BLOOD PROFILES IN WESTERN POND TURTLES (*Emys marmorata*) FROM A NATURE RESERVE AND COMPARISON WITH A POPULATION FROM A MODIFIED HABITAT

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A Thesis

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Master of Science

In

Biology

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by

Ninette R. Daniele

Summer 2014

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ABSTRACT

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Freshwater turtles worldwide are declining due to a variety of human caused impacts. The Western Pond Turtle (*Emys marmorata*) is native to the pacific coast of the North America and is also in decline. Many Western Pond Turtles live in human-modified habitats. The ecophysiology of this species in impacted habitats is largely unknown and blood profile baselines from healthy populations in natural habitats are lacking. Western Pond Turtles were sampled from a nature reserve (n=61) and wastewater treatment facility (n=37) and blood profiles were performed, which included determination of hematocrit and thirteen serum chemistry analytes. Baseline blood profiles for a subset of clinically healthy *E. marmorata* from a highly natural habitat were documented, including values for

gravid females, with significant differences noted between males and females for 8 chemistries. Blood profiles were also compared between all sampled male turtles from the nature reserve and the wastewater facility populations; with significant differences found in ten blood analytes between populations at these habitats. The blood profile baselines from the nature reserve population will be helpful to wildlife veterinarians in evaluating disease in this species and possibly other Emydid turtles. Furthermore, the differences in blood profiles between populations suggest that turtles from altered habitats may have impacted physiologies and that blood profiles, with further study, may be useful in assessing the suitability of modified habitats. Managers may use this work in assessing the health of Western Pond Turtle populations and planning conservation strategies in altered landscapes.

CHAPTER I

STUDY INTRODUCTION

Summary

Anthropogenic changes to landscapes, including urbanization, habitat loss, agriculture, non-native species introductions, and pollution, have contributed to the decline of turtle populations throughout the world, across many taxa (Turtle Conservation Fund 2002; Kiester and Olson 2011). Turtle populations are generally thought of as being susceptible to human impacts because of a limited ability to cope with the loss of mature individuals (Crouse et al. 1987; Heppell 1996, 1998). Turtle life histories include low juvenile recruitment, late maturity, and a long-lived adult stage with high survivorship (Congdon et al. 1993). As such, the stability of turtle populations hinges on the expectation that adult members will continually recharge the population through reproduction over a long lifespan (Crouse et al. 1987; Congdon et al. 1993; Heppell 1996, 1998). Landscape changes that affect adult survival or fitness are likely to have deleterious consequences for populations (Crouse et al. 1987). Negative effects often demonstrate time lags, and since detection of juveniles is low at best, some populations that superficially seem to be doing well, may actually be "ghost populations" consisting of aging adults with little recruitment or breeding (Browne and Hecnar 2007). Urbanization, land conversion to agricultural uses, alterations to waterways, and other potentially detrimental land use changes can be expected to rise in the coming years as

the human population continues to grow. Given these circumstances, it is important to examine the potential for modified habitats to sustain turtle populations for the long-term.

The Western Pond Turtle (*Emys marmorata*) is the only native freshwater turtle in California and populations are declining throughout much of its range (Jennings and Hays 1994; Holland 1994; Hays et al. 1994; Ernst and Lovich 2009). *Emys marmorata* commonly live in modified areas, such as constructed park ponds, wastewater treatment oxidation water bodies, and golf course lakes (Jennings and Hayes 1994; Spinks et al. 2003; Germano 2010; Bury et al. 2012a). As populations seem to persist in these areas, there is concern as to whether these habitats are suitable to support viable *E. marmorata* populations in the long term (Germano 2010). The conditions that are often present in many modified habitats are complex, and trade-offs in benefits versus hindrances to wildlife are likely to occur. In some instances, habitat modification may prove to be beneficial for turtles (Bondi and Marks 2013), such as when modified habitats provide conditions that result in optimal growth (Germano 2010). While in other instances, habitat modification(s) may be harmful, such as when the outcome includes compromised immune function (Polo-Cavia et al. 2010). Consequently, disentangling the potentially harmful consequences of habitat modification on populations from potentially beneficial aspects is challenging.

Because many freshwater turtle populations are in serious decline (Kiester and Olson 2011), society should examine the potential for physiological changes caused by anthropogenic habitat modifications. Conservation biologists may also come to embrace mounting evidence that many turtles may be able to persist in altered habitats, provided that negative consequences are understood and minimized (Spinks et al. 2003; Lambert et

al. 2013). An enhanced understanding of the impacts of habitat alterations on turtle health may provide answers that may allow these animals to persist in the face of human development.

Human Impacts on Freshwater Turtles

The threats to aquatic turtle populations posed by habitat modification are likely multifactorial, additive, and interactive, as have been shown repeatedly in other wildlife species (Munns 2006; Boone et al. 2007; Laurance and Useche 2009). As human developments tend to cluster around water, modification of aquatic habitats can be more common and more extensive than in terrestrial habitats. Human-caused modifications, both to core habitat and the landscape matrix surrounding core habitat, can affect turtle populations directly and indirectly.

Changes to the landscape matrix surrounding core aquatic habitat can influence turtle populations in a variety of direct and indirect ways. Turtles undergo migrations to find mates, nesting sites, and higher quality resources. Anthropogenic land use in the matrix surrounding core habitat may affect turtles through direct mortality and instances of maiming as they migrate between core habitats. Many times sex ratios are skewed towards males in modified habitats, because females are likely to be killed (Connor et al. 2005, Browne & Hecnar 2007) and sustain more injuries than males (Marchard & Litvitis 2004), presumably because females migrate into terrestrial areas more frequently to nest, placing them at greater risk of predatory attacks or road strikes. Patrick and Gibbs (2010) found that decreased road density within 500m of core habitat was associated with higher numbers of females. In addition, high road densities were

associated with smaller size biases in populations nearby, suggesting reduced adult survivorship (Patrick and Gibbs 2010). Agricultural land use in matrix habitat can affect turtles similarly to roads. For example the use of heavy farm equipment has been associated with both maiming and deaths in Wood turtles (*Glyptemys ensculpta*; Samure et al. 2007). In addition, agricultural and urban land uses in areas surrounding core habitats can lead to runoff of contaminants and atmospheric transport of chemicals, such as pesticides and herbicides, into aquatic habitats utilized by turtles (Snodgrass et al. 2008; Meyer et al. 2013).

Other indirect effects of human modification on the surrounding landscape can include artificially high numbers of subsidized predators and population isolation. Urban and suburban land uses in the surrounding matrix may cause increased spillover of generalist predators, such as raccoons, that flourish with access to resources found near human habitations. These subsidized predators can prey heavily on turtle nests (Steen and Gibbs 2004; Brown and Hecnar 2007; Ner and Burke 2008) and have the potential to devastate reproductive efforts (Ner and Burke 2008). In addition, connectivity and quality of core habitat patches may also affect turtle population dynamics. High quality patches often include larger areas of permanent water, thus changes to the local wetland hydrology are likely to affect resident turtles (Roe & Georges 2007). High connectivity also tends to increase colonization and decrease local extinction probability (Cosentino et al. 2010).

Changes to core habitat in wetlands and streams, can also have direct effects on turtle populations. Among the most prominent factors are nutrient loading, pollution,

alteration to riparian vegetation, and the introduction of non-native species (Cadi and Joly 2003; Marchand and Litvaitis 2004; Steen and Gibbs 2004; Browne and Hecnar 2007; Moss at al. 2009; Peterman and Ryan 2009; Bishop et al. 2010; Germano 2010; Yu et al. 2011). Modified environments often have increased water temperature and nutrient levels, which can increase resource abundance for turtles (Marchand and Litvaitis 2004; Germano 2010). For example, Polo-Cavia and colleagues (2010) found that *Emys marmorata* from waste-water oxidation ponds held higher body condition scores than turtles from a nature reserve. However, modified habitats tend to harbor more environmental pollutants (Snodgrass et al. 2008); mercury, lead, and persistent organic pollutants have been shown to bioaccumulate in freshwater turtles (Moss at al 2009; Bishop et al. 2010; Yu et al. 2011). Also, modified habitats may have alterations to riparian vegetation, such as decreased incidences of trees falling into the water that may reduce the availability of suitable basking sites. Peterman and Ryan (2009) showed that when vegetation cover was mowed for canal management, turtles abandoned basking sites that had been hidden by the vegetation. In addition, more introduced predators, such as American Bullfrogs (*Lithobates catesbiana*), Smallmouth and Largemouth Basses (*Micropterus* spp.), and domestic dogs (*Canus lupus familiaris*), can be present in modified habitats (Browne and Hecnar 2007, Steen and Gibbs 2004), as well as native subsidized predators, such as raccoons (*Procyon lotor*) and skunks (*Mephitis mephitis*). Bullfrogs and large-mouth bass are voracious, gape-limited predators and have been suspected to ingest hatching and immature *E. marmorata*, which could additionally decrease the naturally low recruitment of juveniles into the adult population (Moyle

1973; Hays et al. 1999). In addition, historic watershed alterations, such as dams and water diversions, have changed aquatic habitats dramatically in the last century (Bodie 2001) and have been shown to affect turtle populations negatively in some cases (Ursuda et al. 2012). These watershed alterations often increase stream channelization, and reduce braiding and oxbows in floodplains, which have been implicated in salmon (*Onchorynchus* spp.) declines (Limm & Marchetti 2009). This has likely had a negative impact on *E. marmorata* (Reese 1996), because side channels and oxbows also provide habitat for adult turtles, refugia during flushing floods, and excellent nurseries for juveniles.

