for WPTs have general trends including high solar radiation for thermal ecology, nearby wetlands, and suitable basking spots (Horn and Gervais 2018, pp. 10-16). Human-impacted environments are not necessarily unsuitable and should not be disregarded as potential habitats for WPTs. In addition, suitable sites may be found in areas once considered unsuitable including high elevations sites, dried wetlands, and human-altered habitats such as wastewater treatment plants (Germano and Riedle 2015, entire; Germano 2016, entire; Germano 2010, entire).

2.3.3 Diet

WPTs have a generalist diet which mainly consists of small aquatic invertebrates, vertebrates, carrion, plant material and algae. Juvenile diet is insectivorous while adult diet includes a larger portion of plant material (Bury *et al*. 2012, p. 12). The generalist diet is exemplified at wastewater treatment facilities where they feed on human food waste.

2.3.4 Predators

WPTs have a diverse set of predators including raccoons and coyotes. Hatchlings' predators include fish and giant water bugs, but recruitment is increasingly hurt by invasive bullfrog populations. Many population sites are biased towards large, old turtles and have populations of invasive bullfrog populations which prey on hatchling turtles (Sloan 2012, p. 30).

WPTs are thought to exhibit early flight response or high wariness to predators. A study at the UC Davis Arboretum waterway compared WPT flight response with red-eared slider flight response and found WPTs fled at greater approach distances (Costa 2014, entire). The approach distance is the distance at which a basking turtle abandons its site and flees into the water as a potential predator approaches the turtle. Greater flight response distances mean the predator is further away, and therefore that the species is more wary of predators. However, the difference in predator response may have important ecological consequences as it means that the invasive red-eared sliders may have a competitive advantage for basking spots where both species co-occur.

2.3.5 Basking

Perturbations to natural temperature regimes could impact various aspects of WPT ecology, including basking. In order to increase body temperature, WPTs bask in the sun. Residents of warmer climates participate in aquatic basking (resting in algal mats) (Bury *et al*. 2012, pp. 9-10). Unusually, algae has recently been observed growing on WPTs (Bury *et al*. 2015, entire). This increase is thought to be a result of turtles basking less in warmer waters, and algal accumulation on shells because individuals are not basking out of the water which generally serves to limit algal growth on turtle shells. The effects of algal growth on turtles is unknown, but the origin of the algae may be from invasive red-eared sliders (Bury *et al*. 2015, p. 152).

2.3.6 Overwintering

WPTs overwinter annually either on land or underwater. Turtles from riverine habitats preferentially overwinter on land (Bury *et al*. 2012, pp. 10-11). Overwintering is when the turtle buries itself and enters a form of hibernation to survive winter. As mentioned previously, populations in intermittent streams spend more time on land because overwintering occurs earlier in those populations compared to populations in perennial bodies of water and aquatic habitats are not suitable for overwintering.

Section 3: Species Separation

As previously mentioned, recent research has identified two species of WPT: *E. marmorata* from Washington through the San Joaquin Valley and *E. pallida* from the central coast range to Baja California (Spinks *et al.* 2014, entire). This most recent genomic-scale study and analysis of the WPT helps clarify the confusing taxonomy that potentially limits WPT conservation efforts. Recognition that the WPT consists of two species suggests that the status of each species must be assessed separately as each may be experiencing different threats and have differences in viability.

In separating any set of species, an important component is identifying a morphological characteristic that can be used to field identify an individual to species. For *E. marmorata* and *E. pallida*, a characteristic proposed for identification is the presence or absence of the inguinal scute (Figure 2).

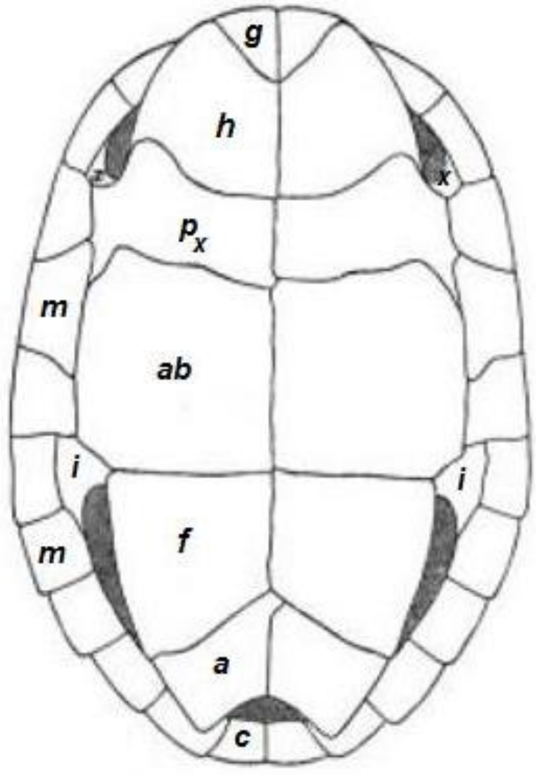


Figure 2. Plastron view of the WPT with inguinal scute (i) (Baruah *et al.* 2016, p. 116)

3.1 Early History of Species Separation by Inguinal

As early as 1945, the presence and size of the inguinal was proposed as a morphological character to identify the subspecies *Clemmys marmorata* and *C. m. pallida*. The 1945 paper by L. M. Seeliger highlighted the geographic variation of the then one species, *Clemmys Marmorata.* At this point in time, there were differences attributed to *Clemmys marmorata pallida*, however it was considered a subspecies. Seeliger determined a “definite geographic variation in the form of the inguinal plate” between individuals from Lower California (southern California) and individuals from the rest of the species range (Seeliger 1945, p. 155). An inguinal was seen in 89% of individuals north of San Francisco Bay. However, the inguinal plate was found to be extremely small or non-existent for individuals from the southern part of California. Individuals found along the coast south of the San Francisco Bay were found to lack inguinal plates in the same manner as individuals from the southern portion of the state lacked the inguinal plates. Finally, individuals from central California seemed to have inguinal plates of varying degrees, suggesting areas of integration between the two types of WPT (Seeliger 1945, pp. 155-156). Ultimately, Seeliger determined that *Clemmys marmorata* could be distinguished from the southern subspecies (*E. pallida*) by the presence of a pair of triangular inguinal plates (Seeliger 1945, p. 158).

3.2 Current Species Separation

While Seeliger utilized the inguinal plate as a primary way to differentiate between the northern and southern varieties of WPT, genomics were utilized by Spinks, Thomson, and Shaffer to delineate two distinct species of WPT. Using the DNA of WPTs from across the range, Spinks *et al.* found two distinct clusters of WPTs. The first cluster consists of a northern batch that ranges from the southern San Joaquin Valley all the way north to Washington state. The second cluster consists of a southern batch stretching from the Central Coast Range south to Baja California and including the Mojave River. Integration of the two species is restricted to 16 of the 923 individuals studied and occurs in a stretch of habitat from the northern central coast range south to the foothills of the Sierra Nevada mountains (Spinks *et al.* 2014, p. 2232). According to the authors, the first northern cluster is *E. marmorata* while the southern cluster in *E. pallida*. Based on the methods and results of the Spinks paper, the ranges of *E. marmorata* and *E. pallida* are similar to the ranges proposed by Seeliger based on the inguinal plate. However, a main area of difference is the wide area of integration Seeliger suggested in central California. Spinks suggests this is really limited to the northern central coast range south to the Sierra foothills.

3.3 Novel Data: Inguinal Presence and Absence

The similar WPT range posited by both inguinal presence and molecular genetic data advances the notion that the inguinal plate is an important morphological characteristic that can be used to distinguish *E. marmorata* and *E. pallida*. As a result, the presence of an inguinal may coincide with the identified range of what has been established as *E. marmorata* by both Seeliger and Spinks while the absence of an inguinal may coincide with the identified range of what has been established as *E. pallida* by Seeliger and Spinks. To verify if the inguinal is a good characteristic to identify the two species going forward, museum data was collected on the presence and size of inguinals in 463 specimens.

Overall, approximately 84% of *E. marmorata* specimens had inguinal plates present, while around 13% of *E. pallida* specimens had inguinal plates present (Figures 3 and 4). While we are dividing species based on counties (Table 1), one indication of these results could be that we are not drawing the correct lines to divide the species. To our knowledge, the presence of the inguinal plate is not a perfect method of identifying the two species, but it is one of the best morphological differences to distinguish *E. marmorata* and *E. pallida*.

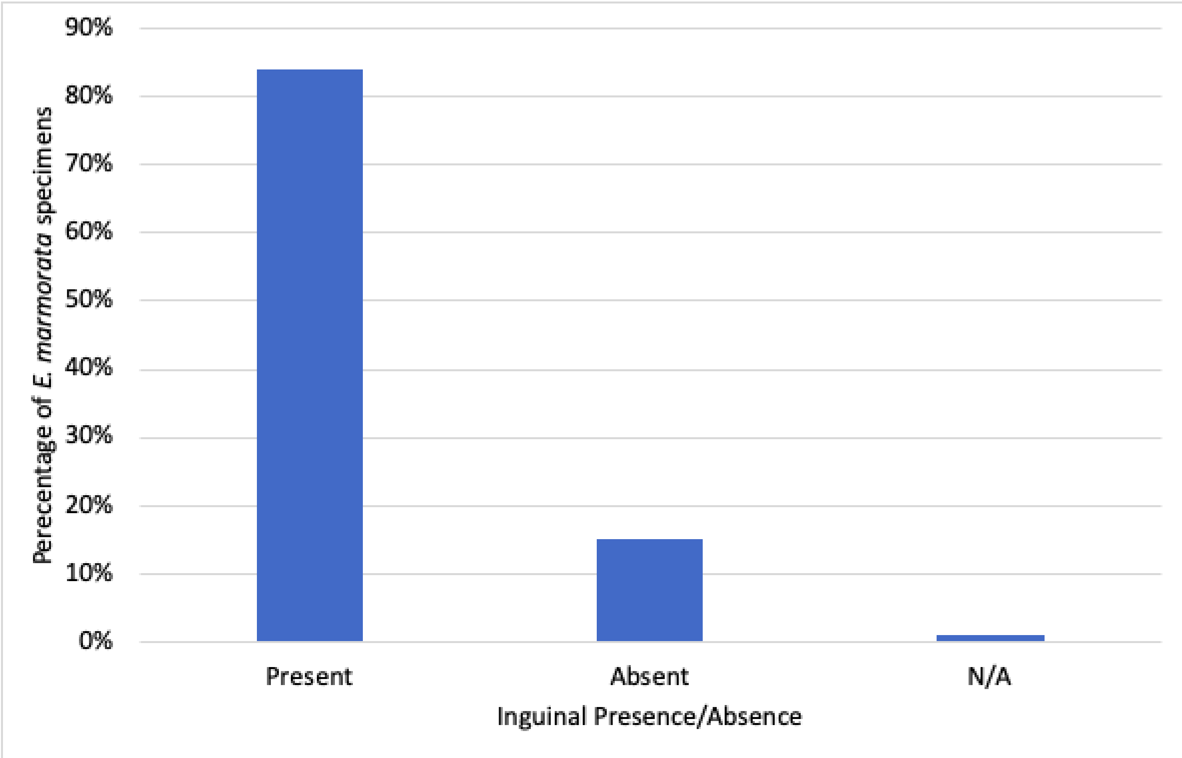


Figure 3. Inguinal plate presence in percentage of total *E. marmorata* museum specimens.

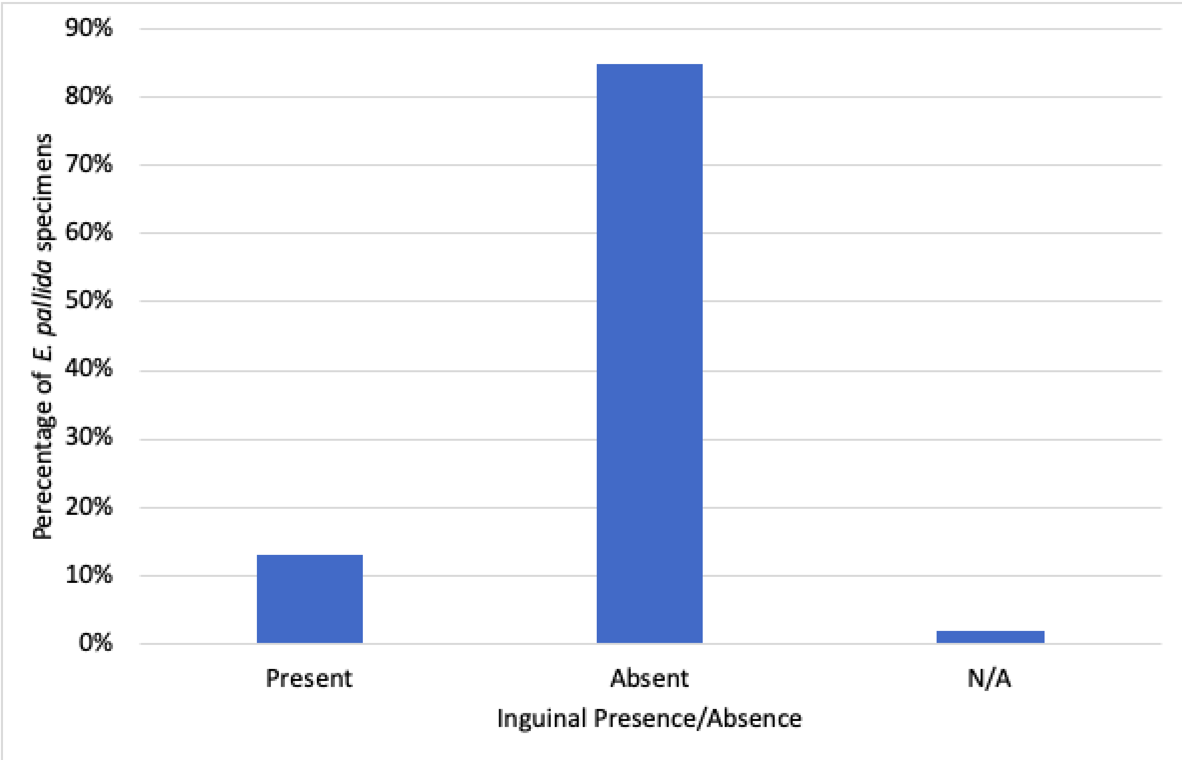


Figure 4. Inguinal plate presence in percentage of total *E. pallida* museum specimens.

To determine why some specimens did not fit the expected presence or absence of inguinal based on its range, the counties of these specimens were extracted from the museum data. This was done for both the specimens unexpectedly with inguinals in *E. pallida* counties and for specimens unexpectedly without inguinals in *E. marmorata* counties (Figures 5 & 6). To further explore this issue, we first determine the breakdown by county of *E. pallida* with inguinals and *E. marmorata* lacking them (Figures 7 & 8).

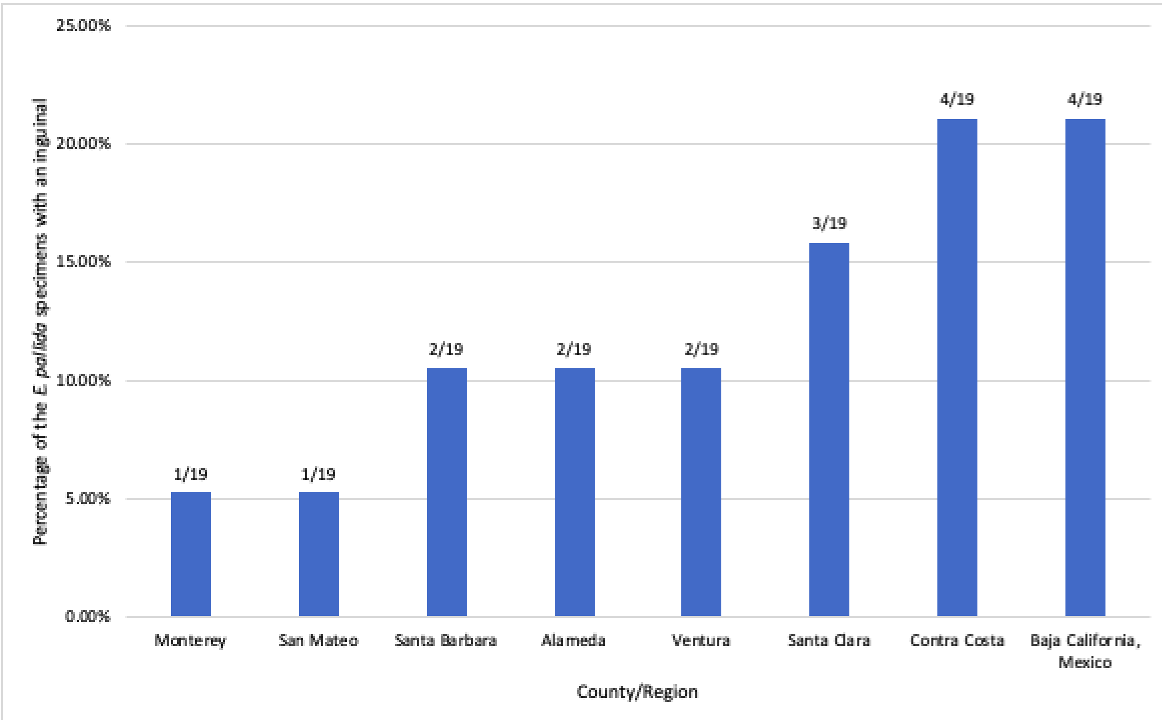


Figure 5. A break-down of the counties of *E. pallida* in which the inguinal plate was present. The y-axis is the percent of the *E. pallida* specimens with inguinals present from each county out of all the *E. pallida* specimens with inguinals present. For example, out of the 13% of *E. pallida* specimens with inguinals present, approximately 6% come from Monterey county.

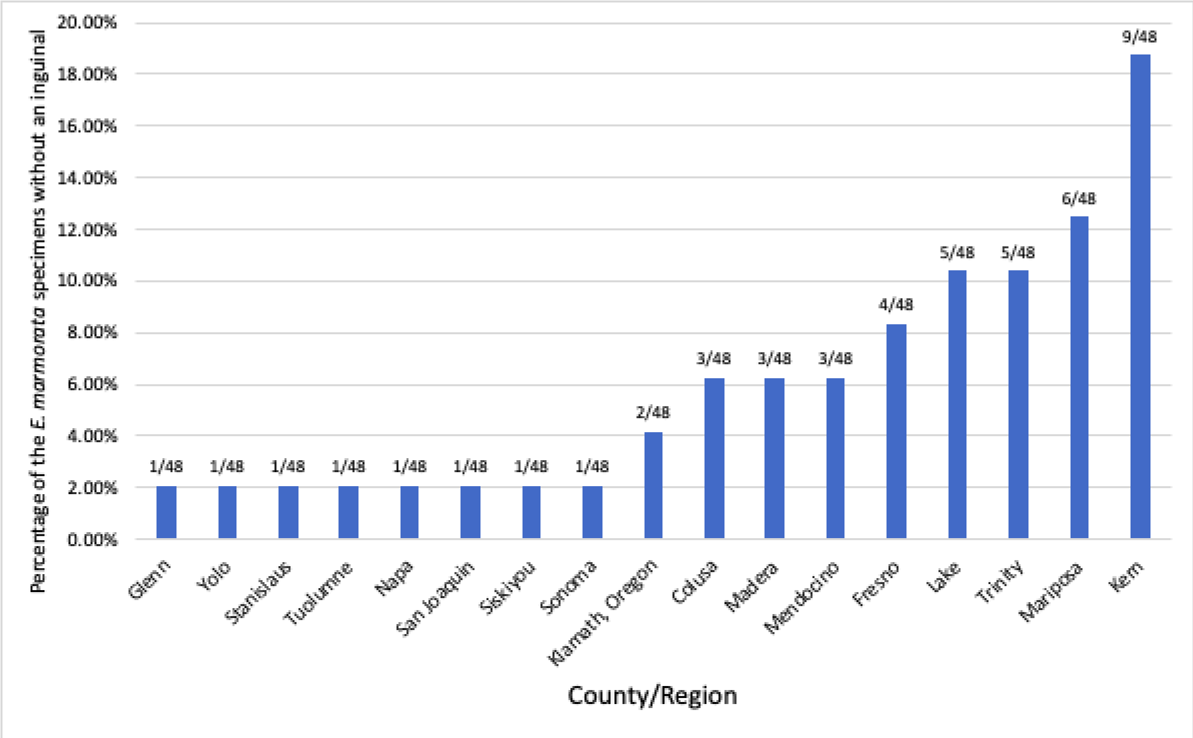


Figure 6. A break-down of the counties of *E. marmorata* in which the inguinal plate was absent. The y-axis is the percent of the *E. marmorata* specimens with inguinals absent from each county out of all the *E. marmorata* specimens with inguinals absent. For example, out of the 16% of *E. marmorata* specimens with inguinals absent, approximately 2% come from Glenn county.

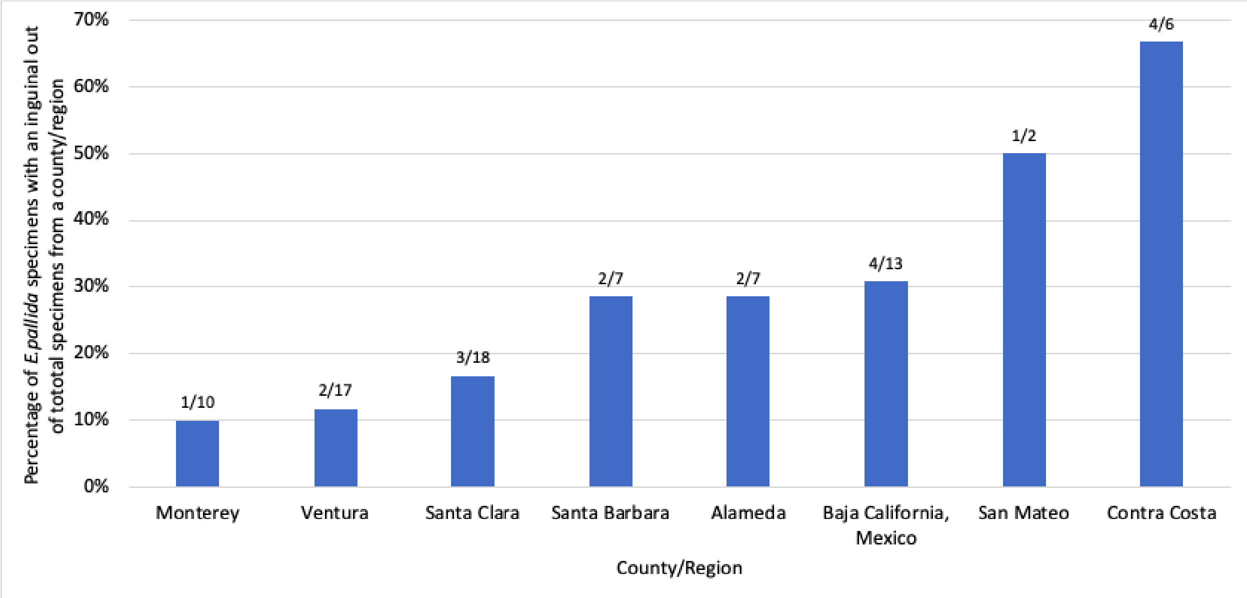


Figure 7. Frequency of inguinal presence by county (*E. pallida*). The percentage occurrence of inguinal presence for each county which showed inguinal presence unexpectedly in the range of *E. pallida* was calculated and forms the y-axis. Each county was labeled with the ratio of specimens with inguinal presence to total specimens in the county.

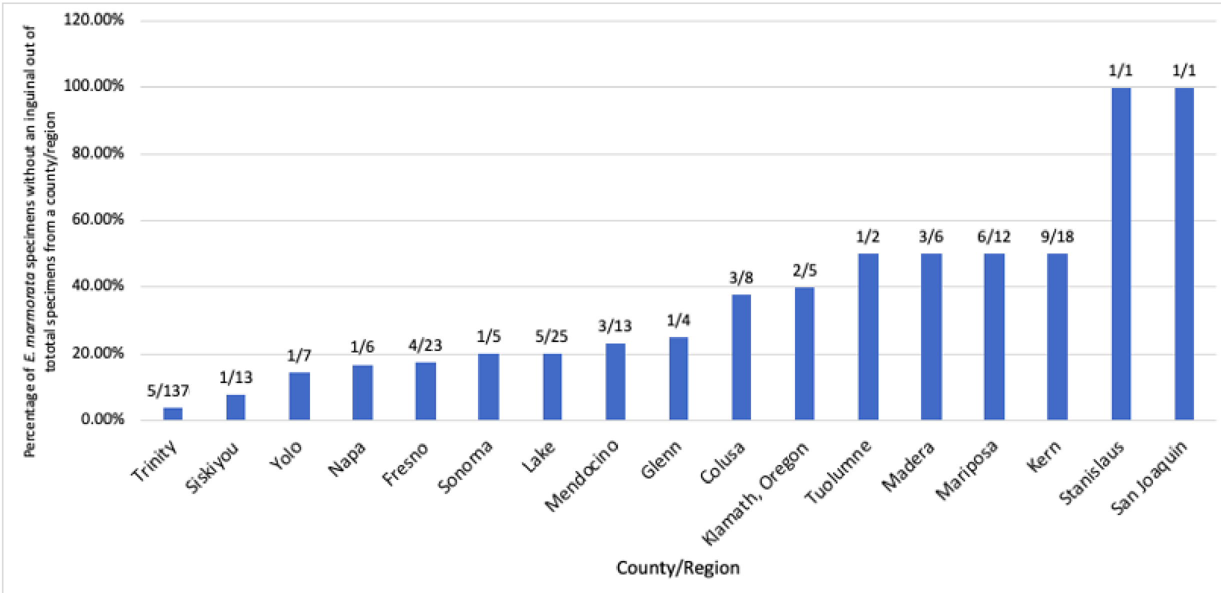


Figure 8. Frequency of Inguinal Absence by County (*E. marmorata*). The percentage occurrence of inguinal presence for each county which showed inguinal absence unexpectedly in the range of *E. marmorata* was calculated and forms the y-axis. Each county was labeled with the ratio of specimens with inguinal absence to total specimens in the county.

Clearly, county lines are not completely accurate in separating species as species' ranges do not correspond to county lines. Thus, some counties have both species of WPT within their boundaries, as certain mixing zones occur. For our analyses, specimens from counties that overlap with the majority of *E. pallida*'s range were identified as such and vice versa for *E. marmorata*. This is illustrated by counties such as Kern County. Half of the museums specimens from Kern did not have inguinals even though we considered it as exclusively containing *E. marmorata*. As the western portion of Kern County is likely a mixing zone, these specimens could actually be *E. pallida* and fit the correct morphological characteristic for its species. Thus, narrowing down the inaccuracies for inguinal presence and absence can help eliminate some biases in the analysis.

Overall, our results show that inguinal scutes are the best morphological feature for identifying the two species of WPTs apart. In the future, we would like to look more closely at the geographical distribution in the counties with the "wrong" inguinal to improve the range delineation for the two taxa (see Appendix A).

Section 4: Risk Factors Affecting *E. pallida* and *E. marmorata*

Table 2. Risk Assessment for *E. pallida*

Risk	Sum	Average	Number of observations
Drought	12	1.2	10
Predation by Non-Native Species	8	1	8
Land Conversion	6	1.2	5
Contaminants	6	1	6
Flood	5	1.25	4
Fire	4	1.33	3
Disease	4	1	4
Invasive Species Competition	3	1	3
Vehicle Traffic	1	0.5	2
Natural Predation	1	0.5	2
Dams and pumping of water	0	0	2
Artificial Ecosystems	0	0	1
Consumption and Pet Trade	NA	NA	0
Climate change	NA	NA	0

Table 3. Risk Assessment for *E. marmorata*

Risk	Sum	Average	Number of observations

Disease	11	1	11
Contaminants	11	1	11
Land Conversion	8	0.57	14
Predation by Non-Native Species	7	1	7
Drought	6	1.5	4
Vehicle Traffic	4	0.67	6
Invasive Species Competition	4	1	4
Natural Predation	4	1	4
Consumption and Pet Trade	2	2	1
Climate Change	2	1	2
Dams and pumping of water	1	0.33	3
Fire	0	0	5
Artificial Ecosystems	0	0	5
Flood	NA	NA	0

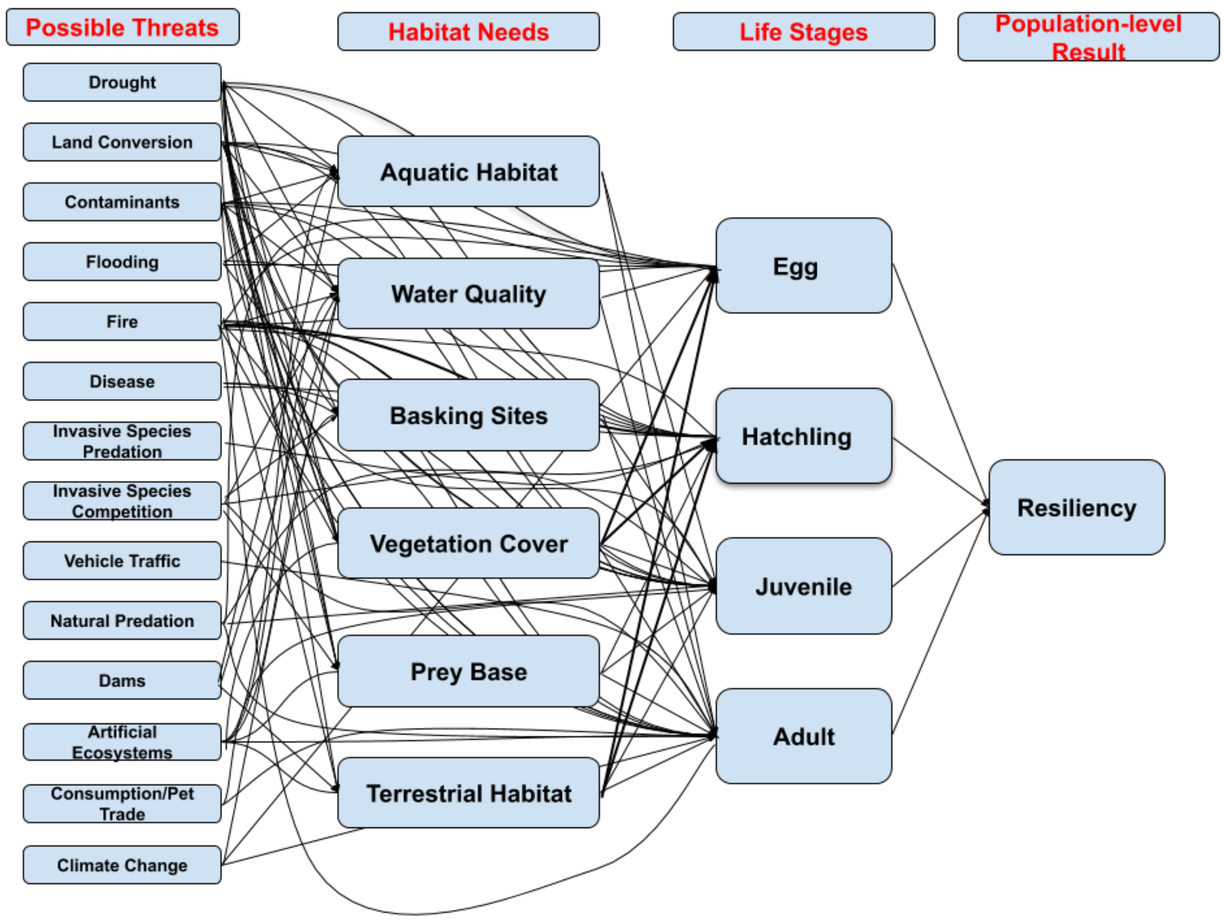


Figure 9. Risk conceptual model showing direct and indirect effects of each risk on the habitat needs and life stages of the WPT.

4.1 Methods

After an initial literature review, 14 risks were identified for the long-term survival and viability of WPT populations: drought, fire, invasive competitors, invasive predators, disease, natural predators, climate change, land conversion, pollution, dams, flash floods, commercial use, road traffic, and artificial ecosystems. Following an initial review, a comprehensive literature review was performed for each risk. Within each risk category there are a range of observations. An observation is defined as a population identified or studied in a published article that was affected by at least one of the 14 risks. This means that an article with one population could result in multiple observations if that population was subjected to multiple risks. Furthermore, an article could have multiple populations and thus multiple observations. The number of observations for each species was recorded under each risk category.

Each observation was given a score which corresponded to the degree of risk. The scores are: 0 for no effect on population size, 1 for negative effect or a decrease in population size, 2 for complete extirpation of a population derived from that threat, and -1 for a positive effect or an increase in population size in cases where what was deemed a risk was actually beneficial. After being scored, the observations for each risk were grouped by species, *E. marmorata* or *E. pallida*, based on the county where the observation occurred (Table 1). A consistent nomenclature was often not used in the articles since only the most recent literature acknowledges the separation of the WPT into *E. marmorata* and *E. pallida*. The scores were then summed under each risk category for each species. The risks with the highest sums were deemed the most impactful to a species. Therefore, we tabulated risk scores organized by sum from highest to lowest. Finally, this sum was divided by the number of observations to calculate an average score for the effects of the risk on populations of each species.

In addition to assigning scores for each risk, we created conceptual models to visualize each risk's effect on both the habitat needs and life stages of the WPT. Figure 9 is an assemblage of all risks and shows the effects of each risk on habitat needs and life stage. Figures 9-23 are conceptual models for each individual risk and also show the effects of each risk on habitat needs and life stage. There are two methods in which our conceptual models show the effects of each risk. The first method is when a risk affects a habitat need which in turn effects a life stage. This is shown by an arrow pointing from the risk to the specific habitat need and then from the habitat need to the affected life stage. The second method is when a risk directly affects a specific life stage. This is shown by an arrow bypassing the habitat needs and directly pointing to a life stage. No matter the method, the risks always affect resilience at the population level. We based our models on the literature we reviewed for both the risk assessment and the entire species assessment. In some cases we also utilized our general knowledge on the species to infer what habitat needs and life stages would be affected.

