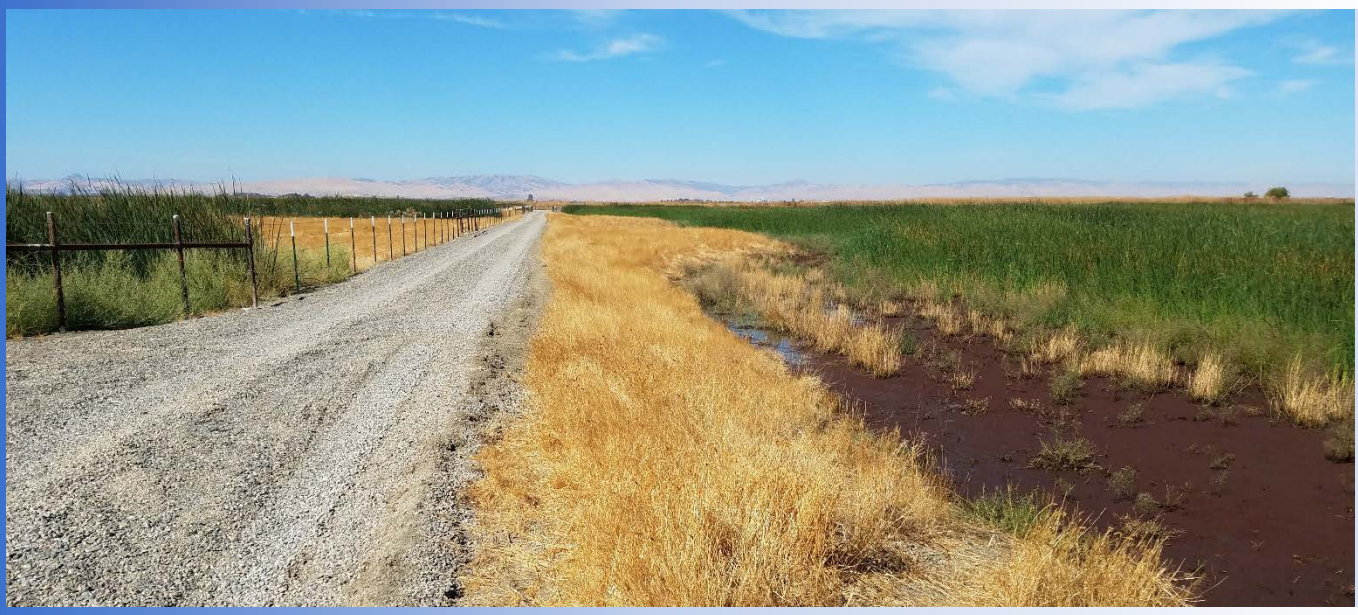
Distribution of the Giant Gartersnake (*Thamnophis gigas*) in the Volta Area of the San Joaquin Valley



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Executive Summary

This study was undertaken to address the status of giant gartersnake (*Thamnophis gigas*) distribution and demography in the Volta region of the San Joaquin Valley (SJV). Through funding from other sources, the geographic scope was expanded in 2016 to include the Mendota region, thus providing a more comprehensive examination of *T. gigas* status in the SJV. *T. gigas* is a federal- and state-listed species endemic to California's Great Central Valley. The species was historically associated with low-gradient streams and the wetlands and marshes of the valley floor. However, the loss of historical habitat for *T. gigas* has resulted in extirpations or serious declines throughout the southern two thirds of its former range. Recent drought and resulting surface water depletion in the SJV pose an immediate threat to regional population persistence. Management actions are likely to be sub-optimal if distributional and demographic information are unavailable, and the lack of information regarding the current extent of Volta area *T. gigas* hinders the development of an informed regional conservation strategy, as well as the targeting of water deliveries for maximum conservation benefit. *T. gigas* is a visually elusive species and appears to occur at low densities in much of the SJV, which results in low detection probability and poor understanding of occupancy patterns throughout much of its range. This study directly targeted Research Priority 1 of Funding Opportunity Number R14AS00050 for the Central Valley Project Conservation Program (CVPCP) and Central Valley Project Improvement Act Habitat Restoration Program (CVPIA HRP), with main components consisting of examining the distribution, abundance and demography of *T. gigas* in the Volta area; evaluating existing sampling protocols with the goal of maximizing detection probability; field testing procedures; and designing a sampling framework facilitating species management and conservation.

The scope was designed primarily to evaluate habitat suitability and to ascertain the presence, distribution, and relative abundance of *T. gigas* using standard trapping techniques, but also included collecting environmental DNA samples to augment and/or validate trap survey results. Consistent with regional efforts conducted over the past decade, visual encounter and trap surveys failed to detect *T. gigas* in most areas despite the presence of putative habitat. Conversely, environmental DNA sampling, which was developed independently over the course of this study, indicates that *T. gigas* are indeed still present at several locales where extirpations have been suspected. Because these newly developed detection methods do not include physically handling the target organism, detailed information regarding distribution and relative abundance remains unavailable in areas where snakes were not physically captured. Regardless, the fact that intensive trapping surveys failed to detect *T. gigas* suggests that densities, and therefore capture probabilities, are lower now than in prior years and that declines are occurring more rapidly in the SJV than they are in the northern extent of the species' range.

Estimates of survival probability were generally higher for the population of *T. gigas* at Volta relative to populations in the Sacramento Valley. However, 95% confidence intervals around the estimates in the two valleys broadly overlapped, indicating no statistical difference. Similarly, estimates of two components of fecundity, the probability of breeding and the number of fetuses given breeding, for the population at Volta were similar to estimates from several populations in the Sacramento Valley. The low detection probability of *T. gigas* DNA resulting from our limited

sampling numbers precluded inferences about patterns in the spatial distribution of *T. gigas* in the San Joaquin Valley and prevented us from comparing occupancy probability and covariates of occupancy in the two portions of the species' range. However, this newly-developed method offers great promise for elucidating patterns of occupancy with greater efficiency and at far less cost than trapping methods, particularly where capture probabilities are low.

This work contributes to ongoing efforts funded by the CVPIA HRP (Hansen 2007, 2008) and CVPCP (Hansen et al 2011) by utilizing inferences resulting from robust and innovative survey and analytical techniques. We have developed abundance, survival, and fecundity estimates for *T. gigas* at the Volta WMA and compared them with estimates for populations in the Sacramento Valley. We have separately developed new sampling techniques and applied them here, providing new insight on the status of *T. gigas* in the Volta and Mendota areas. We have utilized inferences resulting from these surveys and remotely-sensed data to generate a map of occupancy probability (e.g. Dickson et al. 2013) for select areas of the San Joaquin Valley both presently and formerly occupied by *T. gigas*. Finally, we have identified a preliminary list of covariates that are associated with the probability of occupancy at a location. These tools will be useful to land managers for a variety of reasons, including identifying locations for future surveys where *T. gigas* are most likely occur and determining locations in the study area where maintaining habitat for *T. gigas* is most critical.

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GLOSSARY

Akaiki's Information Criterion (AIC) – an estimator of the relative quality of statistical models for a given set of data. Given a collection of models for the data, AIC estimates the quality of each model, relative to each of the other models. Thus, AIC provides a means for model selection.

alfisol – a soil order in USDA soil taxonomy, alfisols form in semiarid to humid areas, typically under a hardwood forest cover. They have a clay-enriched subsoil and relatively high native fertility. "Alf" refers to aluminum (Al) and iron (Fe).

analyte – a substance whose chemical constituents are being identified and measured.

ardisol – a soil order in USDA soil taxonomy; of an order comprising typically saline or alkaline soils with very little organic matter, characteristic of arid regions.

Bayesian – a method of statistical inference in which Bayes' theorem is used to update the probability for a hypothesis as more evidence or information becomes available. Bayesian inference is an important technique in statistics, and especially in mathematical statistics.

Bernoulli random variable – the probability distribution of a random variable which takes the value 1 with probability pand the value 0 with probability $q = 1 - p$ — i.e., the probability distribution of any single experiment that asks a yes–no question; the question results in a boolean-valued outcome, a single bit of information whose value is success/yes/true/one with probability p and failure/no/false/zero with probability q. It can be used to represent a coin toss where 1 and 0 would represent "head" and "tail" (or vice versa), respectively. In particular, unfair coins would have $p \neq 0.5$.

brumation – a term used for the hibernation-like state that cold-blooded animals utilize during very cold weather. On the other end of the spectrum is a state known as aestivation, which like brumation, provides a way for reptiles to handle temperature extremes.

caudal scutes – the ventral (belly) scales in front of the vent or cloaca (i.e., in front of the tail)

conductivity (EC, or specific conductance) – of an electrolyte solution is a measure of its ability to conduct electricity. The SI unit of conductivity is siemens per meter (S/m). In many cases, conductivity is linked directly to the total dissolved solids (T.D.S.), serving as an indicator of analyte presence.

covariate – a variable that is possibly predictive of the outcome under study. A covariate may be of direct interest or it may be a confounding or interacting variable. The alternative terms explanatory variable, independent variable, or predictor, are used in a regression analysis.

demography – study of the size, structure, and distribution of populations, and spatial or temporal changes in them in response to birth, migration, aging, and death.

entisol – In USDA soil taxonomy, entisols are defined as soils that do not show any profile development other than an A horizon. An entisol has no diagnostic horizons, and most

are basically unaltered from their parent material, which can be unconsolidated sediment or rock.

environmental DNA (eDNA) – Organisms liberate DNA into their surrounding environment by leaving behind indicators such as slime, scales, epidermal cells or feces containing DNA can be captured and isolated from water (or soil) samples, where purified total DNA can be interrogated for specific species of interest through use of molecular techniques.

extant – still in existence; surviving.

extirpation – Local extinction or extirpation is the condition of a species (or other taxon) that ceases to exist in the chosen geographic area of study, though it still exists elsewhere. Local extinctions are contrasted with global (complete) extinctions.

fecundity – the actual reproductive rate of an organism or population, measured by the number of gametes (eggs), seed set, or asexual propagules.

geocoordinates – A geographic coordinate system is a coordinate system used in geography that enables every location on Earth to be specified by a set of numbers, letters or symbols. The coordinates are often chosen such that one of the numbers represents a vertical position, and two or three of the numbers represent a horizontal position. A common choice of coordinates is latitude, longitude and elevation.

hurdle model – a class of models for count data that help handle excess zeros and overdispersion.

hydroperiod – the seasonal pattern of the water level that results from the combination of the water budget and the storage capacity of the wetland.

inceptisol – a soil order in USDA soil taxonomy, inceptisols form quickly through alteration of parent material. They are more developed than entisols. They have no accumulation of clays, iron oxide, aluminium oxide or organic matter.

Incremental Level 4 (IL4) – Incremental Level 4 water is the difference between full Level 4 and Level 2 water supply.

Level 2 (L2) – Level 2 refuge water refers to the average amount of water the refuges received between 1977 and 1984.

Level 4 (L4) – Level 4 water is the amount of water required for full development of the refuges based upon the management goals of individual refuges and wildlife areas, consistent with CVPIA 3406(d)1 and (d)2.

life history – an organism's life history is the sequence of events related to survival and reproduction that occur from birth through death. Populations from different parts of the geographic range that a species inhabits may exhibit marked variations in the traits that affect various investments in growth, reproduction, and survivorship.

Markov Monte Carlo simulation – a technique for estimating by simulation the expectation of a statistic in a complex model. Successive random selections form a Markov chain, the stationary distribution of which is the target distribution.

mollisol – a soil order in USDA soil taxonomy, mollisisols are the soils of grassland ecosystems. They are characterized by a thick, dark surface horizon. This fertile surface horizon, known as a mollic epipedon, results from the long-term addition of organic materials derived from plant roots.

occupancy – in ecological models, the probability that a randomly selected site or sample unit in an area of interest in occupied by a species.

parturition – the action of giving birth to young.

pH (potential of hydrogen) – a scale of acidity from 0 to 14. It tells how acidic or alkaline a substance is. More acidic solutions have lower pH. More alkaline solutions have higher pH. Substances that aren't acidic or alkaline (that is, neutral solutions) usually have a pH of 7.

Poisson random variable – the number of successes that result from a Poisson experiment.

posteriors – In Bayesian statistics, the posterior probability of a random event or an uncertain proposition is the conditional probability that is assigned after the relevant evidence or background is taken into account.

precinctive – restricted to a defined geographical area; originating from and occurring in one place and nowhere else.

priors – In Bayesian statistical inference, a prior probability distribution, often simply called the prior, of an uncertain quantity is the probability distribution that would express one's beliefs about this quantity before some evidence is taken into account.

putative – generally considered or reputed to be.

quantitative polymerase chain reaction (qPCR) – a technique used in molecular biology to amplify a single copy or a few copies of a piece of DNA across several orders of magnitude.

radiography – an imaging technique using X-rays to view the internal structure of an object.

refugia – an area in which a population of organisms can survive through a period of unfavorable conditions.

Snout-Vent-Length (SVL) – measurement of length extending from the tip of the snout to the cloaca, not including the tail.

Universal Transverse Mercator (UTM) – a conformal projection that uses a 2-dimensional Cartesian coordinate system to give locations on the surface of the Earth.

vertisol – In USDA soil taxonomy, a vertisol is a soil in which there is a high content of expansive clay known as montmorillonite that forms deep cracks in drier seasons or years.

zero-truncated Poisson random variable – a certain discrete probability distribution whose support is the set of positive integers. It is the conditional probability distribution of a Poisson-distributed random variable, given that the value of the random variable is not zero.

1. INTRODUCTION

1.1. Project Setting and Purpose

The federal- and state-listed giant gartersnake (*Thamnophis gigas*) is precinctive to California's Central Valley. Historically associated with low-gradient streams and the wetlands and marshes of the valley floor, the loss of historical habitat for *T. gigas* has resulted in extirpations or serious declines throughout the southern two thirds of its former range. The species is critically imperiled in the San Joaquin Valley (SJV), where populations have declined or are missing from formerly occupied habitats that otherwise appear to retain suitable physical characteristics (e.g., presence of water, sufficient hydroperiod, emergent vegetation, and terrestrial refugia). Physical characteristics, however, may not be the only drivers of *T. gigas* occupancy in the SJV, and there is some evidence that poor water quality may be impacting *T. gigas* and its prey. Recent drought and resulting surface water depletion, along with concentration of potentially hazardous heavy metals in the perched water table, may also pose an immediate threat to regional population persistence. Presently, the *T. gigas* population at the Volta Wildlife Management Area (WMA) is the only putative self-sustaining population known in the SJV.

Diminished water availability has been the principal cause of this species' decline throughout its range. The Central Valley Project (CVP) was developed in 1933 to distribute water throughout the Central Valley by storing water in reservoirs in the water-rich northern half of the state, and transporting it to the water-poor SJV through a series of canals, aqueducts and pump plants. The development and delivery of water through the CVP facilitated urbanization and agricultural conversion of historic freshwater marshes in the Central Valley, which in turn affected aquatic ecosystems and directly destroyed and fragmented historic *T. gigas* breeding, feeding, and sheltering habitat. To ameliorate the environmental consequences of wetland conversion resulting from the CVP, the Central Valley Project Improvement Act (CVPIA) of 1992 requires that the Department of the Interior acquire additional water supplies to meet optimal waterfowl and wildlife habitat management needs on refuges within California's Central Valley. These refuges collectively encompass National Wildlife Refuges, State Wildlife Management Areas and the Grassland Resource Conservation District (GRCD).

Recent, severe drought conditions have exacerbated threats in the SJV. For example, South-of-Delta (SOD) CVPIA wildlife refuges were allocated only 40% of their full Level 4 (combined Level 2 and Incremental Level 4 [1]) water supply for Water Year 2014. Servicing the Volta area, Grasslands Resource Conservation District (GRCD) recently experienced drastic curtailments to CVPIA water deliveries, receiving just 53% (95,684 acre-feet) and 54% (96,612

[1] Level 2 refuge water refers to the average amount of water the refuges received between 1977 and 1984. Level 4 water is the amount of water required for full development of the refuges based upon the management goals of individual refuges and wildlife areas, consistent with CVPIA 3406(d)1 and (d)2. Incremental Level 4 water is the difference between full Level 4 and Level 2 water supply.

acre-feet) of its full Level 4 allotment in 2014 and 2015. Much of this water is delivered in fall to provide water for migratory waterfowl and is not available to *T. gigas* during the critical summer months. Water supplies in the GRCD did not improve until 2016 and 2017, when Level 4 water supplies increased to 86% (155,606 acre-feet) and 100% (180,000 acre-feet), respectively. Level 4 water supplies are critical for the optimization of seed and biomass production, the health and survival of locally breeding shorebirds and waterfowl, *T. gigas*, and the productivity of the largest of California's remaining wetlands comprising the GRCD. If not supplemented, continued curtailments of this magnitude and duration could potentially eliminate the active season habitat required to sustain remaining *T. gigas* populations in the San Joaquin Valley. This threat of rapid and widespread extinctions is unprecedented in this portion the species' range.

Management actions are likely to be sub-optimal if distributional and demographic information are unavailable, and the lack of information regarding the current extent of Volta area *T. gigas* hinders the development of an informed regional conservation strategy and the targeting of water deliveries for maximum conservation benefit. *T. gigas* is a visually elusive species and appears to occur at low densities in much of the SJV, which results in low detection probability and poor understanding of occupancy patterns throughout much of its range. This study directly targeted Research Priority 1 of Funding Opportunity Number R14AS00050 for the Central Valley Project Conservation Program (CVPCP) and Central Valley Project Improvement Act Habitat Restoration Program (CVPIA HRP), with main components consisting of examining the distribution, abundance and demography of *T. gigas* in the Volta area; evaluating existing sampling protocols with the goal of maximizing detection probability; field testing procedures; and designing a sampling framework facilitating species management and conservation.

Conducted from June 2015 through October 2016, the purpose of this project was to assess the status of *T. gigas* in the Volta area of Merced County. Expanded through other funding sources to include portions of Merced County, this project also utilizes additional data collected from October 2016 through October 2017. Building on work funded by the CVPCP and CVPIA HRP from 2007 through 2008, this effort assists with developing the information required for project planning and species recovery in the San Joaquin Valley Recovery Unit.