Status, Distribution, and Life History of *Emys marmorata*

The Western Pond Turtle (*Emys marmorata*) is a declining freshwater turtle native to the Pacific Northwest states of the USA, and Baja California. This turtle is primarily aquatic, coming out of the water to bask on nearby structures or to nest and hibernate in adjacent upland habitats (Reese 1996; Holland 1994). The species frequents slower moving stretches of rivers and streams, as well as ponds and lakes. *E. marmorata* is a generalist omnivore, with the bulk of its diet comprised of aquatic invertebrates and insect larvae; however, they have also been known to eat aquatic vegetation, larval amphibians, and carrion (Bury 1986). Western Pond Turtles are the only native, freshwater turtle currently persisting in California and are known to have suffered notable population declines and extirpations throughout their range (Jennings and Hayes 1994; Ernst and Lovich 2009).

Emys marmorata is listed as “endangered” in the state of Washington,

"sensitive-critical" in Oregon, and is listed as a "species of special concern" in California. Due to the declining status of the turtle, head-start programs have been implemented to bolster wild populations. These efforts have been undertaken by many entities, including the San Francisco Zoo, the Oregon Zoo, and California State University, Sonoma. Although extensive work has been done describing the natural history and ecology of the species, relatively little is known about the physiology of this species in modified habitats, as compared with natural habitats. In the wild, these turtles frequently live in degraded habitats or human modified habitats, and thus learning about their physiology in natural and modified habitats is important to the conservation biology of this declining species.

Background Ecophysiology Relevant to the Conservation of *Emys marmorata*

Increasingly, attention is turning to assessments of physiological function to measure how turtles are coping with environmental change. For example, blood mercury concentration in wild turtles was correlated with increased hematocrit (packed cell volume or PCV) and creatine kinase (CK) levels, and decreasing aspartate aminotransferase (AST) levels and lymphocyte count (Day 2007). In addition, heavy metals have been correlated with indicators of immunosuppression in wild Red-eared Sliders (*Trachemys scripta*) living near a chemical plant (Yu et al. 2011) and exposure to polychlorinated biphenyls (PCB's) has been linked to increased mortality in wild, juvenile Common Snapping Turtles (*Chelydra serpentina*; Eisenrich et al. 2009). Organochlorine pesticides were associated with disease in Eastern Box Turtles (*Terrapene carolina carolina*; Tangredi et al. 1997). Further, decreased

immunocompetence and increased shell abnormalities were documented in Yellow-blotched Sawback Turtles (*Graptemys flavimaculata)* living in a modified habitat where heavy boat traffic disrupted natural basking behaviors (Selman et al. 2013).

Even minor detrimental changes to the physiology of individuals, may lead to changes in reproduction or longevity, which could hamper population stability (Salice et al. 2013). There are many mechanisms through which habitat modification may lead to changes in physiology that could affect fitness. Many studies of *E. marmorata* in altered landscapes focus on population structure and growth, or body condition indices to assess populations in modified habitats (Spinks et al. 2003; Germano 2010). However these approaches may not accurately reflect the influence that habitat alteration may have on health, and research focused on the ecophysiology of *E. marmorata* in modified habitats is beginning to accumulate.

Recent research examined the potential for altered physiology in *E. marmorata* in modified habitats as a result of hydrological changes, exposure to pathogens, environmental stressors, and contaminants. Hydrological changes have been shown to cause changes to growth, body condition, and thermal ecology of *E. marmorata* (Ashton et al. 2011). Turtles on a dammed fork of the Trinity River exhibited many physiological changes attributed to cooler water in this altered habitat, such as lower growth rates and sizes, increased body condition in females, and increased basking behavior (without achievement of similar body temperature) when compared with turtles from an undammed fork (Ashton et al. 2011). In addition to examining the physiological effects of altered hydrological regimes, recent work has examined impacts of Red-eared

Sliders on the health of *E. marmorata*. Although evidence suggests that Red-eared Sliders may have introduced disease to *E. marmorata* populations in the past (Hayes et al. 1999), recent work did not find evidence of increased *Mycoplasma* infection in *E. marmorata* populations that were sympatric with Red-eared Sliders (Silbernagel et al. 2014). A 2010 study of *E. marmorata* at a wastewater treatment facility in northern California found evidence of immunosupression when this population was compared to a population at a nature reserve, even though turtles from the wastewater facility grew faster, larger, and had better body condition indexes (Polo-Cavia et al. 2010). A few studies have tested *E. marmorata* for pollutants (Henny el al. 2003; Meyer et al. 2013, 2014). Organochlorine pesticides and heavy metals were found in *E. marmorata* eggs (Henny et al. 2003) and a recent study linked thyroid hormone disruption to mercury pollution in *E. marmorata* (Meyer et al. 2014). Furthermore, *E. marmorata* populations that are down-wind from heavily cultivated areas can suffer depressed cholinesterase, a biomarker for pesticide exposure, where alterations in activity may signify deficits in neurological function (Meyer et al. 2013). Although knowledge of the ecophysiology and toxicology of *E. marmorata* in modified habitats has increased, blood profiles have been rarely used to assess health in this species (Keller et al. 2012).

The tendency of wild animals to suppress outward signs of poor health or disease highlights the need for sensitive tests of organ function and assessments of physiological health in wild animals (Knotkova et al. 2008). Blood profiles provide a sensitive, minimally invasive tool to measure physiological conditions within an animal (Campbell 2006). These blood profiles can provide information on the reactions of

individual animals and populations to their environmental conditions (Deem et al. 2009). Researchers can use blood profiles to compare populations and to detect unhealthy individuals within a population (Whiting et al. 2007; Flint et al. 2010; Fazio et al. 2012a). Furthermore, blood profile analytes have been linked to fitness indices, such as maternal success (Perrault et al. 2012). Less is known about clinical blood biochemistry in reptiles, than mammals or birds (Campbell 2006), and there is a lack of scientific works addressing this important topic of research (Irizarry-Rovira 2004). The absence of health information in wild turtles constrains conservation efforts by limiting full assessments of population(s), and the ecosystem status (Chaffin et al. 2008). Only one study documents blood profiles in *Emys marmorata* (Keller et al. 2012), and little is known about baseline (normal) blood profiles in *E. marmorata* from natural habitats or how blood profiles may vary between populations in natural versus modified habitats. The work presented here provides baseline blood profiles for a robust sample of clinically normal, wild Western Pond Turtles sampled from a nature reserve population in northern California, outlining baseline values of male and female turtles. Furthermore, I compared blood profiles between male *E. marmorata* populations sampled at a nature reserve with those from a highly modified habitat at a wastewater treatment facility.

CHAPTER II

MATERIALS AND METHODS

This research was performed under California Department of Fish and Game Scientific Collecting Permit number SC-11130. Research methodology was also reviewed and approved by the California State University Chico (CSU Chico) Institutional Animal Care and Use Committee (IACUC Permit # 201102).