4.2 Drought

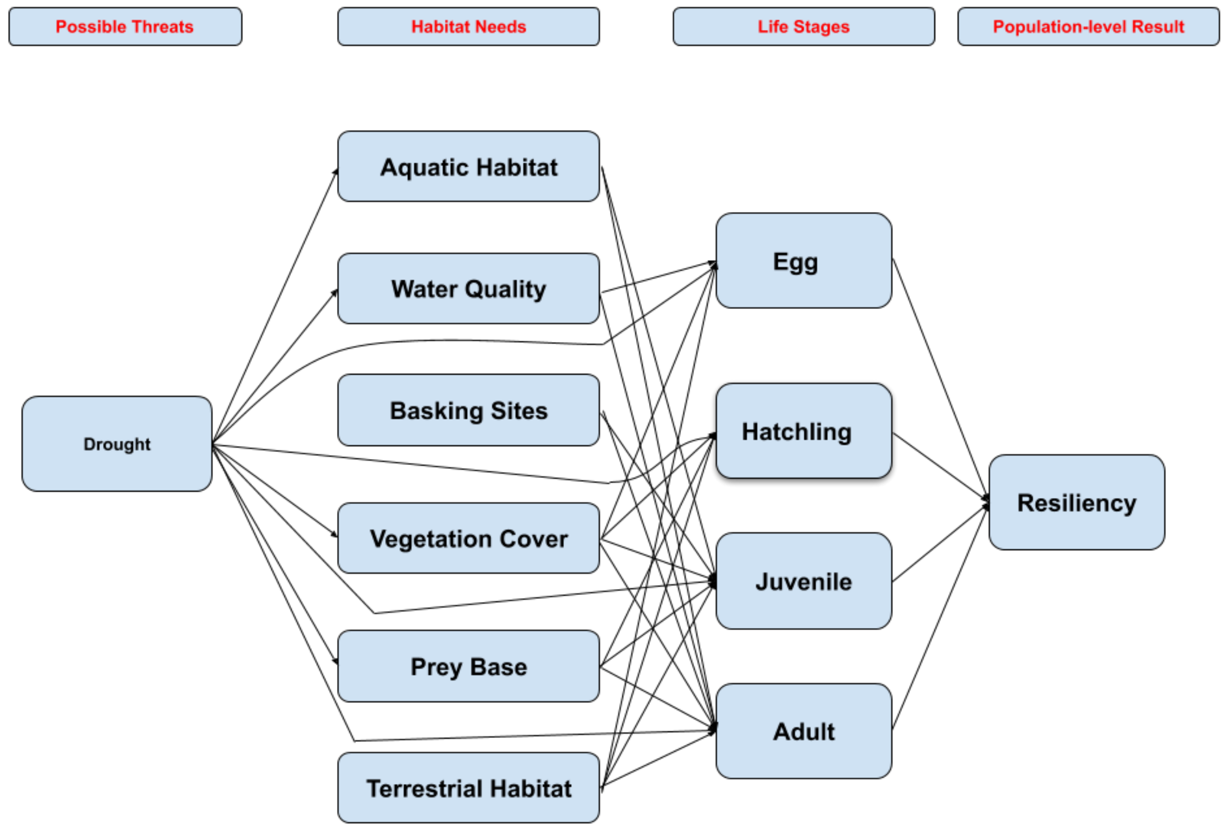


Figure 10. Risk conceptual model showing direct and indirect effects of drought on the habitat needs and life stages of the WPT. Evidence for the direct and indirect effects come from a variety of sources (Holland 1991, p. 65; Lovich *et al.* 2017, pp. 6-8; Purcell *et al.* 2017, pp. 21-23; Leidy *et al.* 2016, p. 73).

Drought is one of the top five risks for both *E. marmorata* and *E. pallida* when considering number of observations and sum of studies. Out of all assessed risks, drought is the top risk for *E. pallida* populations. The severity of drought is high and often leads to mortality events or complete extirpation of populations. For instance, the 1986-1991 drought in southern and central California was devastating to WPT populations especially populations of intermittent streams. In a survey of populations suffering from the drought, the number of WPT carcasses saw an approximately 400% increase when comparing the surveys from 1989-1990 to those of 1987-1988 (Holland 1991, p. 65). Our most recent drought has also seen complete extirpations of robust populations, particularly those of *E. pallida*. While very severe, drought is not a constant risk when compared to other risks such as predation by bullfrogs. All of the recorded observations of drought impacts on WPTs come from the 1989-1990 drought and the 2012-2014 drought. The intensity of the drought also must be factored in. The 2012-2014 drought was 7-9% above the average PET (a combination of temperature, humidity, wind, and insolation) and the average precipitation during the time was the lowest 3 year running record (Williams *et al.* 2015, p. 6822). Nonetheless, droughts will become more common and extended as climate change progresses, particularly in California. Drought has a higher probability of occurring when precipitation deficits (less precipitation than an average year) co-occurs with warm conditions. Anthropogenic warming has increased the probability of this co-occurrence and more droughts similar to the 2012-2014 drought in California are highly probable (Diffenbaugh *et al.* 2015, p. 3931). Thus, drought will not only to continue to be a major risk but will only become more severe.

While clearly a devastating risk, the ultimate cause of WPT mortality due to drought is uncertain. The impacts of drought are numerous and vary depending on the population and habitat. These impacts can either work alone or synergistically to cause mortality. Among causes of WPT mortality, there can be both direct and indirect impacts (Leidy *et al.* 2016, p. 73). Impacts of drought include desiccation, increased vulnerability to predators, depletion of prey, increased distance between aquatic habitats, increased stress on individuals, reduced water quality, complete loss of aquatic habitat, and starvation (Holland 1991, p. 65; Lovich *et al.* 2017, pp. 6-8; Purcell *et al.* 2017, pp. 21-23; Leidy *et al.* 2016, p. 73). One of the most important direct impacts of drought is the drying of bodies of water as turtles will have to spend a larger portion of the year on land. Since WPTs ingest prey in water, the longer the turtles remain out of water, the less energy stores remain in their bodies. This eventually results in the deaths of WPTs due to starvation (Purcell *et al.* 2017, p. 22).

<u>*E. pallida*</u>

Risk	Sum	Average	Number of observations
Drought	12	1.2	10

Over time, drought results in large declines for *E. pallida* populations. During the 1986-1991 drought, two populations of *E. pallida* were completely extirpated and another four populations had high mortality. All had at least a 65% decline in population size (Holland 1991, p. 66). More recently, a robust population of approximately 170 WPTs was completely extirpated at Lake Elizabeth in northern Los Angeles County due to the 2012-2014 drought. The carcasses of the WPTs were all severely emaciated and the water quality was greatly reduced particularly through increased salinity and reduced macroinvertebrate diversity (Lovich *et al.* 2017, pp. 5-6). Also as a result of the 2012-2014 drought, a summer die off of at least 39 WPTs occurred along Coyote Creek in Santa Clara County. Of the carcasses found, 90% had only small remnants of soft tissue remaining in their shell cavities which indicated predators or scavengers fed on them (Leidy *et al.* 2016, p. 73). The WPTs could have died due to vulnerability to predation as water receded. Drought can also result in impacts to populations by creating long term habitat changes. A recent study in Afton Canyon in the Mojave River found the decline of a small population was due to a series of droughts. This reduced the extent of surface water (Lovich *et al.* 2017, p. 13). As drought intensifies, preserving and protecting remaining bodies of water especially perennial ones will become more important for the survival of the WPT. The study conducted along Coyote Creek at the end of the 2012-2014 drought found live WPTs in the refugial ponds remaining after the drought (Leidy *et al.* 2016, p. 74). The turtles congregated in these remaining sources of aquatic habitat. Furthermore, a pond in the Cañada de los Osos Reserve in Santa Clara County did not see high mortality despite the 2012-2014 drought (Smith 2018, p. 3-5). survival may be due to the ability of movement between bodies of water since the reserve has other ponds in the vicinity. Overall, *E. pallida* faces a severe threat in the form of drought, especially since many of their habitats are isolated intermittent bodies of water. Many populations may face extirpation during extended droughts in the future.

<u>*E. marmorata*</u>

Risk	Sum	Average	Number of observations
Drought	6	1.5	4

Drought is a very severe risk to *E. marmorata* and the average severity of drought is worse for *E. marmorata* than *E. pallida*. However, there have been more studies reporting the effects of drought on *E. pallida* which indicates that drought may be a more widespread risk to *E. pallida* as a species than *E. marmorata*. Furthermore, the populations of *E. marmorata* that are most vulnerable to drought are those in the southern range of the species with all of the studies reported being from central California. The populations of *E. marmorata* that are vulnerable face steep declines. Two sites impacted by the 1986-1991 drought experienced greater than 80% declines (Holland 1991, p. 66). Another population in the southern San Joaquin Valley was also extirpated during this drought (Germano and Bury 2008, entire). A study on an *E. marmorata* population in the San Joaquin Experimental Range during the 2012-2014 drought saw the population be practically extirpated due to the drying of a stock pond. The study found WPTs spending more than 400 consecutive days in a terrestrial environment after water dried up with one individual surviving more than 20 months (Purcell *et al.* 2017, p. 22). Some turtles ventured outward in long movements with the goal of reaching other bodies of water but many stayed near the pond to conserve energy (Purcell *et al.* 2017, p. 23). Many WPT carcasses had evidence of predation highlighting how they faced threats other than starvation (Purcell *et al* 2017, pp. 21-23). This study once again illustrates how isolated populations of WPTs lacking other bodies of water are at risk of extirpation if their only source of water goes dry for extended periods of time. While these effects are quite severe, it should be reiterated that data for drought's effects on *E. marmorata* are minimal and come from central California populations where drought can be severe. No studies were found from Oregon, Washington, or Northern California. The northern *E. marmorata* populations, perhaps most of the species' range, may be less impacted by drought, although we lack data.

4.3 Fire

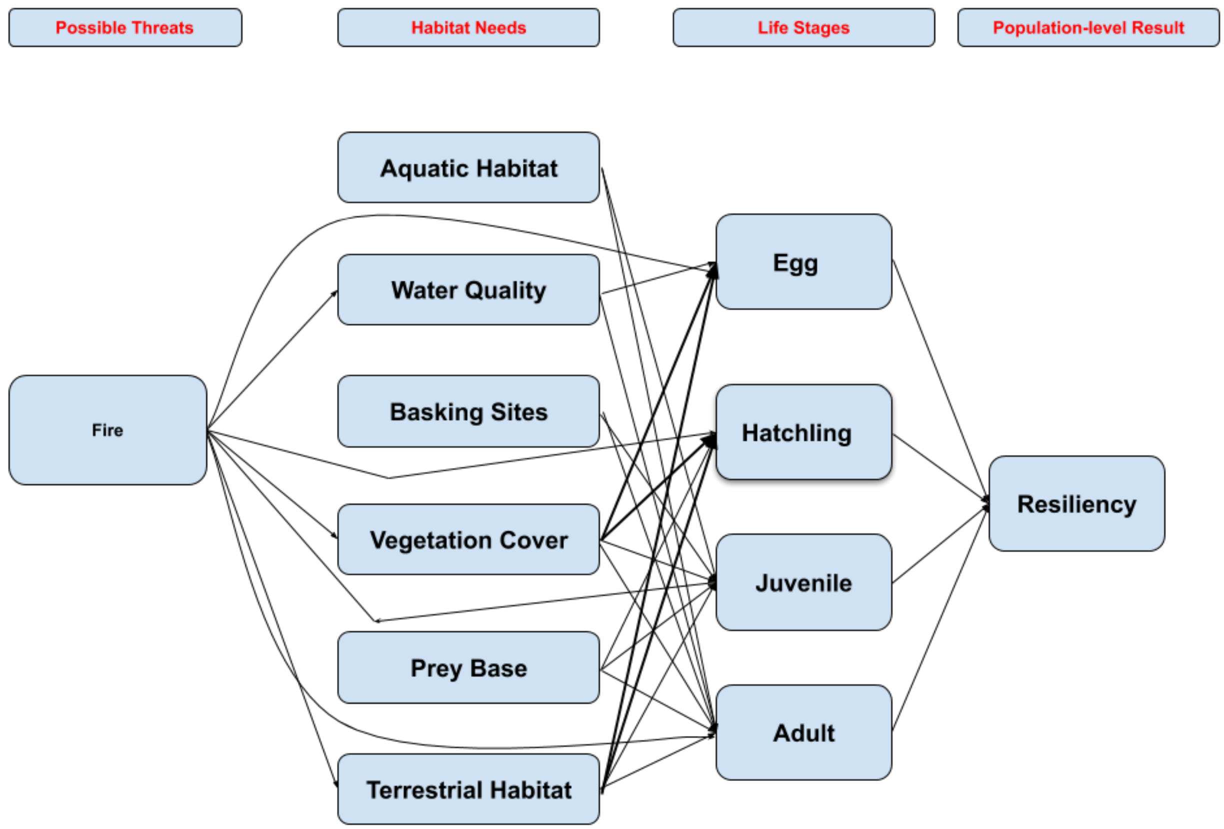


Figure 11. Risk conceptual model showing direct and indirect effects of fire on the habitat needs and life stages of the WPT. Evidence for the direct and indirect effects come from a variety of sources (Lovich *et al.* 2017, pp. 7-8; Holland 1991, p. 79).

Based on current evidence, fire is a medium risk to *E. pallida* populations and not a risk to *E. marmorata* populations. However, as a risk to WPTs, fire may have limited data due to its unpredictability and spatial randomness. Because of this, there are few studies on the effects of fire specifically on WPTs. Furthermore, most publications on the effects of fire are almost entirely on tortoises and not freshwater turtles. The impacts of fire can be broken down into the inputs of ash into bodies of water, reduction in vegetation and leaf litter, and direct mortality of WPTs. Inputs of ash into bodies of water from fire reduce water quality. The changes in water quality include increases in pH, nutrients, turbidity, conductivity, and suspended sediment exports along with decreases in dissolved oxygen and macroinvertebrate density (Lovich *et al.* 2017, p. 7). The decrease in macroinvertebrate density is important to note as a decrease in food resources. Additionally, fire reduces vegetation and leaf litter cover for WPTs in their terrestrial environment (Lovich *et al.* 2017, p. 8). WPTs are more vulnerable without leaf litter cover and can be more exposed to predation. Finally, fire can directly kill WPTS especially overwintering individuals or hatchlings still in their nest (Holland 1991, p. 79).

E. pallida

Risk	Sum	Average	Number of observations
Fire	4	1.33	3

Within the area of burns, fires result in declines of *E. pallida* populations but it is not usually extreme enough to cause extirpation of a population. The Sespe Creek fire in the fall of 1991 resulted in the mortality of hatchlings and hibernating WPTs in an *E. pallida* population but did not result in extirpation (Holland 1991, p. 80). Another fire around the same time, the Santa Barbara fire of 1990 resulted in the decline of an *E. pallida* population indirectly. In response to the fire, channelization of the watercourses in the area was done as a form of flood control and resulted in the loss of WPT habitat and decline of the population (Holland 1991, p. 80). An extreme wildfire in 2013 around Lake Elizabeth in northern Los Angeles county caused the extirpation of a robust *E. pallida* population (Lovich *et al.* 2017, entire). In this case, fire worked synergistically with drought. Both risks reduced water quality and drought directly increased the probability of the wildfire. The drying of the lake due to drought also caused WPTs to spend a larger portion of the year in the terrestrial habitat. On land, the fire was more dangerous to WPTs as a source of direct mortality (Lovich *et al.* 2017, p. 8). Together, the two risks wiped out an *E. pallida* population of 170 WPTs. The severity of wildfires will grow in the future if anthropogenic warming continues. Climate change projections in California chaparral show increased fire activity with both an extended fire season and higher frequency of large fires (Molinari *et al.* 2018, p. 385). As warming occurs, chaparral vegetation mortality will increase and result in more combustible material for fire. Vegetation mortality will be particularly high during droughts which will also increase in frequency. Even as a medium risk, the increases in fires could contribute significantly to future declines in *E. pallida*.

E. marmorata

Risk	Sum	Average	Number of observations
Fire	0	0	5

Unlike *E. pallida*, fire does not appear to have much impact on populations of *E. marmorata*. This could be due to lower probability of fire near *E. marmorata* populations when compared to *E. pallida* populations. Another explanation could be the difference in fire regime as *E. marmorata* may actually benefit from fire to an extent. In Washington, fire suppression has reduced grassland habitat as successional changes result in Douglas fir invasions. For example, less than 10 percent of historical grassland habitat remains in the south Puget Sound region (Crawford and Hall 1997, p. 11). WPTs rely on open habitats for nesting and the increase in shade cover from Douglas fir decreases the amount of suitable habitat for nesting (Hays *et al.*

1999, p. 11). Due to this effect, prescribed burns may actually be beneficial to the survival of *E. marmorata* in Washington and elsewhere (Holland 1991, pp. 120-121). The timing of the burns would be critical as a burn during nesting season could cause mortality to nesting females and hatchlings. The only recorded decline in an *E. marmorata* population due to fire was a strange case in the Sierra National Forest in 1990. During this fire, helicopters would accidentally collect WPTs along with water when filling a bucket to fight the fire. The release of the water in the bucket onto the hotspots of the fire killed the WPTs in the process (Holland 1991, p. 73). While *E. marmorata* populations seem to be free from negative impacts from fire, we reiterate that the available data are limited data. Effects similar to *E. pallida* should be expected when a wildfire occurs in the area of a *E. marmorata* population. A wildfire could still certainly kill individuals through direct mortality and its effect on water quality would also likely occur. With an expected future increase in extreme wildfires, driven by climate change, *E. marmorata* populations may face additional fire-based threats (Cisneros *et al.* 2018, p. 99)

4.4 Floods

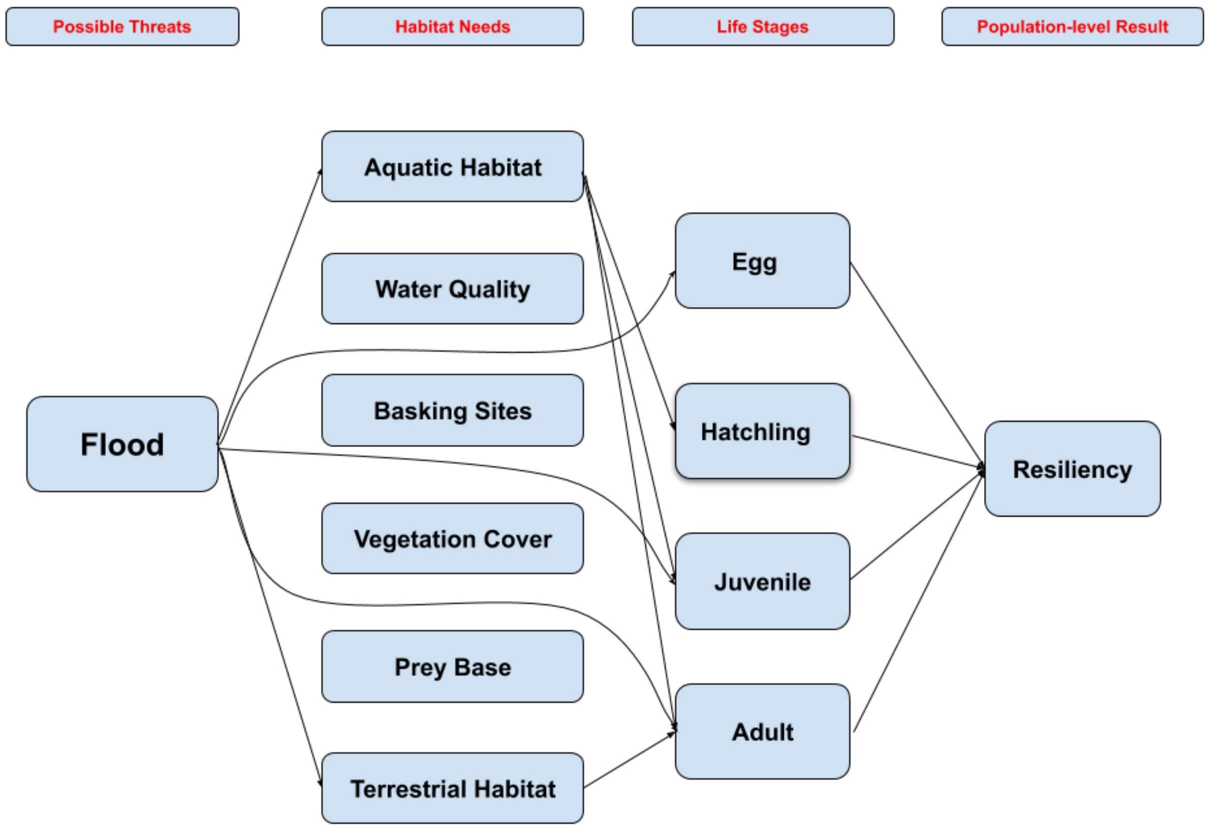


Figure 12. Risk conceptual model showing direct and indirect effects of floods on the habitat needs and life stages of the WPT. Evidence for the direct and indirect effects come from a variety of sources (Alvarez 2005, entire; Rathbun *et al.* 2002, p. 233; Rathbun *et al.* 1992, p. 323).

A major result of climate change in the WPT's range will be the increase of more extreme weather. Flooding is a top five risk for the WPT, specifically *E. pallida*, as the frequency and severity of major floods are expected to increase throughout the species range. There are two primary ways in which the WPT will be adversely affected by increased flooding: flushing of individuals from their aquatic habitat or overwintering spots downstream and inundation of nesting sites with flood waters.

E. pallida

Risk	Sum	Average	Number of observations
Flood	5	1.25	4

While it is not uncommon for WPTs to be flushed downstream by flooding, many WPTs are harmed or killed in the process. In 2005, about 67 WPTs were carried from upstream habitats along the Santa Clara River to downstream areas, some as far as the ocean. Among these WPTs, many had crushed shells, severed legs, smashed heads, internal bleeding due to time spent in ocean water, or were found dead (Alvarez 2005, entire). WPTs living in ephemeral bodies of water will often leave their habitat in late summer and return following winter floods. If these ephemeral bodies of water dry up and are replenished in the winter, WPTs will likely miss the most destructive floods. However, this is not always the case as water bodies do not always dry up (Rathbun *et al.* 2002, p. 233). Remaining WPTs will certainly be washed away during strong flooding events. *Emys pallida* is especially susceptible to the effects of flooding due to the Mediterranean climate of central and southern California that causes arroyos and streams to be prone to flooding in the winter months (Rathbun *et al.* 1992, p. 323). Additionally, all of the literature citing floods as a risk to the WPT is focused on *E. pallida*.

WPT reproduction is another area of concern with respect to flooding as WPT eggs are sensitive to excessive moisture and can be washed away. During floods, streambeds are often scoured of vegetation, sand bars, and mud bars which can drastically alter the structure of the waterway. This scouring would likely flood nearby WPT nests and wash eggs further downstream, potentially to the ocean (Rathbun *et al.* 1992, p. 323). Scouring would affect WPT nests in normal floodplains, however many WPTs nest and take refuge well outside these floodplain areas. However, with more frequent and severe flooding events, areas outside of the typical floodplain may be affected. The flood stage -- the highest level a flooded stream or river reaches-- may become higher as floods become worse and affect typical nesting sites that were once safe from flood events. If eggs are not washed away during flooding, they may still be at risk from increased moisture reaching nesting sites. In addition to the expansion of floodplain areas, the WPT will have to move further away from its aquatic habitat more frequently to avoid the effects of floods (Rathbun *et al.* 2002, p. 228). For nesting, this could be extremely harmful to the WPT as habitat is limited away from streams. Additionally, with development and urbanization there are many more stressors away from aquatic habitat. Again, *E. pallida* nesting behavior is more at risk from floods due to the central and southern California climate and more limited habitat.

E. marmorata

Risk	Sum	Average	Number of observations
Flood	NA	NA	0

Although the range of *E. marmorata* is also expected to experience more extreme floods, there is little to no literature supporting flooding as a major risk to the species. Northern California, Oregon, and Washington typically experience more rain than southern California which might suggest that flooding is not as adverse of an impact in the northern range of the WPT when compared with the southern range. However, the future climate of the WPT's range is highly variable and flooding may prove to be a major threat to *E. marmorata* as well as *E. pallida*.

4.5 Vehicle Traffic

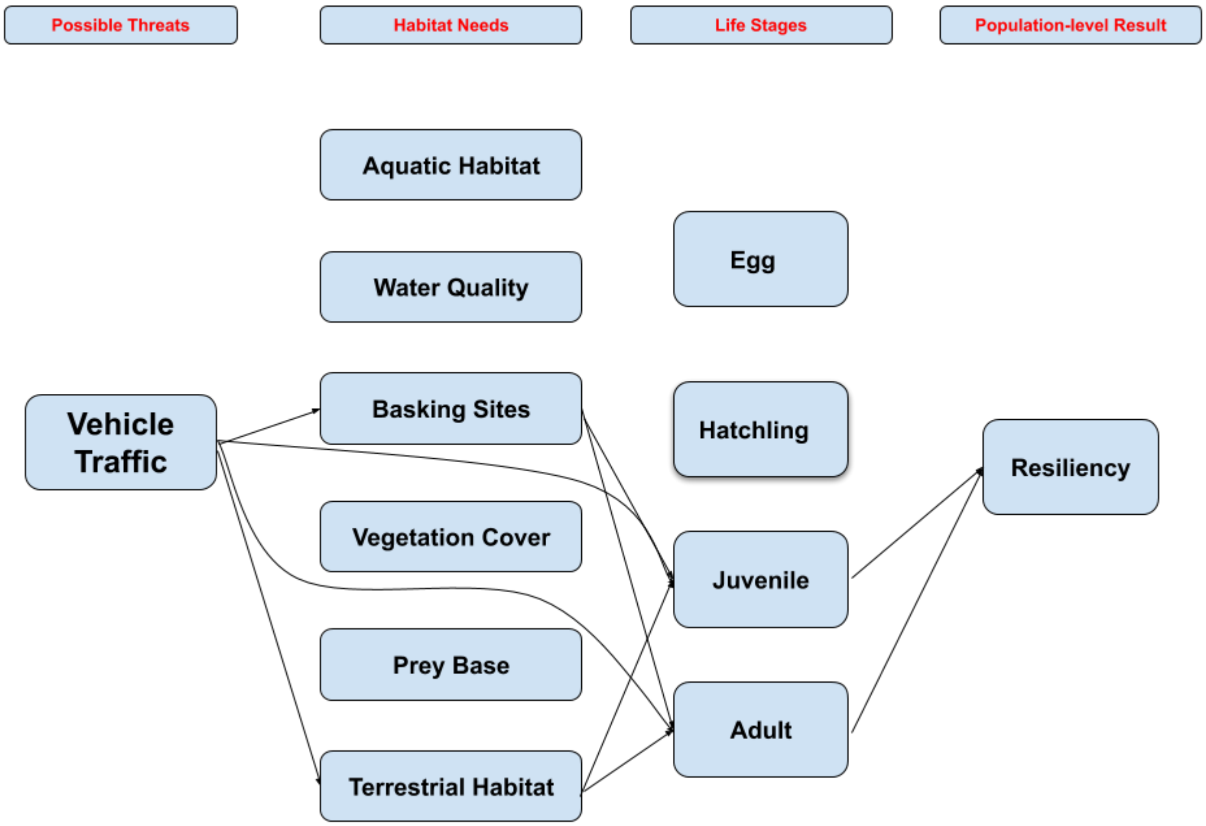


Figure 13. Risk conceptual model showing direct and indirect effects of vehicle traffic on the habitat needs and life stages of the WPT. Evidence for the direct and indirect effects come from a variety of sources (Madden-Smith *et al.* 2005, p. 47; Holland 1994, Sec. 2-13; Brehme *et al.* 2018, p. 928; Gibbs and Shriver 2002, p. 1649; Nyhof 2013, p. 53).

Vehicle traffic is a major threat to the WPT as traffic can directly result in mortality or alter behavior. Roadway traffic is the fifth largest risk for *E. marmorata* and the ninth largest risk for *E. pallida*. According to a review of literature on the threat of road strikes, *E. marmorata* experiences a higher threat with respect to vehicle traffic than *E. pallida*. Mortality occurs when WPTs attempt to cross roads and are struck by passing vehicles. Nesting females are especially prone to being struck by vehicles as they travel long distances away from their aquatic habitats to find nesting sites. However, a New York study on the effects of road mortality on turtle populations across the United States showed that populations of small bodied pond turtles were not expected to be threatened by road mortality anywhere in the United States (Gibbs and Shriver 2002, p. 1649). Despite this, there are many WPT specific studies that suggest vehicle traffic mortality is a significant threat.

E. pallida

Risk	Sum	Average	Number of observations
Vehicle Traffic	2	1	2

While there are not as many *E. pallida* specific studies related to vehicle traffic mortality, this threat still plays a role in the decline of the species. A 2002-2003 study over nine watersheds in San Diego County found that road mortality may play a significant role in reductions in populations of *E. pallida*. Across the nine watersheds, few to no juvenile and female WPTs were captured or documented during the study. The primary cause for the lack of juveniles and females is believed to be many roads across the sites that bisect streams and ponds. As females move around for nesting they are likely struck and killed by vehicles, thus directly reducing the female population and indirectly reducing the juvenile population (Madden-Smith *et al.* 2005, p. 47).

E. marmorata

Risk	Sum	Average	Number of observations
Vehicle Traffic	5	0.83	6

In an E. *marmorata* specific study of the Willamette Valley in Oregon, it was suggested that road crossings are a significant threat to WPT populations. In the study, 25 individuals were found crossing a road, all of which were either injured or dead. Based on the WPT population size of the Willamette Valley, this count represents 3-5% of the total population for the valley (Holland 1994, Sec. 2-13). Roads that parallel bodies of water are especially threatening to the WPT as foraging individuals or nesting females will almost certainly have to cross them (Brehme *et al.* 2018, p. 928). Overall, the relationship between road density and road crossing frequency is strong for turtles with heavily trafficked roads acting as impenetrable barriers to WPTs (Gibbs and Shriver 2002, p. 1649).

Vehicle traffic can also alter the behavior, specifically basking behavior, of the WPT. In a study on *E. marmorata* near a heavily trafficked trail in Mountain View, California, WPT basking behavior was significantly altered in response to motorized vehicle traffic. The passing by of a motor vehicle caused the abandoning of basking site and submergence 45% of the time. In contrast, only 7% of human foot traffic caused disturbance (Nyhof 2013, p. 53). Overall, the length of basking periods was much shorter for disturbed WPTs than undisturbed WPTs. Motor vehicle traffic may be an effective disturbance mechanism that can both cause turtle mortality and reduce basking time. Reductions in basking time can affect the WPTs thermoregulation, leading to stress and more difficulty carrying out normal physiological processes (Nyhof 2013, p. 53). Both road strikes and behavior disturbances are threats to the WPT.

4.6 Predation by Non-Native Species

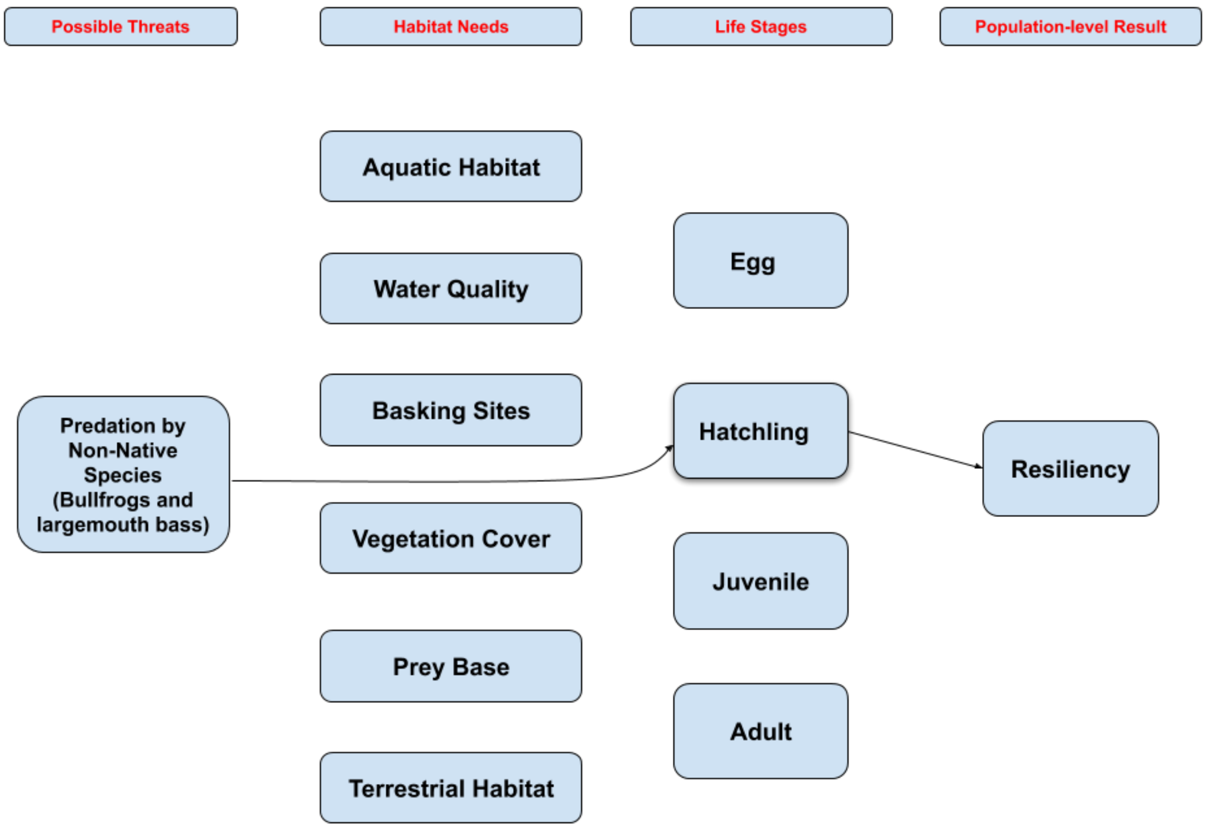


Figure 14. Risk conceptual model showing direct and indirect effects of predation by non-native species on the habitat needs and life stages of the WPT. Evidence for the direct and indirect effects come from a variety of sources (Holland 1994, p. 2-12).