1.2. Project Objectives

- Examine the distribution, and demography of a remnant *T. gigas* population in the Volta area, including aquatic habitat corridors known to connect to other known *T. gigas* population centers (e.g. Los Banos Creek and Mud Slough) within the northern Grasslands Ecological Area.

- Evaluate sampling designs and field collection protocols that maximize the probability of detection and, consequently, minimize the probability that we will fail to detect *T. gigas* when they are present at a location.

- Fit hierarchical statistical models to quantify the extent to which topography, vegetation composition and structure, and other attributes of sampling locations, measured in the

field or from remotely sensed sources, explain and predict variation in occupancy of *T. gigas*.

- Quantify the composition, abundance, or density of *T. gigas* present at sampling locations and measure similarity in these metrics amongst locations.

- Develop outcomes within a framework in which analyses and inferences are straightforward and cost effective to update with new data.

1.3. Project Augmentation

In response to threats to California's natural resources imposed by drought, the Governor's Drought Executive Order 4-25-2014 directed the California Department of Fish and Wildlife (CDFW) to immediately implement projects that respond to drought conditions through habitat restoration and through water infrastructure projects on property owned or managed by CDFW for the benefit of fish and wildlife impacted by diminished water supply. The Executive Order authorized CDFW to enter into contracts to accomplish this work. Because *T. gigas* have declined or are missing from ostensibly suitable habitat formerly occupied in the SJV, it may be that physical characteristics (i.e., presence of water, hydroperiod, emergent vegetation, terrestrial refugia) are not the only drivers of *T. gigas* occupancy. There is some evidence that poor water quality may be impacting the *T. gigas* prey base and potentially the species itself. Recent drought and resulting surface water depletion and concentration of potentially hazardous heavy metals in the perched water table also pose an immediate threat to regional population persistence. Because this study of *T. gigas* distribution, demography, and the habitat variables that predict the species' patterns of occupancy and a separate study of water quality[2] may provide cumulative insight affecting land use planning and resource management on the extensive lands owned and operated by CDFW in the SJV, work was subsequently contracted to complete the following objectives augmenting the ongoing *T. gigas* surveys in 2016:

- Expand the ongoing distribution and demography study to the Mendota Wildlife Area, the Mendota Pool, and suitable habitat corridors connecting the northern and southern Grasslands areas; and

- Expand water quality sampling and analysis across the Grasslands Ecological Area at *T. gigas* survey sites to enable utilizing water quality metrics as covariates in models of occupancy.

1.4. Project Location

From the standpoint of species recovery, *T. gigas* populations within the San Joaquin Valley are represented by three unique management areas; North and South Grasslands (Grasslands Ecological Area, GEA), Mendota Area, and the Lanare/Burrel Area (USFWS 2012). Surveys

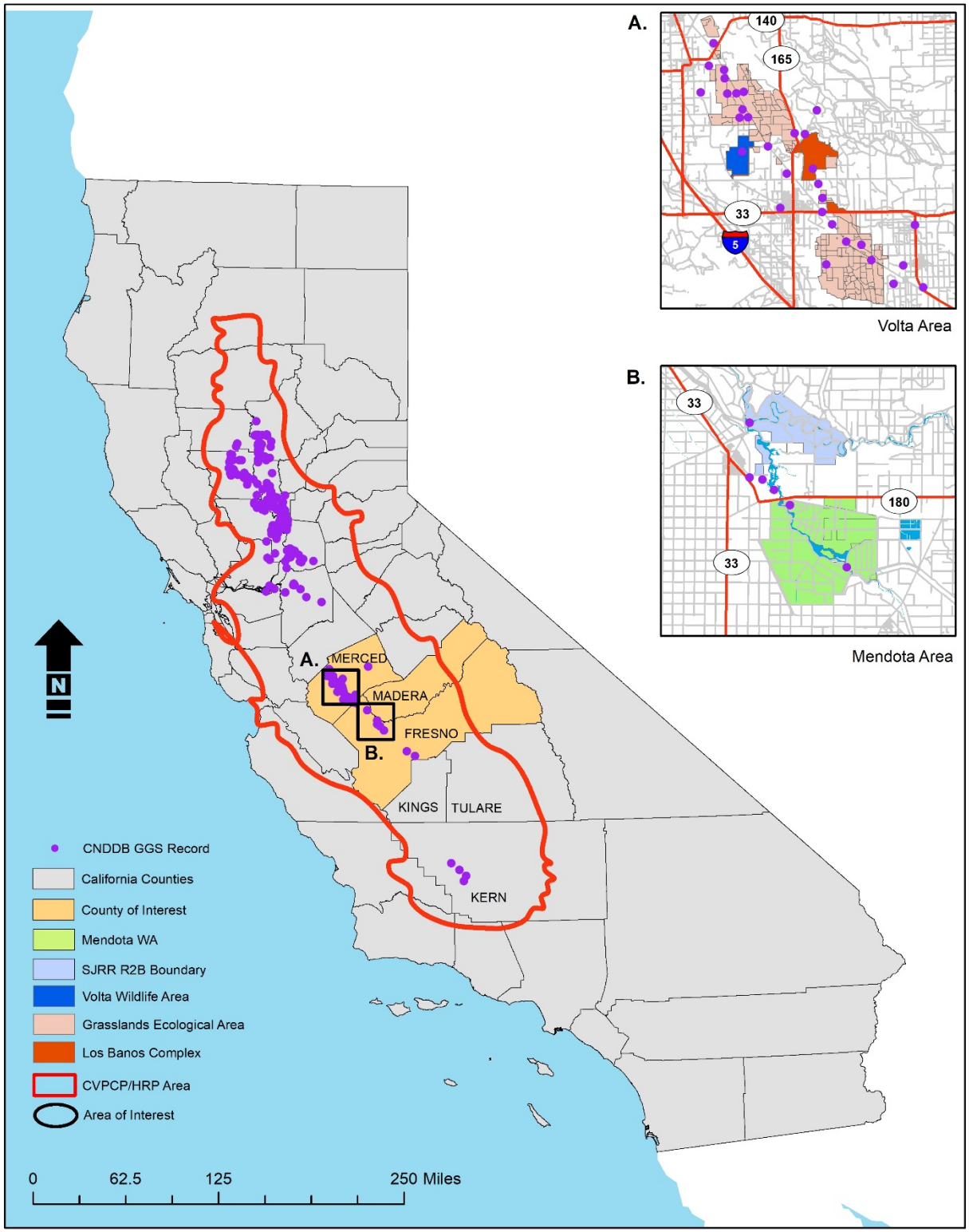
[2] Volta Wildlife Area Level 2 Diversification/ Level 4 Development Project (Pilot Project) - Biological Monitoring. Funding provided by the U.S. Bureau of Reclamation.

were started in mid-June of 2015 near Volta in the GEA, which is comprised of 178,000 acres of Central Valley wetlands and includes federal, state and privately-owned lands including the Volta WMA. Supporting a well-documented population of *T. gigas*, Volta lies approximately four miles northwest of the city of Los Banos along Ingomar Grade and approximately four miles east of Interstate 5 and the town of Santa Nella. The survey area included portions of the GEA including the San Luis National Wildlife Refuge and Grasslands Mitigation Bank (Westervelt Ecological Services), including aquatic habitat corridors (e.g. Los Banos Creek, Mud Slough, and Salt Slough) known to connect to other *T. gigas* population centers. This region supports the only known *T. gigas* breeding population currently recognized within the San Joaquin Valley (Hansen 2008a, 2008b, USFWS 2012).

To facilitate development of a conservation strategy for the local *T. gigas* population, surveys emphasized lands within the north GEA where data are available from CVPCP- and CVPIA HRP-funded sampling occurring from 2006-2008 (Hansen 2007, 2008a, 2008b; Hansen et al. 2011), but also included sites along Los Banos Creek and Mud Slough where robust trapping had not recently occurred. The U.S. Fish and Wildlife Service and Westervelt Ecological Services provided full cooperation and support for this project, as did myriad private landowners within the GRCD. To facilitate development of a drought strategy, emphasis was placed on sites capable of receiving water from the Volta Wasteway L2 Diversification/IL4 Development Pilot Project (Pilot Project) and the Grassland Water District (GWD) IL4 Groundwater Acquisition Pilot Project (GAPP), including aquatic habitat corridors known to connect to other known *T. gigas* population centers. The Pilot Project and GAPP are demonstration projects evaluating the feasibility of groundwater development to diversify a portion of Level 2 supply and to supplement Incremental Level 4 supplies to wildlife refuges within the SJV; the sites that they service are the most likely to receive water when CVP allotments are low.

In 2016, both the Mendota Wildlife Area and Mendota Pool were surveyed contemporaneously under separate funding sources. The resulting data have been incorporated into this report to provide a more comprehensive description of *T. gigas* in the SJV. The Mendota Wildlife Area is comprised of approximately 12,800 acres of mixed seasonal and perennial wetlands managed primarily for waterfowl. The San Joaquin River Restoration Reach 2B project area is comprised of approximately 5,900 acres of private and public lands situated on the Mendota Pool. The Mendota area lies south and west of the San Joaquin River, approximately 40 miles south of Los Banos and 33 miles west of Fresno. Mendota Pool lies immediately east of the town of Mendota, north of Highway 180, and east of Highway 33. Mendota Wildlife Area lies approximately 3 miles southeast of the town of Mendota near Whites Bridge, south of Highway 180 and east of Santa Fe Grade and Highway 33. Sampling locations relative to historic *T. gigas* distribution are illustrated in **Figure 1.1**.

Figure 1.1. Site locator and relationship to giant gartersnake (*T. gigas*) occurrence records in the San Joaquin Valley.



2. SURVEYS AND DATA COLLECTION

T. gigas is a secretive and elusive species that occurs at low density in many locations. Investigations into current distribution must, therefore, use survey and analytical methods (e.g. Halstead et al. 2009, 2011) that can accommodate low expected detection probabilities to produce robust inferences. The size and scope of this project reflects the experience gained by the Principal Investigator (PI, Eric Hansen) over the past twelve years executing rigorous *T. gigas* trapping surveys and developing strong relationships with regional land owners and resource managers in the Volta area (Hansen 2007, 2008a, 2011, 2012, 2013) and seven years of experience with modelling *T. gigas* occupancy and demography (e.g., Hansen et al. 2015, 2017) between the PI and co-PI (Rick Scherer).

2.1. Visual Encounter Surveys and Trapping

Because *T. gigas* is secretive and evasive, it has a low probability of detection (Halstead et al. 2013, Hansen et al. 2017). Visual surveys can fail to detect snakes if they are present in low numbers and/or densities. The core species-level sampling approach was therefore two-tiered, incorporating a staged combination of visual encounter surveys and trapping methods to assess *T. gigas* presence.

2.1.1. Methods

Visual encounter surveys included walking or kayaking along channels, wetlands, and nearby upland areas to search for basking and/or foraging snakes. Surveys occurred primarily during the morning and early afternoon when snakes typically bask. Visual encounter surveys were conducted both independently and during all trap-checking activities, encompassing as many adjacent public rights-of-way as practicable.

To augment visual encounter surveys and to maximize the probability of detecting *T. gigas*, aquatic trapping was conducted by placing as many as 1,100 floating, modified, minnow traps along the edges of promising aquatic features and monitoring them for a minimum of 30 days before relocating them. Traplines consisting of 50 traps spaced at intervals of 5-10 meters were placed in aquatic features where water levels were sufficiently high to ensure that traps were continuously wetted (≥ 10 inches), thereby reducing the risk of desiccation or thermal stress for captured snakes. Sites were also chosen that provided vegetated cover to camouflage traps from public view. The traps used were galvanized 4-mesh eel pots (Tackle Factory [Cuba Specialty Manufacturing], Fillmore, New York, USA) modified to float following the procedures in Casazza et al. (2000). Transect spatial scale was consistent with current methods described by Halstead et al. (2009, 2011), and surveys were timed to maximize the probability of detection by encompassing periods of spring emergence, courtship, and parturition of young (Halstead et al. 2009, 2011, 2013). Trap site locations are depicted in **Figure 2.1**; geocoordinates and survey dates are presented in **Appendix A**.

Data recorded for each trap location included UTM coordinates and environmental characteristics (e.g., vegetation and substrate types). Wherever traps remained in place without interference, organisms within the traps (by-catch) were identified and counted at pre-determined intervals to compare prey composition among trap/transect sites (**Appendix B)**. Water metrics including pH, specific conductivity (EC), and water temperature were measured at each trap site using a portable YSI 556 Multi-Probe unit (**Appendix C)**. Captured snakes were implanted with passive integrated transponder (PIT) tags for permanent identification. As a secondary marking technique, medical cautery units were used to microbrand caudal scutes (e.g., Winne et al. 2006) in a pattern consistent with established scale-clip marking techniques (Brown and Parker 1976). Snakes were released at their point of capture once data was recorded.

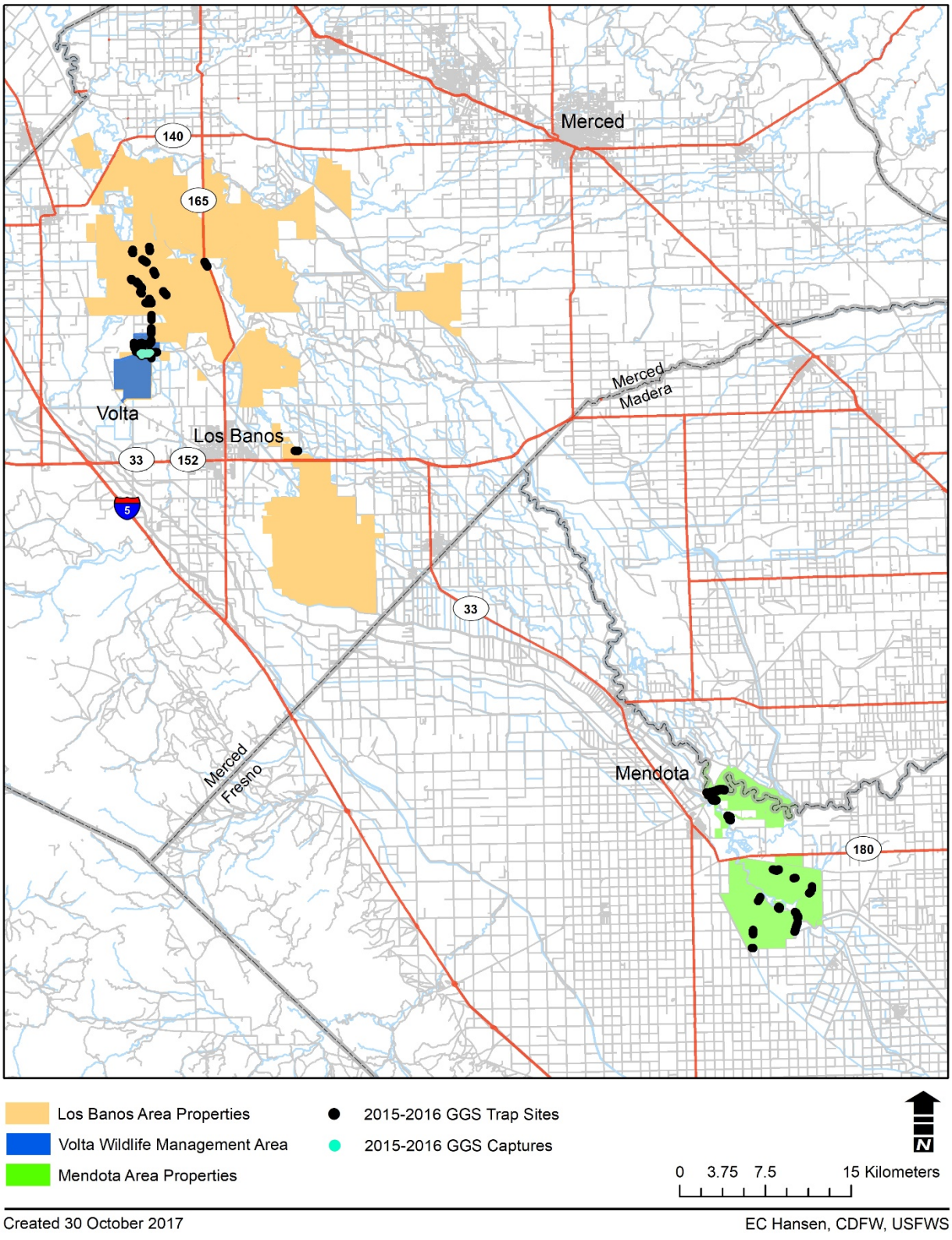
To assess demographics, weight, total length, snout to vent length, and sex were recorded for all *T. gigas* captured. Other physical features such as scars and tumors, as well as identifying characteristics such as scale counts on head and mid-body and measurements of head scales, were also noted. Fecundity and reproductive status was examined through radiograph performed by Dr. Ray F. Wack, DVM, ACZM, staff veterinarian at the Sacramento Zoo. Snakes were transported to the Sacramento Zoo upon capture and released at the point of capture as soon as possible following completion of the radiograph. The average female fecundity for the study population has been calculated, presented with an estimate of precision, and compared to fecundity rates in the Volta WMA and the Sacramento Valley, where fecundity studies are currently being funded through other sources. Results are described in **Section 3.3**.

Surveys were conducted per the terms and conditions of the PI's US FISH AND WILDLIFE SERVICE RECOVERY PERMIT 10(a) (1) (A) ESA TE-018177-7 (valid through 08/06/2019) and DEPARTMENT OF FISH AND GAME SCIENTIFIC COLLECTING PERMIT 003881 (valid through 04/16/2018).

2.1.2. Results

Contrasting with previous efforts (e.g., Dickert 2003, 2005; Hansen 2007, 2008a, 2011; Sloan 2004), no *T. gigas* were captured or observed outside of the Volta WMA area during monitoring in either the 2015 or 2016 season, although sightings and captures of *T. gigas* elsewhere in the Central Valley were frequent during the same period (E. Hansen, unpublished data). Valley gartersnakes (*Thamnophis sirtalis fitchi*) were observed or captured in traps, and other species common to the San Joaquin Valley, including California kingsnakes (*Lampropeltis californiae*) and Pacific gopher snakes (*Pituophis catenifer catenifer*), were frequently either observed directly or identified by shed skins found during visual surveys.