Study Sites

Western Pond Turtles, *Emys* [*Clemmys; Actinemys]marmorata* [hereafter, turtles], were captured from two sites close to Chico, California, which differ greatly in their level of habitat modification and human impacts (Figures 1 and 2). Big Chico Creek, on the Big Chico Creek Ecological Reserve (BCCER) in the Sierra Nevada foothills of California (USA), was chosen as a (relatively) pristine study site, because of its isolation from development and the natural state of its aquatic habitat (Ashton et al. 2012a). The Chico Water Pollution Control Plant (CWPCP) is located in the central portion of the Sacramento River Valley of California (USA) and treats effluent water from the city of Chico (population ~89,000). The nearby oxidation ponds at CWPCP were chosen as a potentially impacted study site, because of their proximity to agricultural and municipal lands, and the highly modified state of the aquatic habitat (see Ashton et al. 2012a). At the BCCER, all turtles were captured along a 3.8 river mile stretch of Big Chico Creek in riverine habitat (Figure 3). At the locations where turtles

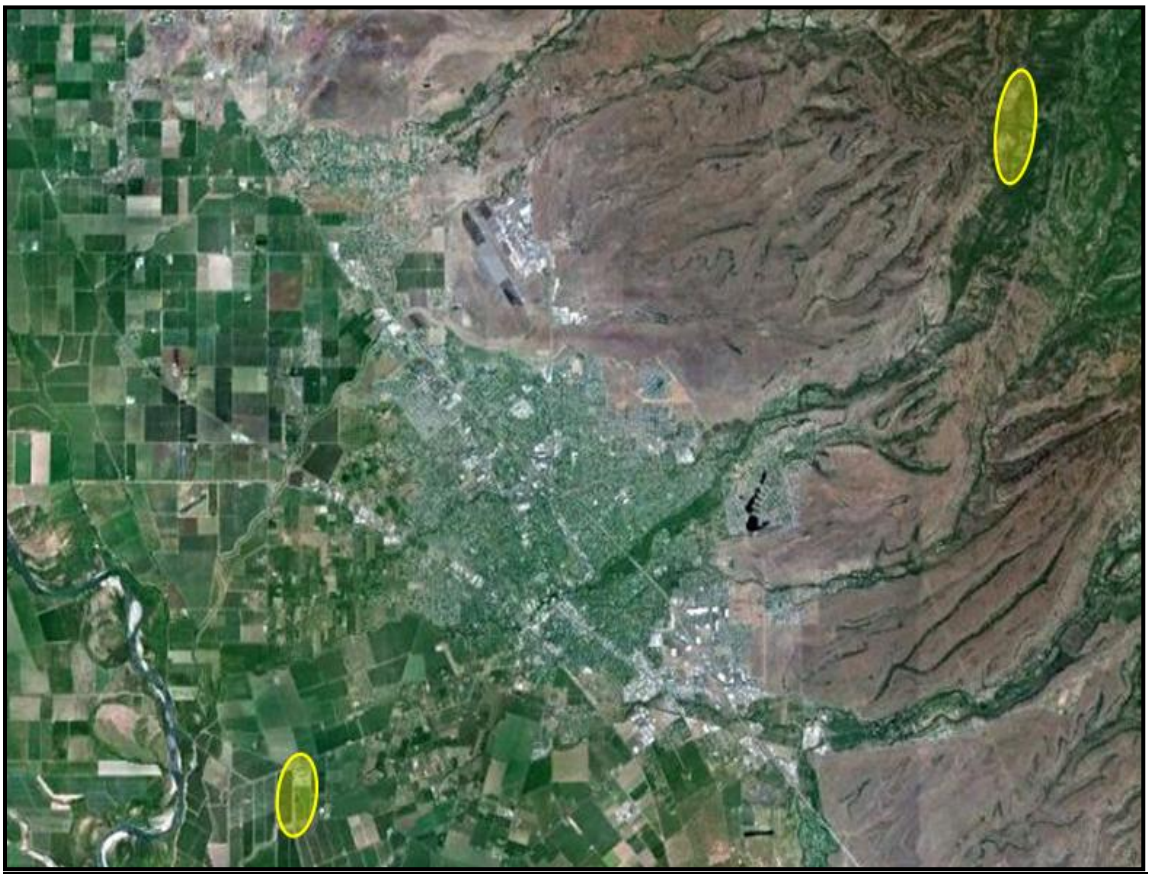

FIGURE 1. Relative locations of field sites on an aerial photograph. Field sites are circled in yellow; the Chico Water Pollution Control Plant (CWPCP) is circled in yellow in the lower left-hand corner and the Big Chico Creek Ecological Reserve (BCCER) is circled in the upper right-hand corner; the city of Chico (California, USA) is in the center.

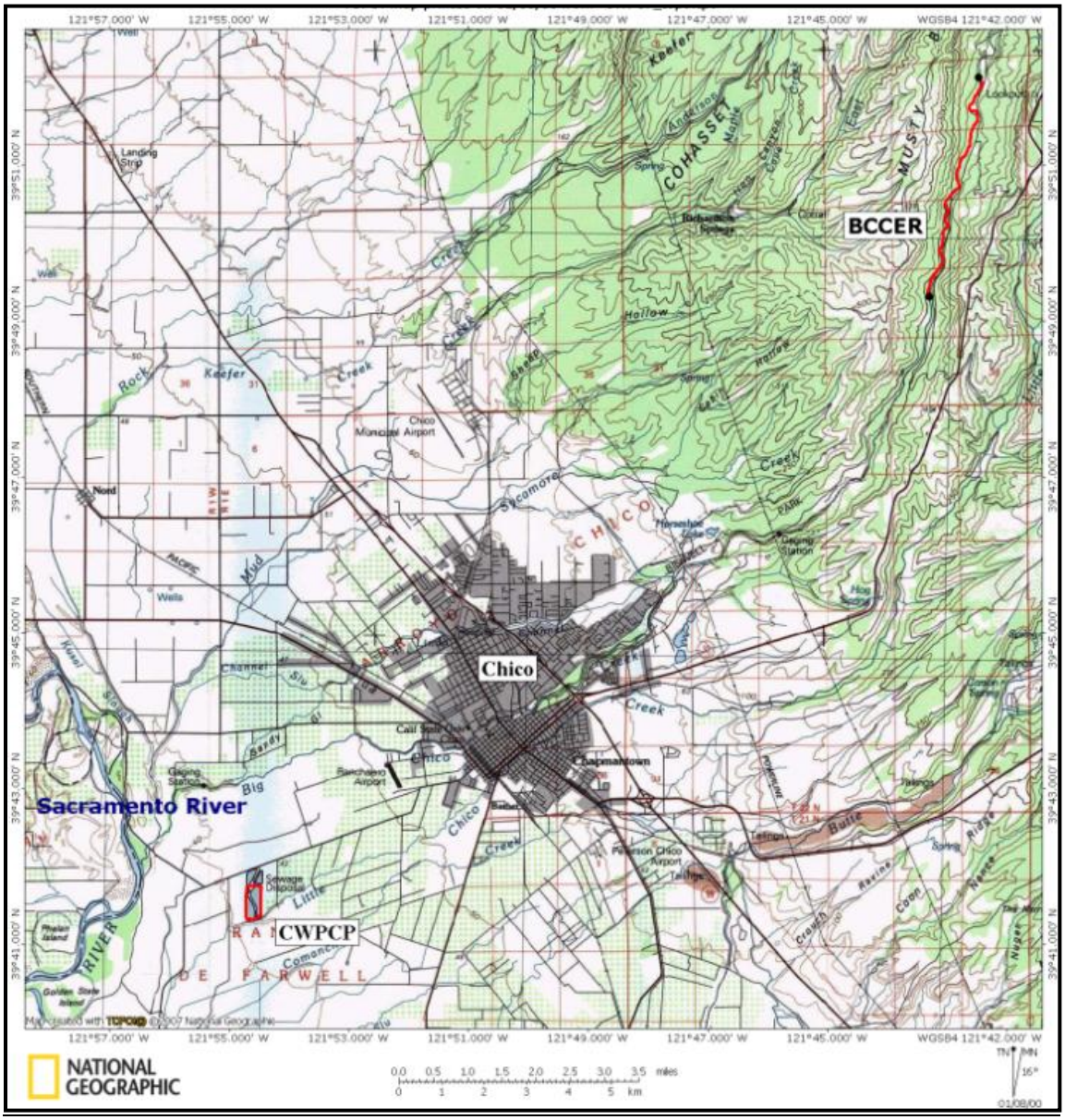

FIGURE 2. Relative locations of field sites a topographic map. Field sites are highlighted in red on the map. The Chico Water Pollution Control Plant (CWPCP) is southwest of the city of Chico (California, USA) in the Sacramento River valley at 38 meters elevation above sea level. The Big Chico Creek Ecological Reserve (BCCER) is northeast of the city of Chico, at 220 to 280 meters elevation; the city of Chico is in the center of the map.