Predation by non-native species is one of the top five risks for both *E. pallida* and *E. marmorata.* American Bullfrogs (*Lithobates catesbeianus*) and Largemouth Bass (*Micropterus salmoides*) are two major non-native predators that can be found throughout the WPT's range [see Appendix B] (Holland 1994, p. 2-12). Hatchlings and juveniles less than 3 years old are the most vulnerable to predation due to their small size (Hallock *et al.* 2017, p. 5). According to Holland, only 10-15% of turtles less than 3 years old may survive annually, whereas an average of 95-97% of adults survive annually (Holland 1994, p. 2-11). Once juveniles reach a carapace length of 120 mm, survivorship increases and appears relatively high (Holland 1994, p. 2-11). Head-started juveniles (raised in captivity) have higher survival rates. In Pierce County, WA, survival rates averaged 77% for newly released, head-started juveniles with carapace lengths of 77-102 mm (3.0-4.0 in.) (Hallock *et al.* 2017, p. 5).

<u>*E pallida*</u>

Risk	Sum	Average	Number of observations
Predation by Non-Native Species	8	1	8

Predation by bullfrogs and bass has been documented at multiple sites throughout *E. pallida*'s range. One site in particular, Cañada de los Osos Ecological Reserve in Santa Clara County, CA had both bullfrogs and bass. In 2018, *E. pallida* were trapped at a series of ponds in the reserve. Trapping efforts at Wilson Ranch pond revealed that the pond contained bullfrogs as well as mature WPTs, but no juveniles were seen or captured (Smith 2018, p. 3). However, a seasonal stream (Coon Hunter's Gulch) contained nine juveniles despite the presence of bullfrogs (the abundance of bullfrogs was not specified) (Smith 2018, p. 6). Despite the presence of bullfrogs at the reserve, there is one pond (Old Corral Pond) that has a robust WPT population. The seasonal nature of the pond is not suitable for bass and bullfrogs, allowing successful reproduction and a robust turtle population (Smith 2018, p. 22).

At Camp Cady in San Bernardino County, *E. pallida* used to be easy to catch and observe in the late 1990s (Lovich and Meyer 2002, entire). The last WPT observation at Camp Cady was in 2014. Today, WPTs have not been seen or captured despite extensive efforts, leading researchers to conclude that they were extirpated from Campy Cady (Lovich *et al.* 2017, p. 8). Bullfrogs, however, are currently present at this location. The original provenance of the turtles at Camp Cady is still under debate (Lovich *et al.* 2017, p. 8). Nearby in Afton Canyon, bullfrogs were present throughout an entire trapping session from April to September in 2017. Only one female turtle was captured throughout the trapping session, suggesting that very few turtles are left (Lovich *et al.* 2017, p. 9).

<u>*E. marmorata*</u>

Risk	Sum	Average	Number of observations
Predation by Non-Native Species	7	1	7

In Washington, *E. marmorata* were historically distributed in central and southern Puget Sound from Snohomish to Thurston counties, as well as along the Columbia Gorge in Skamania and Klickitat counties, and in Clark County (Hallock *et al.* 2017, p. iv). However, due to a combination of habitat loss, overharvest, and introduction of non-native fish and bullfrogs, WPTs were almost completely extirpated in this region. Only about 150 turtles remained in Skamania and Klickitat County by 1994 (Hallock *et al.* 2017, p. iv). The Puget Sound population was almost completely extirpated in the 1990s. The WPTs present there today are the descendants of 12 turtles that were collected and placed into a captive breeding program (Hallock *et al.* 2017, p. iv).

Currently, there are six WPT recovery sites in Washington, including three sites in Skamania County, and one each in Klickitat, Mason, and Pierce counties (Hallock *et al.* 2017, p. 7). However, they are still at risk of predation by invasive species. Bullfrogs continue to be a predator of primary concern due to their widespread abundance in lowland waters of the state

(Hallock *et al.* 2017, p. 9). Five out of the six WPT recovery sites contain bullfrogs and two of the sites contain bass (Hallock *et al.* 2017, p. 9). In Klickitat County, five bullfrogs were found to contain six hatchlings turtles (Rockney 2015, p. 22). Fortunately, bullfrog removal efforts in 2014 resulted in less bullfrogs and increased observations of hatchlings in 2016 (Hallock *et al.* 2017, p. 13-14). While there has been some progress in WPT recovery, natural recruitment remains low due to low hatching success and predation on hatchlings (Hallock *et al.* 2017, p. iv). Without head-starting, predator control, and habitat management, WPTs in this region will revert to near extirpation (Hallock *et al.* 2017, p. 14).

In northwestern California, four out of six lentic (still-watered) sites near the Trinity River were biased towards large, old WPTs (Sloan 2012, p. iii). These four sites had abundant bullfrog populations, while the other two sites completely lacked bullfrogs (Browns Creek and Little Browns Creek) (Sloan 2012, p. 31-32). Again, it is suspected that bullfrogs are consuming hatchlings and inhibiting recruitment. Bass were also found at most of the sites with low percentages of young turtles and may also be responsible for the lack of hatchlings. However, one of the sites with a small percentage of hatchlings lacked bass, suggesting that bullfrogs were the primary cause of low recruitment (Sloan 2012, p. 33).

4.7 Land Conversion

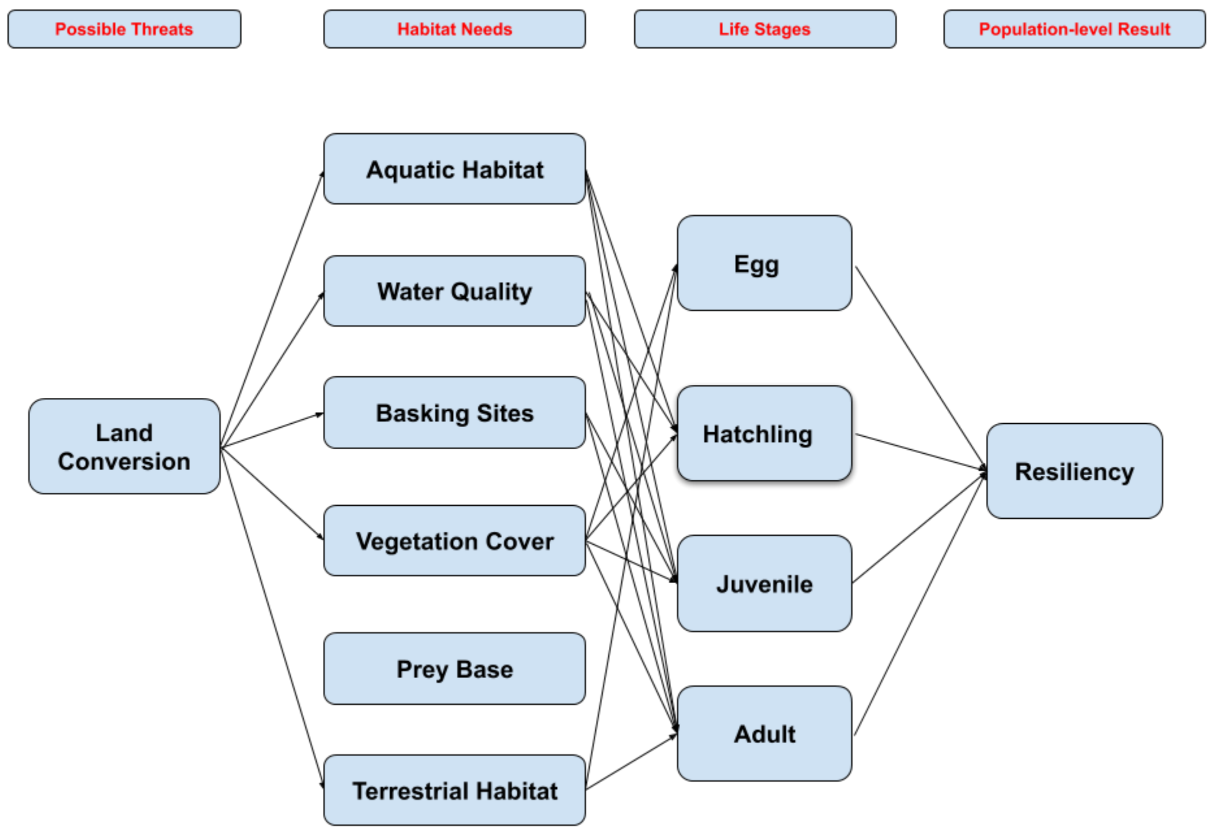


Figure 15. Risk conceptual model showing direct and indirect effects of land conversion on the habitat needs and life stages of the WPT. Evidence for the direct and indirect effects come from a variety of sources (Rosenberg *et al.* 2009, p. 6 and Spinks *et al.* 2003, p. 258.)

WPTs require aquatic habitats (permanent or intermittent) as well as terrestrial habitats (Hallock *et al.* 2017, p. 2*)*. Thus, both *E. marmorata* and *E. pallida* are at risk of decline from factors that can affect either of these habitats or the linkages—areas that serve as movement pathways between them. Loss and alteration of aquatic habitat has been significant throughout the range of WPTs due to human development and agriculture (Rosenberg *et al.* 2009, p. 6). In particular, urbanization has resulted in more channels and silt, a reduction in aquatic vegetation, and fewer or less favorable basking sites (Spinks *et al.* 2003, p. 258). Because WPTs typically nest in upland areas 3-400 meters from water bodies, suitable nest sites have become increasingly scarce and vulnerable to nearby agricultural activities (Reese 1996, p. 105). For example, the Central Valley of California may have once had the greatest WPT density, but draining of wetlands and habitat alteration in the past century has left few suitable aquatic habitats (Germano and Bury 2001, p. 22). Today, populations rarely have densities similar to their historic counterparts and age structures of extant populations tend to be skewed towards adult turtles (Reese 1996, p. 73).

E. pallida

Risk	Sum	Average	Number of observations
Land Conversion	6	1.2	5

In a more recent study, *E. pallida* at Waddell Creek in Santa Cruz County were surveyed to determine age structure, sex ratio, and general abundance in response to potential declines in recruitment (Smith 2018, entire). During earlier studies from 1995 and 1998/1999, turtles less than 100 mm long were common (25-28% of the captures) (Smith 2018, p. 1). However, identified nest sites that were unsuitable for nesting indicated the potential for declines in recruitment. These nest sites consisted of an irrigated lettuce farm, a tomato field that was plowed in fall (when turtle nestlings were still in the nest), a horse corral, and a meadow near the pond (Smith 2018, p. 1). Only one out of the 24 captures in 2018 was smaller than 100 mm, and only one was a female. The single small turtle found in 2018 suggests low recruitment in comparison to 1995-1999 levels (20 out of 72 turtles were less than 100 mm) (Smith 2018, p. 2). Additionally, the single female in 2018 represents a male-biased sex ratio compared to 2007 (19 out of 49 mature turtles were female) (Smith 2018, p. 2). Head-starting the population with young females may restore a reproducing population, but only if there are improved nesting opportunities (Smith 2018, p. 3).

E. marmorata

Risk	Sum	Average	Number of observations
Land Conversion	8	0.57	14

In lowland Puget Sound in Washington, the historic range of the WPT overlaps with 59 percent of the state's human population and is becoming more urbanized (Hallock *et al.* 2017, p. 10). This same pressure occurs along the lower Columbia Gorge. Thus, suitable habitat for the WPT is declining in this region. Additionally, invasive plant species are shading basking and nesting areas, as well as creating potential barriers to movement due to the density of the vegetation (Hallock *et al.* 2017, p. 10). As a result, habitat management is required at the six recovery sites in Washington to keep the vegetation short and maintain suitable nesting areas.

Near Santa Rosa in Sonoma County, *E. marmorata* were tracked in a set of agricultural ponds that consist of portions of creeks that have been artificially ponded by small dams and are surrounded by agricultural lands (Reese 1996, p. 181). Historically, riparian habitats at the Santa Rosa site were a more continuous web of creeks. Their transformation into discrete ponds requires that turtles include multiple ponds in their home ranges and travel overland to obtain resources (Reese 1996, p. 226). Fragmentation of aquatic habitat also creates smaller populations that are more subject to inbreeding and loss of genetic variability (Reese 1996, p. 238-239). For example, gene flow along aquatic routes that have since disappeared may account for the genetic similarity within Columbia River Gorge populations (Gray 1995, p. 1251). Populations with low genetic variation are less able to adapt to changing environments and are more vulnerable to agriculture and urban growth (Gray 1995, p. 1251).

In the Central Valley, habitat loss due to agriculture resulted in the decline of many WPT populations, the majority of which were *E. marmorata* (Germano and Bury 2001, entire). Furthermore, agricultural and vegetation management activities may result in nest destruction and mortality to adult females (Rosenberg *et al.* 2009, p. 6). In 1992, a petition to the U.S. Fish and Wildlife Service declared that the WPT needed protection and listing under the Endangered Species Act, especially in the Central Valley where the remaining populations were predominantly old, non-reproducing adults. To determine the status of the WPT, 55 aquatic habitats on the valley floor of the Central Valley were surveyed in 1999 (Germano and Bury 2001, entire). WPTs were seen or caught at 15 sites and were suspected to occur at many other sites, at least in low numbers (Germano and Bury 2001, p. 22). According to the survey, WPTs were abundant at five sites in the Central Valley, each of which consisted of many young, large turtles. Thus, it was concluded that despite the large population declines in the last century, WPTs in the Central Valley still persist at a number of sites and have sufficient recruitment to maintain numbers (Germano and Bury 2001, p. 28). Abundant WPT populations occurred around irrigated agriculture, receiving water from agricultural runoff. For example, Goose Lake in Kern County was a favorable site for WPTs despite its seasonality. When Goose Lake dries up, adjacent canals which contain water all year provide alternative habitat for WPTs (Germano and Bury 2001, p. 28). Unfortunately, it has been two decades since a similar survey has been conducted in the Central Valley. Additional work must be done in order to understand the long-term effects of land conversion on these populations.

4.8 Invasive Species Competition

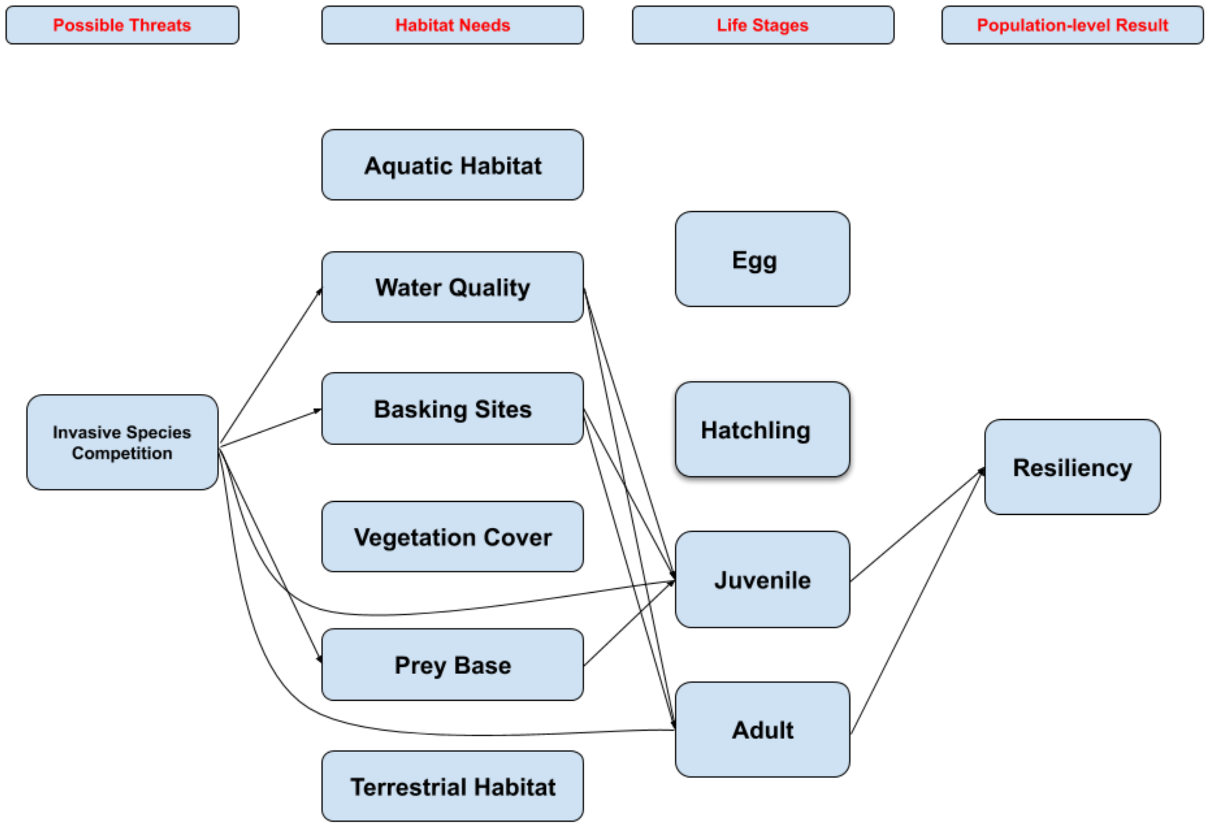


Figure 16. Risk conceptual model showing direct and indirect effects of invasive species competition on the habitat needs and life stages of the WPT. Evidence for the direct and indirect effects come from a variety of sources (Hollingsworth and Stepek 2015, p.61; Brown *et al.* 2015, p.14; Bettelheim 2011, p.8; Costa 2014;Hallock *et al.* 2017, p.12).

Invasive species competition, although not a top risk for *E. marmorata* or *E. pallida* respectively, plays a crucial role in population stability. An invasive species is not native to the ecosystem they occupy. The introduction of an invasive turtle or fish species can initiate competition for the native WPT. The effects of competition are usually delayed, but can be studied looking at fitness components, such as growth, reproduction, and survival rates, and how they are affected by the presence of an invasive competitor (Pearson *et al.* 2015, p.1). In a study by Pearson, invasive juvenile red-eared sliders out-compete native juvenile red-bellied turtles when resources were low. Red-eared sliders were able to adapt to low resources, as seen in their quicker diet change adaptability (Pearson *et al.* 2015, p.3). With the introduction of more red-eared sliders and other invasive species, future WPT populations will suffer from low recruitment rates and decreased population size.

E. pallida

Risk	Sum	Average	Number of observations
Invasive Species Competition	3	1	3

For *E. pallida*, limited basking areas are the outcome of invasive species competition. In San Bernardino County near Mojave Narrows Regional Park red-eared sliders, western painted turtles, and softshell turtles affected WPTs access to basking and other resources. In another study in Orange County at the Naval Weapons Station Seal Beach, interactions between *T. scripta* and *E. pallida* were studied. Since the *T. scripta* was larger in body size than *E. pallida*, it displaced *E. pallida* for suitable feeding and nesting areas. Cattle caused habitat disturbance along with behavioral changes in basking, foraging and mating in the WPT (Hollingsworth and Stepek 2015, p.61). In the study area of Sycuan Peak Ecological Reserve in San Diego County, sunfish (*Lepomis* spp.) caused reduced food resources for juvenile WPTs. When sunfish and other invasive species were removed from the study site they saw an increase in juvenile population along with the amount of WPTs basking (Brown *et al.* 2015, p.14). Published data and studies for *E. pallida* was limited which implies more studies on this species of WPT needs to be done.

E. marmorata

Risk	Sum	Average	Number of observations
Invasive Species Competition	4	1	4

The effects of invasive species competition in *E. marmorata* are, reduction of resources such as basking sites and food, and spread of disease from non-native species. The red-eared slider (*Trachemys scripta*) occupies similar niches to *E. marmorata*. *Trachemys scripta* are also known to grow faster and larger, along with laying larger and more frequent egg clutches. *Trachemys scripta* is also known for being aggressive to WPTs when basking (Bettelheim 2011, p.8). Since the WPT relies on basking as a thermoregulatory mechanism, a threat to this would cause optimal body temperatures to shift and alter vital fitness components (Costa 2014, entire). In a study at the UC Davis arboretum in Yolo county, when the WPT is in the presence of *T. scripta*, it can alter their optimal basking times. The invasive species also initiated a fear response, causing the WPT to leave its basking site (Costa 2014, p.2). Over time, the decision to bask or flee will initiate costs associated with predation and expending unnecessary energy. Some fish can also threaten juvenile WPTs since they share the same food resources. Sunfish compete with juveniles for small invertebrate prey, causing the abundance of food left for juvenile WPTs to be reduced (Hallock *et al.* 2017, p.12).

4.9 Climate Change

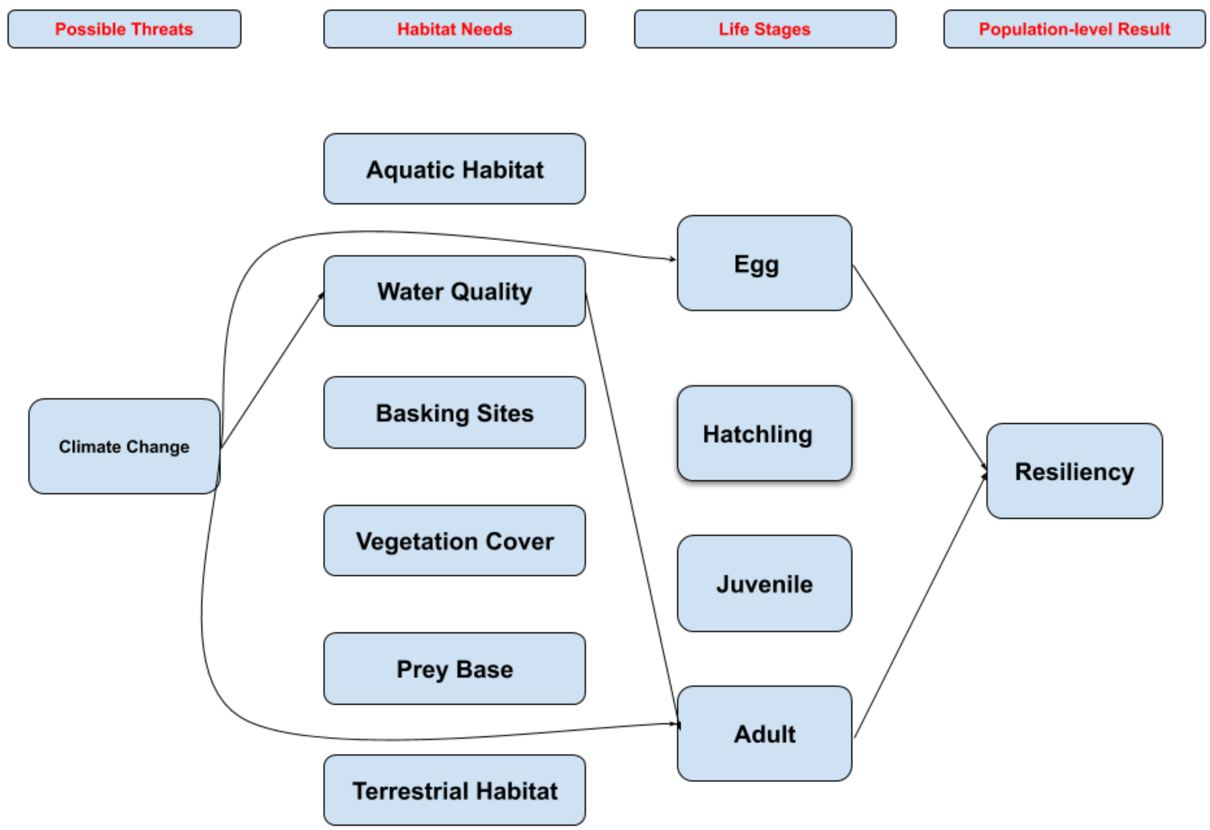


Figure 17. Risk conceptual model showing direct and indirect effects of climate change on the habitat needs and life stages of the WPT. Evidence for the direct and indirect effects come from a variety of sources (Christie and Geist 2017, p.3; Hallock *et al.* 2017, p.18).

Climate change studies for *E. marmorata* are more extensive and researched than that of *E. pallida*. Currently, there are no climate change studies conducted for *E. pallida* to conclude its overall population risk.

E. pallida

Risk	Sum	Average	Number of observations
Climate Change	N/A	N/A	0

Currently, there are no studies on *E. pallida* related to water temperature and climatic alteration. Further experiments need to be conducted to ensure this risk does not threaten the species.

E. marmorata

Risk	Sum	Average	Number of observations
Climate Change	2	1	2

There are many components that contribute to climate change, but water temperature is the key component that will be focused on in this section. Alteration of freshwater temperature and its effects have been studied for *E. marmorata* in California and Washington. The Nature Conservancy Reserve in Lake County found that water temperatures had significant effects of sex determination in *E. marmorata*. Those who spent 30% more time in water cooler than 29℃ had offspring more likely to be male, and those that were above 29℃ were more likely to be female (Christie and Geist 2017, p.3). The Washington *Periodic Status Review for the Western Pond Turtle* for Washington explains that if water temperature increases, even minimally, it could influence sex ratios. When temperatures are inconsistent, future sex ratios of *E. marmorata* can be altered and result in a male dominant population (Hallock *et al.* 2017, p.18).

4.10 Artificial Ecosystems

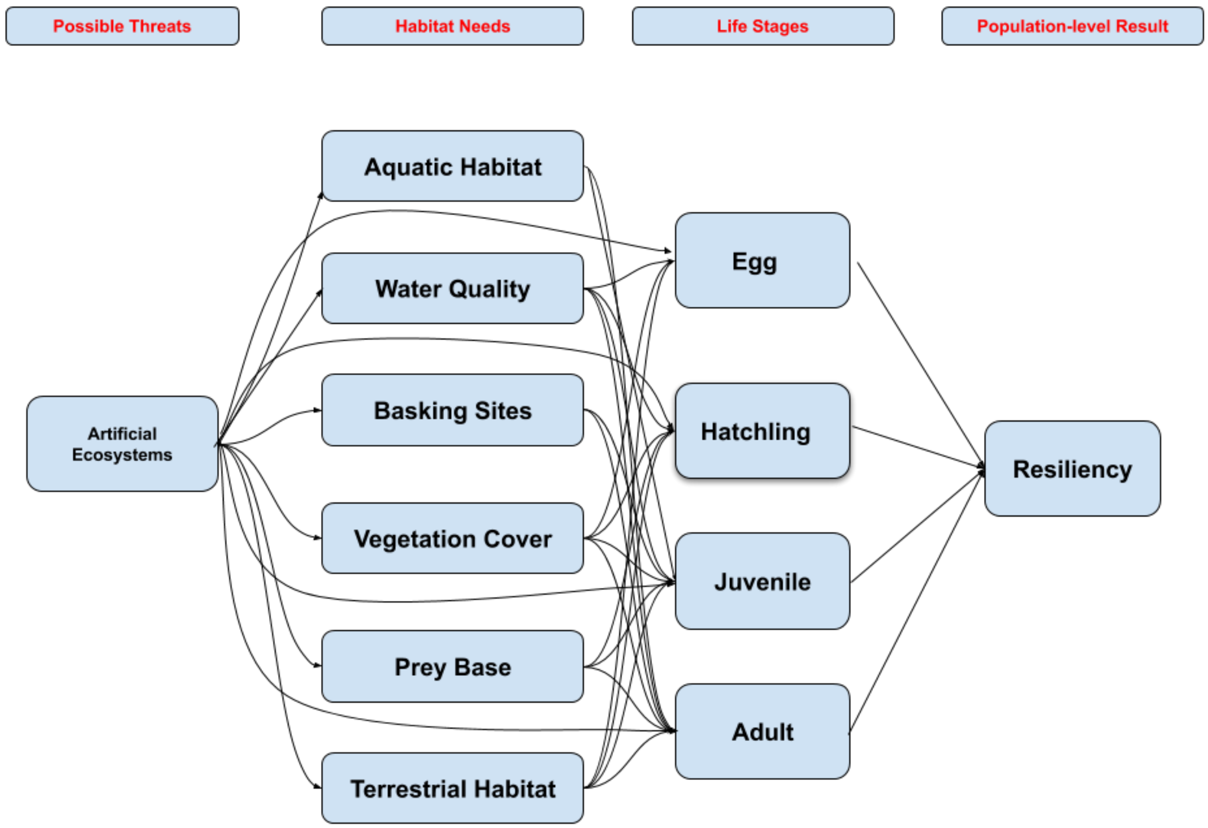


Figure 18. Risk conceptual model showing direct and indirect effects of artificial ecosystems on the habitat needs and life stages of the WPT. Evidence for direct and indirect effects comes from a variety of sources (Germano and Rathbun 2008, p. 192; Germano 2010, pp. 90-95; Polo- Cabia *et al.* 2010, p. 259-261).

Artificial ecosystems, although not found to be a top risk for *E. marmorata* and *E. pallida*, are still an important factor that can affect populations of WPTs. An artificial ecosystem is classified as a man-made system consisting of plants, animals, and people living together. Throughout its range the WPT can be found in various artificial ecosystems, such as sewage treatment plants, human-made waterways, and military bases. Access and use of this area by the public is limited or restricted, therefore this area are relatively undisturbed, making them relatively suitable habitat for wildlife. The degree to which artificial ecosystems pose a threat to WPTs varies from one type of artificial ecosystem to another. However, similar trends are observed between common ecosystems.

E. pallida

Risk	Sum	Average	Number of observations
Artificial Ecosystems	0	0	1

At this time, there is a single study that looks at *E. pallida* with respect to artificial ecosystem. Further studies need to be conducted to fully understand the impact and the types of artificial ecosystem that are prevalent to *E. pallida* in comparison to *E. marmorata*. In Vandenberg Air Force Base in Santa Barbara County *E. pallida* were found to inhabit the area. The area's Mediterranean climate, consisting of dry, warm summers and mild, cold winters, is thought to contribute to the quick growth rate and maturing at a younger age observed in the WPTs at the military base. Furthermore, a high proportion of young turtle was observed, suggesting high reproduction rate among the turtle (Germano and Rathbun 2008, p. 192). Although the study looks at the population dynamics (growth, population structure, reproduction) it fails to study the correlation, if there is any, between the military base and the population dynamics observed.

E. marmorata

Risk	Sum	Average	Number of observations
Artificial Ecosystems	0	0	5

In the San Joaquin Valley, much of the land has been converted for agricultural use leading to the removal of wetlands, large freshwater lakes, and marshes that were historically found in the area. However, despite the loss of the WPTs natural habitat the species can still be found in modified habitats, such as sewage treatment facilities. *Emys marmorata* inhabiting the Fresno- Clovis Regional Wastewater Reclamation Facility and Hanford Wastewater Treatment Facility were captured, their body mass, length of carapace, sex, age, and general conditions were recorded before being released (Germano 2010, p. 90). A total of 321 turtles were captured among the two sites. Data collected on the WPTs at both sites yield similar findings. For example, it was determined that female WPTs at the Hanford facility had an average size clutch of 8.5 and female WPTs found at the facility in Fresno had an average clutch size of 8.2. Moreover, on average males were significantly larger and grew faster than females (Germano 2010, p. 91). As a result, the researchers concluded that WPTs living in sewage treatment plants will typically grow faster, have successful recruitment, and produce large clutches. The ability of WPTs to persist in sewage treatment plants appears to be a result of high nutrient content in the water and higher water temperatures that aid in increasing growth rates (Germano 2010, p. 92-95). In short, sewage-treatment facilities have the ability to serve as suitable, although highly managed, habitats for WPTs.

Body size and body conditions can serve as an indicator when assessing the fitness of a species; however, it can also be misleading. Body size may not serve as a good indicator of physiological health. In a study conducted on a population of *E. marmorata* at the Chico Water Control Plant (CWCP) researchers looked beyond body structure, and assessed health status by analyzing immune system variables— T-cell-mediated immune (CMI) response and heterophil/ lymphocyte (H/L) ratio. Although the WPTs were larger and heavier in comparison to WPTs inhabiting natural reserves they were not necessarily healthier. WPTs in the CWCP were found to have lower CMI responses than WPTs found in nearby reserves. These findings indicate that these WPTs are facing higher numbers of challenging infections than those in natural settings. Furthermore, H/L ratios were high in WPTs inhabiting the CWCP, indicating that the WPTs were not healthier despite their larger body mass (Polo- Cabia *et al.* 2010, p. 259-260). The differences in levels of CMI and H/L ratios found between WPTs from the CWCP and reserve may be attributed to cumulative effects of exposure to pollutants and stressors experienced throughout out the species life, that may be prevalent in one habitat but not the other. There is an increasing need to go beyond body structure assessment (body conditions, size, mass, growth rate) and conduct physiological assessment when assessing the effects of alteration of ecosystem on species (Polo- Cabia *et al.* 2010, p. 261).