Figure 2.1. Overview of San Joaquin Valley giant gartersnake (*T. gigas*) trap locations and results



Prey species observed included crayfish (*Procambarus clarkii*) and adult and larval bullfrogs (*Lithobates catesbeianas*). Other common species observed included black bass (*Micropterus* spp.) and sunfish (*Lepomis* spp.). Capture per unit effort (number of organisms per trap day; CPUE) of bycatch is provided in **Appendix B**.

Water chemistry metric ranges were generally consistent with those supporting *T. gigas* at occupied sites throughout the species' range (Hansen et al. 2011), although some localized measurements of pH and EC were higher than those found for the majority of sites (e.g., adjacent to the VWMA at Grasslands Mitigation Bank). Measured water chemistry metrics are provided in **Appendix C**.

2.2. Maximizing Detection Probability

Species are rarely detected with certainty in studies of spatial distributions (Gu and Swihart 2004), and as we noted above, detecting *T. gigas* at locations where they occur can be particularly challenging. Accounting for imperfect detection, therefore, is an essential component of the sampling design and analytical approach. Trapping methods were implemented to increase detection probability by increasing encounter rates and decreasing escape rates. Where a clear terrestrial interface or foraging pathway is lacking (e.g., within perennial marsh), drift fences with traps modified to maximize surface contact were used to increase encounter rates (e.g. Hansen et al. 2010). Additionally, flexible screen mesh covers extending below the waterline were used on the aperture of each trap funnel to reduce escape rates (Hansen, unpublished data). Resulting detection probabilities are discussed in **Section 3.1**.

2.3. Environmental DNA Surveys

Detection probabilities from trapping surveys may be inadequate when population densities are exceptionally low. Trapping is also hindered by theft and tampering in areas of public access, potentially impacting survey results and endangering the health of the animals present in the census population. Such areas often are not trapped, resulting in gaps in our understanding of *T. gigas* distribution.

We used environmental DNA (eDNA) based methods to obtain population occupancy data that complements or surpasses current visual encounter and aquatic trapping surveys, which are associated with low or imperfect rates of detection (Halstead et al. 2011). Environmental DNA methods provide a means of addressing limitations of visual and trapping surveys, because they: 1) are cost-effective and feasible to deploy over a large survey area; 2) are unambiguously identify target organisms; and 3) are sensitive to trace amounts of DNA in sampled material (Jerde et al. 2011; Thomsen et al. 2010). Given that

molecular diagnostic techniques may be more sensitive than visual methods (Wilcox et al. 2016), information from eDNA was used to obtain the critical presence/absence data that trapping surveys may fail to provide.

The presence of cryptic species is ascertained by using molecular genetic assays to detect DNA that has been shed into the environment. The eDNA approach differs from traditional sampling in that a given survey does not capture the target organisms themselves, but the biological material those organisms leave in their vicinity that contain a "signal" of their genetic identity. Organisms liberate DNA into their surrounding environment by leaving behind indicators such as slime, scales, epidermal cells or feces (Janosik and Johnston 2015). Biological material containing DNA can be captured and isolated from water (or soil) samples, where purified total DNA can be interrogated for specific species of interest through use of molecular biology techniques (Jerde et al. 2011). These techniques are associated high detection probabilities, making this approach suitable for monitoring the performance and compliance of species protection efforts. Preliminarily, given the scientific literature and recent experience of our team in doing this work for *T. gigas* surveys, a reasonable assumption is that the probability (p) of detecting individuals is high (>0.90) at 100 meters in a uni-directionally flowing system such as toe drains, agricultural canals, and other waterways. If detection probability is >0.90, then the probability of detecting species at least once during a survey (p*) is 99 percent when duplicate samples are taken (k=2) at a collection event.

Because this method was not adapted for use with this species until this project was well underway, budget was unavailable for applying a rigorous, standardized sampling approach. Rather, to balance funding limitations with the desire to maximize the geographic extent of our sampling efforts, testing was limited to 2-3 filters per site at a sampling event, spatial separation between sites was often many kilometers, and replications over time were few. Although this approach reduces the probability of detection, it serves not only as a test of the method's potential for improving the precision of data used to develop conservation strategies, but also provides us with information that trapping often does not.

2.3.1. Methods

Environmental DNA sample collection occurred in late September and early October of 2016 and 2017. Field sampling and laboratory protocols followed procedures described in Bergman et al. (2016). Water samples were collected along bank margins or by boat at 100-meter intervals at each sampling location. For each sampling event, water was filtered directly from the water body at an approximate depth of 6 inches below the surface using sterile Saint Gobain XL-60 silicon tubing (Tygon®; internal diameter 6.3mm), and a portable Masterflex1 L/S Easy-Load II peristaltic pump (Cole-Parmer®) powered by a cordless hand drill. Water samples were filtered through a Millipore Sterivex™-GP 0.45µm sterile filter unit (EMD Millipore). No water was transported or stored during sampling nor was any water transported between sampling sites; instead all filtration occurred directly on the bank or boat at each site. Sample filtrate was captured and measured in graduated flasks to verify the

volume of each sample. Filtered water was poured out after completion of sampling at each site. To eliminate cross contamination between sites due to equipment or the investigator, sterile gloves and all sampling materials were pre-packaged and discarded after one use. Tubing and gloves were immediately disposed of after each use into a sealed trash bag on board. All filters were likewise considered single use. After filtration, the cylindrical filters were capped at each end, labelled with location ID, placed into a sterile secondary container, sealed, and immediately placed on ice. All filters were kept on ice in a cooler for the duration of the sampling event, after which they were transferred to a -20°C laboratory freezer. The filters were stored within individually sealed secondary containers at -20°C until DNA extraction.

To ensure that field equipment was free of contamination, DNA field controls were taken for each sample day. Each field control consisted of Sterivex™ filtered ultra-pure water processed in the same fashion as the field samples. The field controls were processed for the presence of giant gartersnake DNA in parallel with all samples. DNA extractions were conducted using PowerWater Sterivex™ DNA Isolation Kit (Mo Bio Laboratories, Inc.) following the manufacturer's recommended guidelines. A DNA extraction negative control was processed in parallel to ensure sample integrity throughout extraction procedure. The DNA extraction control consisted of Sterivex™ filtered ultrapure water only. DNA extraction controls were processed using the same equipment utilized to extract DNA from all samples. Each sample and all controls were analyzed in triplicate for the presence of the giant gartersnake DNA using a quantitative polymerase chain reaction (qPCR) primer (PCR is a technique used in molecular biology to amplify a single copy or a few copies of a piece of DNA across several orders of magnitude) and probe set developed by Cramer Fish Sciences (Schumer et al., *in review*).

Each qPCR replicate consisted of a 5 ul reaction volume. Each 5 ul qPCR reaction was composed of 1x Applied Biosystems TaqMan Universal PCR Master Mix, No AmpErase UNG (Applied Biosystems™), 900nm final primer concentration, 60nm final probe concentration, and 1 ul DNA template. Thermocycling was performed using a Bio-Rad CFX 96 Real time System (Bio-ad Laboratories, Inc.) with the following profile: 10 minutes at 95°C, 40 cycles of 15 second denaturation at 95°C and 1-minute annealing-extension at 60°C. Six template control (NTC) reactions were run on the plate with the samples template controls consisted of 1ul of ultrapure water replacing DNA template within reaction volume. Three positive control reactions consisting of 20ng/ul giant gartersnake DNA template were also tested in parallel to ensure consistent PCR performance. All PCR master mixes were made inside a UV PCR enclosed workstation. DNA template was added to master mix outside of the UV PCR workstation on a dedicated PCR set up workbench. All PCR reactions were conducted on instruments located outside of the main lab in a separate portion of the building. Results of the qPCR reactions were analyzed using BioRad CFX manager v3.1 (Bio-Rad Laboratories, Inc.). A sample was considered positive for the presence of *T. gigas* DNA if any one of the three replicates showed logarithmic amplification within 40 quantification cycles (Cq).

2.3.2. Results

T. gigas DNA was detected in 28 of the 52 locations sampled, indicating presence at 8 of the 17 trap sites as well as sites where trapping proved infeasible. Distribution of DNA suggests that *T. gigas* are present throughout the geographic extent sampled. Results of eDNA analyses are provided in **Appendix E**; sampling locations and results are depicted in **Figure 2.1 and Figure 2.2**.

2.4. Water Quality

Water samples were collected from representative locations at each study site in three batches temporally distributed over the survey season to enable utilizing water quality metrics as covariates in models of occupancy and habitat suitability.

2.4.1. Methods

Water samples were collected from the middle of the water column in sterile 50 mL centrifuge tubes. Water and sediment samples were transported on wet ice in insulated coolers and stored in a freezer until extracted for analysis. Sample analyses were completed by the California Animal Health & Food Safety Laboratory, University of California, Davis.

Arsenic, selenium, total mercury, and boron were measured in samples of water by first adding hydrochloric acid. They were then filtered and analyzed by inductively coupled plasma mass spectrometry (ICP-MS). Quality control samples, including method blanks and certified reference materials (TORT-2 lobster hepatopancreas and DOLT-4 dogfish liver), were run with each set of samples.

2.4.2. Results

Only arsenic and boron were detected among all sites. Values for all analytes are reported in parts per million (ppm), with representative limits of 0.01 ppm for selenium, total mercury, and boron. The reporting limit is the lowest routinely quantified concentration of an analyte in a sample. The analyte may be detected, but not quantified, at concentrations below the reporting limit.

Results are reported in **Appendix D** and are used as a set of covariates in occupancy models described in **Section 4**. Water quality sampling locations are depicted in **Figure 2.3**.

Figure 2.1. Overview of Volta area giant gartersnake (*T. gigas*) eDNA sampling locations and results

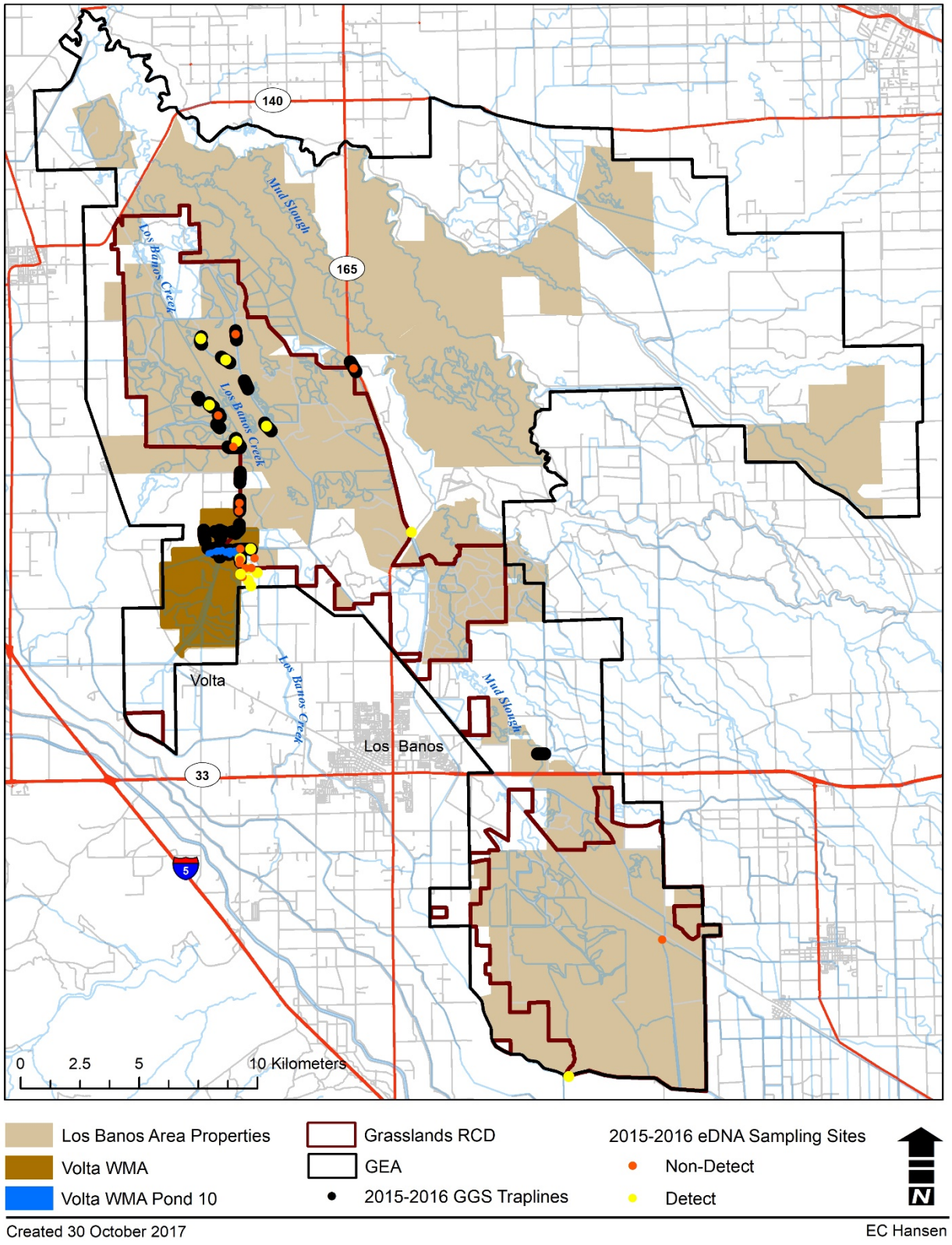


Figure 2.2. Overview of Mendota area giant gartersnake (*T. gigas*) eDNA sampling locations and results

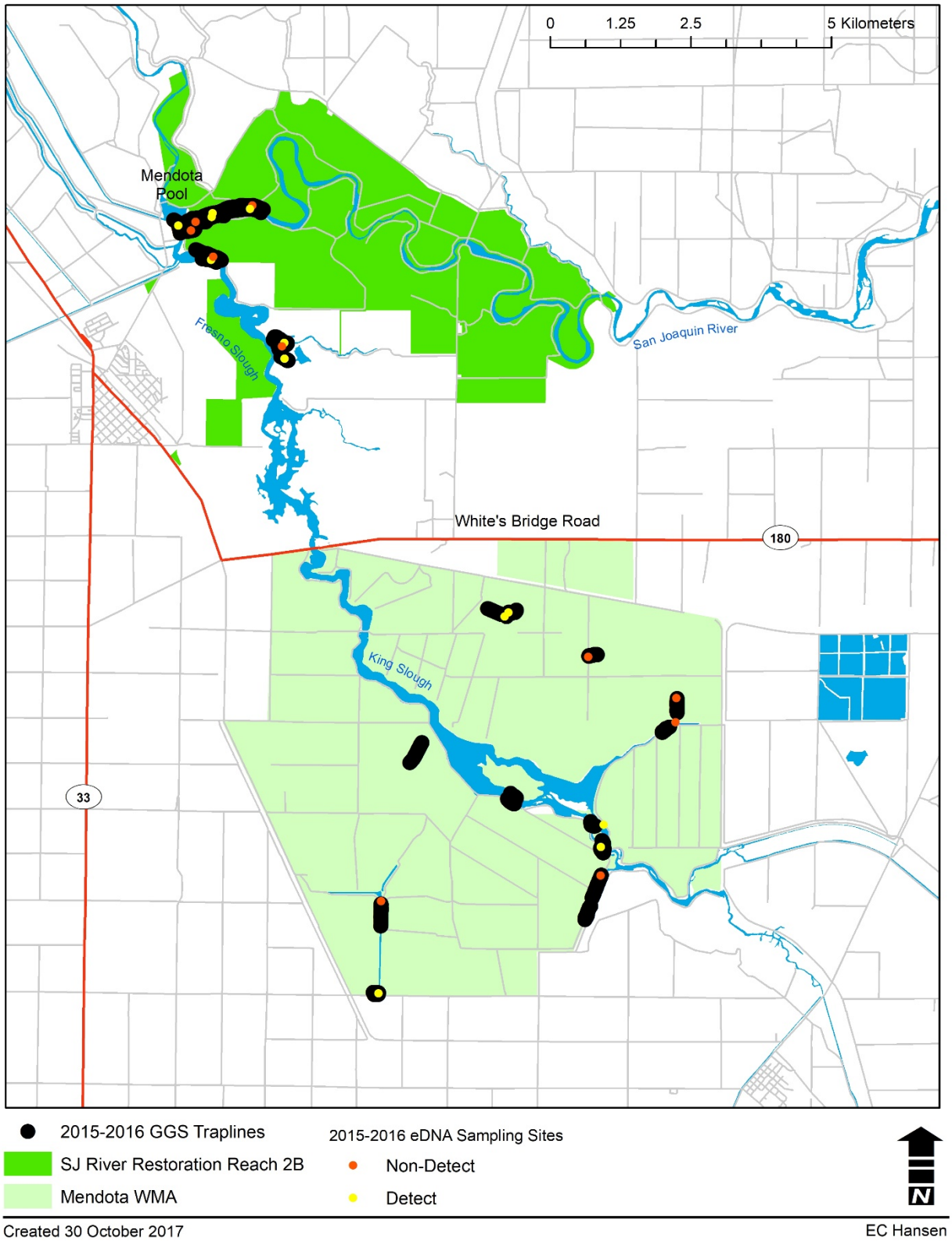
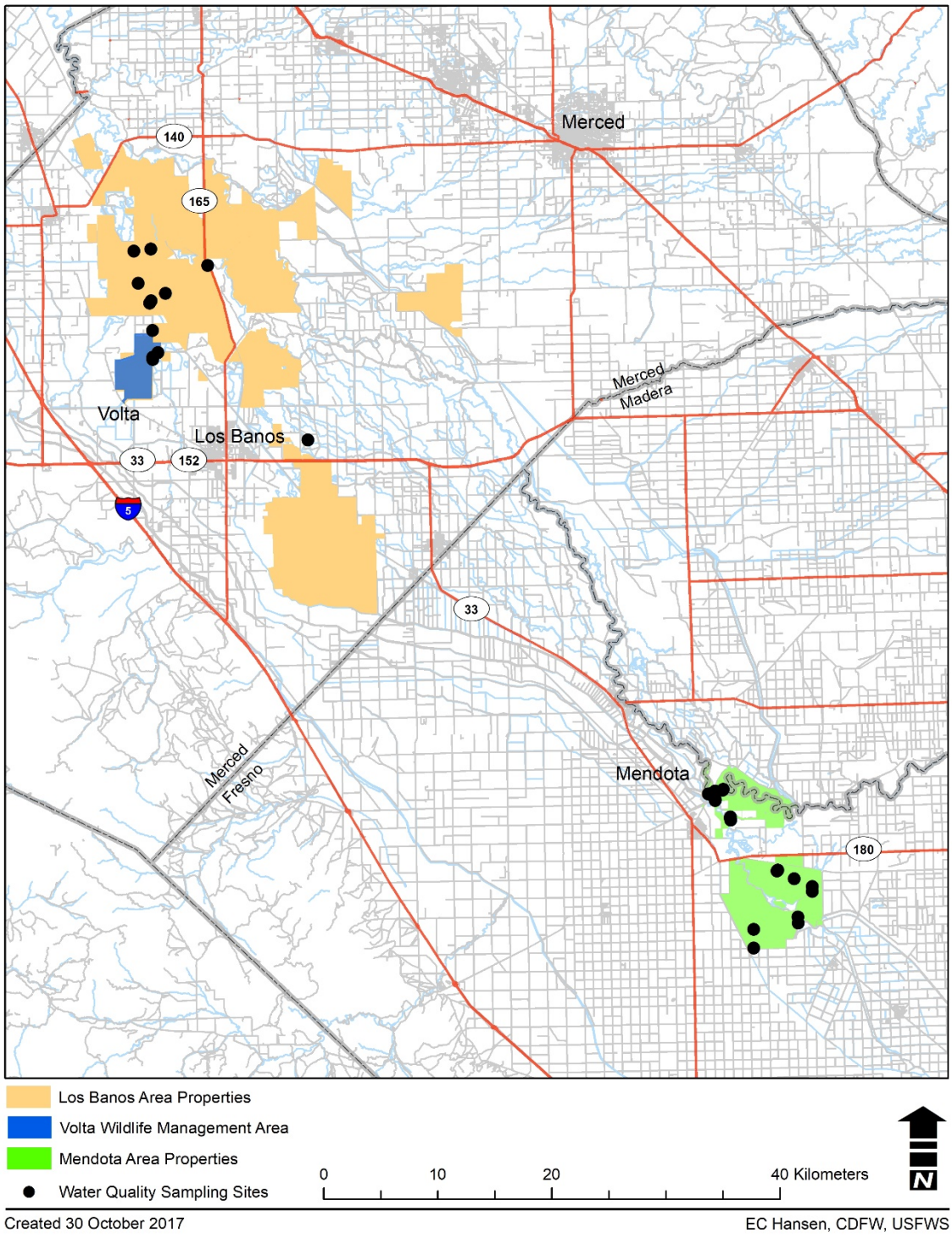