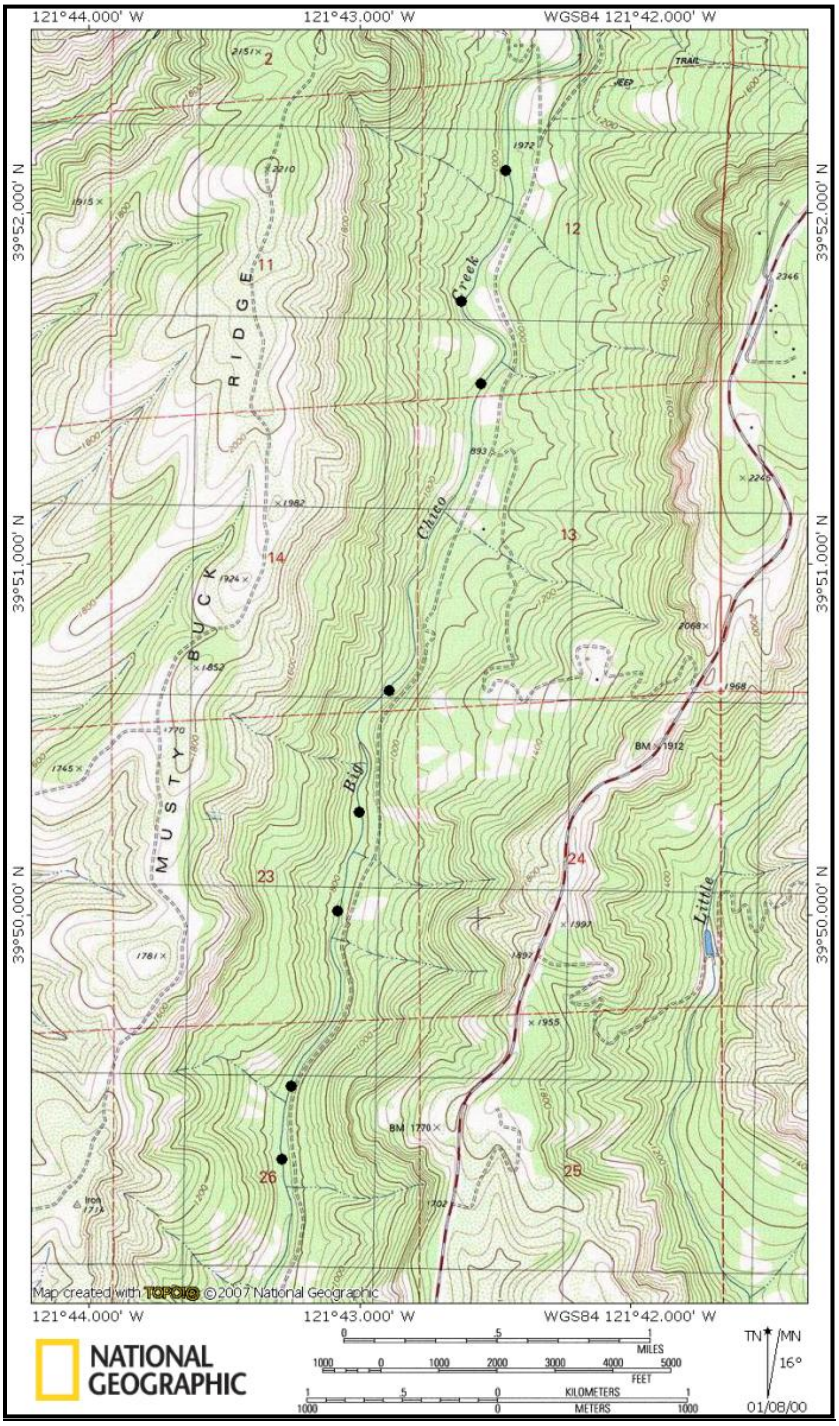

FIGURE 3. Topographic map of sampling locations within Big Chico Creek Ecological Reserve (BCCER) in northern California, USA. Turtle capture locations along a 3.8 mile reach of Big Chico Creek are marked with black dots.

were caught, Big Chico Creek was a fourth order stream and most turtles were captured in slow moving pools. The elevation, where turtles were captured varied from 220 meters to 280 meters. The immediately surrounding vegetation was native riparian trees and shrubs, bordered in the upland habitat by mixed conifer woodland and oak woodland. The nearest public access road to the BCCER is California Highway 32, which is at least 0.55 miles from Big Chico Creek and up a substantial grade of nearly 300 meters (Figure 3). Within the BCCER, vehicle access to the creek is allowed by dirt roads and is restricted to research and administrative users, as well as intermittent restricted access by hunters. While hiking onto the property is allowed; visitors may not swim in the creek or hike with dogs, which reduces disturbance to turtles and other aquatic wildlife. Big Chico Creek supports many native fishes and sensitive or threatened aquatic species, including multiple runs of Chinook Salmon (*Onchorynchus tshawytsha*), Steelhead Trout (*Onchorynchus mykiss*) and Foothill Yellow-legged Frogs (*Rana boylii*). There are no invasive Basses (Family: Centrarchidae) or Red-eared Slider Turtles (*Trachemys scripta elegans*) present on the BCCER. However, American Bullfrogs (*Lithobates catesbiana*) are present, but have been observed very infrequently on the property. Generally, turtles living at the BCCER are found basking on logs and boulders emerging from the stream.

The CWPCP is lower in elevation (38 meters) and the habitats are heavily modified in comparison to the BCCER (Figures 1, 2, and 3). At the CWPCP, turtles live in three oxidation ponds where nutrients settle from effluent that was fully treated by the wastewater plant (Figure 4). The ponds provide waterfowl and wading bird habitat year-round. As a result, there is an access trail and a bird blind on the property that are