Aside from sewage-treatment facilities WPTs can be found in man-made waterways. The effects of waterways on WPTs vary depending on the dynamics of the ecosystem. A study looking at *E. marmorata* in the Howard Slough Unit, a heterogeneous wetland in Glenn county with slow moving sloughs and a number of irrigation canals used to take and bring water to rice fields, found high numbers of WPTs inhabiting the man-made canals. Water flowing from the rice fields into the canals provides a large number of nutrients that contributes to fast growth rates for the turtles living in the canals. Nutrients found in the canals can also be used up by prey of the WPT, contributing to a large supply of food for the for the turtle. Moreover, studies have shown that organic, mud bottom substrates such as that found in the canals also aid in faster growth rate of turtles. Were as turtles living in inorganic, sandy bottom substrates have lower growth rates (Lubcke and Wilson 2007, p. 110-112).However, further studies ay need to be conducted to fully support the notion that canals are suitable ecosystem for WPTs to understand how difference in temperature, water flow, and other important characteristics of a canal are aid or harm the survival of WPTs in this type of ecosystem.

The ecosystem dynamics for *E. marmorata* found in the UC Davis Arboretum Waterway differ from those found in the Howard Slough Unit. Development of the waterway can contribute to less basking and nesting sites causing lower levels of recruitment and higher levels of mortality. Overtime this can lead to an unsustainable population. Moreover, *T.s. elegans are also found to* inhibit the UC Davis Arboretum Waterway. *T.s. elegans* are an invasive species that poses a threat to the WPT, they have the ability to outcompete *E. marmorata* for basking sites and can be vectors for respiratory diseases. Without the proper management of this waterway this population of WPTs can be impacted greatly (Spinks *et al.* 2003, p. 263-265).

4.11 Contaminants

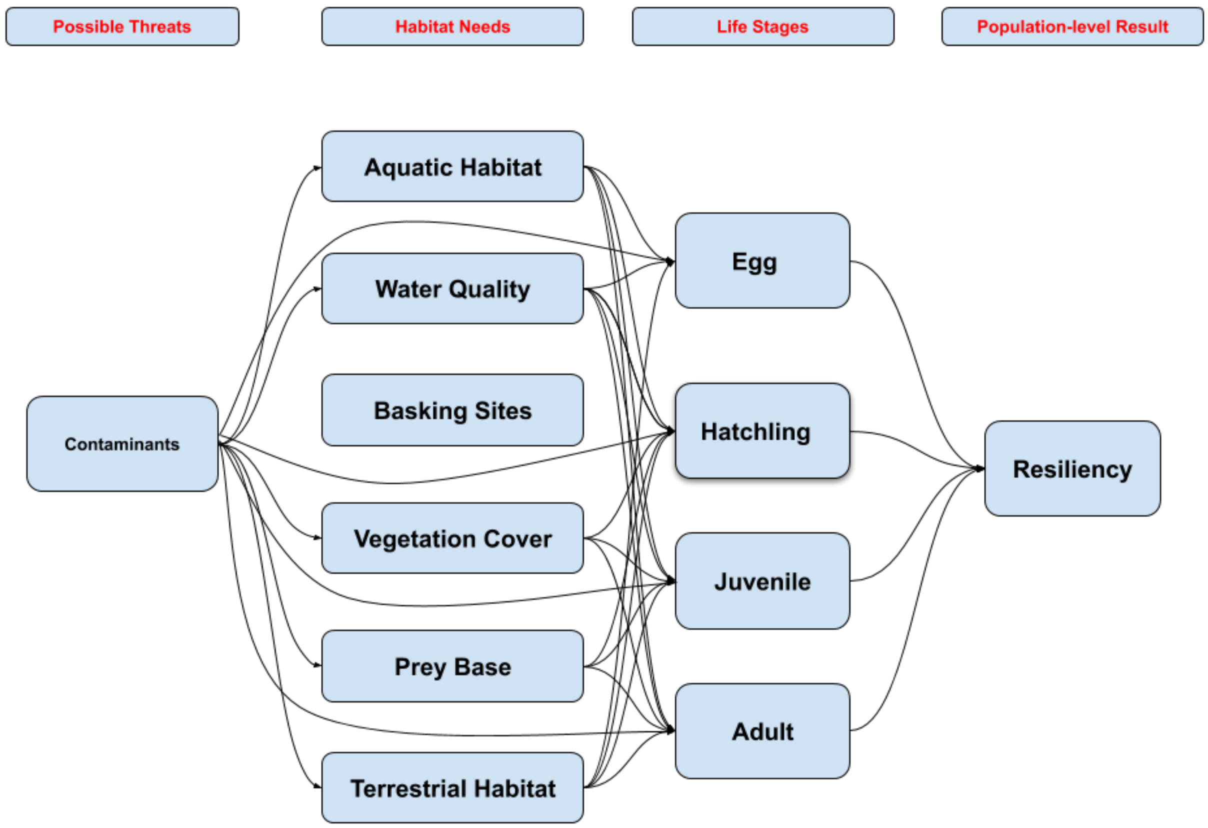


Figure 19. Risk conceptual model showing direct and indirect effects of contaminants on the habitat needs and life stages of the WPT. Evidence for the direct and indirect effects come from a variety of sources (Denchak 2018, entire; Meyer *et al.* 2012, p. 692; Meyer *et al.* 2016, p. 326)

Contamination is among the top five risks to both species of WPTs. In a 2008-2009 survey evaluating national water quality by the U.S. Environmental Protection Agency it was determined that approximately half of the U.S. rivers and over one-third of lakes are polluted (Denchak 2018, entire). There are various ways in which pollutants— chemicals, nutrients, heavy metals, etc— are introduced into water supplies. Chemical waste from factories may be dumped into rivers or lakes. Pesticides applied on agricultural land may be carried by runoff water into surface water and groundwater. These are just a few of the several ways water supplies may be contaminated. Once a pollutant enters the water system removing it becomes a difficult process. Some substances may quickly degrade into harmless chemicals, others may persist in the water and become hazardous to the health of humans and wildlife (American Geosciences Institute 2019, entire). The effect a hazardous substance has on a species varies by the amount of exposure and the type of contaminant.

Among the most prevalent form of contaminants affecting *E. marmorata* and *E. pallida* throughout their ranges are pesticides, some of which are classified as semi-volatile organic compounds (SVOC). SVOC have the potential of causing a series of heath effect— headaches, liver damage, cancer (Environmental Protection Agency 2017, entire). Pesticides can be distributed beyond the area they are applied to via overspray, drift, volatilization, wind-blown erosion, and in runoff water. As a result, even areas classified as pristine like national parks may have exposure to hazardous pollutants (Meyer *et al.* 2016, p. 326). In 2010, two million kilograms of organophosphate and carbamate insecticides alone were put on agricultural land throw-out California (Meyer *et al.* 2012, p. 692). Then in 2011 and 2012 more than 73 million kilograms of pesticides were sprayed on agricultural land, accounting for approximately half of the state's total agricultural pesticide use for the two years(Meyer *et al.* 2016, p. 327).

<u>*E. pallida*</u>

Risk	Sum	Average	Number of observations
Contaminants	6	1	6

Emys pallida is exposed to most of the same contaminants as *E. marmorata*— current-use pesticides (CUPs), historic-use pesticides (HUPs), heavy metals, etc. However, levels of exposure to contaminants may differ among the region and thus health effects may also differ. More studies need to be conducted analyzing *E. pallida* individually from *E. marmorata*. As present studies are looking at exposure to contaminants on both species of WPTs, it is not known if contaminants affect the two species differently.

Moreover, there are many more contaminants found in the habitats of WPTs that have not been studied. Furthermore, the few contaminates that have been studied for their effects on the WPT remains incompletely understood. Therefore, further studies need to be conducted on risk of exposure to contaminants on WPTs, particularly those that are known to cause serious health effects on humans. As it is more than likely to pose a health risk to WTPs and other wildlife, also it is difficult to study every single contaminant that has ever entered an ecosystem.

<u>*E. marmorata*</u>

Risk	Sum	Average	Number of observations
Contaminants	11	1	11

CUPs like organophosphates and carbamates are known to be inhibitors of cholinesterase enzymes (ChE) in wildlife, altering neurotransmission and resulting in numerous deleterious behaviors. A study conducted throughout various rivers and creeks (Jose creek, North Fork Kaweak river, Sycamore creek, Tyler creek, Mad river, South Fork Trinity river, Clear creek, Sequoia National Park, Whiskeytown National Recreation Area, Six River National Forest) throughout the northern and southern regions of California found evidence of ChE activity through analysis of plasma collected from samples of blood. It was found that the total ChE activity in WPTs from the southern region of California was significantly depressed by 31%, having a p-value of 0.005, then in the northern region (Meyer *et al.* 2012, p. 695). Despite evidence of the presence of ChE depression occurring in the WPTs its effects are not known at the individual nor population level of the species. However, ChE inhibition induced by chlorpyrifos has been been shown to reduce spontaneous swimming in Coho salmon attributed depression of acetylcholinesterase in the brain and the muscle. Similar loss of neurotransmission and neuromuscular functions may be observed in WPTs (Meyer *et al.* 2012, p. 696-697). For this reason, further studies need to conducted analyzing the physiology of the WPT when exposed to chemicals that cause ChE depression.

Moreover, SVOC like chlorinated HUPs, polychlorinated biphenyl (PCBs), and polycyclic aromatic hydrocarbons (PAHs), have been observed to lead to deleterious impacts on wildlife that result in numerous effect such as immunosuppression, genotoxicity, and loss of secondary sex characteristics. Throughout Sequoia National Park, Whiskeytown National Recreation Area, Six River National Forest CUPs, HUPs, PAHs, and PCBs were found across most WPTs captured. However, the number of contaminants found in the turtle's blood plasma samples were below the known thresholds levels— the level of exposure for all chemicals that would be a health risk to humans. Thus, no concrete conclusion of the effects of continents on the WPTs could be drawn. However, this bring into question the potential health effects that can developed due to exposure to multiple contaminants at low levels interacting with one another (Meyer *et al.* 2016, p. 333).

In addition to pesticides, mercury has also been observed to threaten the health of wildlife by causing impairment of physiological process. There is a large body of evidence showing that mercury (Hg) has endocrine disrupting capability. California's historical mining activities are associated with the use of Hg and release of Hg in mines. Today, Hg is still found in ecosystems surrounding many mining sites throughout California. A single study conducted from August to October 2011 assessed Hg exposure in WPTs in Jose creek, North Fork Kaweak river, Sycamore creek, Tyler creek, Mad river, South Fork Trinity river, and Clear creek. Blood plasma analysis of WPTs were negatively correlated with triiodothyronine (T3) and positively correlated with thyroxine (T4), thyroid hormones responsible for the regulation of body temperature, metabolism, and heart rate. Irregular levels of T3 and T4 indicate that exposure to Hg in WPTs may influence its endocrine system particularly by the species' thyroid hormone response. However, further studies need to be conducted to fully support this notion (Meyer *et al.* 2014, p. 2993-2994).

4.12 Dams

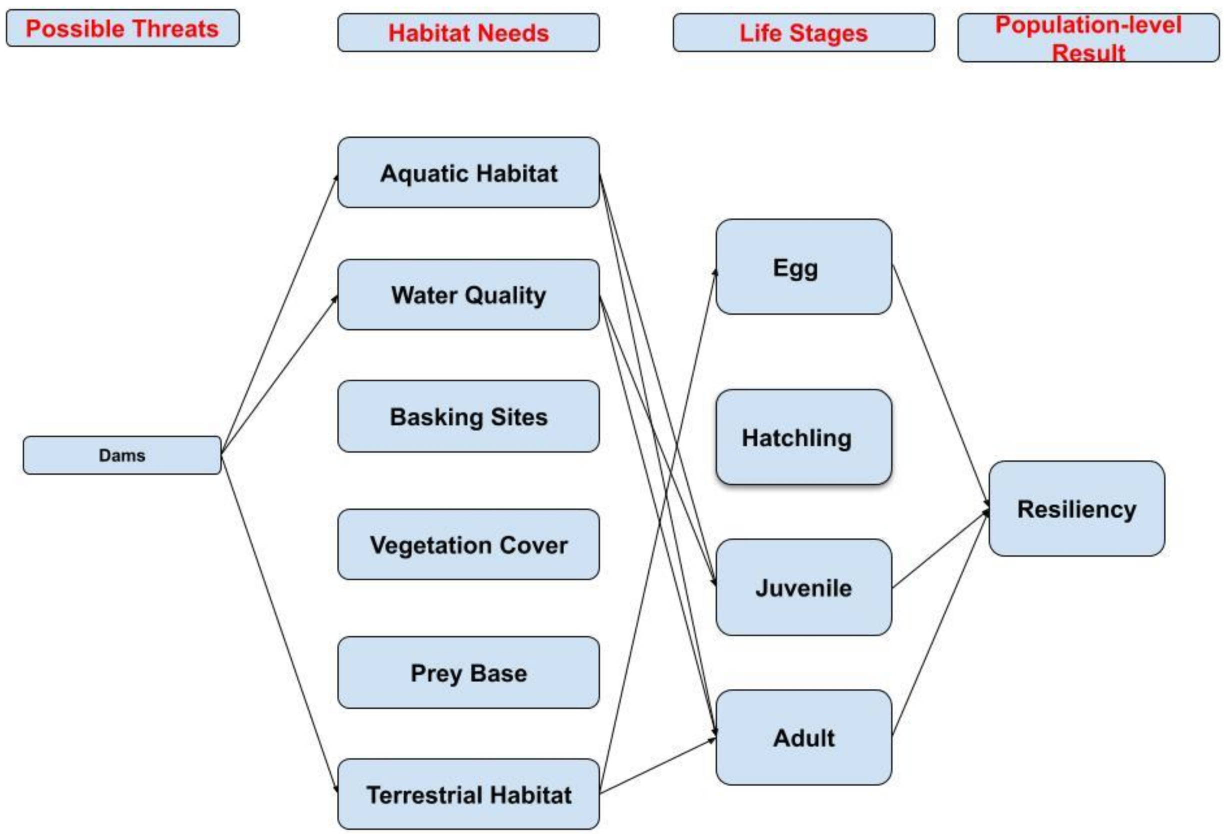


Figure 20. Risk conceptual model showing direct and indirect effects of dams on the habitat needs and life stages of the WPT. Evidence for the direct and indirect effects come from a variety of sources (Alho 2011, p. 600; Lees 2016, p. 459; Norris *et al.* 2018, p. 12; Lovich 2017, p. 3; Reese and Welsh 1998, p. 505; Ashton *et al.* 2015, p. 622; Bondi and Marks 2013, p. 151; Cook 2018, p. 2).

Out of our identified risks, dams rank close to the bottom. However, this may be due to lack of research in regard to the WPT throughout its range. The effect of dams on turtle populations has been more widely researched in the Amazon where hundreds of large-scale hydroelectric dams are being built. Researchers that study turtle populations in the Amazon have found that dams push turtles out of their natural habitats (Alho 2011, p. 600), change the river qualities heavily affecting native species (Lees 2016, p. 459), and flood nesting habitat (Norris *et al.* 2018, p. 12). Because turtles are heavily affected by dams in the Amazon, it's possible they could affect WPT in a similar way; however due to a lack of studies that has yet to be confirmed.

E. pallida

Risk	Sum	Average	Number of observations
Dams	0	0	2

There has not been much research into how dams affects *E. pallida*. In one study of two rivers (both with dams) at Mojave Ranch in San Bernardino County, the dams were found to have no effect on *E. pallida* (Lovich 2017, p. 3). While the general adverse effects of dams on riparian life is well documented and accepted, few studies have addressed its effects on *E. pallida*, however this does not mean that dams do not threaten the species.

E. marmorata

Risk	Sum	Average	Number of observations
Dams	1	0.33	3

Studies on *E. marmorata* populations over a span of twenty years have identified one negative effect. A three-year investigation around the Trinity River dam in Trinity County found that WPT populations below the dam had significantly less juveniles than populations above the dam. This led researchers to believe that the dam is detrimental to young turtles (Reese and Welsh 1998, p. 505). Since then, researchers have performed similar studies along the Trinity River forks and found that there were differences in body size between WPTs along the damned and undammed portions of the river. The populations below the dam had smaller body sizes compared to the populations along the non-damned forks (Ashton *et al.* 2015, p. 622). Ashton hypothesized that the cold water released from the dam was decreasing the amount of prey available for the WPTs, resulting in smaller body sizes. Ashton also found that the dam flooded nesting habitat, similar to the dams in the Amazon.

However, an opposite effect was found in the nearby Mad River; WPT populations below the dam were actually larger than their counterparts above the dam. Here, since the dam changed the river from intermittent to perennial it was increasing feeding time and allowing the WPTs to grow larger. However, populations below the dam had fewer juvenile turtles than those above the dam, which was also observed at Trinity River (Bondi and Marks 2013, p. 151). In a more recent study of the Russian River in Sonoma County, dams were found to have no effect on the turtle populations (Cook 2018, p. 2).

4.13 Natural Predation

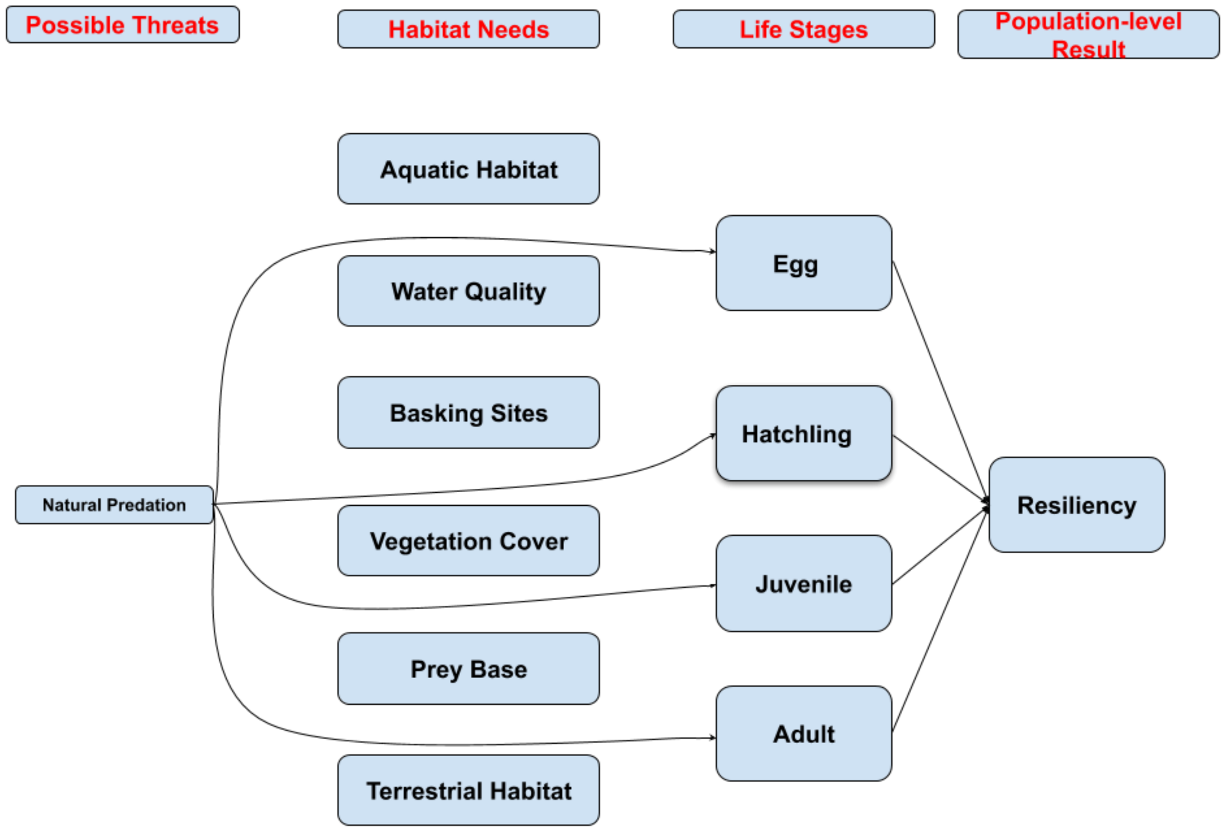


Figure 21. Risk conceptual model showing direct and indirect effects of natural predation on the habitat needs and life stages of the WPT. Evidence for the direct and indirect effects come from a variety of sources (Holland 1991, p. 40; Ernst and Lovich 2009, p. 180; Germano and Bury 2008, p. 7; Holland 1991, p. 40; Holland 1994, p. 50; Germano and Bury 2008, p. 7; Hallock *et al.* 2017, p. 10)

Natural predation does not rank highly among the risks to *E. marmorata* or *E. pallida*. WPTs evolved alongside their natural predators and have developed defenses to mitigate their effects, making extirpation unlikely. However, as urbanization takes place, the abundance of raccoons and other predators tends to reach unnaturally high levels, causing more predation of WPTs and putting populations at risk of extirpation. While these threats may rise in urban areas, predation from natural predators in the wild has been found to pose little risk to WPT populations (Holland 1991, p. 40; Ernst and Lovich 2009, p. 180).

E. pallida

Risk	Sum	Average	Number of observations
Natural Predation	1	0.5	2

There has been practically no investigation of predation of the effects of natural predators on *E. pallida*. We do know the risk of predation by raccoons is worse in urban areas where human trash allows rodent populations to reach numbers higher than those found naturally in the wild (Germano and Bury 2008, p. 7). However, while that is the only study relating directly to *E. pallida* it is likely they suffer the same effects as *E. marmorata*.

<u>*E. marmorata*</u>

Risk	Sum	Average	Number of observations
Natural Predation	4	1	4

River otters are one of the major predators that often prey on WPTs by severing one or more limbs. Although WPTs are not always killed in the process, those with missing limbs are highly unlikely to survive, especially during winter (Holland 1991, p. 40). In another study, Holland found heavy predation of *E. marmorata* nests, most likely due to raccoons and other terrestrial animals (Holland 1994, p. 50). This threat is increased in urban areas where human influences allow predators such as raccoons to reach unnaturally high densities (Germano and Bury 2008, p. 7). In Pierce county Washington local officials found it necessary to start controlling otter and raccoon levels due to the predation of six adult female turtles they had reintroduced (Hallock *et al.* 2017, p. 10).

4.14 Consumption and Pet Trade

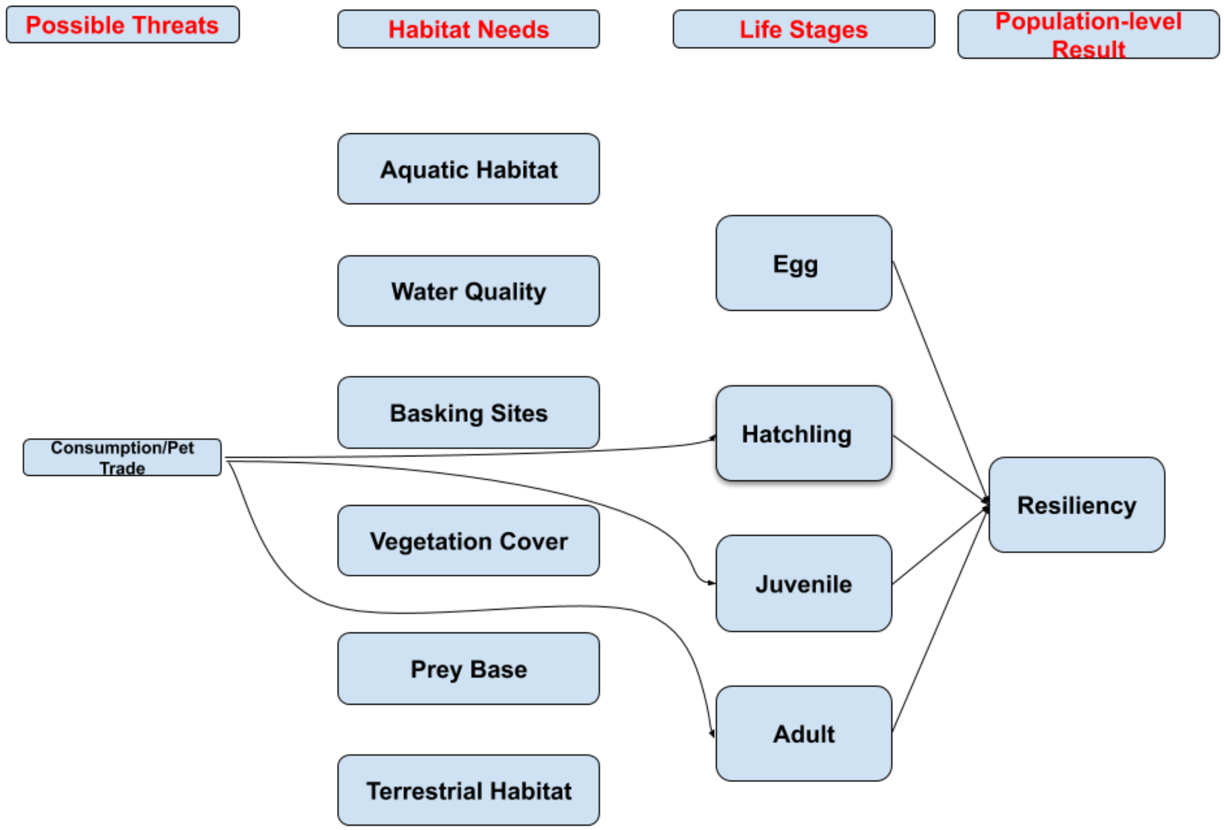


Figure 22. Risk conceptual model showing direct and indirect effects of consumption and pet trade on the habitat needs and life stages of the WPT. Evidence for the effects of consumption and pet trade came from (Bettelheim 2011, p. 32) as well as online offers/listings.

Consumption and pet trade does not rank highly for either *E. marmorata* or *E. pallida*. While WPT's were consumed historically, they are no longer consumed commercially in the present day. As for pet trade, while there online listings for WPT's they were few and far between, and sites that did have a listing either had very few turtles for sale and had been sold out, or had been out of stock for a long period of time. It is highly unlikely that either consumption or pet trade would increase in severity or occurrence in the future.

E. pallida

Risk	Sum	Average	Number of observations
Consumption and Pet Trade	N/A	N/A	0

Currently, there are no studies on *E. pallida* related to consumption and pet trade. Further experiments need to be conducted to ensure this risk does not threaten the species.

E. marmorata

Risk	Sum	Average	Number of observations
Consumption and Pet Trade	2	2	1

Commercial use does not rank highly among the risk to the WPT as it is in the bottom five risks for *E. marmorata* and was not present for *E. pallida* . While consumption and trade of the WPT today is mostly a non-issue, historically it was a small, but present, staple in the diets of many northern Californians. Turtle dishes, particularly soups, were often advertised as a luxurious dish, especially for the wealthy. During the late 19th century and early 20th century, fisheries almost exhausted *E. marmorata* populations in northern California, particularly for consumption in San Francisco, with up to 18000 WPT being consumed per year in the late 19th century (Bettelheim 2011, p. 32). Although consumption of the WPT rarely, if ever, occurs in the present-day, many of the WPTs are still captured and sold in the pet trade. The WPT can easily be found online as we were able to find at least three websites that specialized in amphibians and reptiles that had recently posted offers for the WPT. Prices ranged between the three sites with the lowest price being $495 for a juvenile WPT, and the highest price being $1600 for an adult male. However all websites were out of stock and seemed to have been for an extended period of time, with one website stating that they had only been able to hatch or acquire three WPTS. This could possibly indicate that sellers have had little success in breeding and hatching WPTs and were resorting to attempting to capture them in the wild. Both consumption and pet trade only affected *E. marmorata* populations, as the WPT was a staple for northern Californians and the websites only listed *E. marmorata* for sale and not *E. pallida*. However, this could be due to the fact that the separation of the two species occurred recently. Ultimately, consumption and trade is not a prevalent risk for the WPT and will likely remain a non-issue in the future as long as demand for the WPT remains stagnant, save for a few individuals being captured and sold for the pet trade.

4.15 Disease

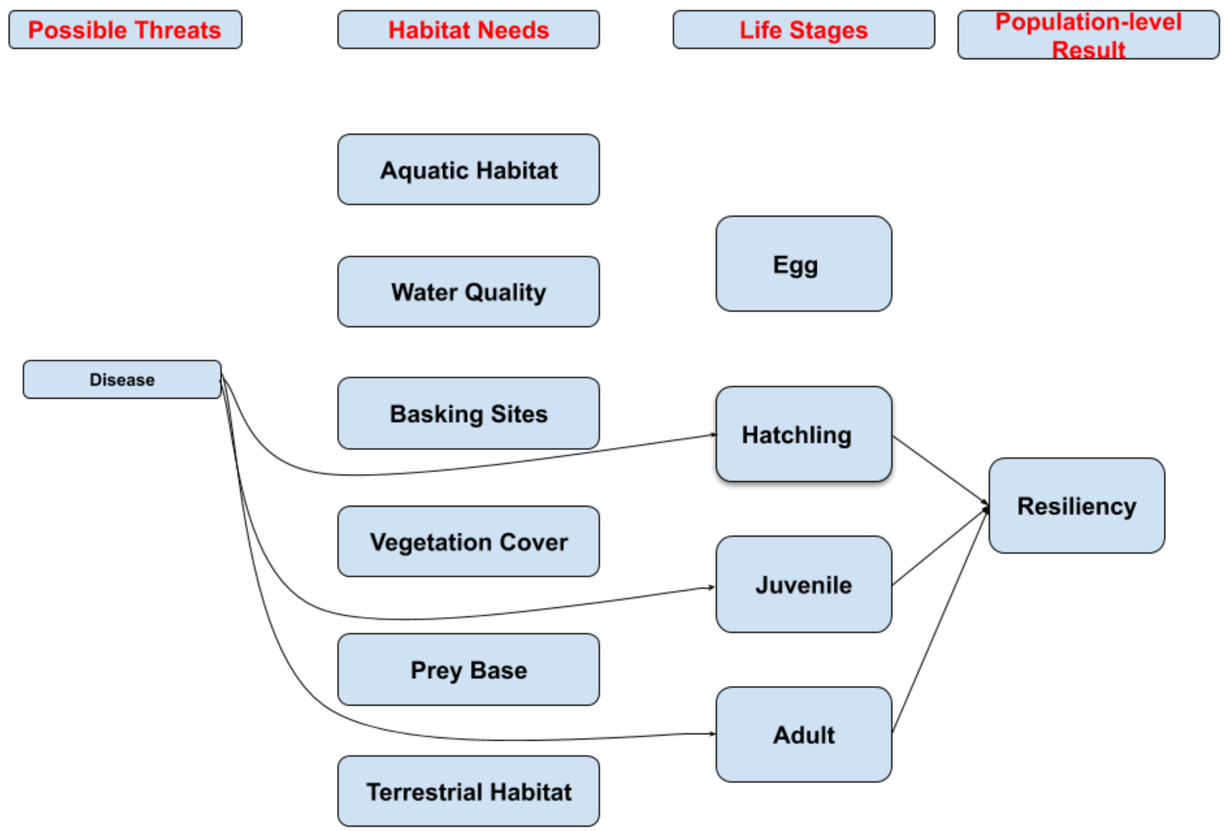


Figure 23. Risk conceptual model showing direct and indirect effects of disease on the habitat needs and life stages of the WPT. Evidence for the effects of disease on life stages came from a variety of sources (Wallach 1975, pp. 25-27), (Silbernagel *et al.* 2013, pp. 42-43), and (Pramuk *et al.* 2012, p. 19).

Disease ranks highly for the WPT, given our data it was the highest risk for *E. marmorata* and was in the top 7 risks for *E. pallida*. Disease for the WPT fell under two main categories: shell disease and pathogen infection. Ulcerative shell disease generally occurs when the shell of a WPT is injured or harmed in some fashion. This damage allows bacteria, fungus, or other pathogens to enter the living tissue that is under the hard outer layer of the WPT's shell. Shell disease has both a wet form and a dry form. The wet form is due to bacterial infection, and the dry form is due to fungal infection. Symptoms of shell disease include shell unevenness, lifting up of the shell plates, bodily discharge under the shell, pitting of the shell, and in extreme cases the shell can even fall off completely. Treatment of the disease depends on the type of disease afflicting the WPT. The dry form is treated with antifungal medication, and the wet form requires the removal of the infected scute and then subsequent antibiotic treatment. Ulcerative shell disease increases mortality of the WPT as the breaks in the shell allow for secondary infections that can often lead to death (Wallach 1975, pp. 25-27). Pathogen infection referred to any sort of pathogen afflicting the WPT, whether it be bacterial or viral.

E. pallida

Risk	Sum	Average	Number of observations
Disease	4	1	4

As for *E. pallida* populations, based on the literature, only pathogen infection was a significant risk. Bacterial infection was found in southern and central California populations, with the two pathogen found being Mycoplasma and Enterobacteriaceae (Silbernagel *et al.* 2013, pp. 42-43). Infection rates in these populations were much higher than *E. marmorata* with rates between 13.3% and 16.7% depending on the site. As with the case with *E. marmorata* there were no demographic factors associated with infection rates in these population, but a significant association between infection and body weight was found in populations infected with Mycoplasma (Silbernagel *et al.* 2013, pp. 42-43). As with *E. marmorata* the paper also hypothesized that lower weight could be due to the infection itself or due to genetic or environmental factors that contributed to lower resistances to infection. However it is important to note that the number of populations studied for pathogen infection in *E. pallida* was much less than for *E. marmorata*.

Ultimately, disease was one of the most prevalent risks for the WPT, with it being the number one risk for *E. marmorata* and a prevalent risk for *E. pallida* based on our collected data. Given the current status of the WPT it is likely that disease will continue to be a high ranking risk for the WPT, especially given the association between environment and water quality and disease infection. Throughout the literature it was shown that environmental quality, especially water quality had a positive correlation with disease infection rates. Environmental degradation and pollutant contamination are major issues currently plaguing California ecosystems, and show no signs of being solved in the near future. It is likely that degradation and pollution levels will continue to increase, as will the rates of disease and infection. It is likely that mortality will continue to increase along with these increases in infection and environmental degradation.