Figure 2.3. Overview of San Joaquin Valley water quality sampling locations



3. ABUNDANCE AND DEMOGRAPHY

3.1. Abundance

The most commonly used methods for estimating abundance of animal populations, while accounting for imperfect detection, are based on capture-recapture data or data from distance sampling. Estimating abundance or density for a population requires relatively rich data sets, and the models used to generate these estimates are generally characterized by restrictive assumptions. Failure to acquire sufficient data can lead to poor precision of estimates, and the inability to meet the assumptions of the model can lead to bias. For many species, the amount of sampling effort required to obtain adequate data for estimating abundance is considerable. In fact, the challenges associated with estimating abundance, particularly for multiple populations of cryptic species over large areas, is part of the motivation for the development of occupancy models.

We did not expect that we would be able to acquire adequate data for deriving estimates of *T. gigas* abundance; therefore, annual abundance estimates were not included in our proposal. Occupancy models were proposed instead. We did, however, generate estimates to evaluate the status of *T. gigas* at Pond 10 in the Volta WMA (hereafter, Volta), the only population for which we have acquired captures since 2008. These estimates could serve as baseline estimates for continued monitoring of *T. gigas*, which could be used to evaluate the effects of management actions on the population. Given that the population at Volta is one of the few remaining in the San Joaquin Valley, continued monitoring may be particularly important.

3.1.1. Methods

We collected data from 2010 to 2016 from the population of *T. gigas* at Pond 10. The primary goal was to estimate annual abundance, and we used a capture-recapture model for closed populations (Otis et al. 1978) to separately analyze the data for each year. These models are appropriate, because they account for the fact that, for most species, a proportion of the individuals in a population are not captured on sampling occasions, regardless of the method of capture and the intensity of effort (a phenomenon referred to as imperfect detection). Imperfect detection is particularly relevant to species of snakes, because their wariness, coloration and other behaviors make them difficult to observe and capture (Lind et al. 2005, Breininger et al. 2012). Therefore, counts of the number of individuals that are trapped will nearly always be smaller than the actual number of individuals in a population. Capture-recapture models for closed populations estimate the probability of capturing individuals, which allows abundance to be estimated. Because traps were checked daily, we treated each day as a sampling occasion, and our estimates of capture probability pertain to each day during the sampling period.

Estimates of abundance from models for closed populations can be negatively biased when capture probability varies among individuals (Williams et al. 2002), and variation in capture probability can be addressed using multiple modeling approaches (Kery and Schaub 2012). We fit the logistic-normal, mixture model to the data (Kery and Schaub 2012). The model included a random effect by treating the capture probabilities of individuals as if they arose from a normal distribution with an estimated mean and variance. We analyzed the data in a Bayesian framework. Prior to fitting models, we augmented the capture-recapture data by adding many zero-only capture histories. Data augmentation is necessary to fix the dimensions of the parameter vector for Markov Chain Monte Carlo simulation, a central feature of Bayesian methods (Kery and Schaub 2012).

We fit all models using OpenBUGS, an open source version of WinBUGS (Lunn et al. 2000). We used Uniform (0, 1) priors on all probability parameters. We ran models on 3 chains for 200,000 iterations each after a burn-in of at least 50,000 iterations. We thinned the chains by a factor of 5, which resulted in a total of 120,000 samples in each posterior. We visually inspected trace plots to ensure chains were well-mixed, and evaluated convergence for each model using the $\hat{R}$ statistic. We concluded that convergence had been achieved if $\hat{R}$ was less than 1.1 (Gelman et al. 2004). Finally, to determine if the posteriors contained enough samples, we examined the ratio of Monte Carlo error to the standard deviation of the posterior for parameters (Kery and Schaub 2012). If the ratio was less than 0.05, we concluded that the number of samples was adequate.

3.1.2. Results

The duration of the sampling period in each year ranged from 55 days in 2010 to 115 days in 2014 and 2016 and exceeded 90 days in all other years. The numbers of captured snakes were generally small and ranged from 10 in 2010 to 34 in 2015 (Table 1), and over 60% of snakes were captured a single time within a sampling period. Therefore, the low estimates of mean daily capture probability were expected. The estimates ranged from 0.002 to 0.014, indicating that less than 1.5% of the population was sampled on a given day.

Annual estimates of population size indicated that the population at Volta is small, and due to the low capture probabilities, the estimates were imprecise (**Table 3.1**). Although estimates suggest a general increase in abundance from 2010 to 2016, the poor precision of the estimates precluded robust inference regarding a trend in abundance. Except for 2010, credible intervals around estimates of abundance broadly overlapped (Table 2).

Table 3.1. Annual numbers of captured snakes and estimates of mean, daily capture probability and annual abundance for the population of giant gartersnakes (*T. gigas*) at the Volta Wildlife Management Area from 2010 to 2016 (95% credible intervals are shown in parentheses).

Year	Number of Captures	Estimate of Mean, Daily Capture Probability	Estimate of Annual Abundance
2010	10	0.014 (0.003-0.0300)	20.58 (11-34)
2011	18	0.004 (0.00004-0.010)	65.63 (27-139)
2012	12	0.003 (0.0003-0.010)	60.24 (18-139)
2013	23	0.004 (0.0013-0.009)	78.80 (38-141)
2014	19	0.007 (0.0008-0.014)	44.04 (22-113)
2015	34	0.003 (0.0003-0.009)	142.60 (56-315)
2016	18	0.002 (0.0003-0.006)	104.40 (34-231)

3.2. Survival

The primary goal of the analysis was to estimate survival probability for the population of *T*. *gigas* at the Volta WMA and compare the estimates to estimates of survival probability from *T*. *gigas* populations in the American and Natomas Basins of the Sacramento Valley.

3.2.1. Methods

We collected capture-recapture data from the population from 2010-2016 using the trapping described above. We structured the capture–recapture data for the Cormack-Jolly-Seber (CJS; Cormack, 1964; Jolly, 1965; Seber, 1965) model by pooling data across all days of sampling in each year. Therefore, individuals were recorded as captured or not in each year. We fit models to the data in Program MARK (White and Burnham, 1999). The two parameters in the CJS model are apparent survival probability and recapture probability, where *i* indexes year. Apparent survival probability is the probability that an individual survives and remains in the sampled population from sampling in year *i* to sampling in year *i* + 1 (Lebreton et al., 1992). This parameter is referred to as apparent survival, because an individual that dies or permanently emigrates from the sampled population appears as a death. If an individual permanently emigrates and survives outside the sampled population, estimates of survival probability from the CJS model will be lower than true survival probability. The second parameter, recapture probability, is the probability that a marked individual is captured during sampling in year *i*.

We fit models of recapture and survival probabilities to the data that included: i) no effects (i.e., a null model), ii) fixed effect of year, iii) fixed effect of sex, and iv) additive effects of sex and year. The final set for inference included 16 models. We assessed the support in the data for each model on the basis of Akaike's Information Criterion

corrected for small sample size (AICc) and Akaike weights (w_i), where *i* indexes model (Burnham and Anderson, 2002). We used model averaging to generate annual estimates of survival probability and compared them to estimates of survival from populations in the Natomas and American Basins in the Sacramento Valley from Hansen et al. (2015). In models that include fixed effects of year, estimates for recapture and survival probability are not separately identifiable in the last year. Therefore, we do not report estimates of recapture probability for 2016 or survival probability from 2015-2016.

3.2.2. Results

Across the duration of the study, we recorded 134 captures of 100 individuals. The highest-ranked model included no effects, indicating no support for effects of sex or year on recapture or survival probabilities (**Table 3.2.1**). Model-averaged estimates of recapture probability were low, ranging from 0.28 to 0.34 (**Table 3.2.2**). These estimates are generally lower than estimates from populations in the Sacramento Valley (Hansen et al. 2015).

Model-averaged estimates of survival probability ranged from 0.63 to 0.70 (**Table 3.2.1**) and were generally higher than estimates from the American and Natomas Basins. Estimates of apparent survival probability for *T. gigas* in the American Basin ranged from 0.34 to 0.62, and they ranged from 0.23 to 0.54 in the Natomas Basin. Although the estimates were higher, 95% confidence intervals around estimates overlapped across all estimates from Volta and the Sacramento Valley. Similarly, the estimates from Volta were generally higher than estimates of survival probability reported by Halstead et al. (2012) for large, radio-marked females in the Sacramento Valley, but confidence intervals overlapped.

Table 3.2.1 Model-selection results for models of recapture and survival probability from the population of giant gartersnakes (*T. gigas*) at the Volta WMA from 2010-2016. AICc = Akaike's Information Criterion adjusted for small sample size, ΔAICc = the difference between the AICc value for a model and the lowest AICc value, wi = Akaike weight, k = the number of estimable parameters in the model, and -2log(L) = -2 times the log of the likelihood function at its maximum value.

Model	AICc	ΔAICc	w_i	*k*	-2log(L)
Survival (no effects); Recapture (no effects)	194.4	0.0	0.38	2	190.3
Survival (no effects); Recapture(sex)	195.9	1.5	0.18	3	189.7
Survival (sex); Recapture (no effects)	196.0	1.6	0.17	3	189.8
Survival (sex); Recapture(sex)	198.0	3.6	0.06	4	189.6
Survival (year); Recapture (no effects)	198.2	3.8	0.06	7	183.2
Survival (no effects); Recapture(year)	198.3	3.9	0.05	7	183.3
Survival (sex+year); Recapture (no effects)	200.5	6.1	0.02	8	183.1
Survival (sex); Recapture(year)	200.5	6.1	0.02	8	183.1
Survival (no effects); Recapture (sex+year)	200.5	6.1	0.02	8	183.2
Survival (year); Recapture (sex)	200.5	6.1	0.02	8	183.2
Survival (year); Recapture (year)	201.5	7.1	0.01	11	176.9
Survival (year); Recapture (sex+year)	202.5	8.1	0.01	12	175.5
Survival (sex+year); Recapture (sex)	202.8	8.4	0.01	9	183.1
Survival (sex); Recapture (sex+year)	202.8	8.4	0.01	9	183.1
Survival (sex+year); Recapture (year)	204.0	9.6	0.00	12	176.9
Survival (sex+year); Recapture (sex+year)	204.9	10.5	0.00	13	175.3

Table 3.2.2. Estimates of recapture and apparent survival probability for the population of giant gartersnakes (*T. gigas*) from 2010-2016 at the Volta Wildlife Management Area. SE represents the standard errors of the estimates, and LCI and UCI represent the lower and upper bounds of 95% confidence intervals around the estimates.

	Recapture Probability					Apparent Survival Probability				
	Year	Estimate	SE	LCI	UCI	Year	Estimate	SE	LCI	UCI
Female	2011	0.34	0.14	0.13	0.64	2010	0.70	0.16	0.34	0.91
	2012	0.30	0.09	0.16	0.49	2011	0.65	0.12	0.39	0.84
	2013	0.31	0.08	0.17	0.49	2012	0.70	0.13	0.40	0.89
	2014	0.31	0.08	0.17	0.49	2013	0.70	0.13	0.41	0.89
	2015	0.33	0.14	0.13	0.62	2014	0.65	0.13	0.38	0.85
Male	2011	0.32	0.15	0.11	0.64	2010	0.68	0.18	0.29	0.92
	2012	0.28	0.10	0.13	0.50	2011	0.63	0.14	0.34	0.85
	2013	0.28	0.10	0.13	0.50	2012	0.68	0.16	0.34	0.90
	2014	0.29	0.10	0.14	0.50	2013	0.68	0.15	0.35	0.89
	2015	0.31	0.14	0.11	0.63	2014	0.63	0.15	0.34	0.86

3.3. Fecundity

The purpose of this task was to examine fecundity in *T. Gigas*. We generally define fecundity as the number of offspring produced by a female, and we separated it into two components: i) the probability a female is pregnant, and ii) given a female is pregnant, the number of fetuses produced. Our goal was to estimate the probability of breeding and the number of fetuses for females at Volta and compare them to estimates from populations of *T. gigas* in the Sacramento Valley.

3.3.1. Methods

We collected data from one population of *T. gigas* in the San Joaquin Valley and eight populations in the Sacramento Valley (**Table 1**). In each population, we captured *T. gigas* using one or more sets of funnel traps as described above. After marking and measuring, each female was transported to the Sacramento Zoo for radiography. To assess the presence of eggs or fetuses, two orthogonal-view diagnostic radiographs were taken of each female. Each radiograph was viewed and the number of eggs or developing fetuses was counted by a board certified veterinary specialist, and the image was optimized for soft tissue visualization.

We used a Bayesian hurdle model (Cragg 1971) to analyze the data, because the fetus counts were zero-inflated (over 70% of the observations were zeroes), and it allowed us to treat the data as if they were outcomes from two ecological processes (Du et al. 2005). The first process determines an individual's pregnancy status (pregnant or not), and the second process determines the number of offspring, given that an individual is pregnant. We treated the pregnancy status of female *i*, b_i (0 = not pregnant

[no fetuses] and 1 = pregnant [at least one fetus]), as a Bernoulli random variable. Because we sampled females over the entire active period, which included periods before fetuses were sufficiently developed to be detected with radiography and after parturition, we hypothesized that the date of the radiograph would influence the probability of being pregnant, and there would be a peak in the probability of being pregnant during the active season. Therefore, we included an effect of day of year as a fixed effect in the model and represented the effect as a second order polynomial. We also hypothesized that the probability of being pregnant would be higher for longer females and included snout-to-vent length (SVL) as a fixed effect (Sparkman et al. 2007).

Both day of year and SVL were centered and scaled to have a mean of zero and unit standard deviation to minimize posterior correlation between fixed effect estimates and the intercept. Finally, we included random effects of female, population and year in the model to account for unmodeled heterogeneity at each of these levels. To restrict estimates of p from 0 to 1, we used the logit link function.

We treated the number of fetuses in a female, conditional on the female being pregnant, as a zero-truncated Poisson random variable. We hypothesized that the number of fetuses would be positively associated with SVL and treated SVL as a fixed effect. We used the same process as described above to evaluate fixed effects of rice on the number of fetuses. We also included random effects of female, population and year in the model.

To allow coupling between the two model components (pregnancy status and number of fetuses), we modeled the female, population and year random effects as bivariate normal. This specification allowed variation in the probability of breeding, among females, populations and years, to be correlated with the expected number of offspring conditional on being pregnant (i.e., it accommodates the situation where the average probability of breeding is related to the average number of fetuses, conditional on breeding). Without this correlation parameter, the random effects for breeding and the number of fetuses would be treated as independent, other than the shared response to SVL.

We used the half-Normal (0, 1) prior distributions for all standard deviation parameters, and LKJ(2) priors for the correlation parameters, which are mildly regularizing (Lewandowski, et al., 2009). We specified improper uniform distributions, from negative to positive infinity, as prior distributions for the fixed effects, β_p and β_λ. We sampled from the posterior distribution of the parameters using Stan's No-U-Turn Sampler and ran four chains for 2000 iterations each. We discarded the first 1000 iterations as warm-up. We assessed convergence by visual inspection of traceplots and by the $\hat{R}$statistic and used $\hat{R}$ < 1.1 as our criterion.

We estimated the probability of breeding and the number of fetuses per female for five populations: Volta, Badger Creek, American Basin, Natomas Basin, and the Westervelt Conservation Bank in Sutter Basin. Due to small sample sizes, we combined the data from Prichard Lake Preserve and Willy Wetlands Preserve with the data from the Natomas Basin. Pritchard Lake and Willy Wetlands are near the populations from which females were sampled in the Natomas Basin. The populations at Yolo Basin and White Slough also had small sample sizes. However, they were not close enough to combine their data with the data from other populations. For all estimates of the probability of breeding and the number of fetuses, we report 95% credible intervals in parentheses.