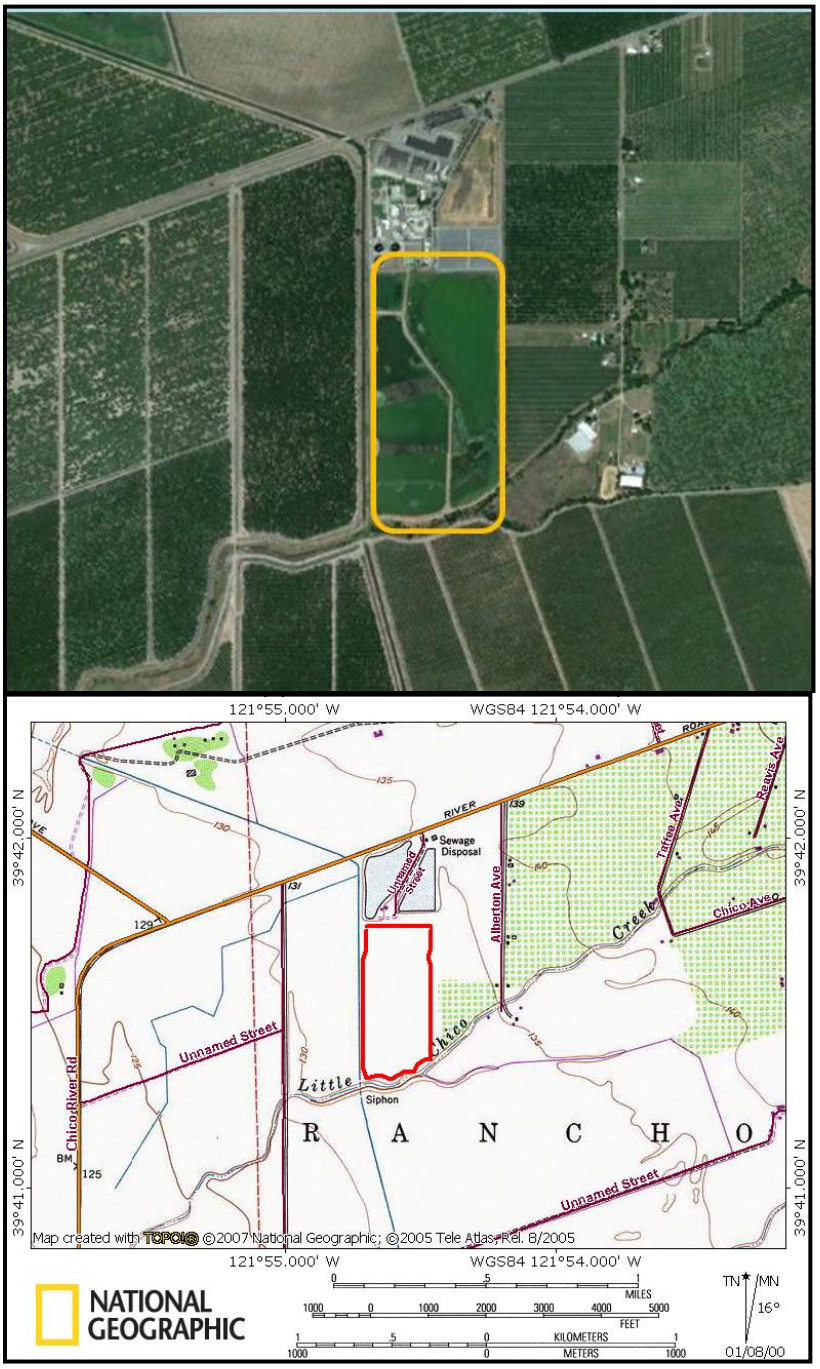

FIGURE 4. Arial and topographic maps of the Chico Water Pollution Control Plant (CWPCP) in northern California, USA. The oxidation ponds, where turtles were trapped, are outlined by the yellow (top) and red (bottom) rectangles, respectively. Little Chico Creek lies along the southern border of the CWPCP site, the water treatment plant facility complex is to the north, and there is a general agricultural matrix surrounding the oxidation ponds.

commonly used by bird enthusiasts. The oxidation ponds also support robust populations of non-native Mosquitofish (*Gambusa affinis*), American Bullfrogs (*L. catesbiana*), Red-eared Slider Turtles (*T. scripta elegans*), Signal Crayfish (*Pacifastacus leniusculus*) and native mesocarnivores, such as North American Raccoons (*Procyon lotor*). The ponds are eutrophic, shallow, and warm. Emergent vegetation is predominately Hard-stemmed Bulrush (*Schoenoplectus acutus*), sedges (Family: Cyperacae) and non-native grasses (Family: Poaecae). Directly surrounding the ponds, there is predominately bare ground (i.e., private dirt access roads) and vegetation is mostly thistle (Family: Cynareae), non-native grasses, and a few willows (*Salix spp.*). The oxidation ponds are bordered to the north by the CWPCP wastewater treatment facility (Figure 4). The remaining surroundings are predominately agricultural areas, comprised of orchards, private dirt roads, and irrigation canals (Figure 4). The nearest public paved road is a moderately busy highway that borders the northern boundary of the CWPCP facility; it is 430 meters from the nearest oxidation pond. Additional aquatic habitats utilized by the turtles lie close to the oxidation ponds. Little Chico Creek, a tributary to Butte Creek, lies within 30 meters of two of the oxidation ponds along the south, but may dry during the summer in years with low precipitation. In addition, a perennially flooded irrigation ditch lies with 20 meters of the ponds to the east (Figure 4).

Data Collection

Due to differences in feasibility at each study site, we used different methods to capture turtles: At BCCER, turtles were captured by hand during snorkel surveys from July to September in 2011. Snorkel surveys were conducted between 10am and 3pm,

when turtles were expected to be foraging. At the CWPCP, turtles were captured using baited funnel traps from April to September in 2011, and in April and May 2012. Traps were baited with sardines or chicken liver placed inside a plastic jar with small holes to prevent turtles from ingesting the bait. Traps were checked twice a day, at approximately 12 hours intervals, following Bury et al. (2012b). Because of the lower capture rate of trapping at CWPCP in 2011, compared with snorkeling at the BCCER, an extra field season was added to the capture effort at the CWPCP.

To minimize stress after capture by hand or trap, turtles awaited processing under shade, in water from the collection site using a mesh bag or bucket. The time between capture and data collection was usually less than 45 minutes, but occasionally up to a 2 hours. When processing turtles, blood was taken before other measurements and marking to avoid changes to the blood profiles induced by handling stress. We used a 0.5 ml Monoject brand tuberculin syringe outfitted with a 25-gauge needle (Thermo Fisher Scientific, Pittsburg, PA) to draw approximately 0.6 ml blood from the axillary vein on the forelimb of each turtle (Hnizdo 2011). Blood was only taken from adult turtles because laboratory analysis generally requires at least 0.5 ml of whole blood to give sufficient serum volume. The amount of blood taken from each turtle was well under the maximal recommended volumes suggested by chelonian veterinarians to ensure that no deleterious consequences occurred due to removal of too large a blood volume (Lloyd and Morris 1999, Wilkinson 2004, Norton 2005, Campbell and Ellis 2007). Blood samples that appeared to have lymph contamination (recognized by clear fluid being drawn into the syringe), or those with a volume less than 0.5 ml, were not included in the

study. After blood was drawn, the puncture site was held with light pressure until bleeding ceased.

Whole blood was used immediately after being drawn from each turtle to determine blood glucose (hereafter BG) and packed cell volume (hereafter PCV; also known as hematocrit). For the PCV analysis, a small amount of whole blood was drawn into two plastic heparinized micro-hematocrit tubes (Thermo Fisher Scientific, Philadelphia, PA) and sealed at one end with Critoseal (Thermo Fisher Scientific, Pittsburg, PA). For the BG determination, one drop of whole blood was placed into a portable glucometer (Accu-Chek Aviva, Rite Aid, Chico, CA). The remaining whole blood was placed in a 0.5 ml serum separator tube (BD Microfuge, Thermo Fisher Scientific, Pittsburg, PA) and held on ice during transport to CSU Chico.

After the venipuncture procedure, turtles were uniquely marked, physically examined, and measured for standard morphometric data (minimum carapace length, weight, etc.). Each turtle was given a unique identification number by filing marginal scutes and marked using an additive numbering system (Holland 1994). Following marking, each turtle was thoroughly examined for parasites, injuries, body damage, and shell damage, which were noted in detail if present. Based on these examinations, animals were classified as clinically normal or sick/injured for future analyses. After examination, each turtle was weighed to the nearest gram and photographed. We used calipers (Hagloff, Sweden) to measure minimum carapace length (MCL), which is the straight line measurement across midline of the long axis of the top shell. All turtles over 135 mm in standard carapace length were considered adults and sexed based on tail and

plastron morphology (Holland 1994; Ashton et al. 2012b). Female turtles were palpated for calcified eggs and considered "gravid" if calcified eggs were detected. Turtles were released back into the water close to the site of captures within 2.5 hours of being caught.

Blood Sample Preparation and Analysis

Within 4 hours of collection, blood samples were taken into the lab for processing. Hematocrit tubes were placed into a hematocrit centrifuge at 10,000 revolutions per minute for 3 minutes in order to ensure that serum and red blood cell components separated completely. The packed cell volume (PCV) was read using a Critocaps micro-hematocrit capillary card reader (Leica Microsystems, Buffalo Grove, IL). This yielded an estimate of hematocrit level (volume % of red blood cells). When there were two tubes present for a given sample, the average PCV value was reported. The serum separator tubes were spun at 10,000 revolutions per minute for 3 minutes to separate the red blood cell fraction completely from the serum fraction. The serum fraction was decanted off and frozen until analysis. Serum samples that appeared to be contaminated with hemolysed red blood cells (identified by serum with a reddish appearance) or appeared lipemic (identified by a cloudy appearance to serum) were discarded from the study, as lipemia and hemolysis confound the analysis of some blood profile analytes (Cray 2004). Serum samples that were less than 250µl were also discarded, as volumes less than 250 µl are insufficient for laboratory analysis without employing additional dilution procedures.

Serum samples were analyzed by Quality Veterinary Lab (Davis, CA) to determine total protein (TP), alanine aminiotransferase (ALT), aspartate aminotransferase

(AST), alkaline phosphate (ALP), lactate dehydrogenase (LDH), creatine kinase (CK), calcium (CA), inorganic phosphorus (PHOS), creatinine (CREAT), cholesterol (CHOL), uric acid (UA), and bile acids (BA) concentrations (see Table 1 for a summary of analytes and specific determination methods for each). The laboratory charge was $68 per sample (each turtle) to run the 12 analytes listed above; the charge for BA comprised almost half of this cost. Serum samples were thawed and run on a calibrated Olympus AU400E chemistry autoanalyzer. Calcium to phosphate ratio (C:P) was calculated by dividing calcium levels by phosphorus levels for each individual.