E. marmorata

Risk	Sum	Average	Number of observations
Disease	11	1	11

Based on our data, both shell disease and pathogen infection were significant risk for *E. marmorata* populations. A population and habitat viability assessment of the WPT in Washington found that shell disease affected up to 30% of the introduced WPT population in Washington (Pramuk *et al.* 2012, p. 19). Three sites in Pierce county, Mason county, and the Columbia River Gorge all had significant numbers of infected WPTs (Pramuk *et al.* 2012, pp. 6, 41). The paper hypothesized that this disease has negative effects on WPT mortality, lowering the overall fitness, survival, and reproductive success of an infected WPT (Pramuk *et al.* 2012, pp. 6-7). They also hypothesized that hatchlings could be more susceptible to the disease because of suboptimal shell growth caused my inadequate nutrition, poor water quality, and pollutants (Pramuk *et al.* 2012, p. 62). As for pathogens, bacterial infection was also found to be a prominent issue as bacterial infection was found in populations of *E. marmorata*. The two bacterial pathogens that were found were Mycoplasma and Enterobacteriaceae, and infection rates in these populations ranged from 4.8% to 14.3% depending on the site. There were no demographic factors associated with infection risk in these populations, however a significant association between infection and body weight was found in populations infected with Mycoplasma (Silbernagel *et al.* 2013, pp. 42-43). The paper hypothesized that lower weight could be due to the infection or due to a genetic or environmental factor associated with lower resistances to infection. Blood values of infected WPTs were compared to other infected species and it was found that similar blood values were linked with reduced growth and infection susceptibility indicating that there is a link between infection and increased mortality (Silbernagel *et al.* 2013, p. 43).

Section 5: Historical & Current Conditions

5.1 Introduction to Museum and U.S. Geological Survey Data

Historically, WPT populations were widespread ranging from Baja California to Washington state, USA. Today, WPT populations are less abundant throughout their range (Reese 1996, p. 73). Two ideas have been proposed for the decrease in WPT populations. First, trapping surveys suggest less recruitment in natural populations is occurring (Smith 2018, entire; Lovich *et al.* 2017, entire; Germano 2010, p. 92). During recent surveys, few hatchlings and juveniles have been caught or observed. However, this may be inconclusive because hatchlings and juveniles are more difficult to trap and sight due to their small size. Additionally, there may be some bias towards certain sex and age classes when using only one trap type during surveys (Tesche & Hodges 2015, entire). However, if recruitment has indeed decreased, then one would expect to see an increase in average age, and average carapace length, over time due to a lack of younger WPTs.

Second, it is believed that the sex ratio of WPT populations have become more male-biased (Spinks *et al.* 2003, p. 264; Polo-Cavia *et al.* 2010, p. 261). This may be due to the disproportionate impact roadway mortality has on female WPTs. Roadway mortality disproportionately affects females because nesting females have a higher probability of being struck and killed by vehicles as they travel relatively large distances in search of nesting sites. For example, a San Diego County study on *E. pallida* found little to no females across various sites located near roadways. This lack of females is attributed to the various roadways bisecting the streams and ponds of the sites (Madden-Smith *et al.* 2005, p. 47).

In order to assess the validity of these two hypotheses, we analyzed specimen data from three different museums and trapping data from the USGS. If the hypothesis for reduced recruitment is accurate, we expect to see an increase in average age, and size, of individual turtles over time. This trend would be reflected as an increase in average carapace length over time, which can be used as a proxy for age. If the sex ratio hypothesis is true, one would expect to see a trend moving from a 1:1 sex ratio towards increasingly male-biased.

5.2 Museum and U.S. Geological Survey Data Methods

Data Acquisition

We surveyed WPT specimens from three museums — Natural History Museum of Los Angeles County (n = 55), California Academy of Sciences (n = 151), and Museum of Vertebrate Zoology (n = 257) — to assess changes in carapace length and sex ratios overtime. Together, we evaluated a total of 463 individual WPT specimens collected from 44 counties between 1892-2005 (Figures 24 & 25). The following measurements were taken for each specimen (Figure 26): midline carapace length, maximum carapace length, midline plastron length, maximum plastron length, shell width at the bridge, maximum shell width, shell height, and body mass (Iverson 2018, entire). The sex of each specimen was also determined when possible.

Once the data were collected, we accessed the relevant online museum databases to merge the corresponding specimen data with our own. We were particularly interested in extracting the collection date, county of origin, and GPS coordinates for each specimen. In the past, most specimens were classified as *Clemmys marmorata* (or *Actinemys* or *Emys marmorata*) as the WPT was considered one species instead of two (See Species Separation). Using the county of origin, we were able to identify each specimen as either *E. pallida* (n = 147) or *E. marmorata* (n = 316).

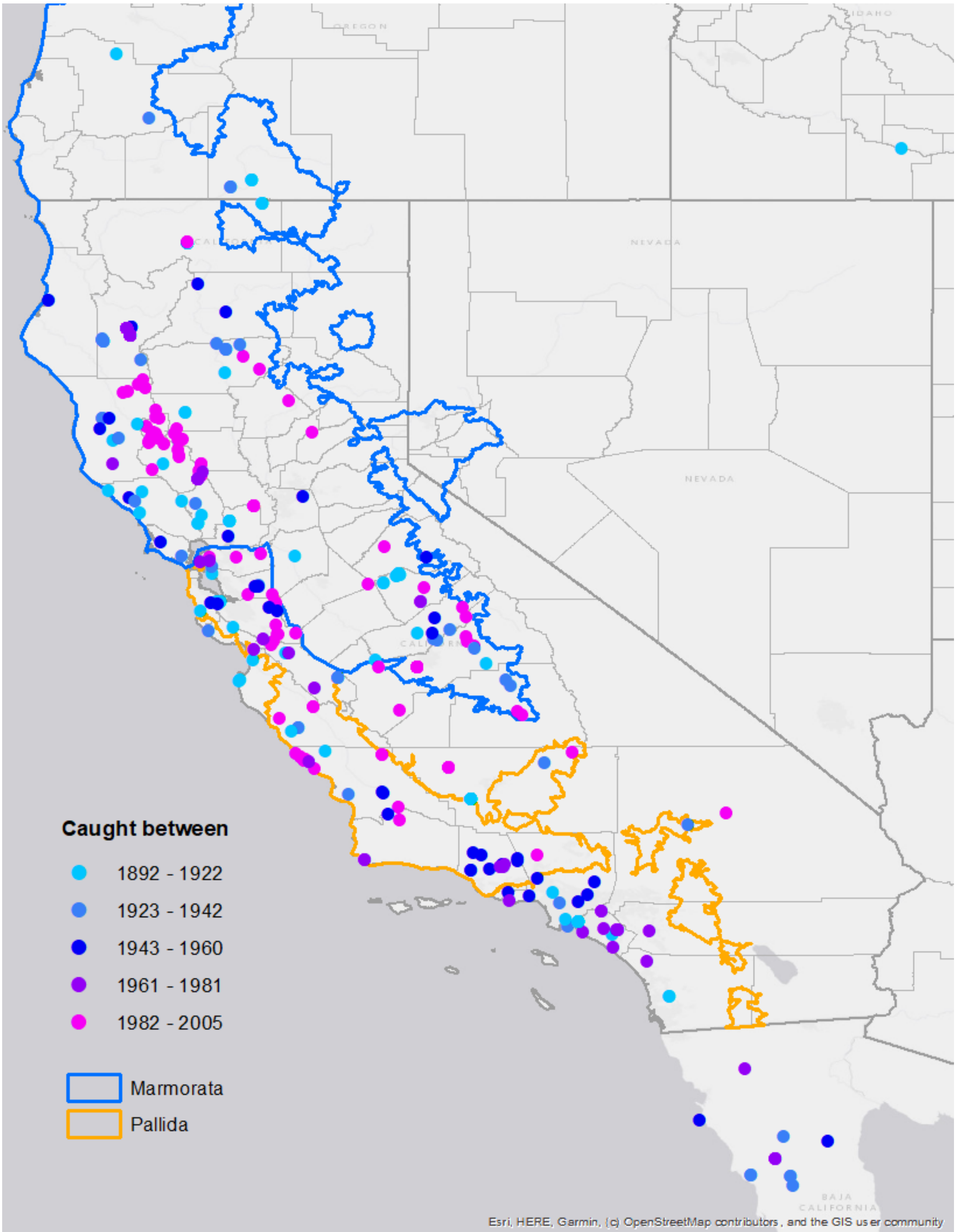


Figure 24. WPT museum specimens caught between 1892-2005. (Source: U.S. Geological Survey - Gap Analysis Project, 2017, Western Pond Turtle).

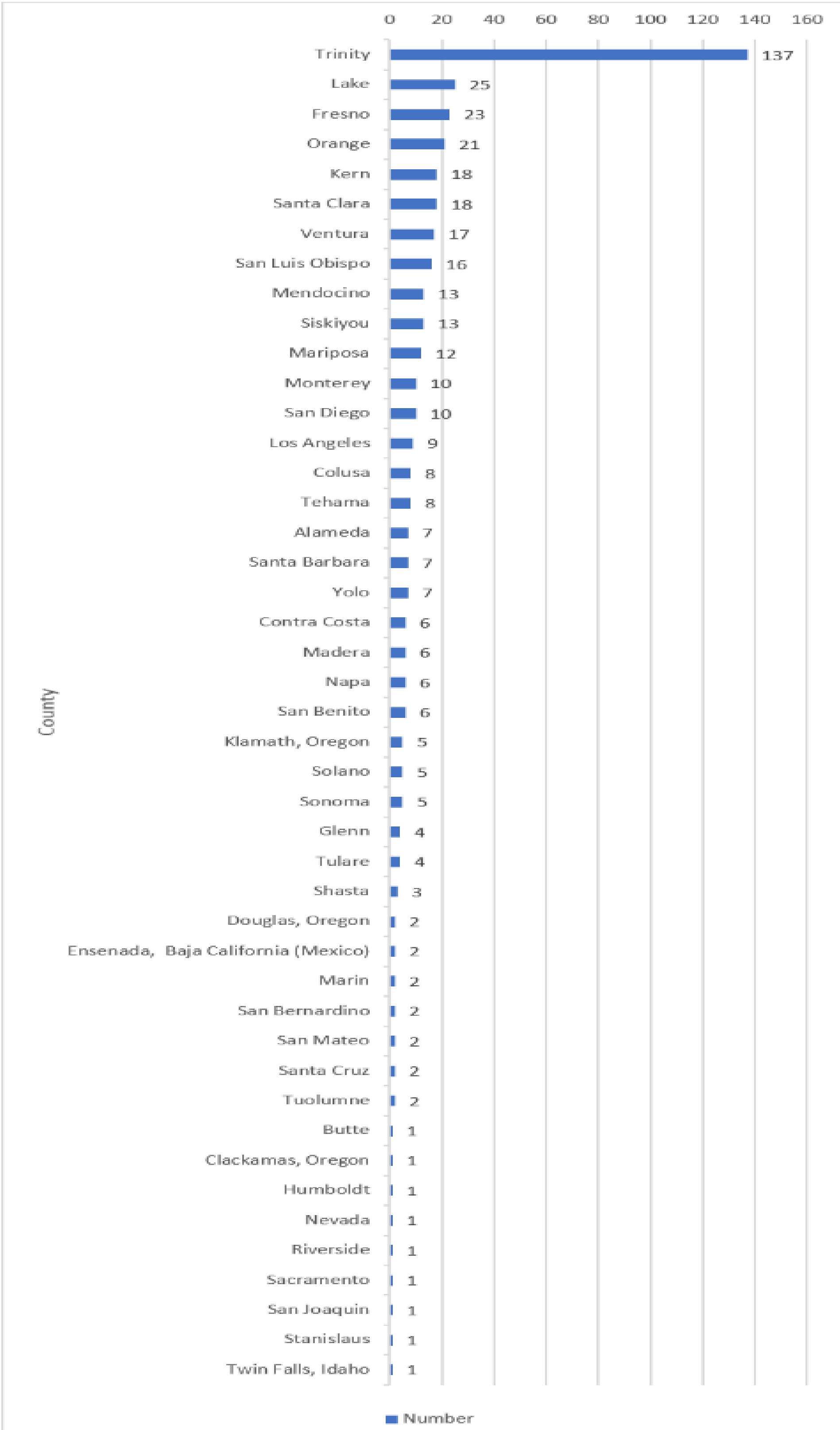

0 20 40 60 80 100 120 140 160
County
Trinity 137
Lake 25
Fresno 23
Orange 21
Kern 18
Santa Clara 18
Ventura 17
San Luis Obispo 16
Mendocino 13
Siskiyou 13
Mariposa 12
Monterey 10
San Diego 10
Los Angeles 9
Colusa 8
Tehama 8
Alameda 7
Santa Barbara 7
Yolo 7
Contra Costa 6
Madera 6
Napa 6
San Benito 6
Klamath, Oregon 5
Solano 5
Sonoma 5
Glenn 4
Tulare 4
Shasta 3
Douglas, Oregon 2
Ensenada, Baja California (Mexico) 2
Marin 2
San Bernardino 2
San Mateo 2
Santa Cruz 2
Tuolumne 2
Butte 1
Clackamas, Oregon 1
Humboldt 1
Nevada 1
Riverside 1
Sacramento 1
San Joaquin 1
Stanislaus 1
Twin Falls, Idaho 1
Number

Figure 25. Count of WPT museum specimens by county (1892-2005) across three museum collections.

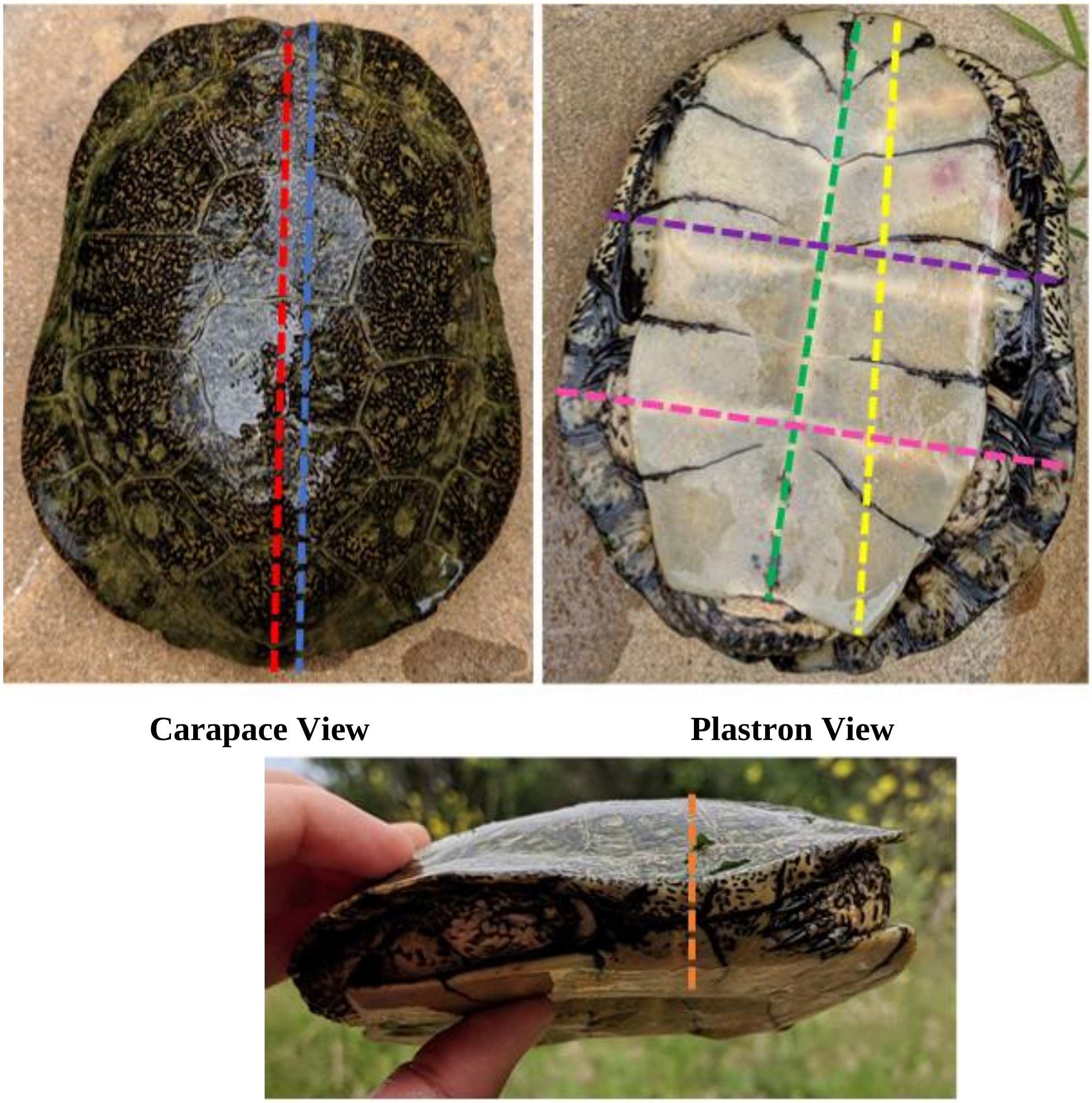


Figure 26. Measurements include midline carapace length (red), maximum carapace length (blue), midline plastron length (green), maximum plastron length (yellow), width at the bridge (purple), maximum width (pink), and maximum shell height length (orange).

Additionally, we acquired trapping data from the years 2006-2018 from the USGS, San Diego office (Fisher and Brown, pers. comm.). In this data set, a total of 799 individual turtles were caught at 56 sites throughout 7 southern California counties (Figure 27). Using the GPS coordinates of each site, we determined that all of these turtles were *E. pallida*. From these data, we extracted the midline carapace length, sex, and location of each turtle. This information was used to supplement the carapace length and sex data for *E. pallida* specimens.

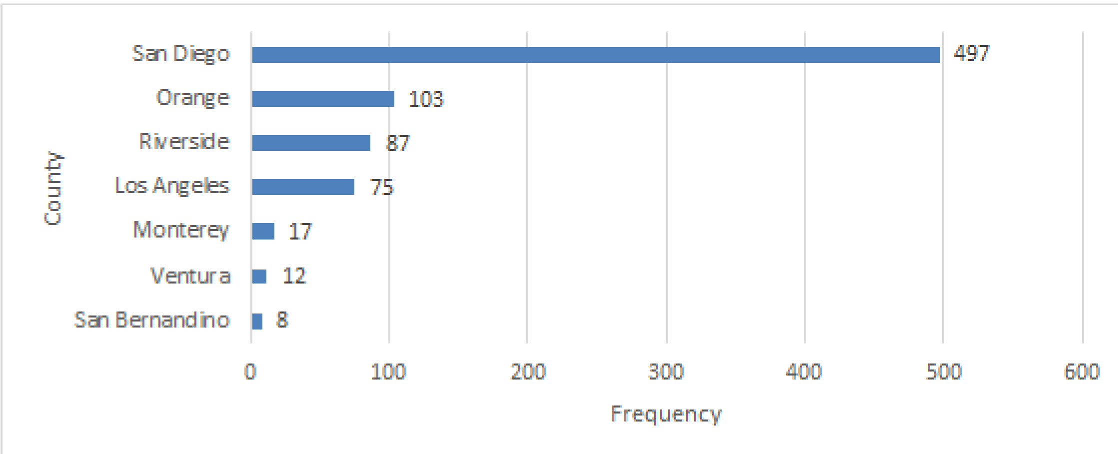


Figure 27. Count of WPTs by county from USGS data from 2006-2018.

Carapace Length Analysis

Given that the USGS data included 220 individuals that were recaptured, data was filtered so that each individual was represented by the most recent capture data. We then combined these data with our museum specimen data for midline carapace length analyses. To quantify changes in carapace length, we used RStudio to plot the measurements over time and fit a line of best fit to each data set using a linear regression model (Figures 28-32). The linear regression was considered a significant fit of the data at a p-value < 0.05 and the adjusted R^2 of this line was considered to infer how much variance in the data was explained by the line of best fit to the data. This analysis was first performed by species, then by sex within each species. For the gender-based analysis, we considered turtles with carapace lengths less than 110 mm as immature and did not include them in our calculations. This cutoff point represents the typical size at which WPTs reach sexual maturity and secondary sexual characteristics become apparent (Holland 1991, p. 11). Our goal was to improve the accuracy of our results, as the sex of young WPTs is difficult to determine. For carapace analyses by species, we included turtles with carapace lengths less than 110 mm because we were not separating by sex.

Sex Ratios Analysis

Similar to the gender-based analysis of the carapace lengths, all turtles with carapace lengths less than 110 mm were considered immature and too small to accurately sex. These individuals were completely removed from the sex ratios analysis. In order to account for the recaptured individuals included in the USGS data (2006-2018), we removed only entries that had the same individual trapped twice within one year. Within a year, the oldest entry was kept for a recaptured turtle. For example if a turtle was caught, processed and documented in March and June of 2016, the June entry in the data would be removed and not included in the sex ratio analysis. We kept repeat turtles for different years because we calculated sex ratios for each year; we decided that the same turtle can count for the sex ratio for different years. In this case, each year has a unique sex ratio from 2006-2018.

We also performed a sex ratio analysis from 1892-2018 using stand alone museum data and the museum-USGS combination data. For this analysis, we calculated the sex ratio (number of males divided by females) for every 20-year period starting in 1890. For the stand alone museum data and the museum-USGS combination data, we combined years to find sex ratios for 20 year periods. Thus, each 20 year period has a unique sex ratio. Combining the museum and USGS data was straightforward because the museum data ended in 2005 and the USGS data began in 2006.

5.3 Results: Carapace Length by Species

E. pallida

Mean carapace length of *E. pallida* museum specimens increased over time from 1892-2005, although not significantly so ($P = .17$) (Figure 28). This coincides with our prediction that less recruitment is occurring throughout *E. pallida*'s range, as an increase in mean carapace length can be interpreted as an increase in average age over time. Overall, juveniles with carapace lengths less than 110 mm made up 47% (27 out of 57) of *E. pallida* museum specimens from 1892-1950 and 45% (39 out of 86) from 1951-2005.

This analysis was also performed using the USGS data for *E. pallida* (2006-2018) in addition to the museum specimen data for *E. pallida* (1892-2005) to provide a more current and comprehensive view of changes in carapace length over time (Figure 29). A plot of the combined data set shows a significant increase in mean carapace length over time from 1892-2018 ($P < .001$). From the USGS data alone, juveniles with carapace lengths less than 110 mm made up 27% (217 out of 798) of *E. pallida* captures from 2006-2018.

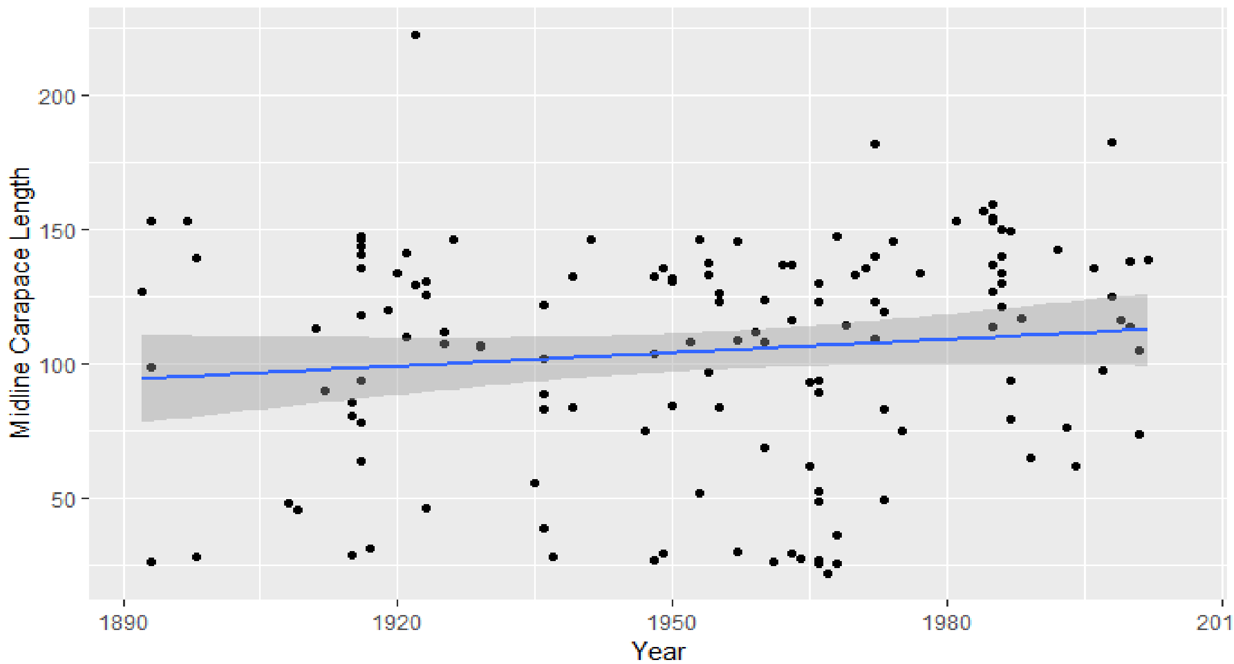


Figure 28. Plot of midline carapace lengths vs. time for *E. pallida* museum specimens from 1892-2005 (n = 147, Adjusted R-squared = 0.0065 , *P* = .17). The blue trend line and grey shading account for the 95% confidence interval.

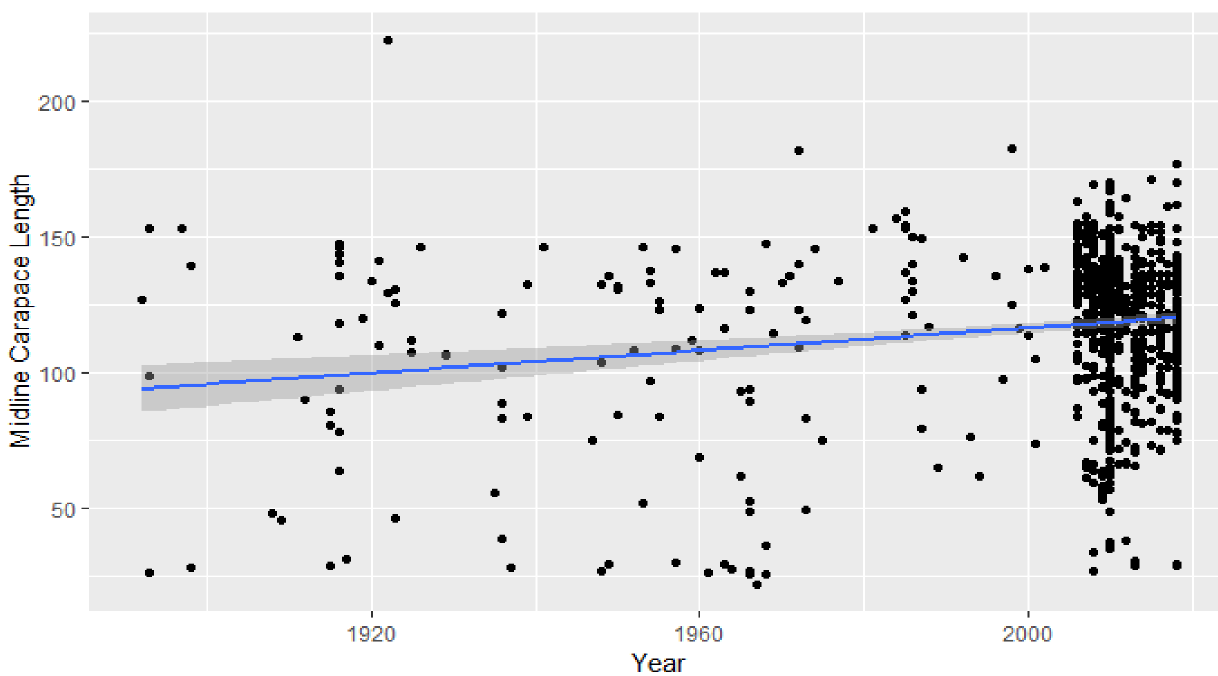


Figure 29. Plot of midline carapace lengths vs. time for *E. pallida* museum specimens from 1892-2005 and USGS captures between the years 2006-2018. (n = 946, Adjusted R-Squared = 0.029, *P* = 1.0e-07). The blue trend line and grey shading account for the 95% confidence interval.

E. marmorata

Mean carapace length also increased in *E. marmorata* from 1894-2005 (*P* = .00056). This increase is statistically significant and provides further support that recruitment has been decreasing over time (Figure 30). Like *E. pallida*, *E. marmorata* hatchlings are at risk of predation by bullfrogs, largemouth bass, and other invasive species. Overall, juveniles with carapace lengths less than 110 mm made up 45% (43 out of 96) of *E. marmorata* museum specimens from 1894-1950 and 27% (59 out of 216) from 1951-2005. The proportion of *E. marmorata* juveniles caught from 1951-2005 was much lower than the proportion of *E. pallida* juveniles caught during the same time interval; however, it is similar to the proportion of *E. pallida* juveniles caught from 2006-2018. Unfortunately, we do not have data available for *E. marmorata* from 2006-2018 to compare.

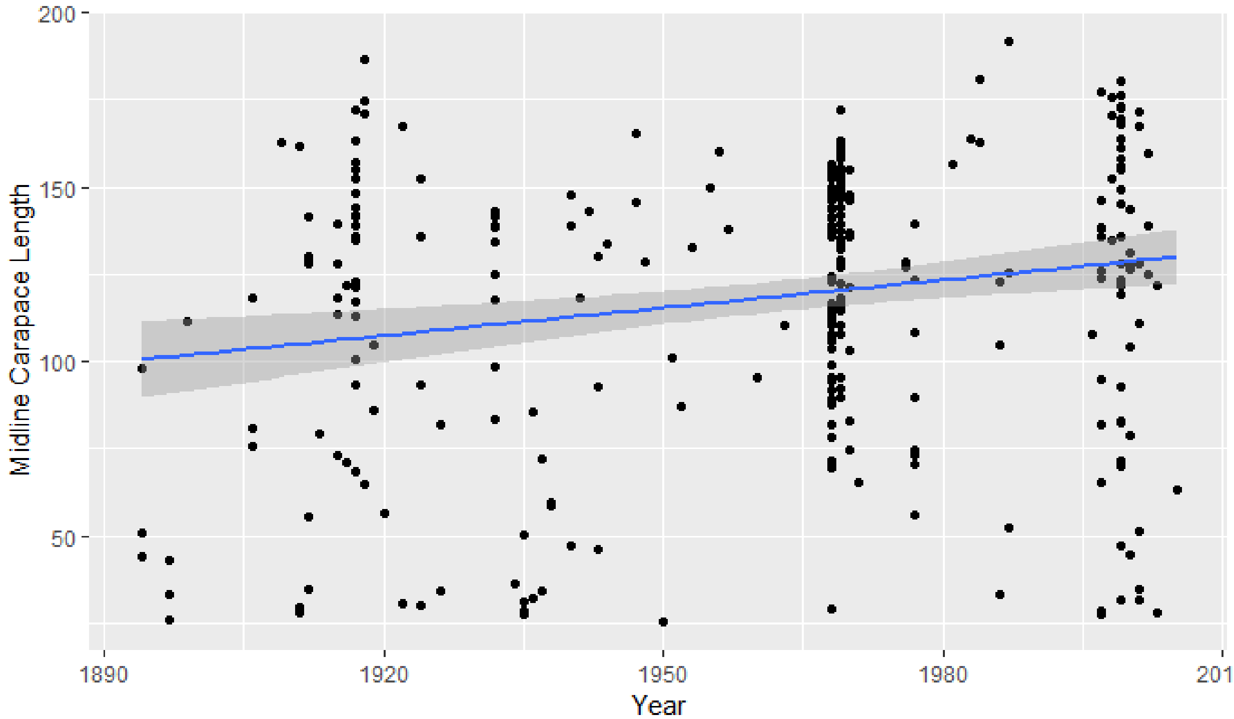


Figure 30. Plot of midline carapace lengths vs. time for *E. marmorata* museum specimens from 1894-2005 (n = 316, Adjusted R-Squared = 0.035, *P* = 0.00056). The blue trend line and grey shading account for the 95% confidence interval.

5.4 Results: Carapace Lengths by Species and Sex

E. pallida

Mean carapace length in adult (> 110 mm) female *E. pallida* specimens and USGS captures decreased from 1893-2018 (P = .030) [Figure 31]. This could be due to female-biased road mortality, which affects nesting females that travel large distances. Mean carapace length also decreased for adult *E. pallida* males from 1892-2018, although less significantly so (P = .046) [Figure 31]. However, we are not sure what could be causing this decrease in males.