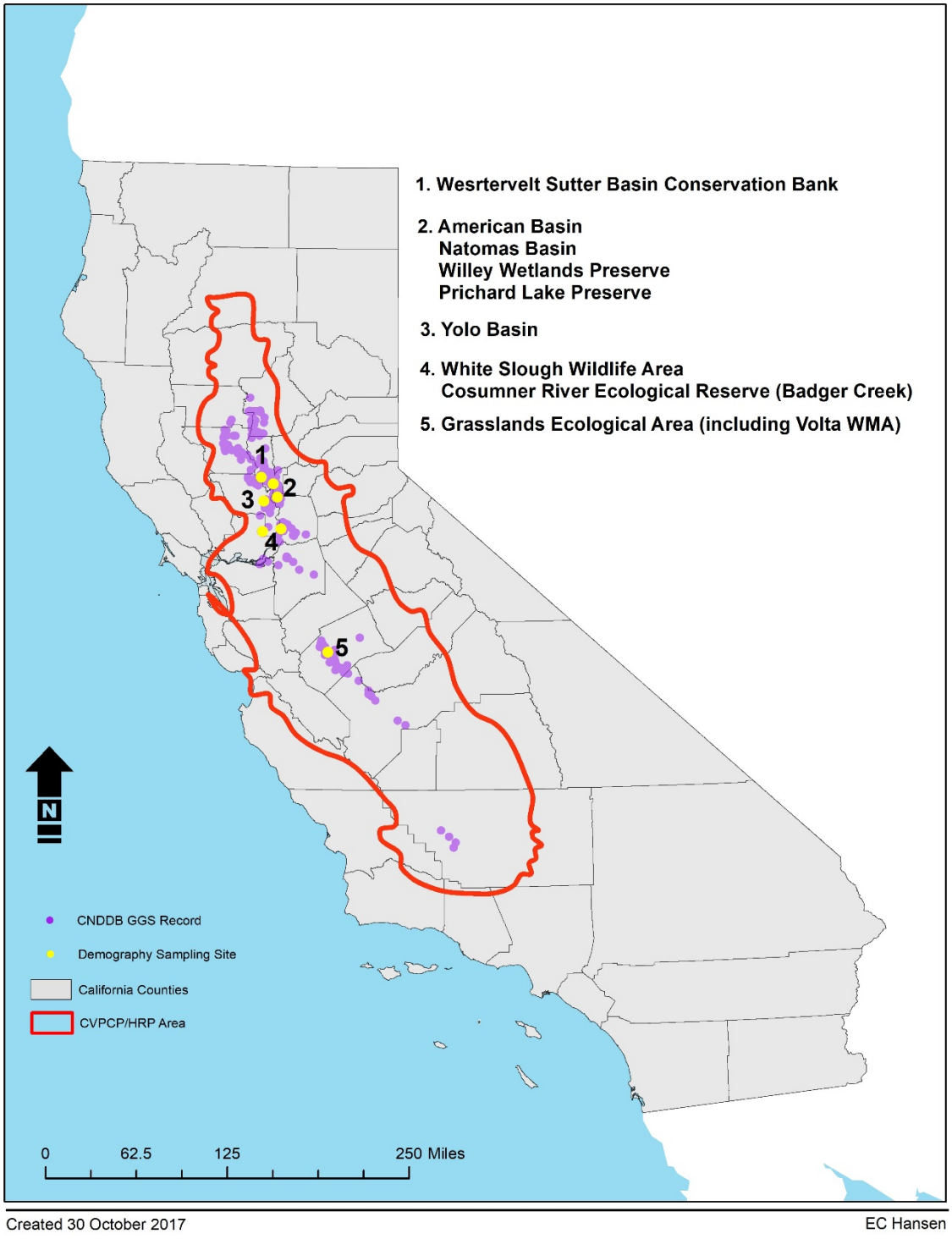
3.3.2. Results

We captured and took radiographs of 258 females over nine years. Estimates of the probability of being pregnant for an average-sized female in the middle of the breeding season (day 202 of the year [i.e., late July]) ranged from 0.23 (0.04 to 0.57) to 0.49 (0.15 to 0.85) (**Figure 3.3.1**). However, due to the low precision of the estimates, their 95% credible intervals broadly overlapped, suggesting that the estimates are not statistically different (**Figure 3.3.2**). The estimates of the number of fetuses were nearly identical, and their 95% credible intervals also broadly overlapped (**Figure 3.3.2**).

Table 3.3.1. The populations from which female giant gartersnakes (*T. gigas*) were sampled for the evaluation of fecundity. The populations were in the Sacramento and San Joaquin Valleys of California.

Valley	Population	Sample Size
San Joaquin	Volta Wildlife Management Area	69
Sacramento	American Basin	66
	Cosumnes River Preserve	22
	Natomas Basin	53
	Prichard Lake Preserve	1
	White Slough Wildlife Area	3
	Westervelt Conservation Bank, Sutter Basin	31
	Willy Wetlands Preserve	2
	Yolo Basin	11
	Total	258

Figure 3.1. Sites where data were collected for giant gartersnake (*T. gigas*) demographic analyses



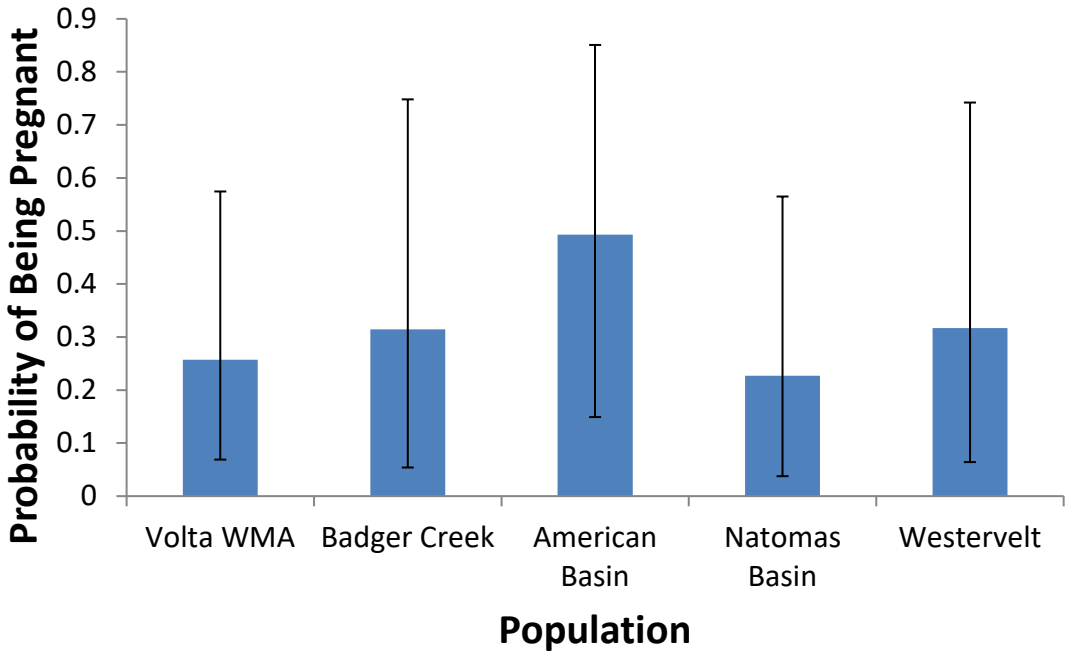


Fig. 3.2. Estimates of the probability of being pregnant for an average-sized female in an average year in the middle of the breeding season (late July [day 202 of the year]) for five populations of giant gartersnakes (*T. gigas*) in the San Joaquin and Sacramento Valleys. Black lines represent 95% credible intervals.

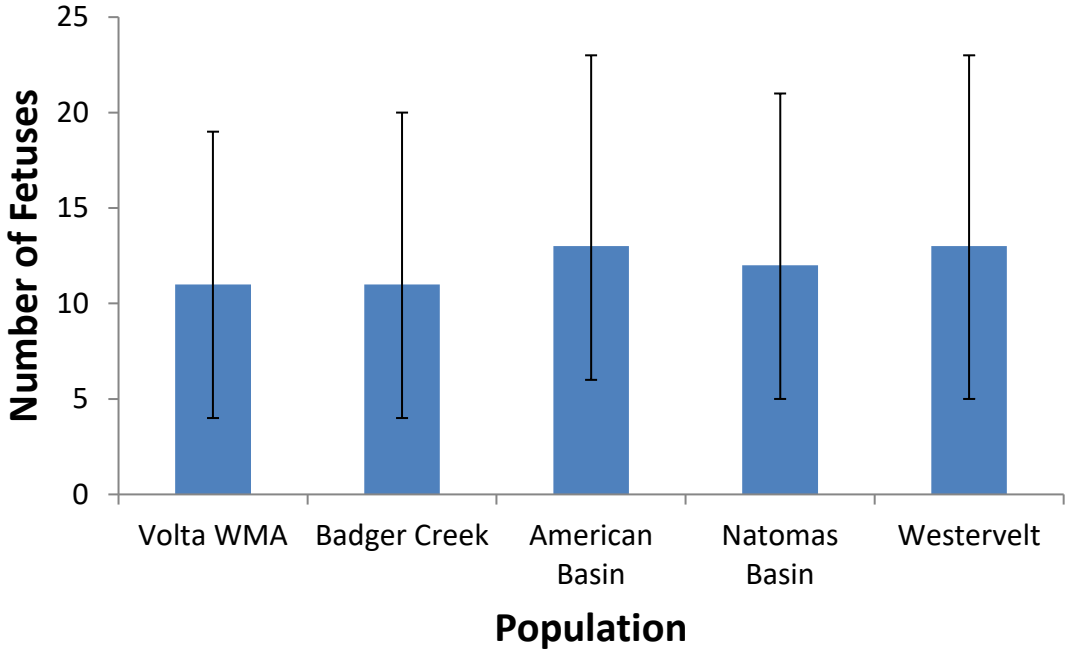


Fig. 3.3. Estimates of the number of fetuses given pregnancy for an average-sized female in an average year in the middle of the breeding season (late July [day 202 of the year]) for five populations of giant gartersnakes (*T. gigas*) in the San Joaquin and Sacramento Valleys. Black lines represent 95% credible intervals.

4. OCCUPANCY

Species are rarely detected with certainty in studies of spatial distributions (Gu and Swihart 2004), and, as we noted above, detecting *T. gigas* at locations where they occur can be particularly challenging. A primary goal of this component of the project is to provide insights regarding the distribution of *T. gigas* in the Volta and Mendota areas (hereafter, study area). We completed two occupancy analyses in the report. In the first analysis, we examined the effects of water quality on the distribution of *T. gigas* in the study area. In the second analysis, we evaluated several hypotheses regarding the effects landscape-level covariates on *T. gigas*. We used the inferences from the second occupancy analysis to develop a map of predicted occupancy in the study area and across the species' range. We used our knowledge of the ecology of *T. gigas* and the results from our studies of the spatial distribution of *T. gigas* in other portions of its range (e.g., Hansen et al. 2017) to develop a set of covariates of occupancy.

4.1. Methods

Like most occupancy work conducted for *T. gigas* to date (e.g., Halstead et al. 2009, 2011; Hansen et al. 2017), this project was designed to use detections and associated covariates recorded in association with trapping to derive estimates of occupancy and identify attributes of wetlands and their landscapes that were correlated with occupancy. However, because trapping efforts failed to produce detections anywhere besides the Volta WMA, we chose instead to utilize the results of the eDNA surveys described above in Section 2.3 and data on *T. gigas* from elsewhere in the species' range. In 2016 and 2017, we collected water samples for environmental DNA at 52 locations. At all locations we collected two filters during one visit. For a subset, we revisited locations and collected one or two additional filters, which resulted in two to four filters at each location. We used a subset of these data to test for associations between occupancy and water quality in the Volta and Mendota areas. We combined the occupancy data from eDNA with data from *T. gigas* trapping at 159 sites in the Sacramento Valley to develop a map of predicted occupancy in the study area and across the species range.

For both occupancy analyses, we analyzed the data with single-season, hierarchical occupancy models in Program MARK (White and Burnham 1999, MacKenzie et al., 2002, 2006). The occupancy models included two parameters: detection probability, and the probability of occupancy (the probability that the species occupied location *i* during any survey in 2016 or 2017). Prior to analyses, we developed sets of hypotheses describing the spatial variation in detection and occupancy probabilities and converted these hypotheses into mathematical models. We describe the hypotheses for each analysis in the following sections. Use of occupancy models to estimate probabilities of detection requires multiple surveys at each location (MacKenzie et al., 2002, 2006), and we treated the multiple filters as replicates for the eDNA data and phases of sampling as replicates for the data from the Natomas Basin (see Hansen et al. 2017).

4.1.1. Water quality

The goal of the first analysis was to evaluate the effects of water quality and water chemistry on the probability of occupancy in *T*. *gigas*. We only collected water quality and chemistry data from 32 of the 52 locations with eDNA data, and consequently, this analysis was conducted on a subset of the full eDNA dataset. Over 80% of the locations (n = 27) from which eDNA were collected were sampled with two filters, 9% (n = 3) were sampled with three filters, and the remaining locations (n = 2) were sampled with four filters. We considered the species to be detected at a given location if *T*. *gigas* DNA was detected in at least one filter.

We fit models to the data in two stages (Lebreton et al., 1992). In the first stage, we focused on identifying the best models of detection probability and included effects of covariates, as well as a model with no covariates (intercept-only or null model). In this stage, the model on occupancy probability was a model with no effects. We hypothesized that detection probability would be higher in areas with higher abundance of *T*. *gigas* and abundance of *T*. *gigas* would be driven by prey and predator abundances. We used data from trapping that occurred at the locations of the water samples to generate indices of prey and predator abundance. As noted above, we captured several species in the traps and used counts of the number of individuals of each species as the indices. Specifically, we used counts of the number of bullfrog tadpoles, mosquitofish, carp, bass, sunfish, and crayfish for each set of traps. We also summed the counts for bullfrog tadpoles, mosquitofish, carp, bass, and sunfish (all putative prey species for *T*. *gigas*). For each species or collection of species, we computed catch-per-unit-effort (CPUE) the number of each species per trap day. We separately fit models with the CPUE for each species or group, as well as a model of no effects on detection probability (a total of eight models). We used Akaike's information criterion adjusted for small sample size (AIC_C) and Akaike weights (w_i) to evaluate the models of detection probability and in all other model selection procedures (Burnham and Anderson, 2002). We considered models with AIC_C values within 2 points of one another to have similar statistical support (Burnham and Anderson 2002). We also examined 95% confidence intervals (CI) around estimates of regression coefficients to quantify the importance of the covariates. If the CIs included 0, we did not use the model with the covariate in the second stage of the analysis.

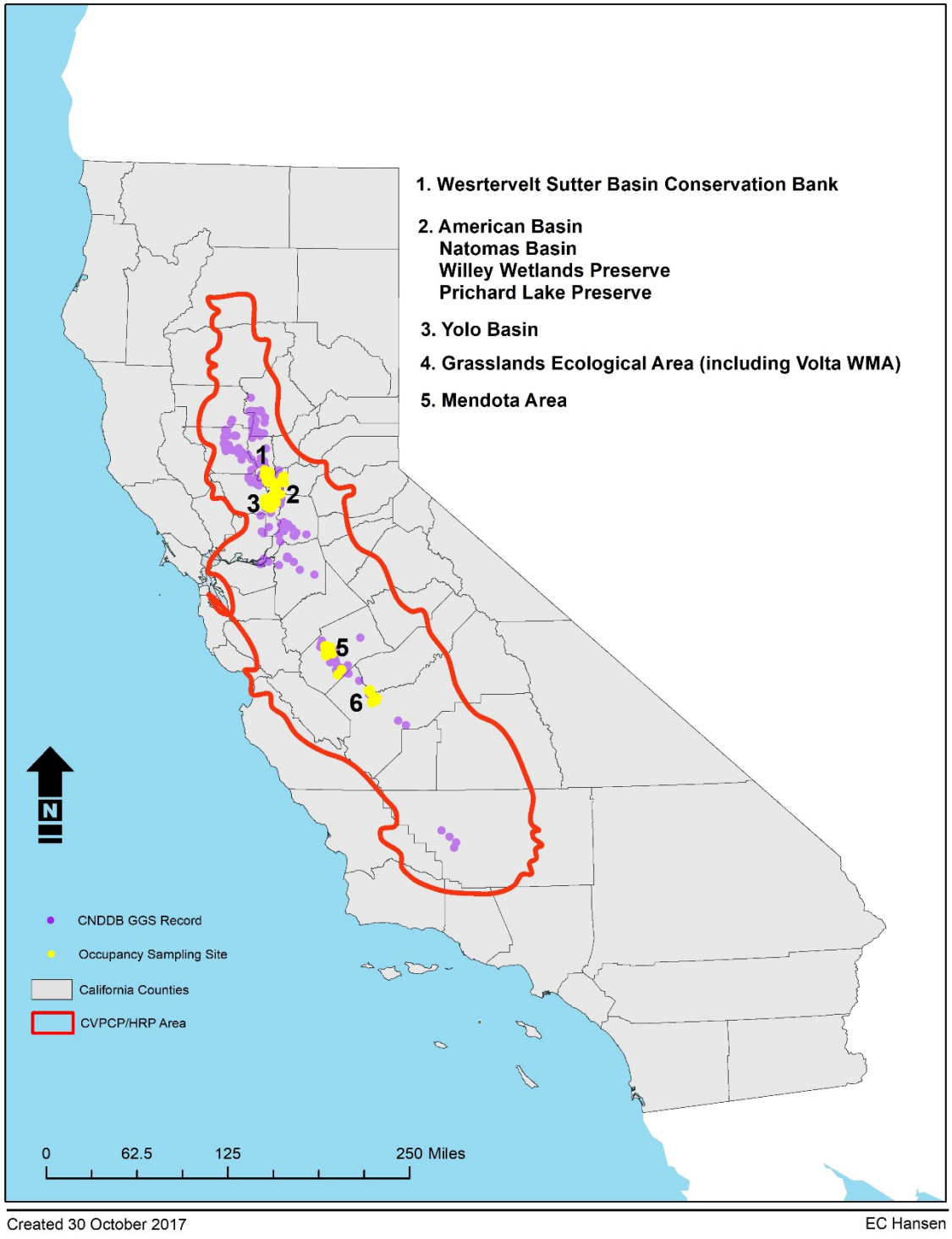
In the second stage, we evaluated models of occupancy probability. We combined the models of occupancy probability with all supported models of detection probability from the first stage. The models of occupancy included five covariates related to water chemistry or quality: boron concentrations (parts per million), conductivity (mS/cm), salinity (parts per trillion), percentage of dissolved oxygen concentration, and pH. We fit models to the data with an intercept-only model of occupancy or an intercept and a single water-related covariate. As above, we evaluated the importance of the covariates of occupancy probability by examining AIC_C, w_i, and the 95% confidence intervals around the estimates of the regression coefficients. In the results that follow, we report estimates with their 95% CIs in parentheses.