TABLE 1. Summary of blood panel analytes and specific determination methods for each.

Blood Analyte	Acronym	Determination Method	Machine Used
Packed cell volume	PCV	Microhematocrit	n/a
Total protein	TP	Biuret method	Olympus AU400E
Blood glucose	BG	Portable glucometer	Accu-Chek Aviva
Alkaline phosphatase	ALP	PPNP with AMP as buffer	Olympus AU400E
Alanine aminotransferase	ALT	Modified IFCC, TRIS, no P5P	Olympus AU400E
Aspartate aminotransferase	AST	Modified IFCC, TRIS, no P5P	Olympus AU400E
Lactate dehydrogenase	LDH	Lactate – pyruvate	Olympus AU400E
Creatine kinase	CK	NAC – activated	Olympus AU400E
Calcium	CA	Arsenazo III dye	Olympus AU400E
Inorganic phosphorus	PHOS	Phosphomolybdate – UV	Olympus AU400E
Calcium to phosphorus ratio	C:P	Calcium divided by phosphorus	n/a
Cholesterol	CHOL	Enzymatic	Olympus AU400E
Uric acid	UA	Uricase	Olympus AU400E
Creatinine	CREAT	Kinetic alkaline picate (Jaffe reaction)	Olympus AU400E
Bile acids	BA	Enzymatic	Olympus AU400E

Data Analysis

Data were assessed for normality using an Anderson-Darling normality test and for equality of variance using a Levene's test. Central tendency was evaluated with a

Mann-Whitney U test. T-test statistics are reported as well to substantiate results, because sample sizes were large enough to compensate for the violations of a normal distribution by the central limit theorem. All analyses were conducted using Minitab 15 statistical software (State College, PA).

Analyses for Baseline Blood Profiles

Blood profiles for turtles captured at the BCCER were presented as baseline values for a population living in relatively natural habitat. Baseline values were presented as median, 95% confidence, and range (mean and standard deviation were reported in Appendices for comparison). Only animals presumed to be healthy based on physical examinations were used in calculating the baseline values. However, extreme outlier values that were more than 3 times the interquartile range from the median were discarded from calculations to reduce the possibility that unhealthy animals (not detected by physical exam) be included in values intended to represent a healthy sub-population. Only adult turtles were analyzed. Because individuals were marked and some were sampled more than once through time, only the most recent blood sample was analyzed. In a few cases where blood serum values were reported below a detection limit from the lab, half of the detection limit was used for analyses. The normality and equality of variance were assessed for each variable prior to testing central tendency; a Levene's test was performed to test equality of variance. Blood parameter means and medians between the sexes were compared using the Mann-Whitney U test and t-test without the assumption of equal variance (Minitab 15, State College, PA).

Analyses for Blood Profile Comparisons

All blood samples, including samples from both clinically normal and from sick/injured turtles, were included in tests comparing BCCER turtles to CWPCP turtles. Outliers were not removed because these are potentially the animals of questionable health and it was important to report all existing variation. Only 11 females were captured at the CWPCP, thus an overview of their blood profiles is given, but not compared to the BCCER females due to small sample size of females at the CWPCP. The blood profiles of male turtles from BCCER were compared to the CWPCP males. Data were tested for equality of variance using a Levene's test. Means and medians in blood serum values between turtles from the BCCER and CWPCP were compared using a t-test assuming unequal variance and using the Mann-Whitney U test. All data were examined and compared using Minitab 15 (State College, PA).

CHAPTER III

RESULTS

Summary of Turtles Sampled at Each Site

In total, 102 adult Western Pond Turtles (*Emys marmorata*) were sampled for this study. This included 65 adult turtles collected from relatively healthy habitat at Big Chico Creek Ecological Reserve (BCCER). In addition, 37 adult turtles were collected from modified habitat at Chico Water Pollution Control Plant (CWPCP). In the following sections, baseline morphometric and blood profile results are reported for normal adult turtles collected from natural habitat within BCCER, in addition to comparisons of blood profiles between all male individuals captured in the BCCER and CWPCP sub-populations.

Baseline Blood Profiles for Normal *Emys marmorata* from a Nature Reserve

In total, blood profiles were collected for 61 clinically normal, wild-caught, *E. marmorata* from the BCCER (24 males and 37 females; Table 2). Four turtles were eliminated from inclusion in the clinically normal sub-population due to injuries. When palpated, 8 of the clinically normal 37 females were determined to be gravid with calcified eggs (hereafter referred to as "gravid females"). Given the physiological differences between male and female turtles, as well as possible variation between non-gravid and gravid females, the blood profile results of each sub-population were analyzed and reported separately as appropriate. The minimum carapace length (MCL) of male

TABLE 2. Summary of morphometric data for wild-caught, clinically normal male and female *Emys marmorata* from Big Chico Creek Ecological Reserve (BCCER) in northern California, USA.

	Minimum Carapace Length (mm)				**Mass (g)**			
Sex (n)	Median	Mean	95% CI	Range	Median	Mean	95% CI	Range
Males (24)	149	151	147–154	138–167	493	498	464–531	370–697
Females (37)	151	152	149–154	137–165	542	547	518–576	400–699
Gravid (8)	*147*	*149*	*144–153*	*141–156*	*527*	*526*	*463–589*	*431–642*
Non-gravid (29)	151	*152*	*149–156*	*137–165*	*550*	*553*	*519–587*	*400–699*

turtles collected from BCCER ranged from 138 to 167 mm (median: 149). The MCL for all female turtles from the BCCER, regardless of gravid status, ranged from 137 to 165 mm (median: 151). The MCL for non-gravid females ranged from 137 to 165 mm (median: 151 mm), and for palpably gravid females ranged from 141 to 156 mm (median: 147 mm; Table 2). The mass of BCCER males ranged from 370 to 697 g (median: 493 g) and the mass of all females collected at the BCCER ranged from 400 to 699 g (median: 542 g). Non-gravid females ranged in mass from 400 to 699 g (median: 526 g), while gravid females ranged from 431 to 632 g (median: 550 g; Table 2). The mean and 95% C.I. of MCL and mass for each sub-population collected at BCCER are also reported (Table 2). Gravid females did not differ significantly from non-gravid females in mass (W=132, P=0.47) or MCL (W=117, P=0.20). Male turtles from the BCCER were significantly lower in mass than females (W=1291.5, P=0.03); males and females did not differ significantly in MCL (W=1186.5, P=0.56). Serum biochemistry parameters and packed cell volume (PCV) values for clinically normal male and female turtles from the BCCER are reported for all 61 adults collected (Table 3). All values for creatinine (CREAT) were below the method detection limit of 0.02 mg/dL, therefore statistical comparisons were not appropriate. Extreme outliers were identified for alkaline

TABLE 3. Blood profiles for wild-caught, clinically normal *Emys marmorata* from Big Chico Creek Ecological Reserve (BCCER) in northern California, USA.

	Males					Females				
Blood Analyte	**Median**	**95% CI**	**Range**	**(n)**	**[Outliers]**	**Median**	**95% CI**	**Range**	**(n)**	**[Outliers]**
PCV (%)	29	26–29	19–41	(24)	N.A.	28*	27–29*	20–41*	(28)*	N.A.
TP (g/dL)	4.4	4.0–4.7	3.4–5.7	(24)	N.A.	4.5	4.3–4.8	3.1–6.1	(37)	N.A.
BG (mg/dL)	83	64–97	40–120	(24)	N.A.	71	61–79	36–114	(36)	N.A.
ALP (U/L)	94	83–112	39–149	(24)	N.A.	77	73–84	42–148	(36)	[168]
ALT (U/L)	10	7–12	5–29	(24)	N.A.	9	7–12	3–26	(37)	N.A.
AST (U/L)	150	123–162	84–272	(23)	[281]	130	113–147	77–216	(37)	N.A.
LDH (U/L)	914	663–1250	217–1867	(24)	N.A.	674	616–822	307–1448	(37)	N.A.
CK (U/L)	343	229–432	77–1373	(23)	[1847]	197	150–255	28–771	(34)	[1576, 1690, 1304]
CA (mg/dL)	10.7	10.1–11.4	6.8–14.7	(24)	N.A.	17.2	14.8–18.8	8.9–27.1	(37)	N.A.
PHOS (mg/dL)	3.5	3.1–3.9	2.6–4.8	(24)	N.A.	4.6	4.1–4.9	3.3–6.6	(37)	N.A.
C:P (ratio)	2.9	2.7–3.5	2.1–4.2	(24)	N.A.	3.6	3.5–4.0	2.2–5.2	(37)	N.A.
CHOL (mg/dL)	101	92–126	71–207	(24)	N.A.	131	107–144	58–267	(37)	N.A.
UA (mg/dL)	1.4	1.1–1.8	0.4–2.6	(22)	[5.5]	1.2	1.0–1.3	0.6–2.0	(33)	[3.8, 2.7]
CREAT (mg/dL)	<0.2	~	~	(24)	N.A.	<0.2	~	~	(37)	N.A.
BA (µmol/L)	12.1	9.9–13.8	3.1–27.3	(23)	[40.4]	11.5	9.5–13.9	2.5–36.6	(37)	N.A.