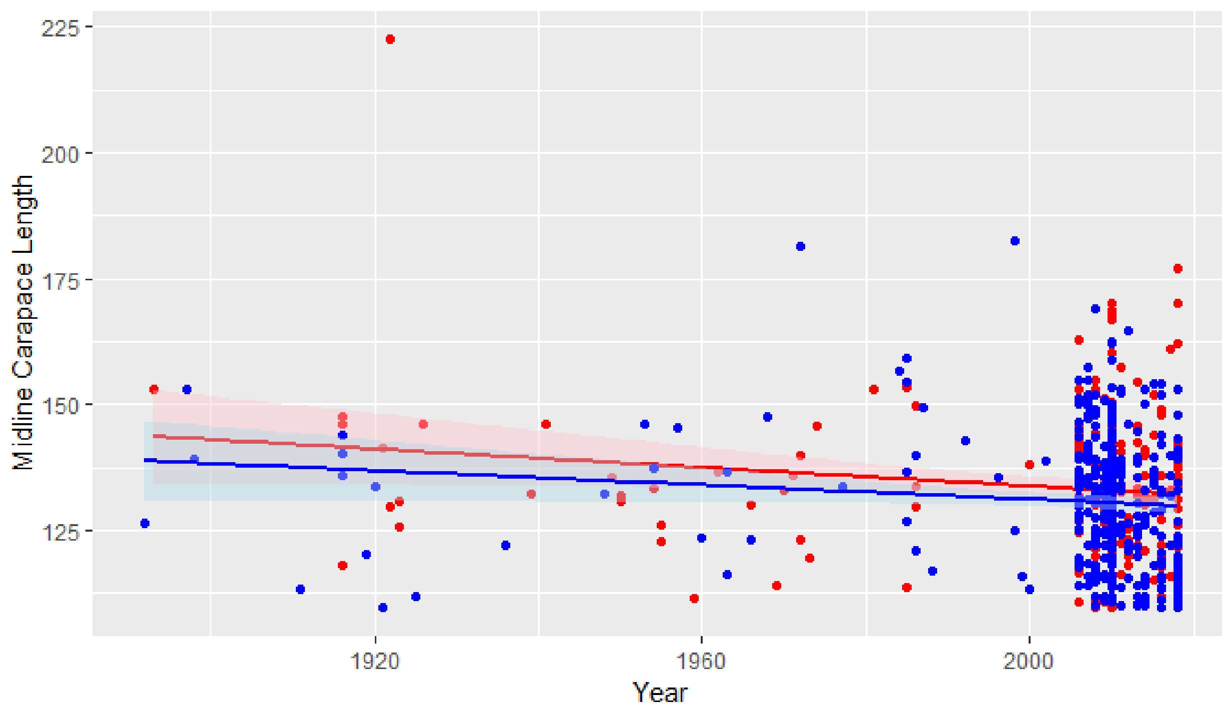


Figure 31. Plot of midline carapace lengths vs. time for *E. pallida* female specimens (red) from 1893-2018 and *E. pallida* male specimens (blue) from 1892-2018. Females: n = 237, Adjusted R-Squared = .016, P = .030. Males: n = 398, Adjusted R-Squared = .0076, P = .046. The blue trend line and grey shading account for the 95% confidence interval. Although the regression models for *E. pallida* males and females are slightly different, they are not significantly so (P = .70).

E. marmorata

The mean carapace length remained relatively constant for adult (> 110 mm) *E. marmorata* females from 1911-2001 (P = .95) [Figure 32]. However, there was a significant increase in mean carapace length for *E. marmorata* males from 1894-2005 (P = .012) [Figure 32]. The increase in males is consistent with the increase in mean carapace length overtime for *E. marmorata* in Figure 30 and may be contributing to that trend. Overall, trends in female carapace lengths for *E. marmorata* are not consistent with our predictions, as we would expect to see a decrease due to female-biased road mortality.

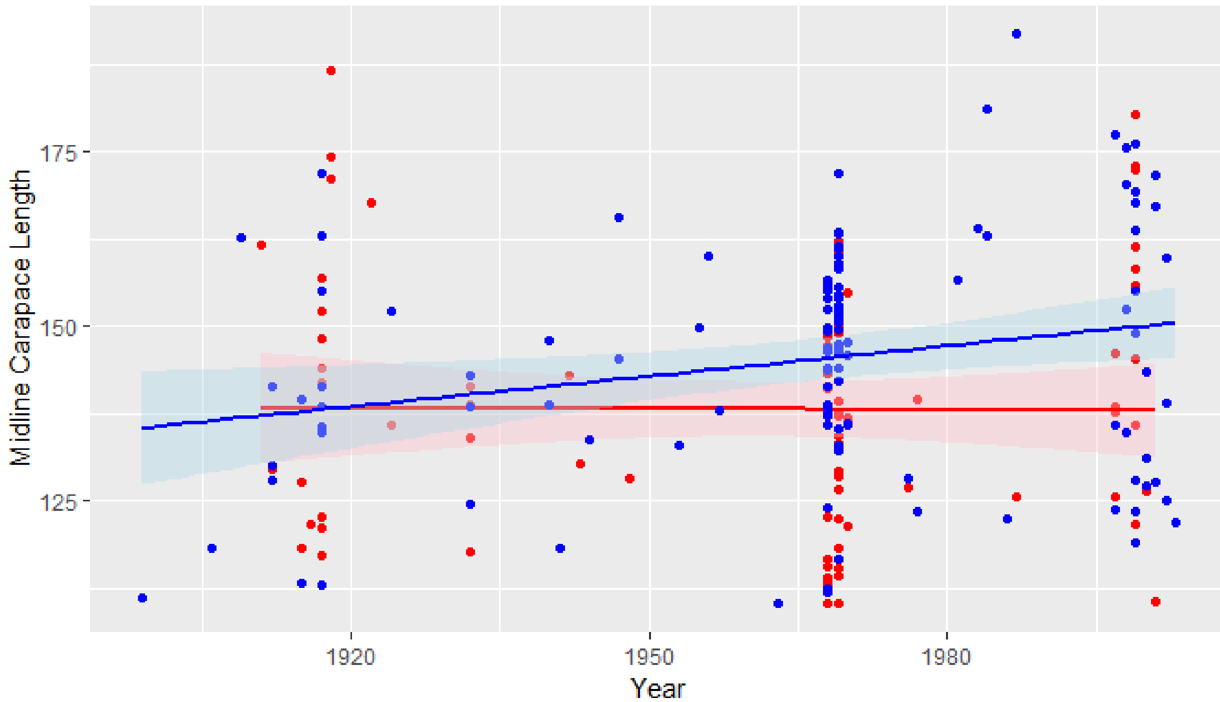


Figure 32. Plot of midline carapace lengths vs. time for *E. marmorata* female specimens (red) from 1911-2001 and *E. marmorata* male specimens (blue) from 1894-2005. Females: n = 87, Adjusted R-Squared = -.012, *P* = .95. Males: n = 123, Adjusted R-Squared = .043, *P* = .012. The blue trend line and grey shading account for the 95% confidence interval. Although the regression models for *E. marmorata* males and females appear to be different, they are not significantly so (*P* = .094).

5.5 Results: Sex Ratios

E. pallida

From 1890 to 1989, the overall sex ratio of *E. pallida* in the three natural history collections we examined was on average 0.8:1 (male:female), with little deviation when broken out into 20-year intervals (Figure 33). Because only five *E. pallida* specimens were collected from 1890-1909, the observed sex ratio may not be indicative of that time period, especially when considering the relatively stable sex ratio in the following 80 years. Out of the five specimens collected, 4 were female while 1 was male, greatly biasing the sex ratio.

In a departure from earlier intervals, for the years 1990-2009 we found a very biased sex ratio with 3.5 males for every female. Contrasting to the earliest time point, the sample size for these years is not dramatically low, and supports a recent deviation from the historical sex ratio for this species. Thus, the change from a relatively 1:1 sex ratio to a high male-biased sex ratio indicates that for *E. pallida*, female-biased road mortality or other types of predation or collection while on land may be shifting sex ratios.

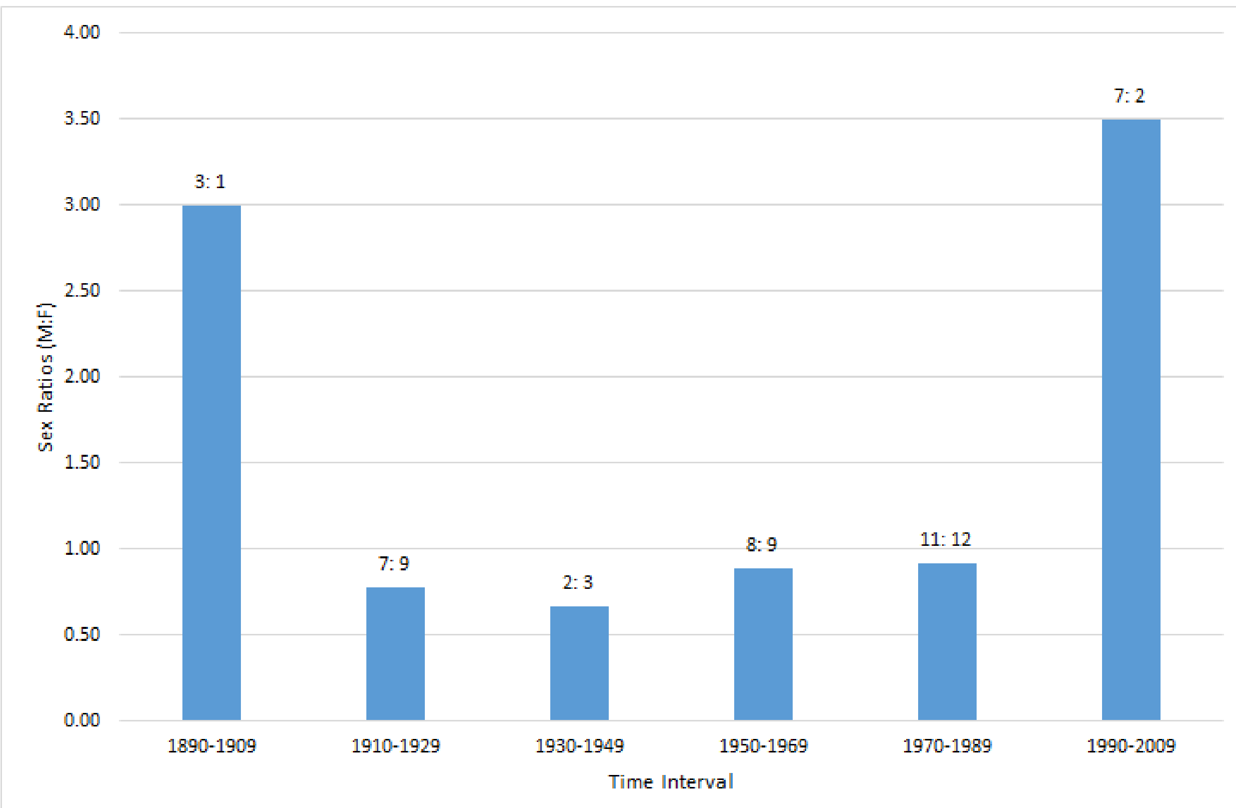


Figure 33. Sex ratios for *E. pallida* calculated from WPT specimens between the years 1890-2009 from museum data, with male:female ratios labeled for each time interval.

Table 4. Overall sex ratios and breakdown for *E. pallida* calculated from WPT specimens between the years 1890-2009 from museum data.

E. pallida Sex Ratios (1890-2009)				
Time Interval	Female Count	Male Count	Ratio (M:F)	Sample Size
1890-1909	1	3	3	4
1910-1929	9	7	0.78	16
1930-1949	3	2	0.67	5
1950-1969	9	8	0.89	17
1970-1989	12	11	0.92	23
1990-2009	2	7	3.50	9

From 2006 to 2018, the overall sex ratio of *E. pallida* in the USGS data hovered between 1:1 and 2:1, with some deviations (Figure 34). The years 2006, 2008, and 2017 all slightly exceeded 2:1, suggesting more male dominance in the WPTs tracked those years. In 2007, there was an extremely low count of females which resulted in a highly male dominated sex ratio. This could be a result of a variety of factors including sampling bias or the disappearance of females. Additionally, the year 2018 was the only female dominated year; however only two more females than males were captured. Since 2007 and 2018 are outliers, they are not indicative of the overall trend of a male biased sex ratio. This new data advances the idea of a shift from a relatively even sex ratio to a male biased sex ratio for *E. pallida*. The current male biased sex ratio for *E. pallida* seen through the USGS data suggests that there may be female-biased road mortality, predation, collection, or other mortality types due to the additional time female WPTs must spend on land for nesting and reproduction.

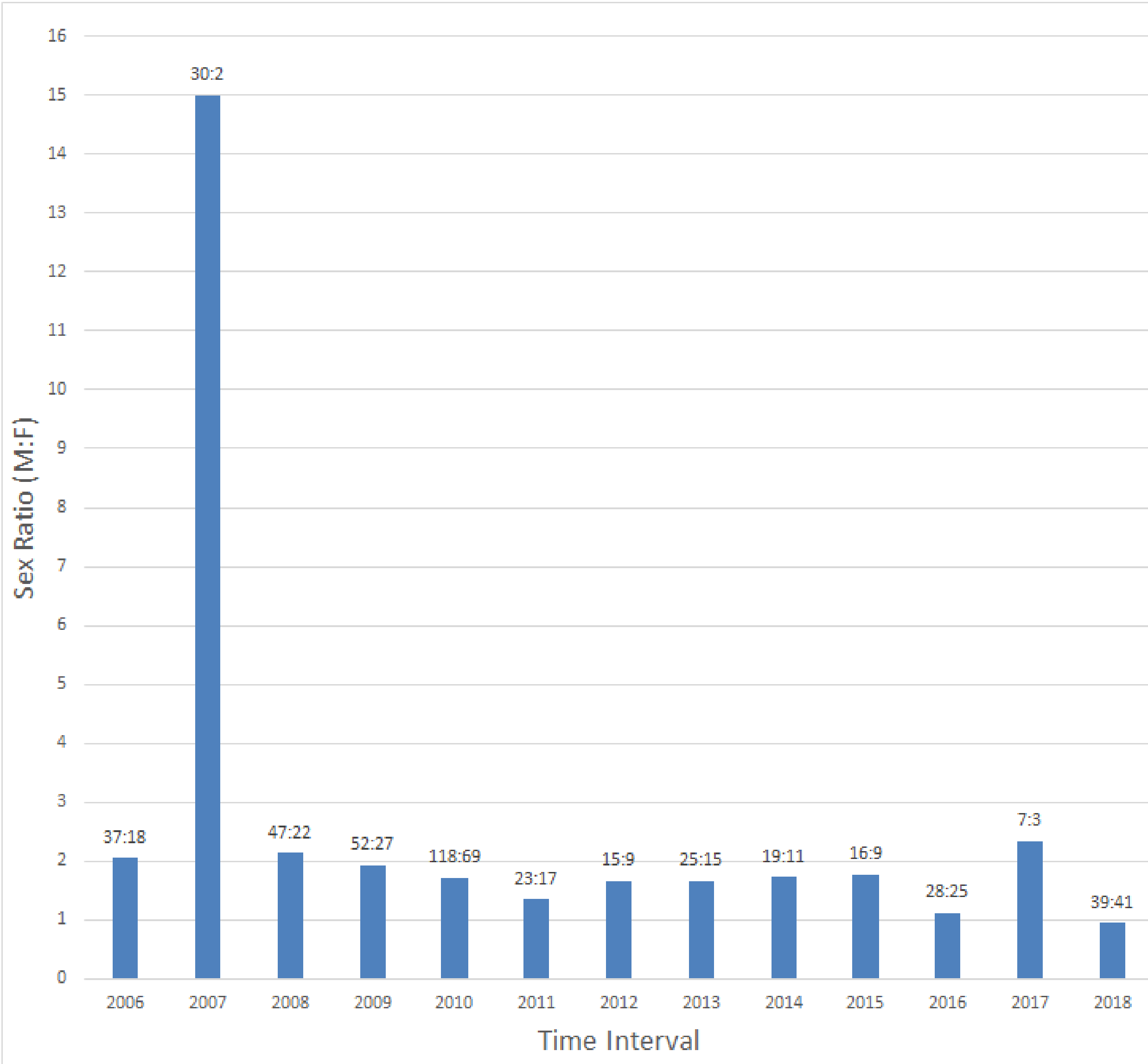


Figure 34. Sex ratios for *E. pallida* calculated from WPT specimens between the years 2006-2018 from USGS data, with male:female ratios labeled for each time interval.

Table 5. Overall sex ratios and breakdown for *E. pallida* calculated from WPT specimens between the years 2006-2018 from USGS data.

E. pallida Sex Ratios (2006-2018)				
Time Interval	Female Count	Male Count	Ratio (M:F)	Sample Size
2006	18	37	2.06	55
2007	2	30	15	32
2008	22	47	2.14	69
2009	27	52	1.93	79
2010	69	118	1.71	187
2011	17	23	1.35	40
2012	9	15	1.67	24
2013	15	25	1.67	40
2014	11	19	1.73	30
2015	9	16	1.78	25
2016	25	28	1.12	53
2017	3	7	2.33	10
2018	41	39	0.95	80

Figure 35 combines the museum data sex ratios from 1892-2005 and the USGS data sex ratios from 2006-2018. This combination allows us to understand the scope of the current condition of *E. pallida* as the museum data lacks records from the most recent time intervals. Placing the USGS sex ratios into a 20 year time interval allows us to easily add it to the museum data intervals and bring our data to present time. By doing this, we see that the sex ratio drops to around 1.5:1 and remains male biased, but to a lesser extent.

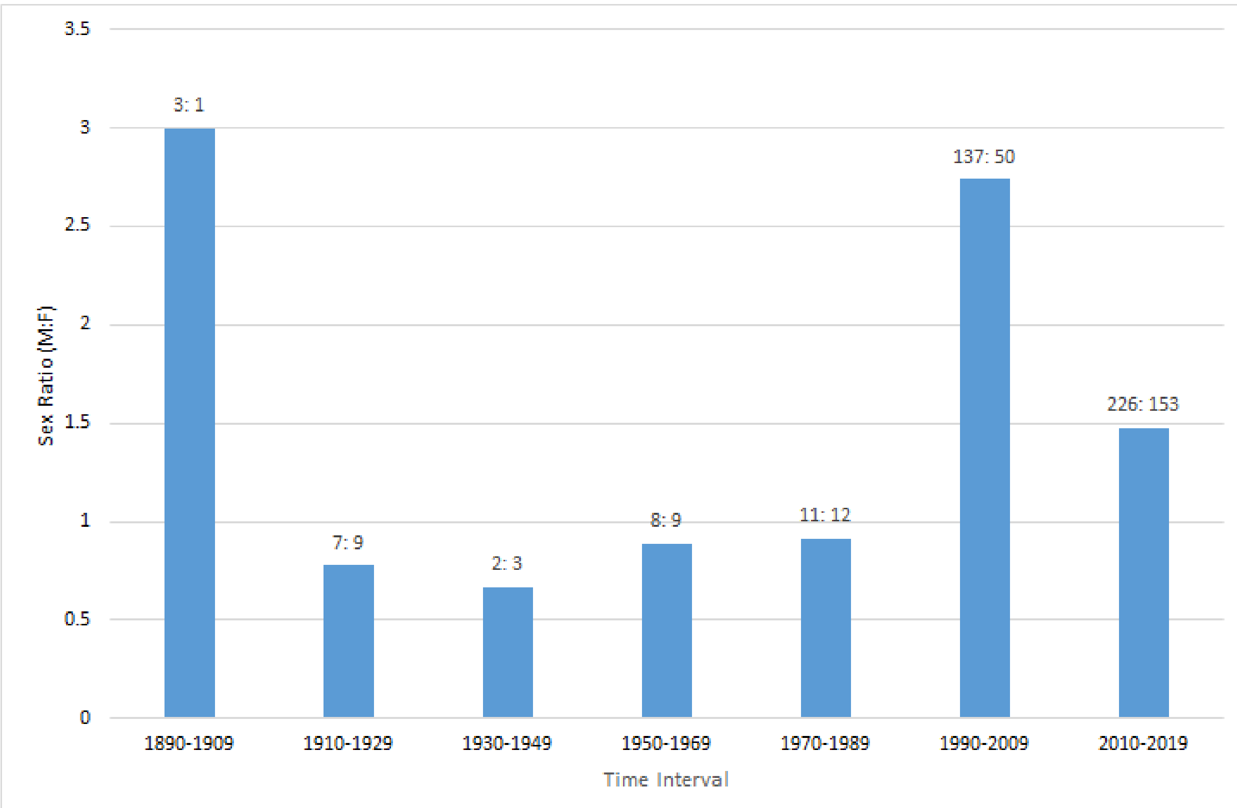


Figure 35. Sex ratios for *E. pallida* calculated from WPT specimens between the years 1890-2019 from museum data and USGS data, with male:female ratios labeled for each time interval.

Table 6. Overall sex ratios and breakdown for *E. pallida* calculated from WPT specimens between the years 1890-2019 from museum data and USGS data.

E. pallida Sex Ratios (1890-2019)				
Time Interval	Female Count	Male Count	Ratio (M:F)	Sample Size
1890-1909	1	3	3	4
1910-1929	9	7	0.78	16
1930-1949	3	2	0.67	5
1950-1969	9	8	0.89	17
1970-1989	12	11	0.92	23
1990-2009	50	137	2.74	187
2010-2019	153	226	1.48	379

E. marmorata

From 1910 to 2005, the overall sex ratio of *E. marmorata* in the three natural history collections was examined and it was found that *E. marmorata* sex ratios have been become more male-biased over time with the current ratio at 1.5:1(Figure 36). The time interval from 1910-1929 shows a female-biased sex ratio at 0.74:1 that is much closer to 1:1. However, every time interval after shows an increase in male-bias until the most recent interval. This indicates that the sex ratio has become slightly more male-biased for *E. marmorata* over time but the increase in male-bias is not as dramatic as the increase seen in *E. pallida*. From the most current interval of 1990-2009, there were 1.5 males for every female. The sample size from this time period is relatively large (N = 43) and is similar to the sex ratio from 1950-1969, which also had a large sample size (N = 96). Thus the most recent sex ratio from our data indicates that there has not been a dramatic shift in sex ratios, as it is in line with the slightly male biased ratios that were historically present.

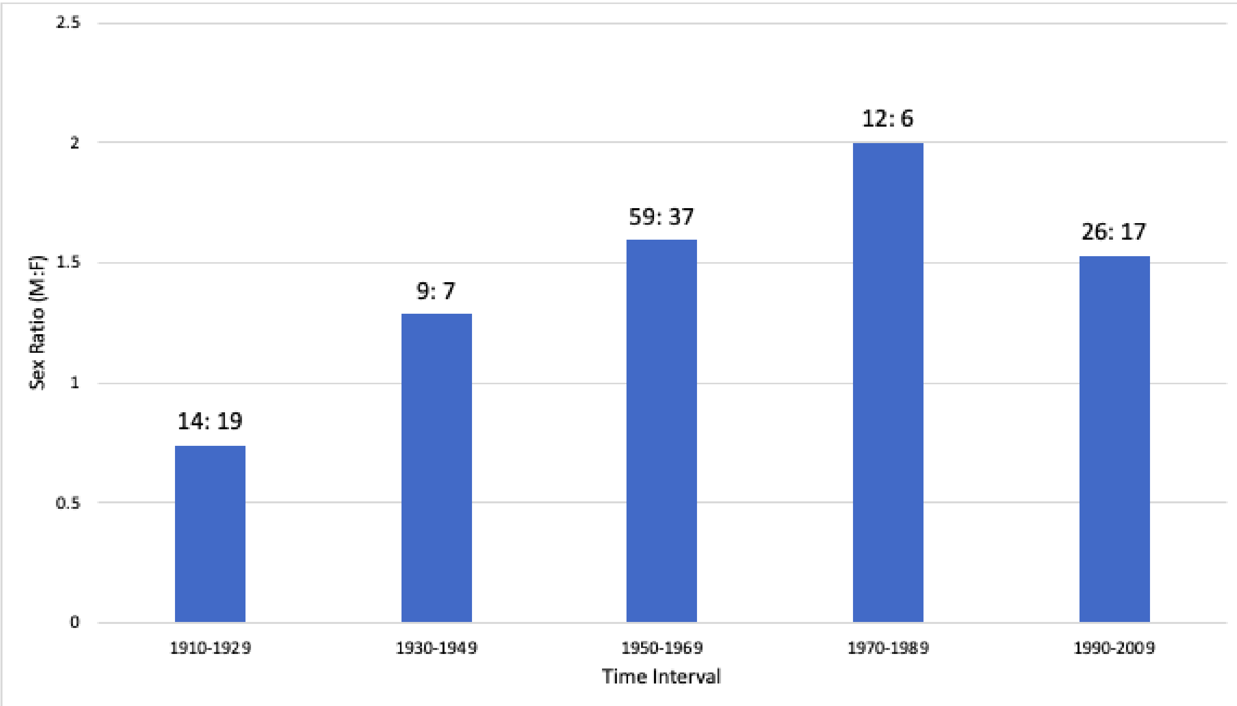


Figure 36. Sex ratios for *E. marmorata* calculated from WPT specimens between the years 1910-2009 from museum data, with male:female ratios labeled for each time interval.

Table 7. Overall sex ratios and breakdown for *E. marmorata* calculated from WPT specimens between the years 1910-2009 from museum data.

E. marmorata Sex Ratios (1910-2009)				
Time Interval	Female Count	Male Count	Ratio (M:F)	Sample Size
1910-1929	19	14	0.74	33
1930-1949	7	9	1.29	16
1950-1969	37	59	1.59	96
1970-1989	6	12	2	18

1990-2009	17	26	1.53	43

5.6 Introduction to Literature Sightings Data

There is a paucity of data showing current range-wide population sizes and population size fluctuations for the WPT. However, there are numerous smaller studies that we can use to infer trends in range-wide populations when analyzed together. In order to visualize current WPT population conditions we reviewed trapping data through field surveys, literature and USGS data within the past few decades. We used these turtle sightings to represent the current WPT distribution.

5.7 Literature Sight Survey Methods

We started off by searching academic publication websites for all current published WPT sightings. We were given additional site surveys and literature from the USFWS and incorporated USGS capture data. The literature obtained was reviewed and important information on the county, species, geographic coordinates of the study site, population size, and year of study was recorded for all the literature analyzed. With site and trapping information from the literature, we created a map showing an estimate of current WPT populations (Figure 37). The map was created using the observations where a single sighting survey was taken and areas where multiple sighting surveys were taken in a given year. There were 41 sites obtained from the current literature and 62 from the USGS data. It is important to note that not all researchers use the same sampling techniques, therefore there is the potential that several sites may have larger populations than what was estimated using the data from the literature and USGS. Thus, the literature and the USGS data only provide limited insight into general population size trends of WPTs throughout its range.

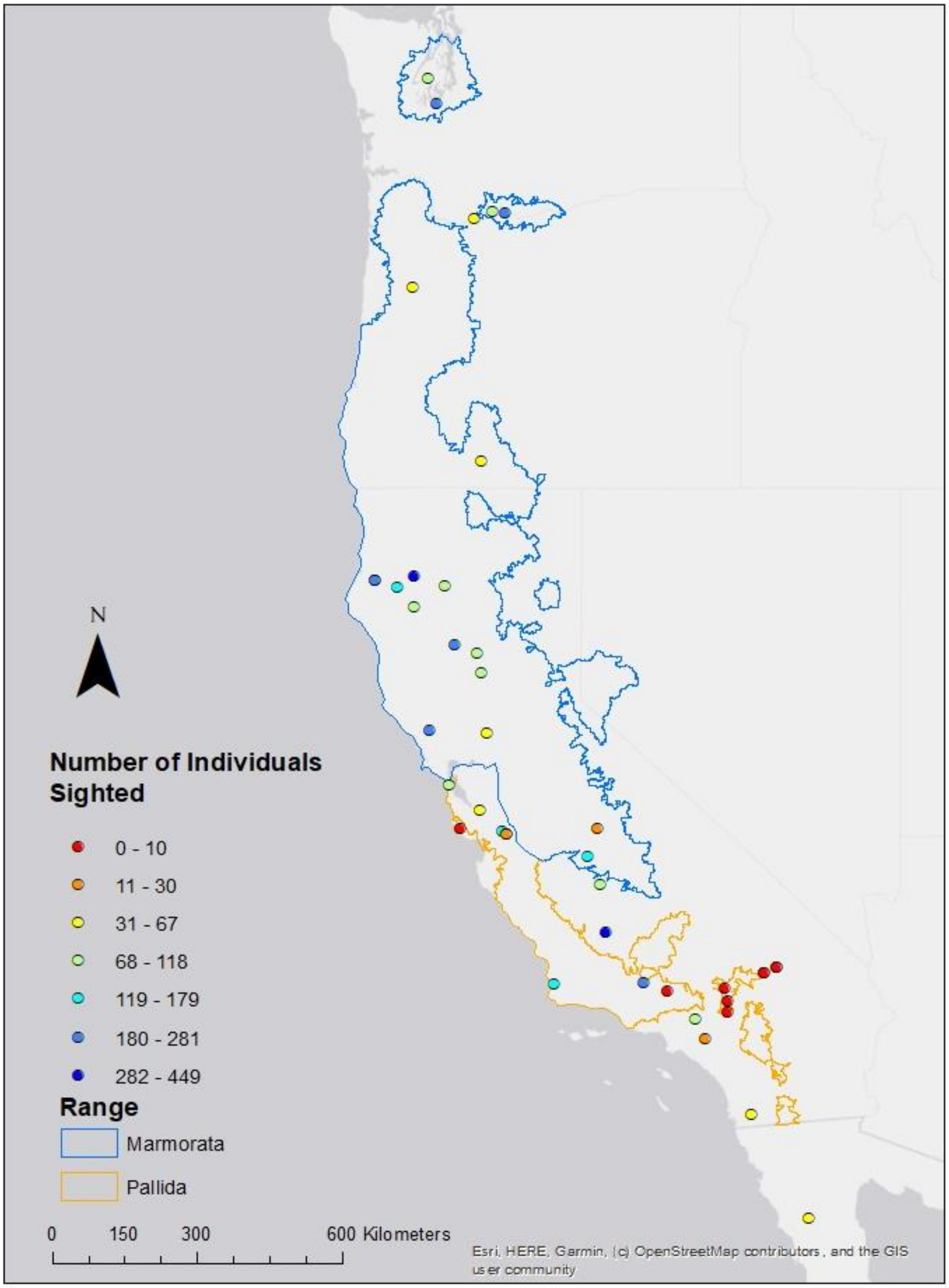


Figure 37. Most recent sightings of *E. pallida* and *E. marmorata* at multiple sites from trapping surveys referenced in primary literature (1993-2018). (Source: U.S. Geological Survey - Gap Analysis Project, 2017, Western Pond Turtle).

5.8 Literature Sighting Survey Results

Analyses of the literature revealed several interesting findings. There was a total of 19 study sites for *E. pallida* from 1993-2018. The maximum number of individual sightings was 215 at a single site in San Diego. Furthermore, the average number of sightings is approximately 59, with a standard deviation (SD) of 79.10. The number of sites that had sightings smaller than the average was 13. It is also important to note that in terms of size, *E. pallida* is smaller than *E. marmorata* and based on our data is more severely threatened than *E. marmorata* as well, all of which could have potentially influenced sightings in the literature.

Moreover, there was a total of 22 *E. marmorata* sites surveyed in the literature between 2002-2017. The maximum number of individual sightings was seen in Goose lake (n=449), which was also the highest number of sightings of the two species. The average number of sightings within this time frame was about 145 and had a SD of 114.4. With the SD being so large, it shows that most sites are further from the average of 145. Only 7 of the 22 sites had observations higher than the average. It is also important to note that *E. marmorata* appears to be less threatened than *E. pallida* which could have potentially influenced literature sightings.

The USGS data had a total of 62 sites surveyed between 2006-2018. The average number of sightings within these sites was about 9 with 13.4 being the SD. Forty-three of the sixty-two sites had sightings that were less than 9. The data from USGS had sightings that were much lower than those found in current literature, showing how sampling techniques can vary across sources.

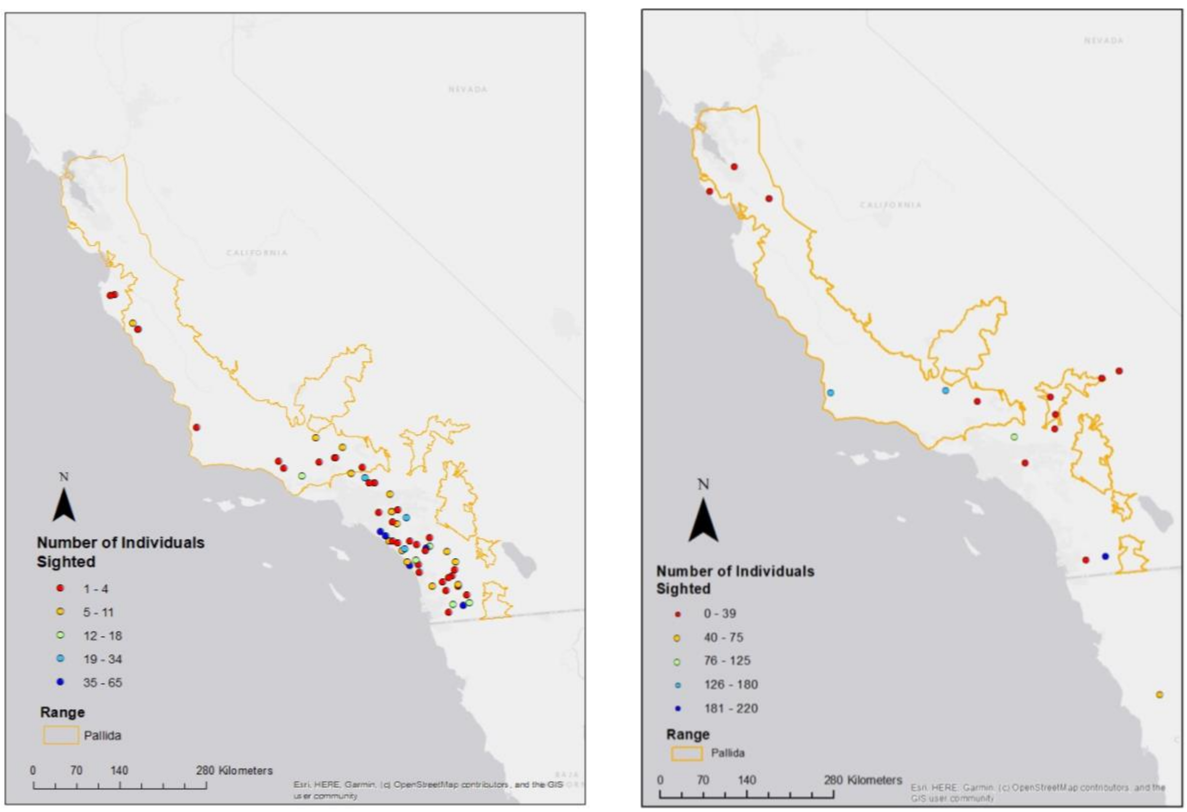


Figure 38. USGS captures data (left) and most recent literature sightings (right). (Source: U.S. Geological Survey - Gap Analysis Project, 2017, Western Pond Turtle).