4.1.2. Landscape

From 2009 through 2012 in the Sacramento Valley, we conducted surveys of *T. gigas* in canals, sloughs, and wetlands using methods identical to those described in **Section 2.1.1**. We sampled 159 sites in the American and Yolo Basins and the southern Sutter Basin (**Figure 4.1)**. The study area covers at least 50% of the current range of *T. gigas* in the Sacramento Valley. In each of the four years of the study, we sampled sites during two phases. The first phase was from early May through mid-June, and the second phase was from mid-July through mid-September. We chose these phases to encompass differences in life histories among sexes and age classes. During the first phase, males recently have left hibernacula and are exposed to traps while searching for mates and food (Coates et al. 2009). During the second phase, females have given birth and are exposed to traps while searching for food. We deployed traps for a minimum of two weeks at each site in a phase, and maintained and checked all deployed traps daily. In 2016 and 2017 in the San Joaquin Valley, we conducted occupancy surveys using environmental DNA as described in **Section 2.3.1.**

We derived eight environmental variables, seven continuous (**Table 4.1**) and one categorical, at each site. To derive four variables related to the land-cover adjacent to sampled wetlands, we used ArcGIS (ArcGIS Desktop, release 10.2, Environmental Systems Research Institute, Redlands, California) to place a grid (30 m by 30 m cells) over a spatial extent that included *T. gigas* estimated historic range. We estimated the percent cover of the four cover classes (**Table 4.1**) for each cell by computing the proportion of cells within a 1600 m neighborhood of the cell. The spatial distributions of these classes changed little among the four years in the Natomas Basin and two years in the San Joaquin Valley. Because all roads were classified by the US Department of Agriculture (2016) as urban, the proportion of urban cover could be overestimated. Therefore, we retained clusters of urban cells and removed narrow strips of urban cells that usually represented roads (Theobald 2013). We used a 30-m digital elevation models to assign elevation to each cell in the study areas. We included elevation, because we expected it to represent environmental conditions at cells that were not represented by the other covariates (e.g., air temperature). We hypothesized that occupancy would be negatively associated with elevation, road density, and the proportions of urban areas, grasslands, and row crops and fallowed fields (Table 1). We also hypothesized that occupancy would positively associated with canal density and the proportions of rice and wetland (**Table 4.1**; Halstead et al. 2010, Hansen et al. 2017).

Figure 4.1. Sites where data were collected for giant gartersnake (*T. gigas*) occupancy analyses



We used data from the National Cooperative Soil Survey (SSURGO) to classify the soils underlying each site as belonging to one of the following orders: vertisols, mollisols, alfisols, entisols, inceptisols, and aridisols. Our previous work indicated that occupancy probability in *T*. *gigas* was not different at sites underlain by vertisols, mollisols, and alfisols and was greater than sites underlain by entisols and inceptisols. Therefore, we pooled sites underlain by vertisols, mollisols, and alfisols into one group and pooled sites underlain by all other soil orders into a second group. One site was underlain by soils in the aridisol order. Based on a description of the order, we pooled this site with the sites on entisols and inceptisols. The majority of the sites (n = 193 of 211 sites) were located on vertisols, mollisols, and alfisols. We hypothesized that historic hydrologic and geomorphologic processes drove soil formation in the valley, and that wetlands and their underlying soil types develop distinct hydrogeochemical signatures that affect habitat selection by *T*. *gigas*.

We pooled the data from the four years in the Sacramento Valley and the two years in the San Joaquin Valley into a single analysis. For data from the Sacramento Valley, we treated surveys during the two phases in each of the four years of the study as replicates, which resulted in eight possible surveys per site. We considered the species to be detected at a given site in a given phase if at least one individual was captured in any trap. For data from the San Joaquin Valley, we treated the data from each filter as replicates, which resulted in five possible surveys per site (as many as two filters at a site in 2016 and three filters at a site in 2017). Locations from which these data were collected are presented in **Figure 4.1.**

We fit models to the data in two stages (Lebreton et al. 1992). First, we combined models of detection probability with a model of occupancy that included a fixed effect of soil order. Models of detection probability for the data from the Sacramento Valley included fixed effects of year or phase. In models that included effects of year, we did not allow estimates of detection probability for the two phases within a year to vary, but allowed estimates to vary among years. In models that included effects of phase, we allowed estimates of detection probability to vary among all phases. Because we did not sample the same sites in all phases and weather conditions varied among phases, we expected detection probability to vary among the phases. For the data from the San Joaquin Valley, we assumed detection probability was the same among filters within a year, because the samples were collected on a single visit to the site within years. We fit models that allowed estimates of detection probability to differ between 2016 and 2017 and to be the same in 2016 and 2017. We used Akaike's information criterion adjusted for small sample size (AIC_C) and Akaike weights (w_i) to evaluate the models of detection probability and in all other model selection procedures (Burnham and Anderson 2002).

In the second stage, we combined all models of occupancy with the highest-ranked models of detection probability. The models of occupancy included the eight environmental variables described above. Initially, we fit models to the data with an intercept-only (i.e., null) model of occupancy or included a single environmental variable.

We identified the variables for which the 95% confidence intervals (CIs) around the estimates of the regression coefficients did not include 0, and combined those variables into models of occupancy probability with multiple predictor variables. However, we did not include two variables in the same model if their correlation coefficient was > |0.60|. Similarly, we did not include multiple land-cover variables in the same model because they were inherently correlated (i.e., an increase in the proportion of one land-cover type decreased the proportions of the other land-cover types). We evaluated the relative strengths of association of the environmental variables with occupancy on the basis of their occurrence in the highest-ranked models and the estimates of their regression coefficients and their 95% CIs. We report the 95% CIs in parentheses for all point estimates.

We generated a map of predicted *T. gigas* occupancy for the species' range using a weighted average of the estimated occupancy probability for each 30-m cell in the grid. We predicted the probability of occupancy for cells based on each model in **Table 4.2**. Therefore, each cell had 10 predicted probabilities of occupancy (one from each model). We derived a weighted average across the 10 predicted probabilities for each cell using Akaike weights (w_i; Table 2) to give greater influence to predictions from models with more support in the data. We evaluated the map of predicted occupancy using data from the California Natural Diversity Database (CNDDB 2016). The data, which are presence-only, include coordinates of observations of *T. gigas* from across the Sacramento and San Joaquin Valleys and provide a useful, independent dataset for evaluating the predictions of the map.

Table 4.1. The continuous covariates we used in submodels of occupancy of giant gartersnakes (T. gigas) in the Natomas Basin and San Joaquin Valley, and sources of spatial data.

Covariate	Abbreviation in model descriptions	Source of data
Elevation	elev	U.S. Geological Survey 30-m digital elevation model (http://nationalmap.gov/)
Proportion of urban cover	urban	2013 U.S. Department of Agriculture National Agricultural Statistics Service Cropland Data Layer (2013 CDL) (http://nassgeodata.gmu.edu/CropScape/)
Proportion of rice or wetland	rice.wetland	2016 U.S. Department of Agriculture National Agricultural Statistics Service Cropland Data Layer (2016 CDL)
Proportion of row crops or fallowed fields	rowcrop.fal	2016 CDL
Proportion of grassland	grassland	2016 CDL
Canal density	canal	2017 National Hydrography Dataset (2017 NHD) (https://nhd.usgs.gov/data.html)
Road density	road	2016 TIGER/Line Shapefiles. Prepared by the U.S. Census Bureau, 2015 (2015 TIGER).

4.2. Results

The model-selection results from the first stage of modeling indicated that detection probability differed among phases and years in the Sacramento Valley and were the same in 2016 and 2017 in the San Joaquin Valley (**Table 4.2**). Estimates of detection probability from the highest ranked model were highly variable across years and phases and ranged from 0.06 (0.01 – 0.34) to 0.85 (0.68 – 0.94). Estimates of detection probability from the eDNA data in the San Joaquin Valley were 0.34 (0.25 – 0.46).

The highest-ranked models included effects of soil and the percentage of row crop and fallowed fields in the landscape around a site (Table 3). In addition, estimates of the 95% confidence intervals around regression coefficients did not include 0 for these two covariates. Therefore, we combined them in a model and fit the model to the data. It was the most supported model, and estimates of regression coefficients indicated that occupancy is higher at sites underlain by alfisols, mollisols, and vertisols relative to the remaining soil types and lower at sites with more row crops or fallowed fields around them. There was little to no support for all other models, suggesting low associations between occupancy probability and the remaining covariates (**Table 4.3.**). The estimated regression coefficient for alsifols, mollisols, and vertisols from the highest-ranked model was 1.96 (0.43 – 3.48), and the estimate for the proportion of row crops and fallowed fields was -0.72 (-1.42 - -0.02). Model-averaged estimates of predicted occupancy ranged from 0.137 to 0.845. A visual inspection of historical observations of *T. gigas* occurrence from the California Natural Diversity Database indicated a suggested the map of predicted occupancy represented a good fit to the data, though indicating that our models likely overestimate occupancy due to the low number of covariates developed using the small dataset.

Table 4.2. Model-selection results for landscape models of occupancy probability of giant gartersnakes (*T. gigas*) in the Sacramento and San Joaquin Valleys. AICC, Akaike's Information Criterion adjusted for small sample size; Δ AICC, the AICC of the model minus the AICC of the highest-ranked model; wi, Akaike weight; -2Log(L), -2 times the value of the likelihood at its maximum. Models shown are only for occupancy probability, and all models have the same structure on detection probability (interaction between year and phase for the Sacramento Valley and the same estimate for 2016 and 2017 for the San Joaquin Valley). Landscape covariates are defined in Table 4.1.

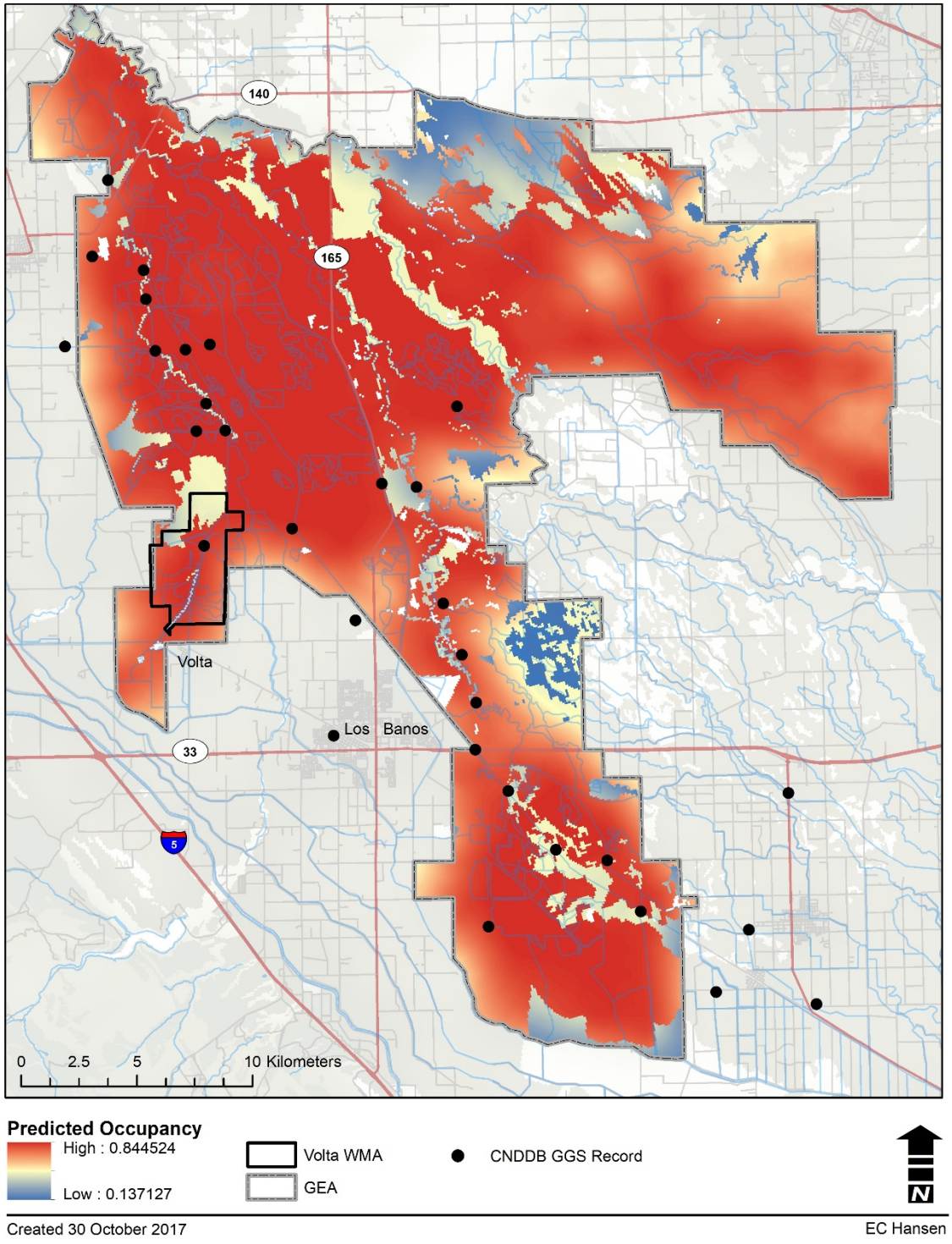
Model	AIC_C	ΔAIC_C	w_i	No. Parameters	-2Log(L)
soil + rowcrop.fal	624.2	0.0	0.65	12	598.6
soil	626.2	2.0	0.24	11	602.8
rowcrop.fal	628.1	3.9	0.09	11	604.7
rice.wetland	633.6	9.4	0.01	11	610.3
urban	634.3	10.1	0.01	11	611.0
Intercept-only	634.8	10.6	0.00	10	613.7
elev	635.4	11.2	0.00	11	612.1
canal	636.3	12.1	0.00	11	613.0
road	636.4	12.2	0.00	11	613.1
grassland	637.0	12.8	0.00	11	613.7

4.2.1. Water quality

The model-selection results from the first stage of modeling provided no support for effects of prey or predator abundances on detection probability of *T. gigas*. Although the highest-ranked model included an effect of the abundance of bass, the CI that represented the effect broadly overlapped 0. The second-ranked model was a model of no effects. The estimate of the probability of detection from the model with no effects was 0.27 (0.18 – 0.38), indicating that environmental DNA (collected under the limitations described in **Section 2.3.1,** above) is expected to detect *T. gigas* in approximately one in four filters when the species is present at a location.

All models of occupancy probability that included water-related covariates failed to properly converge, which was caused by the relatively low detection probability and small number of filters from each location. A primary design recommendation for occupancy studies is to achieve a probability of false absence equal to 0.05 to 0.15 (MacKenzie et al. 2006). Due to low filter-specific detection probability (0.27) and the low number of filters at each location (two or three at most locations), estimates of the probability of false absences from our study were 0.39 to 0.53, much higher than the recommended probability. To achieve the recommended level under the conditions in which these samples were collected (late season with high rates of flow), 7 or more filters per location would be required. Modifications to sampling protocols may also help to increase sensitivity and reduce the number of samples required.

Figure 4.2. Predicted occupancy for giant gartersnakes (*T. gigas*) in the Grasslands Ecological Area



5. DISCUSSION

From the standpoint of species recovery, *T. gigas* populations within the SJV are represented by three unique management areas; North and South Grasslands (GEA), Mendota Area, and the Lanare/Burrel Area (USFWS 2012). Tulare Lake Basin and Kern-Wasco Area populations are presumed to be extirpated, and observations of deteriorating habitat at Burrell-Lanare in 1992 led to the conclusion in the final listing that this population, if it was not already extirpated, was severely and imminently threatened (Hansen and Brode 1980; USFWS 1993). Studies conducted in the Buena Vista region, Fresno Slough, Kern National Wildlife Refuge, Kings River, and North Kings River during 2006 supported this conclusion (Wylie and Amarello 2008).

Reported occurrences of *T. gigas* in the SJV originate south and west of the San Joaquin River where large wetland complexes are still maintained (**Figure 2.1** and **Figure 2.2**). Most of these locality records were described during range-wide status and distribution surveys conducted by George Hansen from 1976 through 1995. Earlier surveys during this period described populations near Mendota and Los Banos as widespread, occurring in densities comparable to those found in the rice growing regions of the Sacramento Valley (Hansen and Brode 1980).

In subsequent surveys conducted from 1986-1987 and in 1995, *T. gigas* were not detected in areas where they had been found previously (Hansen 1988, 1996). Many or most of the sites established in the 1970's had deteriorated in quality and many sites were either maintained without water or without ample vegetative cover during the spring and summer *T. gigas* active season (Hansen 1996). However, *T. gigas* appeared to have declined more rapidly and to a greater extent than had putative habitat, suggesting that factors other than habitat loss may contribute to the decline of *T. gigas* in the San Joaquin Valley (Hansen 1996). Extensive trapping conducted by the U.S. Geological Survey and California Department of Fish and Game in the GEA and Mendota Wildlife Areas of the SJV from 1998 through 2004 confirmed that *T. gigas* were still present in some areas. Rapid declines, however, were still apparent (Dickert 2003, 2005; Sloan 2004; Williams et al. 2004).