Note: All outliers are identified specifically when removed or otherwise noted as not applicable (N.A.). A statistical summary Anderson-Darling and t-tests (not assuming equal variance) as well as the mean and standard deviation of these data can be found in appendix I.

*PCV includes females that are NOT palpably gravid only; palpably gravid females (n=8) were significantly different and therefore were not pooled (see Table 5 and Figure 5).

~ All values for CREAT were below method detection limit of 0.2 mg/dL, so statistical summaries were not possible.

phosphatase (ALP), aspartate aminotransferase (AST), creatine kinase (CK), uric acid (UA), and bile acids (BA) blood analytes; these were removed from statistical calculations (Table 3). Males and females were tested for differences in median, mean, and variance (Table 4). Because results for gravid turtles are rarely reported in the literature, all blood profile results for gravid females were reported here for future comparisons (Table 5). Results for gravid females are pooled with non-gravid females for all analytes, except PCV. PCV varied significantly between females that were not gravid on palpation (non-gravid) and gravid females (W=576, P=0.028; Figure 5A). As a result, these groups were not pooled for PCV results and only non-gravid females were compared to males (Table 4; Figure 5B). For palpably gravid females (n=8), PCV values

TABLE 4. Summary of statistical tests of blood profile analytes for differences between normal male and female *Emys marmorata* collected at the Big Chico Creek Ecological Reserve in northern California, USA (BCCER).

	P-Values		
Blood Analyte	Levene's Test	T-test	Mann-Whitney Test
PCV	0.401*	0.757*	0.8758*
TP	0.878	0.644	0.5557
BG	0.605	0.208	0.2077
ALP	0.111	**0.036**	**0.0292**
ALT	0.498	0.527	0.7342
AST	0.750	0.095	0.0715
LDH	**0.006**	**0.012**	**0.0137**
CK	**0.033**	**0.027**	0.2640
CA	**0.002**	**<0.001**	**<0.0001**
PHOS	**0.046**	**<0.001**	**<0.0001**
C:P	0.899	**0.002**	**0.0028**
CHOL	0.546	0.065	**0.0381**
UA	**0.010**	0.094	0.1082
BA	0.680	0.821	0.7553

Note: P-values <0.05 are **bolded.** *Tested differences in PCV for males and non-gravid females only; gravid females are not included (Table 2; Figure 2).

TABLE 5. Blood profile parameters for 8 normal, gravid female *Emys marmorata* from Big Chico Creek Ecological Reserve (BCCER) in northern California, USA.

Blood Analyte	Median	95% CI	Mean	SD	Range
PCV (%)	23	19–28	24	5.5	18–35
TP (g/dL)	4.6	4.1–5.0	4.5	0.53	3.5–5.1
BG (mg/dL)	74	61–97	76	16.6	50–103
ALP (U/L)	75	60–81	70	14.3	42–88
ALT (U/L)	9	6–16	11	6.2	3–23
AST (U/L)	136	111–201	143	45.2	77–214
CK (U/L)	250	191–314	261	102.2	151–486
LDH (U/L)	744	605–1147	826	282.5	464–1208
CA (mg/dL)	18.1	16.9–20.7	18.9	3.81	14.0–27.1
PHOS (mg/dL)	4.7	4.0–5.3	4.7	0.78	3.9–5.9
C:P (ratio)	4.0	3.5–4.6	4.0	0.54	3.3–4.8
CHOL (mg/dL)	130	96–144	124.4	24.12	90–156
UA (mg/dL)	1.0	0.9–1.4	1.2	0.62	0.9–2.7
CREAT (mg/dL)	<0.2	~	~	~	~
BA (µmol/L)	15.0	9.3–20.0	16.3	9.36	6.2–36.6

Note: ˜ All values were below the detection limit of 0.2 mg/dL, so statistical summaries were not possible.

ranged from 18–35% (median: 23%; Table 5, Figure 5A). Other than levels of PCV gravid females did not differ significantly from non-gravid females for any other blood parameter.

Many serum biochemical parameters differed between clinically normal male and female turtles when testing variance (Levene's test) and central tendency (Mann-Whitney U and T-test not assuming equality of variance). Males and female turtles differed significantly in median ALP, calcium (CA), cholesterol (CHOL), phosphorus (PHOS), calcium to phosphorus (C:P), and lactate dehydrogenase (LDH) levels. There was also a significant difference in the variance of CK, CA, PHOS, LDH, and UA levels between sexes (Table 4). Calcium (CA) in adult female turtles was nearly 2 times higher relative to adult male turtles collected during the same time period at the BCCER (Figure

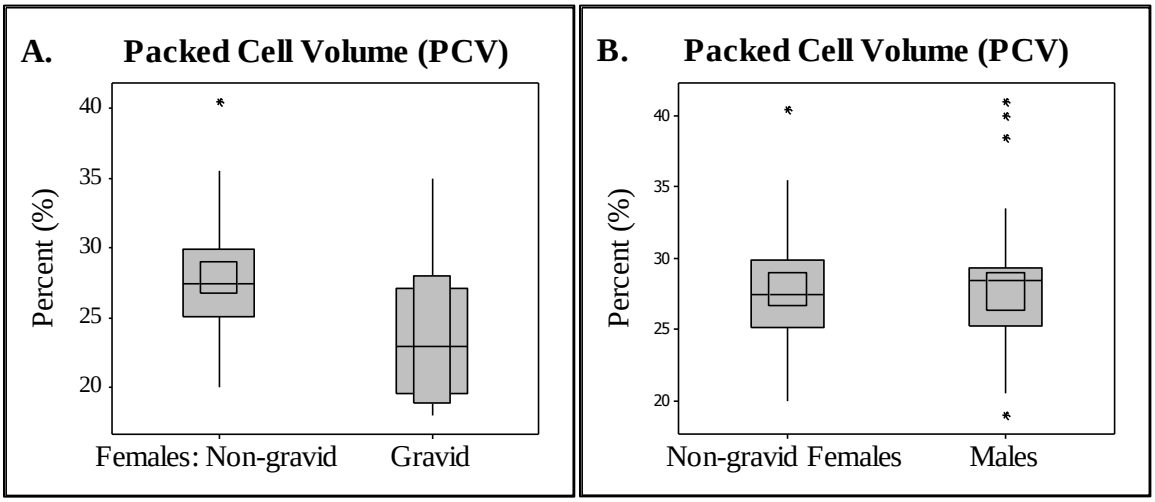

FIGURE 5. Packed cell volumes of normal non-gravid female, gravid female, and male *Emys marmorata* from Big Chico Creek Ecological Reserve (BCCER). Median packed cell volume was significantly lower in females found to be gravid on palpation as compared with females that were not (A). Packed cell volume was not significantly different in median or variance when non-gravid females were compared with males (B).

6A). Similarly, PHOS and the C:P ratios were also elevated in the blood of female turtles compared to adult male turtles at BCCER (Tables 3 and 5; Figure 6). Interestingly, there was also significantly higher levels of variance in CA and PHOS, but not C:P, for female relative to male turtles collected from BCCER (Table 4, Figure 6). The levels of CHOL were also significantly higher in female, relative to male, turtles (Table 4, Figure 6D). However, when gravid and non-gravid females were compared, there were no significant differences for these or other parameters (other than PCV, as noted above).

Median levels of ALP, LDH, and UA were all significantly higher in male relative to female turtles collected at BCCER (Tables 3 and 4; Figure 7). In addition, CK, LDH, and UA also showed significantly higher levels of variation in male relative to female turtles (Table 5; Figure 7). Levels of blood glucose (BG), total protein (TP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and bile acids (BA)

did not differ in median or variance between male and female *Emys marmorata* from the BCCER (Table 4, Figure 8).