Table 8. The sites and number of sightings of *E. pallida* from cited literature (1993-2018)

Number of Individuals Sighted (*E. pallida*)			
Site	**County**	**Year**	**Total (source)**
West Fork of the San Gabriel River	Los Angeles (CA)	1993	115 (Goodman 1997, p. 26)
Chino Hills State Park, Upper Aliso Creek	San Bernardino (CA)	1994	30 (Goodman 1997, p.26)
Vandenberg Air Force Base	Santa Barbara (CA)	1996	179 (Germano & Rathbun 2008, p. 3)
Waddell Creek and Turtle Pond	Santa Cruz (CA)	1998	5 (Crump 2001, p. 30)
Gorman Lake	Los Angeles (CA)	2010	240 (Germano & Riedle 2015, p.105)
Coyote Creek	Santa Clara (CA)	2011	173 (Belli 2015, p. 4)
Sycuan Peak Ecological Reserve, Sweetwater River	San Diego (CA)	2012	38 (Wood 2012, p. 1)
Pine Valley Creek, Upper Site	San Diego (CA)	2013	215 (Brown et al 2015, p. 24)
Diablo Range, Coyote Creek	Santa Clara (CA)	2013	39 (Leidy *et al* 2016, p. 3)
Lake Elizabeth	Los Angeles (CA)	2015	0 (Lovich 2017, p. 8)
Arroyo at San Rafael	Churumuco (MX)	2015	49 (Valdez Villavicencio *et al.* 2015, p. 7)
Waddell Creek	Santa Cruz (CA)	2017	13 (Smith 2018, p. 5)
Camp Cady Wildlife Area	San Bernardino (CA)	2018	0 (Lovich 2017, p. 8)
Afton Canyon, Mojave River	San Bernardino (CA)	2018	1 (Lovich 2017, p. 9)
Las Flores Ranch, Mojave River	San Bernardino (CA)	2018	10 (Lovich 2017, p. 6)
Mojave Narrows Regional Park, Mojave River	San Bernardino (CA)	2018	0 (Lovich 2017, p. 7)
Palisades Ranch, Mojave River	San Bernardino (CA)	2018	0 (Lovich 2017, p. 7)
Canada de los Osos Ecological Reserve, Old Corral Pond	Santa Clara (CA)	2018	67 (Smith 2018, p. 4)
Canada de los Osos Ecological Reserve, Wilson Ranch Pond	Santa Clara (CA)	2018	17 (Smith 2018, p. 5)

Table 9. The sites and number of sightings of *E. marmorata* from primary literature (2002-2017).

Number of Individuals Sighted (*E. marmorata*)			
Site	**County (State)**	**Year**	**Total (source)**
Howard Slough Unit	Butte (CA)	2002	79 (Lubcke & Wilson 2007, p. 4)
Sacramento River	Colusa (CA)	2002	86 (Lubcke & Wilson 2007, p. 4)
Hanford Wastewater Treatment Facility	Tulare (CA)	2002	101 (Germano 2010, p. 91)
Big Chico Creek Ecological Reserve	Butte(CA)	2003	281 (Lubcke & Wilson 2007, p.4)
Goose Lake	Kern (CA)	2005	449 (Germano 2016, p. 5)
Fresno-Clovis Regional Wastewater Reclamation Facility	Fresno (CA)	2007	138 (Germano 2010, p. 4)
Hayfork Creek	Trinity (CA)	2010	174 (Bury *et al.* 2010, p.446)
Hell to Find Lake	Trinity(CA)	2010	94 (Bury *et al.* 2010, p. 446)
Klamath Basin	Trinity (CA)	2010	52 (Bury *et al.* 2010, p. 446)
Whiskey Town	Trinity (CA)	2010	113 (Bury *et al.* 2010, p. 446)
Bergen	Skamania (WA)	2011	86 (Hallock *et al.* 2017, p. 6)
Pierce National Wildlife Refuge	Skamania (WA)	2011	41 (Hallock *et al.* 2017, p. 6)
Trinity River	Trinity(CA)	2012	365 (Sloan 2012, p. 15)
Washington Department of Natural Resources lands	Mason (WA)	2013	98 (Hallock *et al.* 2017, p. 6)
Washington Department of Fish and Wildlife Area	Klickitat (WA)	2014	251 (Hallock *et al.* 2017, p. 6)
UC Davis Arboretum Waterway	Yolo (CA)	2014	47 (Spinks *et al.* 2003, p. 636)
Luckiamute State Recreation Area	Benton (WA)	2015	40 (Bury *et al.* 2015, p. 4)
San Joaquin Experimental Range	Madera (CA)	2015	22 (Purcell *et al.* 2017, p. 21)

Washington Department of Fish and Wildlife Area	Pierce (WA)	2015	254 (Hallock *et al.* 2017, p. 6)
River Fork Ranch, Carson River	Douglas (NV)	2016	118 (NDOW 2016, p. 4)
Russian River	Sonoma (CA)	2017	242 (Cook 2018, p. 2)
Mad River	Humboldt (CA)	2018	59 (GDRC 2018, p. 7)

Table 10. The sites and number of sightings of *E. pallida* from the USGS data (2006-2018):

Number of Individuals Sighted (*E. pallida*)			
Site	**County (CA)**	**Year**	**Total**
Fullerton Arboretum	Los Angeles	2006	1
San Diego Creek	Los Angeles	2006	41
Santiago Creek	Los Angeles	2006	1
Upper Sweetwater River	San Diego	2006	1
Long Canyon	San Diego	2007	8
Ladd Canyon	Los Angeles	2008	6
Cajalco Pools	Riverside	2008	28
Big Tujunga Creek	Los Angeles	2009	29
Bluewater Canyon TRIB 2	Los Angeles	2009	1
San Francisquito Canyon	Los Angeles	2009	1
Cedar Creek	San Diego	2009	1
San Mateo Canyon	San Diego	2009	10
W Temecula Cr	San Diego	2009	1
Aliso Canyon	Los Angeles	2010	1
Aliso Creek	Los Angeles	2010	6
Bernard Biological Field Station	Los Angeles	2010	6
Oso Creek	Los Angeles	2010	2
Pacoima Wash	Los Angeles	2010	11
Gorman Lake	Los Angeles	2010	9
Chileno Canyon	Los Angeles	2010	2
Elizabeth Lake	Los Angeles	2010	5
San Juan Creek	Los Angeles	2010	1
West Fork San Gabriel River	Los Angeles	2010	3
WF San Gabriel RiverTrib 29	Los Angeles	2010	3
San Vicente Creek	San Diego	2010	1
Santa Margarita River	San Diego	2010	18
Las Flores Creek	San Diego	2010	9
Black Canyon	San Diego	2010	2
Orcutt Creek	Santa Barbara	2010	2
Matilija Creek	Ventura	2010	4
San Antonio Creek	Ventura	2010	3
Lusardi Creek	San Diego	2011	8
Santa Ana River	San Bernardino	2011	1
Lower Santa Clara River	Ventura	2011	13
Warm Springs Creek	San Diego	2011	3
San Mateo Creek	San Diego	2012	34
Agua Hedionda Creek Trib1	San Diego	2013	3

Pilgrim Creek Trib2	San Diego	2013	1
Rancho Jamul Ecological Reserve	San Diego	2014	2
Aliso Canyon	Los Angeles	2015	3
Oak Valley Creek	Monterey	2015	14
Pine Valley Creek	San Diego	2015	41
Santa Maria Creek	San Diego	2016	1
Upper San Diego River	San Diego	2016	7
WF San Luis Rey River	San Diego	2016	11
Lower Santa Ysabel Creek	San Diego	2017	3
Murrieta Cr	San Diego	2017	16
100-Dam Reach Extra Habitat San Francisquito	Los Angeles	2018	1
Shady Canyon-Trib6	Los Angeles	2018	65
Arroyo Seco-Monterey	Monterey	2018	7
Carmel River	Monterey	2018	1
Las Gazas Creek	Monterey	2018	2
San Antonio River-Upper	Monterey	2018	1
North Fork San Antonio River	Monterey	2018	2
Middle Sweetwater River	San Diego	2018	17
Scholder Creek	San Diego	2018	2
Cockleburr Canyon	San Diego	2018	40
Middle Piru Creek-Bird	Ventura	2018	1

5.9 Conclusion

______Based on the data gathered from the literature we observe that *E. pallida* contains lower sightings and has a lower average than *E. marmorata.* Many of the sightings observed for E. pallida in the literature were observed to be less than 40.

Analysis of USGS data and literature data could only be done for *E. pallida*, since the USGS data only covered *E. pallida*. Sightings of *E. pallida* in both the literature and USGS data was small. On average, the number of WPT sighted at a single site from the data gathered from the USGS was less than 4 and for the information gathered from the literature it was less than 40.

In brief, our museum data, review of current literature sightings and USGS provides considerable insight into the historical and current demographics for both *E. pallida* and *E. marmorata*.

E. pallida

For *E. pallida*, we found that average male carapace length remained relatively stable over time while average female carapace length decreased over time. We also found a sex ratio shift from a relatively even male to female ratio to a male-biased ratio. Current sightings of *E.pallida* are lower when compared to historical trends and *E. marmorata* sightings.

E. marmorata

For *E. marmorata*, we found that male carapace length increased on average over time while female carapace length remained stagnant. We also found a relatively stagnant sex ratio for *E. marmorata* as it typically remained male-biased. Current sightings of *E.marmorata* have been lower but not as significant as *E.pallida.*

Section 6: Future Conditions

6.1 Population Viability Analysis

To model future projections of WPT populations, we utilized the program Vortex. Vortex is a computer model that simulates the complexities of a species population growth and how population growth can change with different internal and external variables. For each population simulated, specific demographic parameters are required. To the best of our ability, we utilized demographic parameters and mortality schedules for the population being simulated by extracting the parameters from published long-term studies. However, some parameters had to be estimated based on general knowledge of the species from past studies. In some cases parameters were estimated based of a population of the same species that was already the focus of a simulation. For example, after simulating an *E. pallida* population from Coyote Creek, we used many of the same parameters for another *E. pallida* population from Pine Valley Creek. Some variables were also constant throughout every population (Table 11). These variables were generally constant scenario settings such as our extinction definition that are important to understand for the results of our simulations. The following tables list both the variables and parameters used for the PVAs. Unless mentioned, all SD values were kept as the default values provided by Vortex. Each population was simulated as a single population which meant there were no metapopulation values including dispersal. To do this, the option to "run as population-based model" was selected. Multiple simulation inputs were not relevant to these PVAs and were kept without parameters including catastrophes, state variables, harvest, supplementation, and genetics. Inbreeding depression and density dependent reproduction were also not selected for these PVAs. The option to make offspring dependent on their dam for a set number of year was also not relevant and was not selected. Finally, all inputs for carrying capacity other than the carrying capacity itself were not selected or parameterized. This includes future change in carrying capacity and implementing carrying capacity based on a limit on some population variable other than population size. Carrying capacity was assumed to be twice the initial population size for all simulated populations since there was no indication any of the populations were at carrying capacity. Finally, we kept the default order of events in a Vortex year (Table 12).

Table 11. Constants for both *E. pallida* and *E. marmorata*

Variable	Parameter
Maximum Lifespan	45 Years (Holland 1994, p. 2-11)
Initial Sex Ratio	1:1[1]
Maximum Age of Reproduction	45 Years (Holland 1994, p. 2-11)[2]
Mate Monopolization	100%[3]
Number of Iterations	100
Number of Years	100

Duration of Each Year	365 Days
Definition of Extinction	One Sex Remains
Number of Populations	1
Reproductive System	Polygynous
Specify the distribution of number of offspring per female per brood	Use normal distribution
Initial Population Size	Use specified age distribution

[1]: Initial Sex Ratio is assumed as 1:1 even if population starts as male biased because incoming recruitment is assumed as 1:1 due to lack of evidence against sex ratio at birth being different from 1:1
[2]: Assumption of Maximum Age of Reproduction comes from assumption that WPTs can reproduce until death
[3]: 100% of males are in the breeding pool

Table 12. Order of events in a vortex year

EV (Environmental Variation)
Breed
Mortality
Age
Disperse
Harvest
Supplement
rCalc
K truncation
UpdateVars
Census

6.2 Viability Projections for *E. pallida*

6.2.1 PVA #1: Coyote Creek

Table 13. Coyote Creek Population Information

Age of First Offspring (Females and Males)	7 Years (Belli 2015, p. 69)
Maximum Number of Broods per Year	2 (Bury *et al.* 2012, p. 16)
Maximum Number of Progeny per Brood	13 (Holland 1994, p. 2-10)
Adult Females Breeding	39% (Belli 2015, p. 69)[1]
Distribution of Broods Per Year: 0 Broods	0%[2]
Distribution of Broods Per Year: 1 Brood	62% (Belli 2015, pp. 69-70)[3]
Distribution of Broods Per Year: 2 Broods	38% (Belli 2015, pp. 69-70)[3]
Mean Number of Offspring per Brood	7 (Holland 1994, p. 2-10)[4]
Mortality Rate: Age 0-1 (Both Females and Males)	91.25% (Holland 1994, p. 2-11)[5]
Mortality Rate: Age 1-2 (Both Females and Males)	87.5% (Holland 1994, p. 2-11)
Mortality Rate: Age 2-3 (Both Females and Males)	87.5% (Holland 1994, p. 2-11)
Mortality Rate: Age 3-4 (Both Females and Males)	87.5% (Holland 1994, p. 2-11)
Mortality Rate: Age 4-5 (Both Females and Males)	82.5% (Holland 1994, p. 2-11)[6]
Mortality Rate: Age 5-6 (Both Females and Males)	77.5% (Holland 1994, p. 2-11)[6]
Mortality Rate: Age 6-7 (Both Females and Males)	72.5% (Holland 1994, p. 2-11)[6]
Mortality Rate: After Age 7 (Both Females and Males)	4% (Holland 1994, p. 2-11)
Initial Population Size	212, Juveniles were 14.5% of the population (Belli 2015, p. 41)[7]
Carrying Capacity	424[8]

[1]: Calculated by combining data from 2011 and 2012; 13 gravid turtles /33 total turtles = 0.39. The percentage may be biased because turtles were caught opportunistically and many were only checked once to determine if gravid.

[2]: It was assumed that there was 0% of 0 broods because lack of laying any eggs was included in the % of adult females breeding

[3]: The distribution of clutches was found by calculating the proportion of double clutches (5 double clutches found / 13 gravid turtles = 0.38). The proportion of single clutches was what remained

[4]: The mean offspring per brood is the mean of the range of 1-13 offspring possible from Holland 1994
[5]: The mortality rate from age 0-1 was calculated by using Holland's 70% hatching success and the mortality rate of 87.5% for juveniles age 1-3. The overall survival rate is 0.7 x 0.125 = 0.0875. 1 - 0.0875 = 0.9125 = mortality rate.
[6]: The mortality rate after age 3-4 was decreased by 5, an arbitrary but reasonable number chosen in order to create a stable and incremental decrease, each year because Holland mentions the mortality rates should decrease after age 3-4 but does not give new mortality rate numbers until they are mature adults.
[7]: The initial population size used in Vortex was slightly higher than what the literature reports because Vortex requires whole numbers to be entered and by breaking the population down into year classes our closest estimates with whole numbers resulted in a slight increase in population size
[8]: Carrying capacity is assumed to be twice the initial population size since there is no indication the population is at carrying capacity.

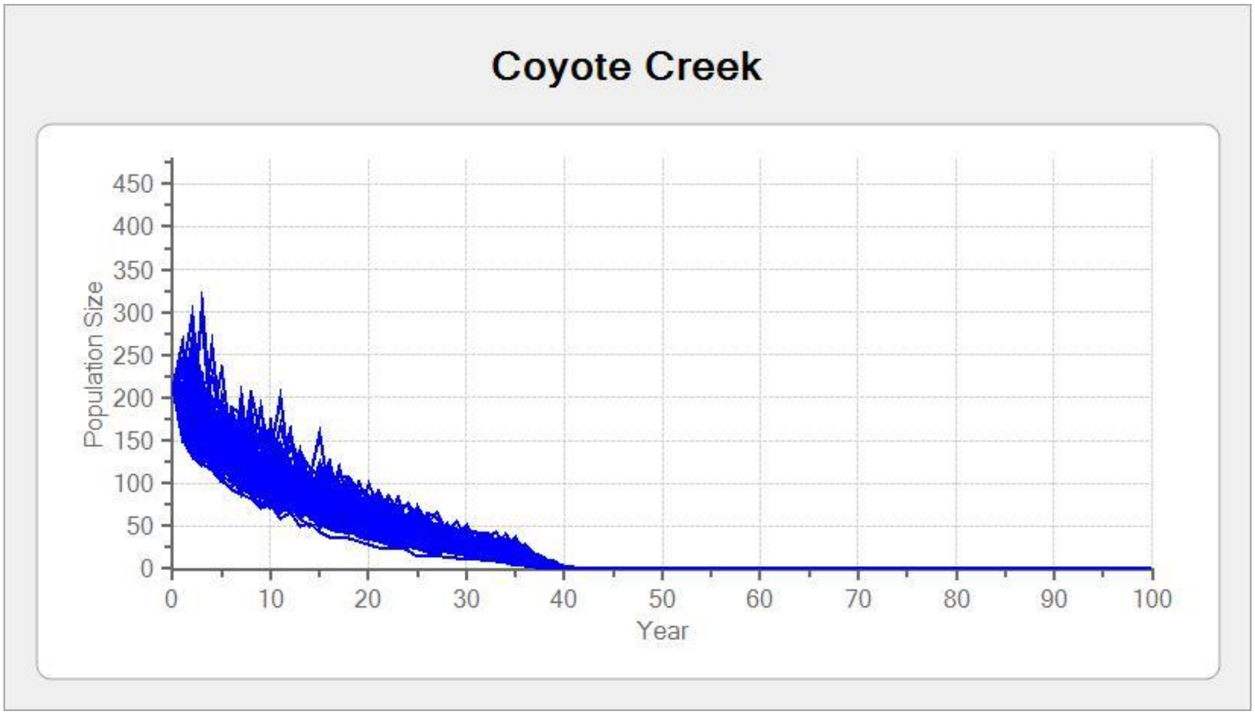


Figure 39. PVA graph for Coyote Creek
Results: r = -0.101, SD(r)=0.239, Pr.extinction = 1.00, N = 0

Coyote Creek is in Santa Clara County, which is in the northern portion of the range of *E. pallida*. Our model predicts that the population will go extinct within 40 years with the known parameters of the population. This is equivalent to the lifetime of all the original WPTs in the population which implies that recruitment is so low, and mortality is so high, that essentially no or very few animals survive to maturity.

6.2.2 PVA #2: Pine Valley Creek

Table 14. Pine Valley Creek Population Information

Age of First Offspring (Females and Males)	7 Years (Belli 2015, p. 69)

Maximum Number of Broods per Year	2 (Bury *et al.* 2012, p. 16)
Maximum Number of Progeny per Brood	13 (Holland 1994, p. 2-10)
Adult Females Breeding	39% (Belli 2015, p. 69)[1]
Distribution of Broods Per Year: 0 Broods	0%[2]
Distribution of Broods Per Year: 1 Brood	62% (Belli 2015, pp. 69-70)[3]
Distribution of Broods Per Year: 2 Broods	38% (Belli 2015, pp. 69-70)[3]
Mean Number of Offspring per Brood	7 (Holland 1994, p. 2-10)[4]
Mortality Rate: Age 0-1 (Both Females and Males)	91.25% (Holland 1994, p. 2-11)[5]
Mortality Rate: Age 1-2 (Both Females and Males)	87.5% (Holland 1994, p. 2-11)
Mortality Rate: Age 2-3 (Both Females and Males)	87.5% (Holland 1994, p. 2-11)
Mortality Rate: Age 3-4 (Both Females and Males)	87.5% (Holland 1994, p. 2-11)
Mortality Rate: Age 4-5 (Both Females and Males)	82.5% (Holland 1994, p. 2-11)[6]
Mortality Rate: Age 5-6 (Both Females and Males)	77.5% (Holland 1994, p. 2-11)[6]
Mortality Rate: Age 6-7 (Both Females and Males)	72.5% (Holland 1994, p. 2-11)[6]
Mortality Rate: After Age 7 (Both Females and Males)	4% (Holland 1994, p. 2-11)
Initial Population Size	454, Juveniles are 25% of the population (Brown *et al.* 2015, p. 24)[7]
Carrying Capacity	908[8]

[1]: Calculated by combining data from 2011 and 2012; 13 gravid turtles /33 total turtles = 0.39. The percentage may be biased because turtles were caught opportunistically and many were only checked once to determine if gravid.
[2]: It was assumed that there was 0% of 0 broods because lack of laying any eggs was included in the % of adult females breeding
[3]: The distribution of clutches was found by calculating the proportion of double clutches (5 double clutches found / 13 gravid turtles = 0.38). The proportion of single clutches was what remained
[4]: The mean offspring per brood is the mean of the range of 1-13 offspring possible from Holland 1994
[5]: The mortality rate from age 0-1 was calculated by using Holland's 70% hatching success and the mortality rate of 87.5% for juveniles age 1-3. The overall survival rate is 0.7 x 0.125 = 0.0875. 1 - 0.0875 = 0.9125 = mortality rate.

[6]: The mortality rate after age 3-4 was decreased by 5 each year because Holland mentions the mortality rates should decrease after age 3-4 but does not give new mortality rate numbers until they are mature adults.
[7]: The initial population size used in Vortex was slightly higher than what the literature reports because Vortex requires whole numbers to be entered and by breaking the population down into year classes our closest estimates with whole numbers resulted in a slight increase in population size
[8]: Carrying capacity is assumed to be twice the initial population size since there is no indication the population is at carrying capacity.

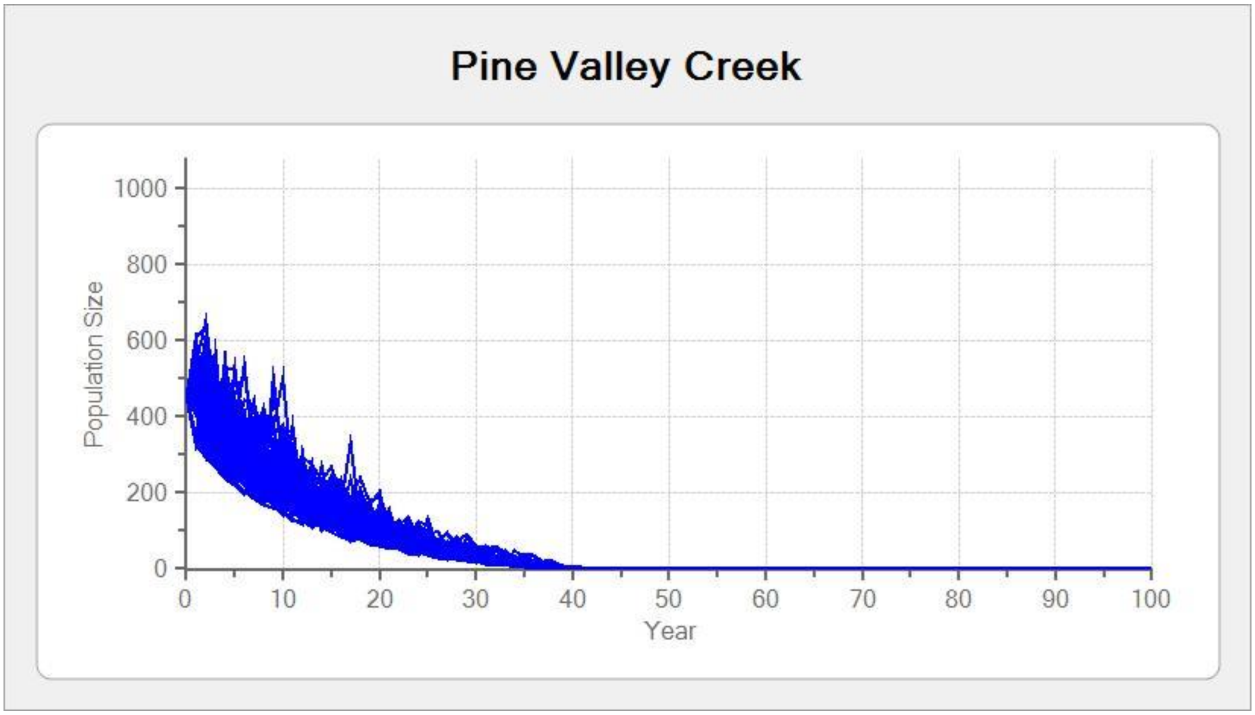


Figure 40. PVA graph for Pine Valley Creek
Results: r = -0.123, SD(r)=0.286, Pr.extinction = 1.00, N = 0

Pine Valley Creek is in San Diego County which is in the southern portion of the range of *E. pallida*. The parameters are the same as the Coyote Creek population except the age structure which comes from Pine Valley Creek. The Coyote Creek parameters are used because it is also an *E. pallida* population so without knowing these parameters from Pine Valley Creek, Coyote Creek was deemed the most population most similar. As with Coyote Creek, this population goes extinct within 40 years with the known parameters of the population. This is equivalent to the lifetime of all the original WPTs in the population which means recruitment must be too low to ensure this population remains viable given these best-estimate population parameters.

6.2.3 Conclusion on E. pallida Future Conditions

Both *E. pallida* populations we simulated showed a low probability of persistence as both populations went extinct within 40 years. This is a result of the low recruitment occurring in the populations due to the simulation utilizing Holland's widely cited values for mortality and hatching success in WPTs. The Coyote Creek study which formed the basis of our Coyote Creek PVA directly cites Holland's mortality rates. Despite being 15 years old, Holland's mortality rates are the predominantly cited mortality rates throughout *E. pallida* studies. Unfortunately, despite being widely cited, Holland's mortality rate is based on unpublished data. The length and location of the data collection are unknown, making it difficult to evaluate the confidence in the mortality rates for different populations. The rates may be accurate, but most studies since 1994 have not attempted to calculate mortality rates in demographic studies to confirm the validity of Holland's mortality rates for their specific population. Holland's hatchling mortality rate is a main cause for the apparent lack of recruitment for *E. pallida*. Here, the hatchling age class refers to WPTs aged 0-1 years. The hatchling mortality rate combines Holland's hatching success rate and 1st year survivorship. Yet, there may be uncertainty in this rate as it is very difficult to estimate. WPT researchers have cautioned against estimating population trends until more data is reported on survivorship of young turtles (Bury *et al*. 2012, p. 19). The reason for the uncertainty is primarily because young turtles are small, cryptic, and stationary (Bury *et al*. 2012, p. 19). These characteristics of young turtles can result in underestimating their numbers and overestimating the mortality rate. With this in mind, uncertainty in the mortality rates of the early age classes may cause uncertainty in the simulated results of our PVAs. The validity of extinction probabilities depends on the quality and appropriateness of parameter values (Reed *et al.* 2002, p. 14). With there being uncertainty in our mortality parameters, it is recommended that modeling be used to find a focus of further research (Reed *et al.* 2002, p.15). In our case, mortality rates are where future research must be done in order to create models with higher confidence. However, if our parameters are correct, *E. pallida* is at a high risk of extinction even without any environmental catastrophes or external pressures. Our two populations cover both northern and southern portions of the range of *E. pallida* which emphasizes the widespread risk of extinction. Furthermore, with appropriate parameter values, PVAs have been found to be very accurate as long-term population studies show the predicted outcomes from PVAs including risk of decline and population size predictions (Brook *et al.* 2000, entire). It must be emphasized that researchers argue that this accuracy will only occur if data is extensive and reliable (Coulson *et al.* 2001, entire). Long-term data may be limited for the *E. pallida* populations we simulated which may increase the uncertainty in our results. Limited information on hatchling survival is of particular cause for uncertainty as hatchling survival is of vital importance to the survival of *E. pallida* populations.

6.3 Viability Projections for *E. marmorata*

6.3.1 PVA #3: Goose Lake (Germano 2016)

Table 15. Goose Lake Population Information

Age of First Offspring Female	4 Years (Germano 2016, p. 667)[1]
Age of First Offspring Male	4 Years (Germano 2016, p. 667)[1]
Maximum Number of Broods per Year	3 (Bury *et al.* 2012, p. 16)
Maximum Number of Progeny per Brood	11 (Germano 2016, p. 668)

Distribution of Broods Per Year: 0 Broods	0%[2]
Distribution of Broods Per Year: 1 Brood	94% (Germano 2016, p. 668)[3]
Distribution of Broods Per Year: 2 Broods	5% (Germano 2016, p. 668)[3]
Distribution of Broods Per Year: 3 Broods	1% (Germano 2016, p. 668)[3]
Mean Number of Offspring per Brood	7 (Germano 2016, p. 668); SD = 1.6 (Germano 2016, p. 668)[4]
Mortality Rate: Age 0-1	49% (Holland 1994, p. 2-11; Germano 2016, p. 670)[5]
Mortality Rate: Age 1-2	26.9% (Germano 2016, p. 670)
Mortality Rate: Age 2-3	16.2% (Germano 2016, p. 670)
Mortality Rate: Age 3-4	16.2% (Germano 2016, p. 670)
Mortality Rate: Female After 4	26.9% (Germano 2016, p. 670)
Mortality Rate: Male After 4	18.7% (Germano 2016, p. 670)
Initial Population Size	625, Juveniles are 41.5% of the population (Germano 2016, p. 667)[6]
Carrying Capacity	1250[7]

[1]: We utilized Germano's graph of carapace length vs. age to determine the age of sexual maturity with 120 mm being the carapace length of maturity.

[2]: It was assumed that there was 0% of 0 broods because lack of laying any eggs was included in the % of adult females breeding

[3]: The proportion of double broods was calculated from the 6 second clutches divided by total clutches which was 113, the remaining proportion was a single brood with 1% going to third broods because it is possible just highly unlikely.

[4]: SD was calculated from the standard error (SE) of 0.15 provided by Germano. The equation of SE = (SD/ square root of n) was utilized. Thus, 0.15 = (SD/ square root of 113).

[5]: The mortality rate from age 0-1 was calculated by using Holland's 70% hatching success and Germano's mortality rate of 26.9% for juveniles age 1-3. The overall survival rate is 0.7 x 0.731 = 051. 1 - 0.51 = 0.49 = mortality rate.

[6]: The initial population size used in Vortex was slightly higher than what the literature reports because Vortex requires whole numbers to be entered and by breaking the population down into year classes our closest estimates with whole numbers resulted in a slight increase in population size

[7]: Carrying capacity is assumed to be twice the initial population size since there is no indication the population is at carrying capacity.

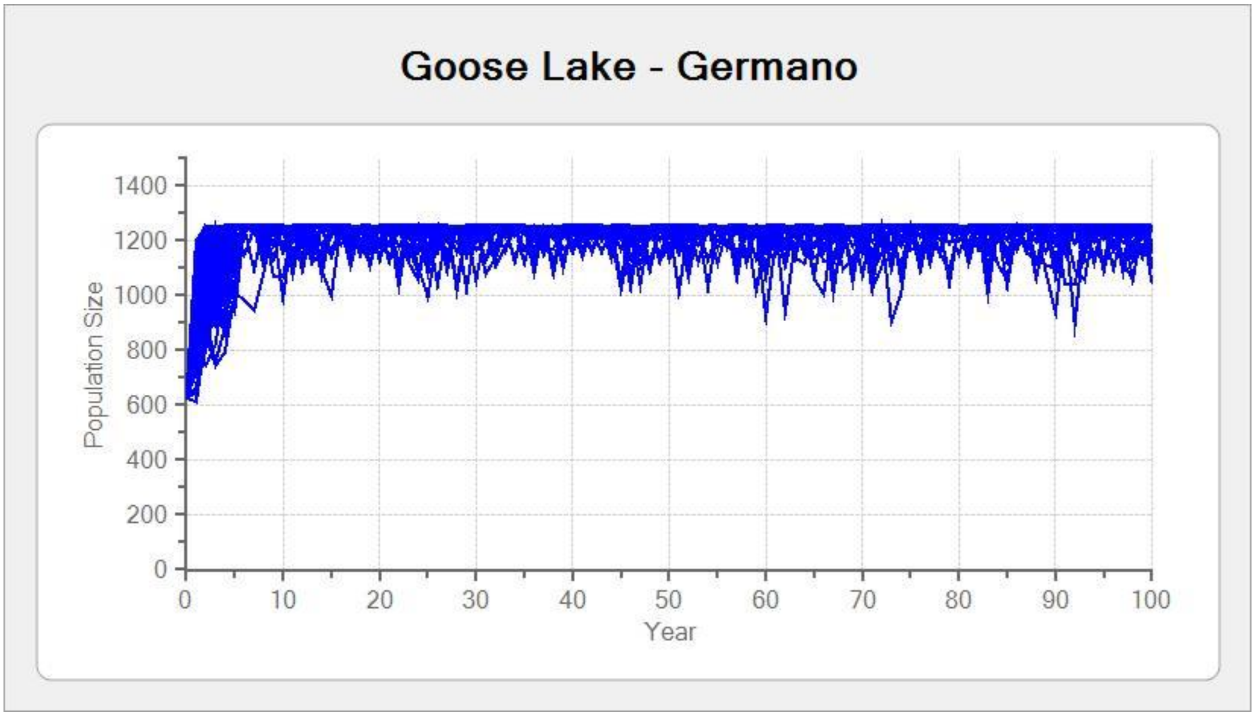


Figure 41. PVA graph for Goose Lake
Results: r = 0.175, SD(r) = 0.130, Percent Extinction = 0, N = 1191

Goose Lake is located in west-central Kern County which is in the southwestern portion of the range of *E. marmorata*. This area of Kern County overlaps with the range of *E. pallida*, thus there is a possibility of mixing in this population or inclusion of *E. pallida*. However, based on Germano's study and the species division by county method of our assessment, Goose Lake is considered a population of *E. marmorata*. The population is stable and persists with a 0% extinction probability over the course of 100 years. This suggests that the population is robust and healthy as was previously suggested by Germano (2016).