Subsequent CVPIA HRP-funded work by Eric Hansen in 2007 and 2008 (Hansen 2007, 2008; Hansen et al. 2011) detected *T. gigas* in the Los Banos area at only three locations, and intensive surveys conducted during 2015 and 2016 have failed to detect any *T. gigas* outside of the Volta Wildlife Area using trapping techniques (E.C. Hansen, unpublished data). Volta now supports the only *T. gigas* breeding population currently known from the San Joaquin Valley (Hansen 2008a, 2008b; USFWS 2012). In the Los Banos area, this could represent the loss of at least half of the known genetic diversity (Wood et al. 2015). Although genetic data and demographic (e.g., survival and fecundity) estimates are available for Volta and Los Banos, no population estimates are available for Mendota and no tissue samples are available for genetic analyses.

While intensive trapping throughout the San Joaquin Valley in 2015 and 2016 supports the conclusions originating from multiple projects over the past decade, new sampling techniques suggest that *T. gigas* may be more broadly distributed in the San Joaquin Valley than previously assumed. Regardless, the cumulative results of this work suggest that remaining *T. gigas* in the San Joaquin Valley persist at very low densities. Recent studies show that San Joaquin Valley *T. gigas* populations are genetically distinct, have experienced significant declines and loss of genetic variation in the recent past, and are the most at-risk populations of the species (Wood et al. 2015). *T. gigas* is a secretive and elusive species clearly occurring at low density in some locations. Surveys addressing current distribution and occupancy must, therefore, include survey and analytical methods (e.g. Halstead et al. 2009, 2011) that account for low expected detection probabilities. Enhancing survey method sensitivity will improve project and recovery planning for *T. gigas*.

A clear progression in sampling technique and detection rates has occurred since *T. gigas* were first identified in the San Joaquin Valley. From the 1940's through the 1970's and 1980's, *T. gigas* in the San Joaquin Valley occurred at densities facilitating routine observations through visual encounter surveys. When visual encounter surveys failed to produce detections, intensive trapping surveys confirmed presence at many sites, though detections have since diminished to the point that *T. gigas* now are presumed extirpated throughout much of the San Joaquin Valley. Techniques utilizing scent detection dogs and environmental DNA, that we have pioneered through collaboration with H.T. Harvey & Associates and Cramer Fish Sciences, respectively, confirm that *T. gigas* are still present at some sites where intensive trapping has been unsuccessful (E.C. Hansen, unpublished data). The low detection probabilities associated with eDNA should not be considered a fatal flaw. Rather, because the low detection probabilities observed in this study are largely attributable to funding and time limitations preventing rigorous field sampling, these results should be used to enhance the sampling design of future distribution studies that make use of eDNA. Because of the work done in this study, future studies will have the information needed to design studies for strong inference. When *T. gigas* densities are low, two to four filters per location may simply be inadequate. Rather, seven or more filters may be required to achieve desirable detection probabilities. In addition, we suspect that adjustments to field methods (e.g., refraining from sampling on days of high water flow) and alterations to filters that allow more water to be pumped through them may also improve detection probabilities. Finally, these newly developed techniques have not been applied across the entirety of the species' putative historic range. Given that low-density populations of *T. gigas* are likely to persist in other locations that have only be sampled by trapping, the full extent of the species' contemporary distribution may be underestimated.

This work contributes to ongoing efforts funded by the CVPIA Habitat Restoration Program, which seeks to determine the extent of the contemporary distribution and the status of populations of *T.* gigas in the SJV. We have developed abundance, survival, and fecundity estimates for *T. gigas* at the Volta WMA and compared them with

estimates for populations in the Sacramento Valley. We have utilized inferences resulting from these surveys and remotely-sensed data to generate a map of occupancy probability (e.g. Dickson et al. 2013) for select areas of the San Joaquin Valley both presently and formerly occupied by *T. gigas*. We have identified a preliminary list of covariates that are associated with the probability of occupancy at a location. These tools will be useful to land managers for a variety of reasons, including identifying locations for future surveys where *T. gigas* are most likely occur and determining locations in the study area where maintaining habitat for *T. gigas* is most critical. Such maps can be progressively expanded as data become available, and can be useful to resource managers for a variety of reasons, including: 1) increased ability to efficiently plan and prioritize maintenance work, particularly in relation to potential mitigation; 2) ability to prepare an avoidance and implementation strategy that is compatible with relevant operations and maintenance activities and can be leveraged into permits; and 3) ability to document increases in populations/distribution and hence the efficacy of avoidance and minimization measures.

6. RECOMMENDATIONS

The work presented here provides new and updated information regarding *T. gigas* distribution in the SJV, yet there is much information vital to successful conservation planning that remains undeveloped. This work helps clarify steps to be taken going forward. A brief list of our recommendations for future work is presented below:

- Establish a mechanism for maintaining reliable water in areas known or likely to support *T. gigas* and develop a method for prioritizing these areas;
- Establish protocols for maximizing eDNA sensitivity/probability of detection;
- Apply eDNA sampling over a broader landscape, including the southern SJV (e.g., areas south of Mendota such as Kern, Buena Vista, and Tulare);
- Use the updated spatial information to refine predicted occupancy for the entirety of the SJV;
- Use predicted occupancy or data derived from other sensitive techniques (i.e., eDNA sampling and scent detection dogs) to focus demographic and genetic studies;
- Use resulting information to refine conservation strategies for the SJV, including, but not limited to, developing a repatriation plan.

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8. APPENDICES

The tables listed in this section provide ready access to variable that are addressed in the text, but do not provide an exhaustive list of all data collected as part of this two-year project. Covariate data, such as environmental data associated with individual traps, daily weather conditions, water temperatures, etc. are managed within an electronic database managed by the PI.

To request copies of these data, you may contact the PI, Eric Hansen, at echansen@sbcglobal.net or through www.hansenbio.org.

Appendix A. 2015-2016 GGS Trapping Quadrat Geocoordinates[1] and survey Dates

Quadrat ID	Centroid Easting	Centroid Northing	Start Date	End Date	Total Days	Total Captures
VWW01	685252	4112697	5/17/2015	8/15/2015	90	1
VWW02	685202	4112614	5/17/2015	8/15/2015	90	3
VWW03	685174	4112524	5/17/2015	8/15/2015	90	3
FDT01	685951	4112787	5/15/2015	8/15/2015	92	5
FDT02	685843	4112712	5/15/2015	8/15/2015	92	7
FDT03	685737	4112696	5/15/2015	8/15/2015	92	4
FDT04	685622	4112680	5/15/2015	8/15/2015	92	5
FDT05	685529	4112669	5/15/2015	8/15/2015	92	2
FDT06	685083	4112700	5/16/2015	8/15/2015	91	1
FDT07	685127	4112821	5/16/2015	8/15/2015	91	1
FDT08	685279	4112811	5/16/2015	8/15/2015	91	2
FDT09	685333	4112794	5/16/2015	8/15/2015	91	3
FDT10	685651	4112780	5/16/2015	8/15/2015	91	0
FDT11	685882	4112727	5/15/2015	8/15/2015	92	3
FDT12	685792	4112641	5/15/2015	8/15/2015	92	9
FDT13	685541	4112615	5/15/2015	8/15/2015	92	1
FDT14	685375	4112685	5/15/2015	8/15/2015	92	3
FDT15	685078	4112795	5/16/2015	8/15/2015	91	0
FDT16	684983	4112719	5/16/2015	8/15/2015	91	1
FDT17	685202	4112717	5/16/2015	8/15/2015	91	0
FDT18	685157	4112773	5/16/2015	8/15/2015	91	2
FDT19	685605	4112796	5/16/2015	8/15/2015	91	1
FDT20	685651	4112780	5/16/2015	8/15/2015	91	2
FDTREF	685835	4112801	6/19/2015	8/15/2015	58	2
GMB4	686275	4112848	5/23/2016	6/29/2016	38	0
GMB5	686025	4112545	5/23/2016	6/23/2016	32	0
MS01	698715	4104204	6/4/2016	6/15/2015	12	0
MWA1	742533	4062686	6/1/2016	7/6/2016	36	0
MWA2	738711	4062040	6/2/2016	6/29/2016	28	0
MWA3	739253	4064967	6/3/2016	7/7/2016	30	0
MWA4	740985	4064140	6/8/2016	8/16/2016	70	0
MWA5	742621	4063315	7/6/2016	9/29/2016	86	0
MWA6	742626	4063477	7/7/2016	9/29/2016	85	0
MWA7	738672	4060651	7/8/2016	8/18/2016	42	0
MWA8	741021	4064223	8/17/2016	9/28/2016	43	0
MWA9	742393	4062254	8/19/2016	9/28/2016	41	0
MWA10	740848	4067501	8/20/2016	9/26/2016	38	0
MWA11	740563	4067514	8/20/2016	9/26/2016	38	0
MWA12	742430	4063774	8/27/2016	9/28/2016	33	0
MWA13	743865	4065934	8/29/2016	9/27/2016	30	0
MWA14	743631	4065473	8/29/2016	9/27/2016	30	0
MWA15	742362	4066797	8/30/2016	9/27/2016	29	0
NG01	684878	4112876	06/22/2015	07/29/2015	37	0
NG02	684498	4113424	06/23/2015	07/30/2015	37	0
NG03	685312	4113575	06/24/2015	08/05/2015	42	0
NG04	684992	4113574	06/25/2015	08/06/2015	42	0
NG05	686022	4115869	07/14/2015	07/29/2015	15	0
NG06	685978	4113661	07/15/2015	07/29/2015	14	0
NG07	685894	4117363	07/15/2015	07/29/2015	14	0

NG08	684823	4118840	07/29/2015	09/15/2015	48	0
NG09	685120	4117999	07/30/2015	09/09/2015	42	0
NG10	685669	4113561	08/05/2015	08/28/2015	23	0
NG11	685189	4113310	08/06/2015	08/29/2015	23	0
NG12	685161	4118446	08/06/2015	09/15/2015	40	0
NG13	685887	4112724	08/15/2015	08/30/2015	15	5
NG14	685129	4112820	08/15/2015	08/30/2015	15	0
NG15	685889	4117115	08/20/2015	09/15/2015	26	0
NG16	684287	4119143	08/21/2015	09/15/2015	25	0
NG17	685816	4117485	09/11/2015	09/15/2015	4	0
NG18	685880	4117377	5/21/2016	7/28/2016	69	0
NG19	685749	4117157	5/22/2016	6/21/2016	31	0
NG20	686006	4114699	5/22/2016	6/25/2016	35	0
NG21	684503	4113399	5/26/2016	6/14/2016	20	0
NG22	685795	4117125	5/26/2016	6/27/2016	33	0
NG23	685783	4112787	5/27/2016	8/17/2016	83	7
NG24	685419	4120821	5/29/2016	6/28/2016	31	0
NG25	690809	4120504	5/29/2016	6/20/2016	23	0
NG26	685871	4121689	6/20/2016	7/30/2016	41	0
NG27	686292	4119742	6/21/2016	8/9/2016	50	0
NG28	685845	4112685	6/22/2016	8/17/2016	57	12
NG29	685165	4112771	6/23/2016	9/13/2016	83	3
NG30	684717	4118955	6/27/2016	6/16/2016	51	0
NG31	684342	4121635	6/28/2016	8/9/2016	43	0
NG32	687191	4117975	6/29/2016	8/9/2016	42	0
SJR1	736029	4074584	6/13/2016	7/13/2016	30	0
SJR2	735991	4074518	6/13/2016	7/13/2016	30	0
SJR3	735322	4074425	6/14/2016	7/14/2016	30	0
SJR4	735313	4074357	6/14/2016	7/14/2016	30	0
SJR5	735323	4073596	6/15/2016	8/15/2016	61	0
SJR6	735359	4073652	6/15/2016	7/15/2016	30	0
SJR7	735645	4074540	7/13/2016	8/14/2016	32	0
SJR8	735685	4074482	7/13/2016	8/14/2016	32	0
SJR9	734968	4074323	7/14/2016	8/13/2016	30	0
SJR10	735028	4074258	7/14/2016	8/13/2016	30	0
SJR11	735104	4073740	7/15/2016	8/14/2016	30	0
SJR12	734723	4074183	8/13/2016	9/12/2016	30	0
SJR13	734951	4074102	8/13/2016	9/12/2016	30	0
SJR14	736675	4072161	8/14/2016	9/12/2016	30	0
SJR15	736634	4072097	8/14/2016	9/13/2016	31	0
SJR16	736547	4072239	8/15/2016	9/13/2016	30	0
SJR17	736687	4071879	8/15/2016	9/13/2016	30	0

1. North American Datum 1983, UTM Zone 10

Appendix B. 2015-2016 Prey Catch Per Unit Effort (CPUE)

Quadrat ID	Trap Days	CPUE (Bullfrog)	CPUE (Mosquitofish)	CPUE (Carp)	CPUE (Bass)	CPUE (Sunfish)	CPUE (Crayfish)
VWW01	)0	)0323	)0508		)0169	)0667	)1111
VWW02	900	0	.00889	0	0	.00444	.01000
VWW03	900	0	.01222	0	0	.00444	.01935
FDT01	610	0	.02787	0	0	.00164	.00492
FDT02	910	0	.05652	0	.00326	.00543	.02391
FDT03	910	0	.06630	0	.00109	.00978	.01848
FDT04	910	0	.01522	0	.00217	.02065	.00652
FDT05	910	.00109	.00326	0	.00217	0	.01304
FDT06	910	0	.00769	0	0	.00330	.00440
FDT07	910	0	.00549	0	0	.00330	0
FDT08	910	0	.02967	0	.00330	.01209	.00110
FDT09	910	0	.01868	0	0	.00769	.00330
FDT10	910	.00110	.00166	0	0	.00167	.01648
FDT11	910	0	.01739	0	.00435	.00761	.01957
FDT12	910	.00109	.07065	0	.00326	.00109	.02283
FDT13	910	.00326	.04783	0	.00435	.01739	.01522
FDT14	910	0	.00435	0	.00217	.00109	.07391
FDT15	910	0	.00549	0	0	.00220	.00220
FDT16	910	0	.00989	0	.00330	.00220	.00879
FDT17	910	0	.00769	0	0	.00110	.00769
FDT18	910	0	.00500	0	0	.00330	.00989
FDT19	910	.00330	.01648	0	.00329	.00769	.01978
FDT20	910	.00220	.03166	0	.00833	.00769	.01209
FDTREF	1450	.00414	.07407	0	0	.00828	.01724
GMB4	1900	.00684	.00684	.00211	.00158	0	.01737
GMB5	1600	.00250	.00563	.00438	0	0	0
MS01	361	.00277	.00277	0	0	0	.21053
MWA1	1800	.00111	.00222	0	.00778	0	.00389
MWA2	1300	.00077	.00692	0	.00462	.00077	.00615
MWA3	1498	.00334	.01335	0	.02069	0	.00935
MWA4	3497	.00743	.01716	0	.00743	.01401	.01087
MWA5	4279	.00047	.00210	.00023	.00070	.00234	.00234
MWA6	4240	.00047	0	0	.00094	.00047	.00094
MWA7	2100	0	.00619	0	0	.00143	.00381
MWA8	2120	.00094	.00377	0	.00236	.00849	.00755
MWA9	2044	.00098	.00049	.00049	.00049	.00098	0
MWA10	1900	.00053	.00316	0	0	.00158	.00737
MWA11	1896	.00158	.01108	.00053	0	.00369	.01530
MWA12	1650	0	.00061	0	.00485	.00303	.00061
MWA13	1499	.00133	.00334	.00133	.00133	.00067	.00667
MWA14	1500	.04133	.00467	0	0	.00333	.01267
MWA15	1450	.01655	.00276	0	0	.00069	.01034

Appendix B. 2015-2016 Prey Catch Per Unit Effort (CPUE)

Quadrat ID	Trap Days	CPUE (Bullfrog)	CPUE (Mosquitofish)	CPUE (Carp)	CPUE (Bass)	CPUE (Sunfish)	CPUE (Crayfish)
NG01	1850	.0865	.01243	0	.00054	.03946	.00649
NG02	1850	.00703	.03135	0	.02270	0	.01027
NG03	2100	0	.00143	0	0	.00095	.01095
NG04	2100	.00619	0	0	0	.00286	.01381
NG05	750	.00133	.04533	0	0	0	.06267
NG06	700	0	.01143	0	0	.01143	.05143
NG07	700	.00143	.02000	0	0	.00714	.04714
NG08	2400	0	.00125	0	0	.00542	.03125
NG09	2100	.00095	.00238	.00625	0	.00190	.01476
NG10	1150	.02000	0	.00429	0	.00957	.03739
NG11	1150	.02174	0	0	0	.03565	.06957
NG12	2000	.00050	0	0	0	.00450	.03700
NG13	750	0	0	.00400	0	0	0
NG14	750	0	0	0	0	0	0
NG15	1300	0	0	0	0	.00077	.02154
NG16	1250	0	.00240	.00077	0	.00400	.04640
NG17	24	0	.50000	.00800	0	0	.08333
NG18	3049	.00098	.01246	.00066	.00197	.00525	.04887
NG19	1550	.00065	.02710	.00065	0	0	.06516
NG20	1750	0	.02686	0	0	.00114	.08057
NG21	940	.00426	.00851	0	0	.00213	.04043
NG22	1561	0	.09673	.00192	0	0	.12044
NG23	4150	.00024	.00024	.00024	.00169	.00024	.00602
NG24	1545	0	.01812	0	0	.00129	.00971
NG25	1150	.00174	.00174	0	0	0	.00957
NG26	2015	0	.00099	0	.00050	.00447	.02581
NG27	2463	0	.00365	.00203	.00528	0	.05156
NG28	2849	.00211	.00176	.00176	.00316	.00140	.00562
NG29	4149	.00145	.00072	.00024	.00121	.00048	.00771
NG30	2549	0	.00706	.00118	0	.00235	.10043
NG31	2150	.3674	.03488	.00047	.00140	0	.01535
NG32	2100	.00048	.02286	.00857	.02810	0	.08619
SJR1	1500	.0020	.0140	.0013	0	.1347	.0267
SJR2	1500	0	.0140	.0093	.0507	0	.0400
SJR3	1500	0	0	0	0	.0020	0
SJR4	1500	0	.0027	.0013	0	.0340	.0413
SJR5	3050	.0029	.0075	.0003	.0128	.0134	.0111
SJR6	1500	0	.0047	0	.0093	0	0
SJR7	1472	.0040	0	.0136	.0190	.0367	.0150
SJR8	1472	.0027	.0007	.0360	.0177	.0550	.0400
SJR9	1500	.0013	.0007	.0127	.0273	.0660	.0260
SJR10	840	0	.0012	.0095	.0238	.0381	.0345

Appendix B. 2015-2016 Prey Catch Per Unit Effort (CPUE)

Quadrat ID	Trap Days	CPUE (Bullfrog)	CPUE (Mosquitofish)	CPUE (Carp)	CPUE (Bass)	CPUE (Sunfish)	CPUE (Crayfish)
SJR11	1140	0	.0017	0	.0035	.0026	.0079
SJR12	1500	.0007	0	0	.0153	.0120	.0513
SJR13	1350	.0044	0	.0015	.0489	0	.0674
SJR14	1380	.0304	.0101	0	.0101	0	.0101
SJR15	1333	.0022	.0112	0	.0555	.0022	.0375
SJR16	1470	.0061	.0122	0	0	.0306	.0353
SJR17	1350	.0052	.0030	0	.0415	.0096	.0518
total	**25357**	**.0036**	**.0052**	**.0047**	**.0189**	**.0254**	**.0279**

Appendix C. 2015-2016 Water Chemistry Metrics

Transect ID	Date	Time	H2O Temp (C)	Conductivity (mS/cm)	Salinity (PPT)	DO % Saturation	pH
VWW01	7/9/2015	10:58	22.92	1.04	.54	96.5	7.28
VWW02	7/9/2015	11:03	23.45	1.05	.54	101.4	7.29
VWW03	7/9/2015	11:06	23.68	1.05	.53	101.5	7.28
FDT01	7/9/2015	9:04	19.07	.994	.56	110.8	7.41
FDT02	7/9/2015	9:17	18.84	1.00	.57	97.7	7.42
FDT03	7/9/2015	9:29	20.59	1.14	.62	104.2	7.28
FDT04	7/9/2015	9:36	19.08	1.03	.58	95.8	7.28
FDT05	7/9/2018	9:48	19.88	1.02	.57	175.8	7.38
FDT06	7/9/2015	10:30	20.56	1.10	.60	81.2	7.31
FDT07	7/9/2015	10:17	21.14	1.04	.56	88.1	7.37
FDT08	7/9/2015	10:10	21.28	1.06	.54	81.3	7.37
FDT09	7/9/2015	10:06	19.31	1.03	.58	85.6	7.37
FDT10	7/9/2015	11:30	22.02	1.06	.56	208.2	7.32
FDT11	7/9/2015	9:12	18.83	1.00	.57	75.3	7.38
FDT12	7/9/2015	9:21	19.64	1.08	.60	102.1	7.41
FDT13	7/9/2015	9:42	20.34	1.05	.58	130.5	7.32
FDT14	7/9/2015	9:57	20.39	1.07	.59	129.2	7.37
FDT15	7/9/2015	10:20	20.56	1.08	.59	83.8	7.30
FDT16	7/9/2015	10.26	21.45	1.18	.63	66.8	7.28
FDT17	7/9/2015	10:46	22.36	1.13	.60	147.5	7.27
FDT18	7/9/2015	10:50	21.91	1.11	.59	171.1	7.27
FDT19	7/9/2015	11:20	25.16	1.13	.56	249.9	7.47
FDT20	7/9/2015	11:27	23.01	1.09	.56	285.9	7.36
FDTREF	--	--	--	--	--	--	--
GMB4	--	--	--	--	--	--	--
GMB5	10/10/2016	10:10:44	18.14	4528	2.43	92.8	9.08

Appendix C. 2015-2016 Water Chemistry Metrics

Transect ID	Date	Time	H2O Temp (C)	Conductivity (mS/cm)	Salinity (PPT)	DO % Saturation	pH
MS01	--	--	--	---	--	--	--
MWA1	10/9/2016	18:06:52	19.68	1195	.60	101.8	8.25
MWA2	10/9/2016	17:41:15	18.79	1050	.52	105.1	7.65
MWA3	10/9/2016	17:17:36	20.19	1003	.50	159.9	8.81
MWA4	10/9/2016	17:23:41	20.48	1354	.68	101.3	8.23
MWA5	--	--	--	--	--	--	--
MWA6	--	--	--	--	--	--	--
MWA7	10/9/2016	18:02:11	22.61	1516	.76	90.9	8.74
MWA8	10/9/2016	17:26:53	20.10	1051	.52	98.0	7.69
MWA9	10/9/2016	17:46:27	19.22	1168	.58	102.2	7.95
MWA10	10/9/2016	16:46:01	22.29	1184	.59	109.7	7.77
MWA11	10/9/2016	16:46:14	23.70	1158	.57	101.1	8.38
MWA12	10/9/2016	17:34:00	21.15	1018	.50	95.5	7.53
MWA13	10/9/2016	16:22:22	20.53	1076	.53	125.2	7.41
MWA14	10/9/2016	16:12:03	26.72	24	.01	113.5	6.91
MWA15	10/9/2016	16:34:49	21.95	1030	.51	109.2	7.89
NG01	7/29/2015	10:42:28	25.59	1170	.58	37.9	8.22
NG02	7/29/2015	11:44:13	22.25	1357	.68	31.7	8.77
NG03	7/30/2015	12:03:54	24.87	1290	.64	51.3	8.60
NG04	7/30/2015	12:18:07	24.46	1313	.65	45.1	8.70
NG05	7/29/2015	13:24:14	28.60	1276	.63	134.1	8.56
NG06	7/29/2015	11:59:53	26.43	1146	.57	100.7	8.58
NG07	7/29/2015	13:31:04	26.20	1473	.74	120.5	8.56
NG08	8/2/2015	11:33:58	25.59	1464	.73	59.3	9.47
NG09	9/16/2015	12:01:00	21.13	1294	.65	94.4	8.76
NG10	8/8/2015	12:42:05	22.61	1003	.50	38.4	9.99
NG11	8/8/2015	13:10:30	23.39	1313	.66	70.3	9.98
NG12	9/16/2015	12:06:37	20.85	1334	.67	98.5	8.81
NG13	8/18/2015	8:21:09	23.11	869	.43	48.1	9.05
NG14	8/18/2015	8:28:00	24.61	826	.41	45.4	9.53
NG15	9/16/2015	11:44:47	20.61	2057	1.05	92.7	8.91
NG16	9/16/2015	11:25:19	21.10	2057	1.07	107.8	8.50
NG17	9/16/2015	11:47:37	19.77	1089	.54	71.1	8.82
NG18	--	--	--	--	--	--	--
NG19	10/10/2016	11:01:49	20.58	1195	.60	87.5	7.80
NG20	--	--	--	--	--	--	--
NG21	10/10/2016	10:49:33	17.56	793	.39	110.2	7.87
NG22	10/10/2016	11:02:21	18.54	735	.36	84.3	8.40
NG23	--	--	--	--	--	--	--

Appendix C. 2015-2016 Water Chemistry Metrics

Transect ID	Date	Time	H2O Temp (C)	Conductivity (mS/cm)	Salinity (PPT)	DO % Saturation	pH
NG24	--	--	--	--	--	--	--
NG25	--	--	--	--	--	--	--
NG26	--	--	--	--	--	--	--
NG27	--	--	--	--	--	--	--
NG28	--	--	--	--	--	--	--
NG29	--	--	--	--	--	--	--
NG30	10/10/2016	11:29:50	23.16	674	.33	99.9	7.57
NG31	--	--	--	--	--	--	--
NG32	--	--	--	--	--	--	--
SJR01	10/09/2016	11:13:58	19.94	252	0.12	230.9	9.49
SJR02	10/09/2016	13:17:54	20.14	643	0.31	99.8	8.34
SJR03	10/09/2016	11:37:08	20.25	652	0.32	94.8	8.17
SJR04	10/09/2016	12:46:06	21.57	250	0.12	106.6	8.82
SJR05	10/09/2016	13:21:32	20.97	641	0.31	109.0	8.37
SJR06	10/09/2016	13:29:16	20.79	643	0.31	107.3	8.42
SJR07	10/09/2016	--	--	--	--	--	--
SJR08	10/09/2016	13:29:16	20.28	644	0.31	105.7	8.36
SJR09	10/09/2016	11:54:40	19.74	644	0.31	99.1	8.29
SJR10	10/09/2016	12:14:24	21.03	633	0.31	99.7	8.29
SJR11	10/09/2016	14:03:29	20.28	629	0.31	129.6	8.57
SJR12	10/09/2016	12:05:46	19.20	642	0.31	97.9	8.41
SJR13	10/09/2016	12:14:34	20.72	628	0.31	97.6	8.26
SJR14	10/09/2016	14:16:20	24.93	--	--	98.4	7.59
SJR15	10/09/2016	14:23:40	21.70	650	0.32	112.7	8.66
SJR16	10/09/2016	14:16:04	25.17	--	--	97.7	7.54
SJR17	10/09/2016	14:54:19	25.54	838	0.41	117.2	7.87

Appendix D. 2016-2017 Water Quality Sampling Locations and Results

Site		Easting	Northing	Date	Aresenic	Selenium	Mercury	Boron
MS	1	698715	4104204	6/10/2016	ND	ND	ND	0.84
MWA	1	742600	4062885	6/10/2016	ND	ND	ND	0.48
MWA	2	738708	4062307	6/10/2016	ND	ND	ND	0.49
MWA	3	740748	4067428	6/10/2016	ND	ND	ND	0.39
MWA	4	686480	4112841	6/10/2016	ND	ND	ND	0.38
GMB	4	686480	4112841	6/12/2016	ND	ND	ND	0.97
GMB	5	686017	4112238	6/12/2016	0.022	ND	ND	6.9
NG	18	685880	4117377	6/12/2016	ND	ND	ND	0.7
NG	19	685749	4117157	6/12/2016	0.016	ND	ND	0.8
NG	20	686007	4114788	6/12/2016	ND	ND	ND	0.81
NG	21	684503	4113399	6/12/2016	0.031	ND	ND	1.3
NG	22	685795	4117125	6/12/2016	ND	ND	ND	1.8
NG	23	686022	4112431	6/12/2016	ND	ND	ND	0.74
NG	24	685419	4120821	6/12/2016	ND	ND	ND	2.2
NG	25	690829	4120469	6/12/2016	ND	ND	ND	0.86
NG	23	686022	4112431	8/10/2016	ND	ND	ND	0.51
NG	26	685847	4121910	8/10/2016	ND	ND	ND	0.76
NG	27	686292	4119742	8/10/2016	ND	ND	ND	0.76
NG	28	685845	4112685	8/10/2016	ND	ND	ND	0.51
NG	29	685165	4112771	8/10/2016	ND	ND	ND	0.53
NG	30	684729	4118917	8/10/2016	ND	ND	ND	0.65
NG	31	684362	4121715	8/10/2016	ND	ND	ND	0.6
NG	32	687142	4118026	8/10/2016	ND	ND	ND	0.71
NG	33	684403	4122010	8/10/2016	ND	ND	ND	0.67
MWA	1	742600	4062885	10/9/2016	ND	ND	ND	0.53
MWA	2	738708	4062307	10/9/2016	ND	ND	ND	0.27
MWA	3	740748	4067428	10/9/2016	ND	ND	ND	0.2
MWA	4	740985	4064140	10/9/2016	ND	ND	ND	0.51
MWA	5	742621	4063315	10/9/2016	ND	ND	ND	0.31
MWA	6	742588	4063394	10/9/2016	ND	ND	ND	0.27
MWA	7	738713	4060672	10/9/2016	ND	ND	ND	1.1
MWA	8	741021	4064223	10/9/2016	ND	ND	ND	0.23
MWA	9	742393	4062254	10/9/2016	ND	ND	ND	0.38
MWA	10	740811	4067503	10/9/2016	ND	ND	ND	0.33
MWA	11	740563	4067514	10/9/2016	ND	ND	ND	0.34
MWA	12	742430	4063774	10/9/2016	ND	ND	ND	0.26
MWA	13	743850	4066078	10/9/2016	ND	ND	ND	0.27
MWA	14	743851	4065645	10/9/2016	ND	ND	ND	0.27
MWA	15	742257	4066760	10/9/2016	ND	ND	ND	0.25
SJR	1	736029	4074584	10/9/2016	ND	ND	ND	0.085
SJR	2	735991	4074518	10/9/2016	ND	ND	ND	0.042
SJR	3	735322	4074425	10/9/2016	ND	ND	ND	0.054
SJR	4	735313	4074357	10/9/2016	ND	ND	ND	0.088

Appendix D. 2016-2017 Water Quality Sampling Locations and Results

Site		Easting	Northing	Date	Aresenic	Selenium	Mercury	Boron
SJR	5	735323	4073596	10/9/2016	ND	ND	ND	0.14
SJR	6	735359	4073652	10/9/2016	ND	ND	ND	0.075
SJR	7	735645	4074540	10/9/2016	ND	ND	ND	0.075
SJR	8	735691	4074485	10/9/2016	ND	ND	ND	0.091
SJR	9	734968	4074323	10/9/2016	ND	ND	ND	0.074
SJR	10	735028	4074258	10/9/2016	ND	ND	ND	0.14
SJR	11	735098	4073742	10/9/2016	ND	ND	ND	0.14
SJR	12	734723	4074183	10/9/2016	ND	ND	ND	0.14
SJR	13	734951	4074102	10/9/2016	ND	ND	ND	0.14
SJR	14	736675	4072161	10/9/2016	ND	ND	ND	0.2
SJR	15	736634	4072097	10/9/2016	ND	ND	ND	0.13
SJR	16	736544	4072241	10/9/2016	ND	ND	ND	0.13
SJR	17	736687	4071879	10/9/2016	ND	ND	ND	0.12
GMB	4	686480	4112841	10/10/2016	ND	ND	ND	1.2
GMB	5	686017	4112238	10/10/2016	0.017	ND	ND	6
NG	23	686022	4112431	10/10/2016	ND	ND	ND	0.25
NG	24	685419	4120821	10/10/2016	ND	ND	ND	0.39
NG	25	690829	4120469	10/10/2016	ND	ND	ND	0.62
NG	26	685847	4121910	10/10/2016	ND	ND	ND	0.4
NG	27	686292	4119742	10/10/2016	ND	ND	ND	0.21
NG	28	685845	4112685	10/10/2016	ND	ND	ND	0.22
NG	29	685165	4112771	10/10/2016	ND	ND	ND	0.16
NG	30	684729	4118917	10/10/2016	ND	ND	ND	0.22
NG	31	684362	4121715	10/10/2016	ND	ND	ND	0.37
NG	32	687142	4118026	10/10/2016	ND	ND	ND	0.37
NG	33	684403	4122010	10/10/2016	ND	ND	ND	0.37

1. North American Datum 1983, UTM Zone 10

Appendix E. 2016-2017 eDNA sampling locations and results

Transect		Visit 1	Visit 2	Visit 3	Visit 4	Easting	Northing	Detect
GMB	1	0	0	0	--	686033	4112862	N
GMB	4	0	1	0	--	686480	4112841	Y
GMB	5	0	0	0	--	686017	4112238	N
GMB	8	0	0	0	--	686640	4112461	N
GMB	12	0	0	0	--	686026	4112385	N
GMB	13	0	1	--	--	686753	4111847	Y
GMB	18	1	0	1	--	686487	4111286	Y
GMB	21	0	1	0	--	686373	4111504	Y
GMB	22	0	1	--	--	686381	4111532	Y
GMB	24	0	0	0	--	686136	4111667	N
GMB	28	0	0	1	--	686388	4112015	Y
GMB	30	0	0	0	--	686512	4112053	N
GMB	32	0	0	--	--	686268	4112038	N
GMB	35	1	0	--	--	686053	4111781	Y
MWA	1	0	0	--	--	742600	4062885	N
MWA	2	0	0	--	--	738708	4062307	N
MWA	3	0	1	--	--	740748	4067428	Y
MWA	6	0	1	--	--	742588	4063394	Y
MWA	7	0	0	--	--	738713	4060672	Y
MWA	10	1	0	--	--	740811	4067503	Y
MWA	13	0	0	--	--	743850	4066078	N
MWA	14	0	0	--	--	743851	4065645	N
MWA	15	0	0	--	--	742257	4066760	N
NG	12	0	0	--	--	685104	4118485	N
NG	18	0	0	0	1	685880	4117377	Y
NG	19	0	0	--	--	685749	4117157	N
NG	20	0	0	--	--	686007	4114788	N
NG	23	1	0	0	--	686022	4112431	Y
NG	24	0	0	0	1	685419	4120821	Y
NG	25	0	0	--	--	690829	4120469	N
NG	26	0	0	--	--	685847	4121910	N
NG	30	0	1	--	--	684729	4118917	Y
NG	31	0	1	--	--	684362	4121715	Y
NG	32	0	0	1	0	687142	4118026	Y
SJR	1	0	0	--	--	736029	4074584	N
SJR	2	0	1	--	--	735991	4074518	Y
SJR	3	0	1	--	--	735322	4074425	Y
SJR	4	0	1	--	--	735313	4074357	Y
SJR	5	1	1	--	--	735323	4073596	Y
SJR	6	0	0	--	--	735359	4073652	N
SJR	10	0	0	--	--	735028	4074258	N
SJR	12	1	0	--	--	734723	4074183	Y
SJR	13	0	0	--	--	734951	4074102	N

Appendix E. 2016-2017 eDNA sampling locations and results

Transect	Visit 1	Visit 2	Visit 3	Visit 4	Easting	Northing	Detect
SJR 14	1	1	--	--	736675	4072161	Y
SJR 15	0	0	--	--	736634	4072097	N
SJR 17	1	1	--	--	736687	4071879	Y
WP 57	0	0	--	--	703855	4096371	N
WP 58	1	0	--	--	699906	4090586	Y
WP 59	1	0	--	--	686479	4112842	Y
WP 67	0	1	--	0	742630	4063787	Y
WP 71	0	0	--	--	685976	4114449	N
WR 165	0	1	--	--	693265	4113546	Y

1. North American Datum 1983, UTM Zone 10