6.3.2 PVA #4: Goose Lake (Holland-Germano Hybrid)

Table 16. Goose Lake (Holland-Germano Hybrid) Population Information

Age of First Offspring Female	4 Years (Germano 2016, p. 667)[1]
Age of First Offspring Male	4 Years (Germano 2016, p. 667)[1]
Maximum Number of Broods per Year	3 (Bury *et al.* 2012, p. 16)
Maximum Number of Progeny per Brood	11 (Germano 2016, p. 668)
Distribution of Broods Per Year: 0 Broods	0%[2]
Distribution of Broods Per Year: 1 Brood	94% (Germano 2016, p. 668)[3]
Distribution of Broods Per Year: 2 Broods	5% (Germano 2016, p. 668)[3]

Distribution of Broods Per Year: 3 Broods	1% (Germano 2016, p. 668)[3]
Mean Number of Offspring per Brood	7 (Germano 2016, p. 668); SD = 1.6 (Germano 2016, p. 668)[4]
Mortality Rate: Age 0-1	91.25% (Holland 1994, p. 2-11)[5]
Mortality Rate: Age 1-2	26.9% (Germano 2016, p. 670)
Mortality Rate: Age 2-3	16.2% (Germano 2016, p. 670)
Mortality Rate: Age 3-4	16.2% (Germano 2016, p. 670)
Mortality Rate: Female After 4	26.9% (Germano 2016, p. 670)
Mortality Rate: Male After 4	18.7% (Germano 2016, p. 670)
Initial Population Size	625, Juveniles are 41.5% of the population (Germano 2016, p. 667)[6]
Carrying Capacity	1250[7]

[1]: We utilized Germano's graph of carapace length vs. age to determine the age of sexual maturity with 120 mm being the carapace length of maturity.

[2]: It was assumed that there was 0% of 0 broods because lack of laying any eggs was included in the % of adult females breeding

[3]: The proportion of double broods was calculated from the 6 second clutches divided by total clutches which was 113, the remaining proportion was a single brood with 1% going to third broods because it is possible just highly unlikely.

[4]: SD was calculated from the SE of 0.15 provided by Germano. The equation of SE = (SD/ square root of n) was utilized. Thus, 0.15 = (SD/ square root of 113).

[5]: The mortality rate from age 0-1 was calculated by using Holland's 70% hatching success and the mortality rate of 87.5% for juveniles age 1-3. The overall survival rate is 0.7 x 0.125 = 0.0875. 1 - 0.0875 = 0.9125 = mortality rate.

[6]: The initial population size used in Vortex was slightly higher than what the literature reports because Vortex requires whole numbers to be entered and by breaking the population down into year classes our closest estimates with whole numbers resulted in a slight increase in population size

[7]: Carrying capacity is assumed to be twice the initial population size since there is no indication the population is at carrying capacity.

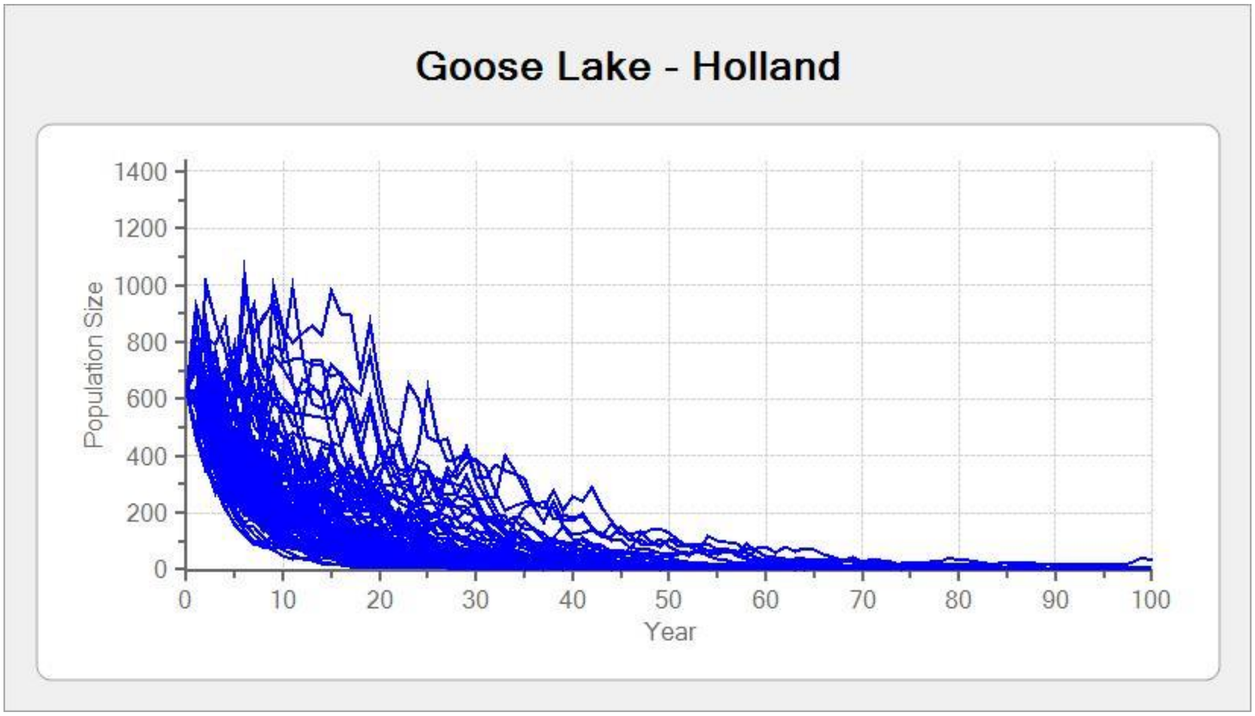


Figure 42. PVA graph for Goose Lake (Holland-Germano Hybrid)
Results: r = -0.103, SD(r) = 0.223, Pr. Extinction = 1.00, N = 0

When the hatchling mortality rate is changed to the highly cited Holland mortality rates, the Goose lake population goes extinct. This is a stark change from the robust population resulting from Germano's mortality rates. Most iterations of the population goes extinct within 40 years. This is equivalent to the lifetime of all the original WPTs in the population which means recruitment must be too low to keep the population extant. Because the only variable changed was the hatchling mortality rate, it is clear the hatchling mortality rate is a critical variable to species persistence.

6.3.3 Goose Lake Sensitivity Analysis

Table 17. Goose Lake Sensitivity Analysis of Juvenile Mortality Rates

Mortality Rates: Age 0-1, Male & Female	Percent Extinction
50%	0.00
55%	0.00
60%	0.00
65%	0.00
70%	0.00
75%	0.00

80%	0.00
82%	0.00
83%	0.01
84%	0.04
85%	0.15
86%	0.34
87%	0.52
88%	0.78
89%	0.88
90%	0.93
91%	0.99
92%	1.00

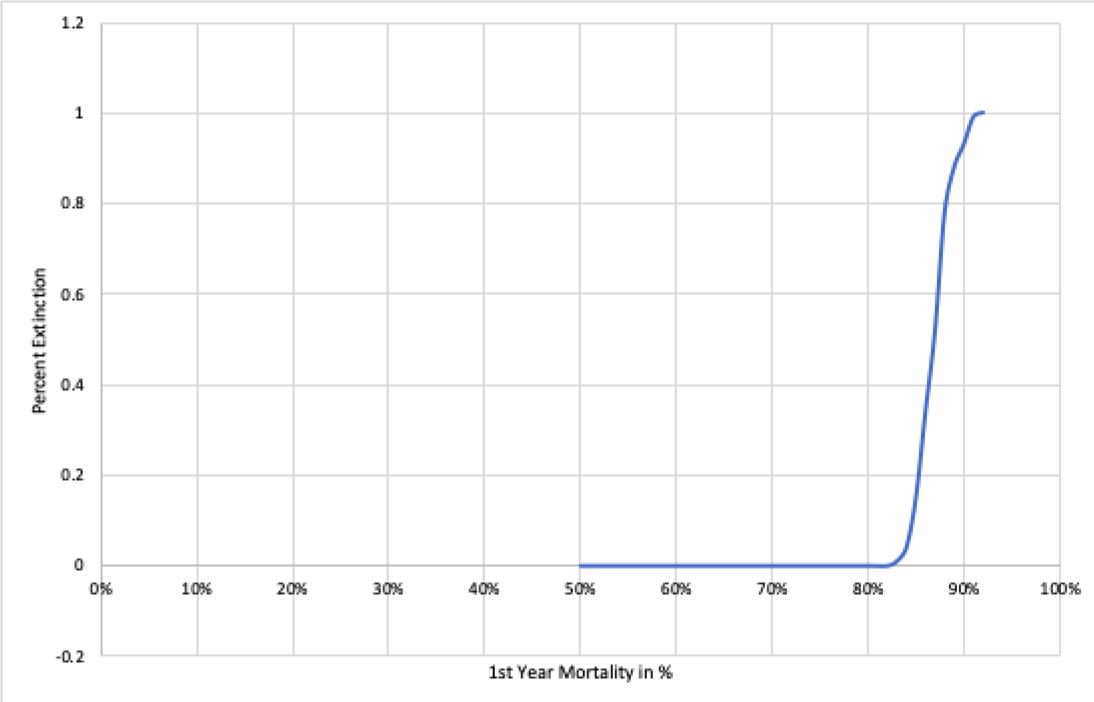


Figure 43. Goose Lake Sensitivity Analysis of Juvenile Mortality Rates

Upon conducting the PVA's for Goose Lake with Germano's and Holland's different mortality rates for WPTs aged 0-1 year, we found very different results. Using Germanos 49% mortality rate, the population for Goose Lake appeared stable with a 0% chance of extinction. When we used Holland's 91.25% mortality rate, the population for Goose Lake was not stable and had a 100% extinction probability. We conducted a sensitivity analysis to explore the effects of an increasing mortality rate at Goose Lake. Upon conducting the sensitivity analysis, we found that extinction probability began to rise when juvenile mortality reached 83%. Here there was a 1% chance of extinction. From there, the probability of extinction continued to rise with the inflection point occurring between 85% and 88% mortality. Extinction probability begins to rapidly rise following 85% mortality and levels off after 88% mortality. 100% extinction probability is reached between 91% and 92% mortality. From this analysis, we conclude that a population can be relatively stable, without any other risk factors, under 87% mortality for WPT's aged 0-1 and that survival of WPTs aged 0-1 is extremely important to the survival of the population.

Table 18. Goose Lake Sensitivity Analysis of Adult Female Mortality Rates

Mortality Rates: Adult Females (Over age 4)	Percent Extinction

30%	0.00
35%	0.00
40%	0.00
45%	0.00
50%	0.00
55%	0.00
60%	0.00
65%	0.00
70%	0.00
75%	0.00
80%	0.00
82%	0.01
84%	0.02
86%	0.07
88%	0.08
90%	0.17
92%	0.34
94%	0.37

96%	0.68
98%	0.75
100%	0.79

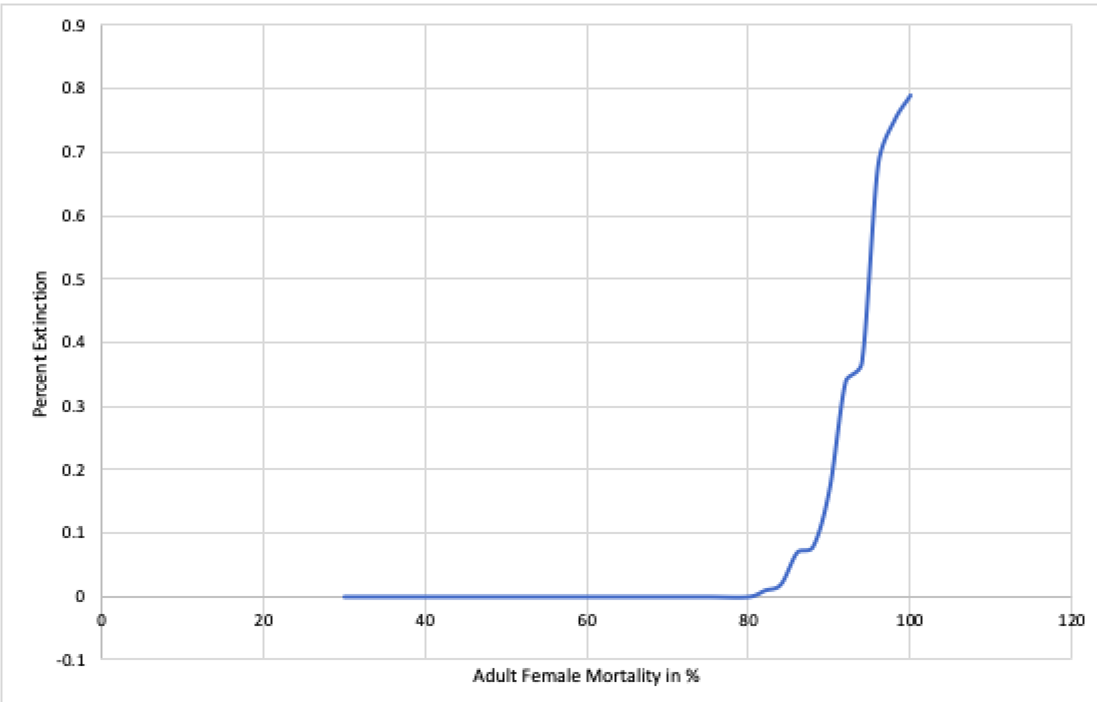


Figure 44. Goose Lake Sensitivity Analysis of Adult Female Mortality Rates

Following the sensitivity analysis on the mortality rate for WPT's aged 0-1, we conducted a similar analysis for the mortality of adult female WPTs at Goose Lake. For this analysis we used a juvenile mortality rate of 91.25% (Holland 1994, p. 2-11) for ages 0-1. Adult female mortality has traditionally been thought of as a parameter that defines the stability of a population. It has been suggested that road mortality on females is a major risk for WPTs and an important reason for their decline (Madden-Smith *et al.* 2005, p. 47). However, upon conducting the sensitivity analysis for adult females, we found that even with an extremely high mortality rate after age 4, populations are stable. There was essentially no chance for extinction until the adult female mortality rate reached 82%. Even when the mortality rate of adult females reached 100%, the population only reached a 79% probability of extinction. This is because mortality occurs after reproduction in a Vortex year which allows WPT females to reproduce once when they become adults and then die. This was confirmed by changing the order of mortality and reproduction to have mortality occur first. With the shift, the population went extinct within 20 years when the mortality rate of adult females was at 100%. From our sensitivity analysis on adult female mortality, we conclude that adult female mortality is not a major factor in the survival or stability of a WPT population. This also suggests that survival of younger WPTs, especially those aged 0-1, is more important than the survival of adult females for the stability of a population.

6.3.4 PVA #5: Russian River: E. marmorata

Table 19. Russian River Population Information

Age of First Offspring Female	6 Years (Cook 2018, p. 2)
Age of First Offspring Male	6 Years (Cook 2018, p. 2)
Maximum Number of Broods per Year	3 (Bury *et al.* 2012, p. 16)
Maximum Number of Progeny per Brood	11 (Germano 2016, p. 668)
Distribution of Broods Per Year: 0 Broods	0%[2]
Distribution of Broods Per Year: 1 Brood	94% (Germano 2016, p. 668)[3]
Distribution of Broods Per Year: 2 Broods	5% (Germano 2016, p. 668)[3]
Distribution of Broods Per Year: 3 Broods	1% (Germano 2016, p. 668)[3]
Mean Number of Offspring per Brood	7 (Germano 2016, p. 668); SD = 1.6 (Germano 2016, p. 668)[4]
Mortality Rate: Age 0-1 (Both Females and Males)	91.25% (Holland 1994, p. 2-11)[5]
Mortality Rate: Age 1-2 (Both Females and Males)	87.5% (Holland 1994, p. 2-11)
Mortality Rate: Age 2-3 (Both Females and Males)	87.5% (Holland 1994, p. 2-11)
Mortality Rate: Age 3-4 (Both Females and Males)	87.5% (Holland 1994, p. 2-11)
Mortality Rate: Age 4-5 (Both Females and Males)	82.5% (Holland 1994, p. 2-11)[6]
Mortality Rate: Age 5-6 (Both Females and Males)	77.5% (Holland 1994, p. 2-11)[6]
Mortality Rate: After Age 6 (Both Females and	4% (Holland 1994, p. 2-11)

Males)	
Initial Population Size	242, Juveniles are 15.8% of the population (Cook 2018, p. 2)[7]
Carrying Capacity	484[8]

[1]: We utilized Germano's graph of carapace length vs. age to determine the age of sexual maturity with 120 mm being the carapace length of maturity.

[2]: It was assumed that there was 0% of 0 broods because lack of laying any eggs was included in the % of adult females breeding

[3]: The proportion of double broods was calculated from the 6 second clutches divided by total clutches which was 113, the remaining proportion was a single brood with 1% going to third broods because it is possible just highly unlikely.

[4]: SD was calculated from the SE of 0.15 provided by Germano. The equation of SE = (SD/ square root of n) was utilized. Thus, 0.15 = (SD/ square root of 113).

[5]: The mortality rate from age 0-1 was calculated by using Holland's 70% hatching success and the mortality rate of 87.5% for juveniles age 1-3. The overall survival rate is 0.7 x 0.125 = 0.0875. 1 - 0.0875 = 0.9125 = mortality rate.

[6]: The mortality rate after age 3-4 was decreased by 5 each year because Holland mentions the mortality rates should decrease after age 3-4 but does not give new mortality rate numbers until they are mature adults.

[7]: The initial population size used in Vortex was slightly higher than what the literature reports because Vortex requires whole numbers to be entered and by breaking the population down into year classes our closest estimates with whole numbers resulted in a slight increase in population size

[8]: Carrying capacity is assumed to be twice the initial population size since there is no indication the population is at carrying capacity.

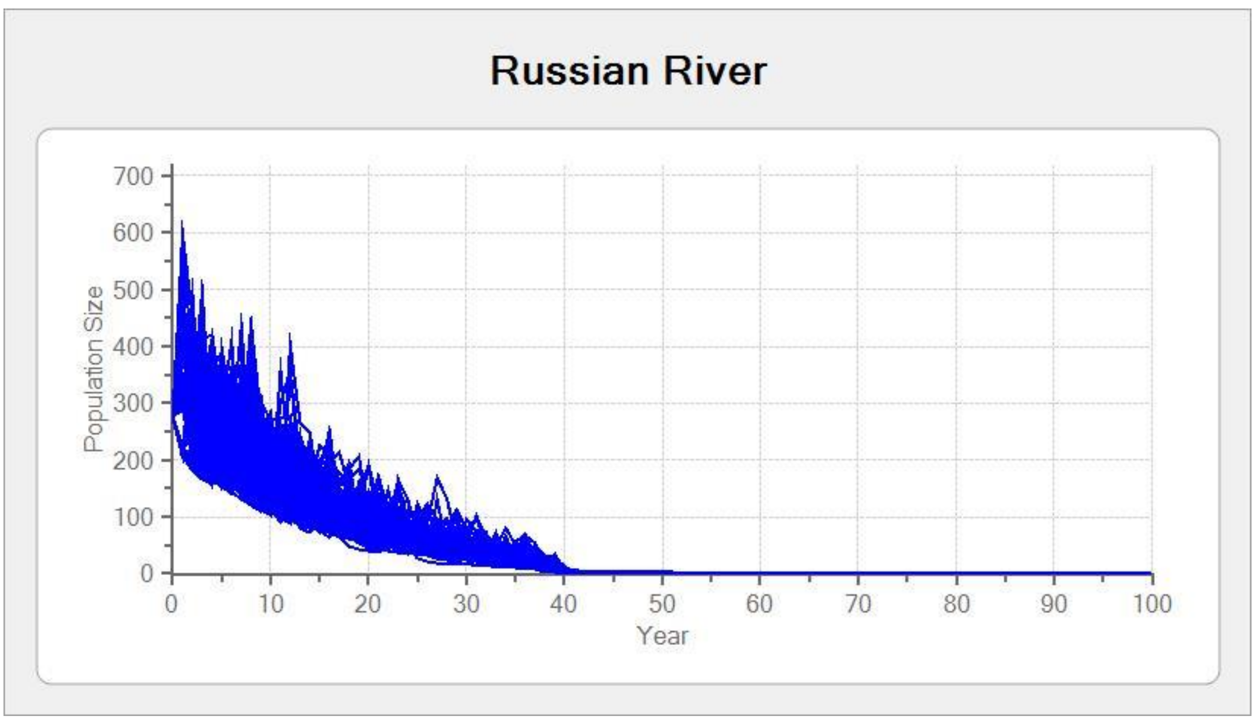


Figure 45. PVA graph for Russian River

Results: r = -0.101, SD(r)=0.369, Pr.extinction = 1.00, N = 0

The Russian River is in Sonoma County in the south-coastal portion of the range of *E. marmorata.* To conduct this PVA, we not only included the aforementioned constant values, but also made use of many of the parameters used for Goose Lake. Goose Lake is in the southern San Joaquin valley desert and the Russian River in the moist inner coast range of Sonoma County, and this may mean that some of these parameter values are not directly transferable. However, they represent our best estimates for *E. marmorata* at this time. This included the brood progeny and distribution data, as well as the mortality rates used for the Germano-Holland PVA model. The data specific to the Russian River population was the age at which males and females are able to produce offspring, the age structure and the initial population size. Carrying capacity for the population was set to be double the initial population size. Using these data, we ran the model and found that the population had a 100% extinction probability within 100 years. Most iterations of the population goes extinct within 40 years.This is equivalent to the lifetime of all the original WPTs in the population which means recruitment must be too low to keep the population viable. Because the only variable changed was the hatchling mortality rate, it is clear the hatchling mortality rate is a critical variable to species.

6.3.5 Conclusion for E. marmorata Future Conditions

Our PVAs based on the best estimates for population demography and mortality schedules and sensitivity analyses conducted for *E. marmorata*, we believe the high rates of extinction for the populations are due to the high mortality rate estimates for hatchlings. Again, hatchlings are WPTs in the 0-1 year age class. Regardless of the population's age structure or initial size, when the Holland-based hatchling mortality rate is used in a PVA, the population experiences a 100% extinction probability within 100 years, and often much sooner. According to this mortality parameter, there is not sufficient recruitment to keep any population relatively stable with this high initial mortality rate. However, upon conducting a PVA for Goose lake with the hatchling mortality rate from Germano (49%), the population had a 0% probability of extinction. This suggests that a lower hatchling mortality rate can lead to a stable population. This is supported by the Russian River PVA where all parameters, including Hollands death rates, were kept from the Goose Lake PVA except the age structure, initial population size, and age at which the turtles begin breeding. Even with these changes to age structure, initial population size, and the age at which the turtles begin breeding the PVA still predicted 100% extinction probability within 100 years, showing that these are not the variables leading to extirpation. It was only when hatchling mortality was reduced that the populations began to survive. This again suggests that hatchling mortality rates are extremely important in the survival and stability of populations of *E. marmorata* (Hays *et al.* 1999, p. 11).

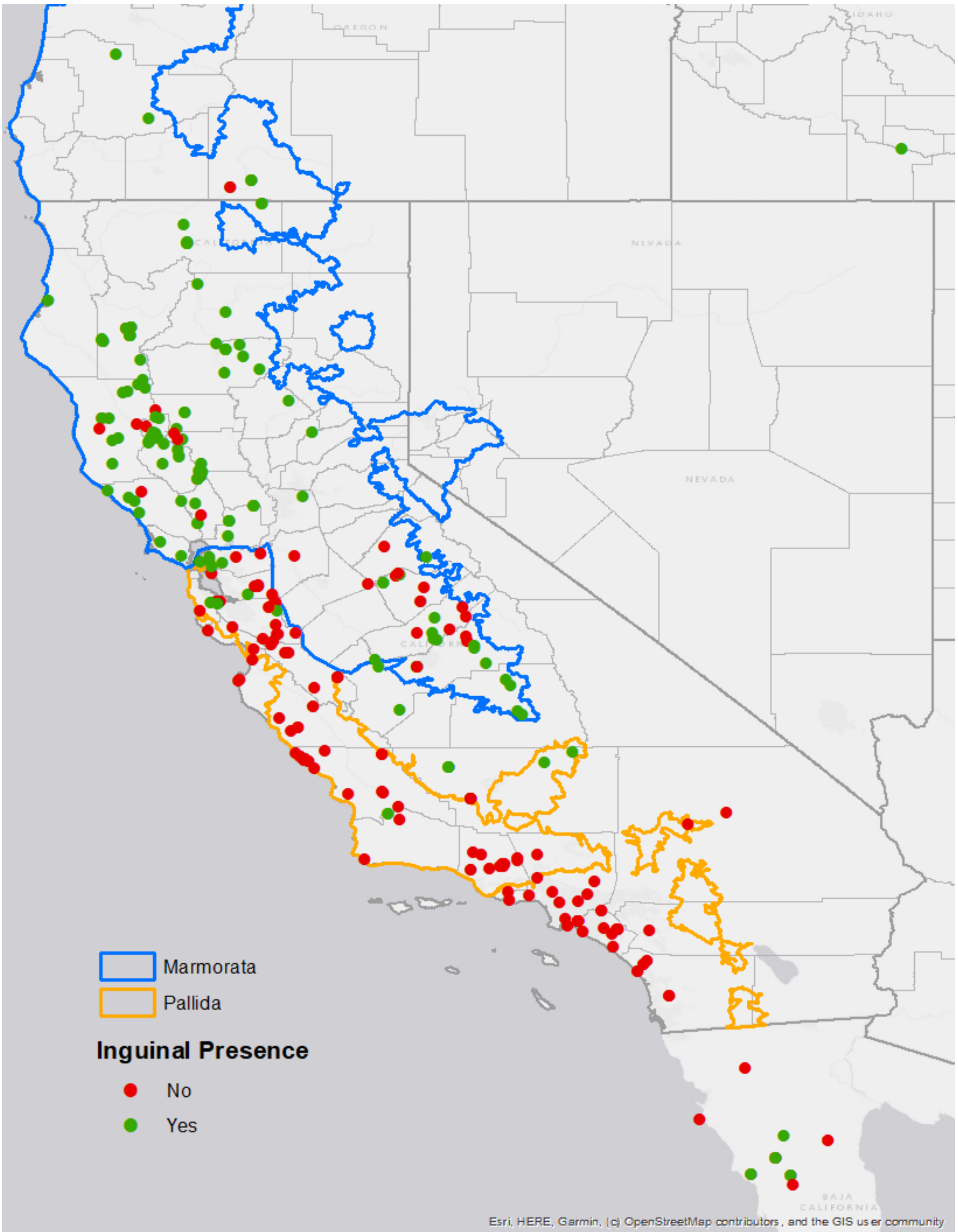
Section 7: Species Risk Conclusions

Both *E. pallida* and *E. marmorata* are facing declines across their historic range. The question is whether populations of these species will face extirpation in the future. Our analysis of historical and current trends indicate both species have seen decreased recruitment. Highlighting the importance of this loss of recruitment, our PVAs show that hatchling and juvenile recruitment are critical to population viability. If our parameters are correct, populations without high recruitment may go extinct within 40 years and we may be seeing the last generation of WPTs for many populations. Our analysis of historical and current trends also indicate that both species have become male-biased over time which may further impair recruitment and the viability of a population, especially if the number of females continues to decrease within a population. Assessing the most current number of WPT sightings for populations of both species indicates that *E. pallida* has very low numbers. However, *E. marmorata* has a higher number of sightings in northern California, which indicates that they may be doing better within that region. Finally, our risk assessment suggests the decline of both species may be further exacerbated by a variety of risks. In particular, *E. pallida* faces more severe risks which are more difficult to manage, such as drought and flood.

Based on our research and evidence, *E. pallida* and *E. marmorata* have declined significantly and appear to be on course for further declines. The majority of our research points toward lack of recruitment being a primary reason for population decline. While adult survival and WPT longevity may allow for the persistence of a population, the lack of recruitment will eventually lead to the extirpation of many populations. Although there are many other factors for the decline of the WPT, the lack of younger individuals may be the most important. However, we came across some healthier and more robust populations of WPTs that have the potential to be utilized as a model for conservation efforts. From our research and personal experience, the healthiest WPT populations occur in areas protected from many of the manageable risks we assessed, particularly protection from non-native juvenile predators, invasive competitors, and vehicle mortality. These populations may hold the key to the survival of the WPT.

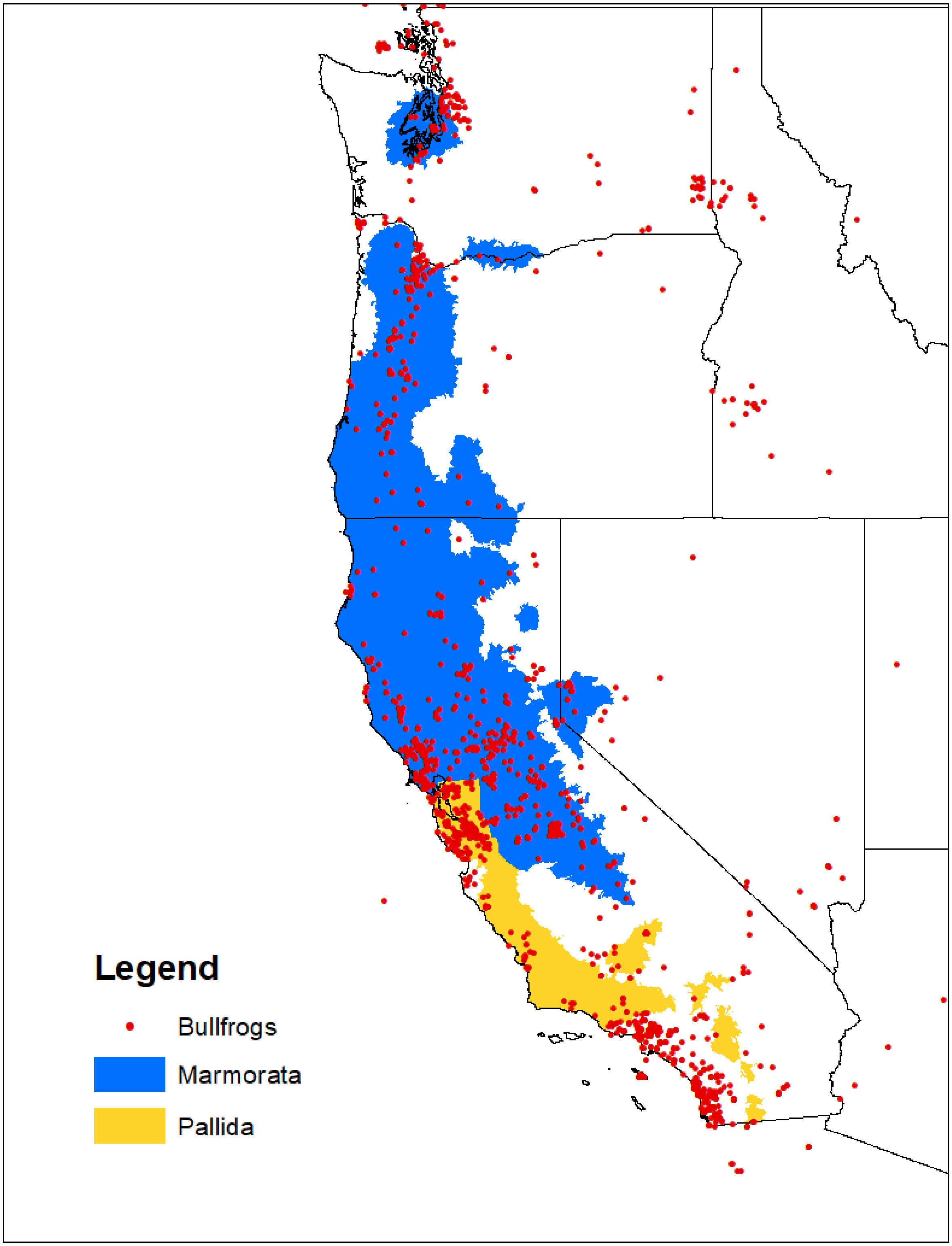
While the purpose of this assessment was not to argue for the listing of the WPT under the ESA, it is hard to ignore the fact that this species is in danger of true decline. This paper synthesises novel data from the field and museums, foundational WPT literature from the past, the most recent literature on the WPT, and population viability analysis into one cohesive report that gives insight into the current state of both *E. pallida* and *E. marmorata*. Although we will not formulate a decision for the WPT under the ESA, it is hard to argue for the WPT not to receive additional protections if it is to persist for future generations.

Appendix A: Inguinal Presence and Absence



Inguinal presence and absence in museum specimens caught between 1892-2005. (Source: U.S. Geological Survey - Gap Analysis Project, 2017, Western Pond Turtle).

Appendix B: Spatial Risk Assessment



Bullfrog sightings from iNaturalist throughout the WPT's range.

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