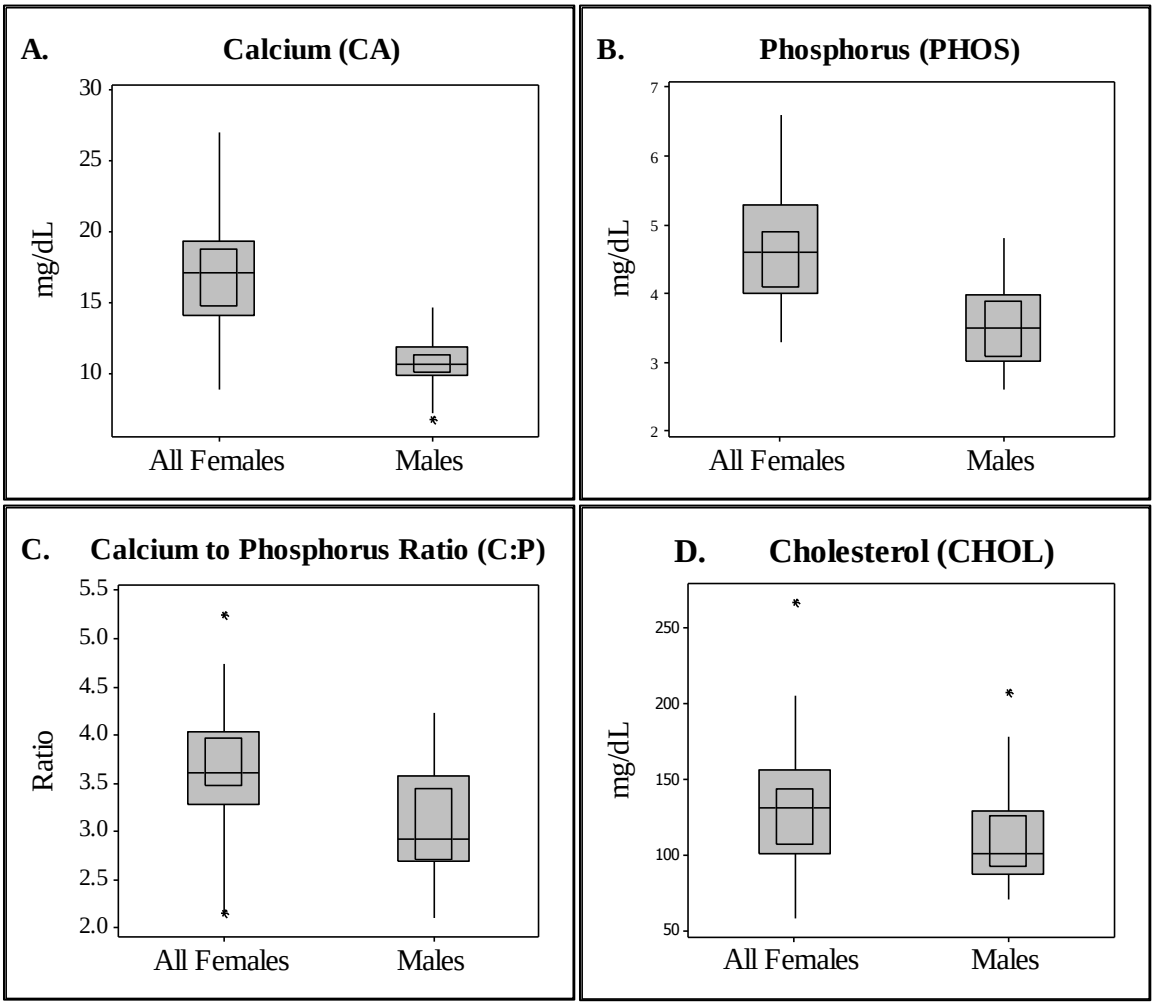

FIGURE 6. Significantly higher median and/or variance in 4 analytes in normal female, relative to male, *Emys marmorata* from Big Chico Creek Ecological Reserve (BCCER). Median calcium (A), phosphorus (B), calcium to phosphorus ratio (C), and cholesterol (D) were significantly higher in females relative to males. Calcium (A) and phosphorus (B) levels were also significantly more variable in female turtles as compared with males.

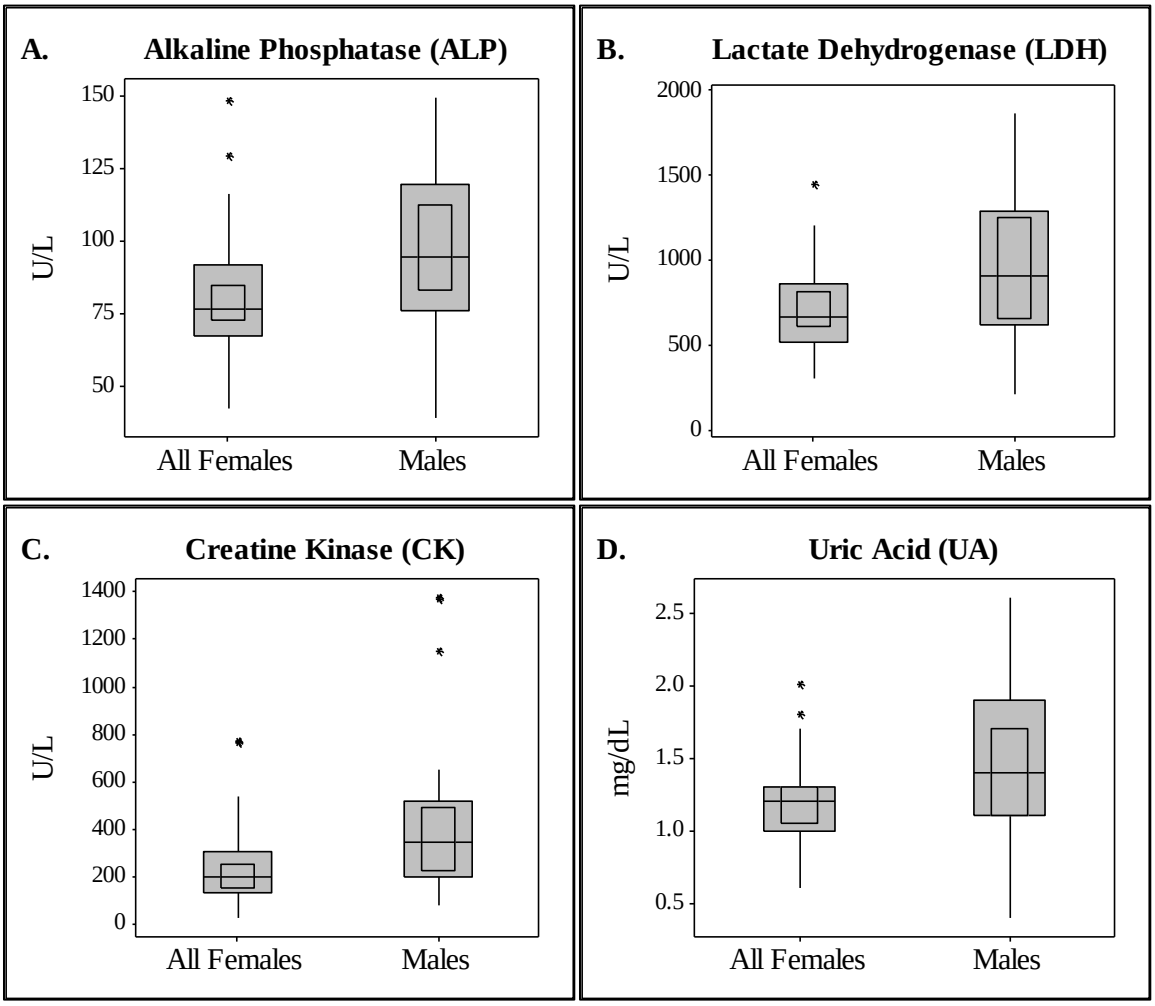

FIGURE 7. Significantly higher median and/or variance in 4 analytes in normal male, relative to female, *Emys marmorata* from Big Chico Creek Ecological Reserve (BCCER). Median alkaline phosphatase (A) and lactate dehydrogenase (B) were significantly higher in males relative to females; lactate dehydrogenase (B) was also significantly more variable in males. Creatine kinase (C) and uric acid (D) were significantly more variable in male, relative to female, turtles.

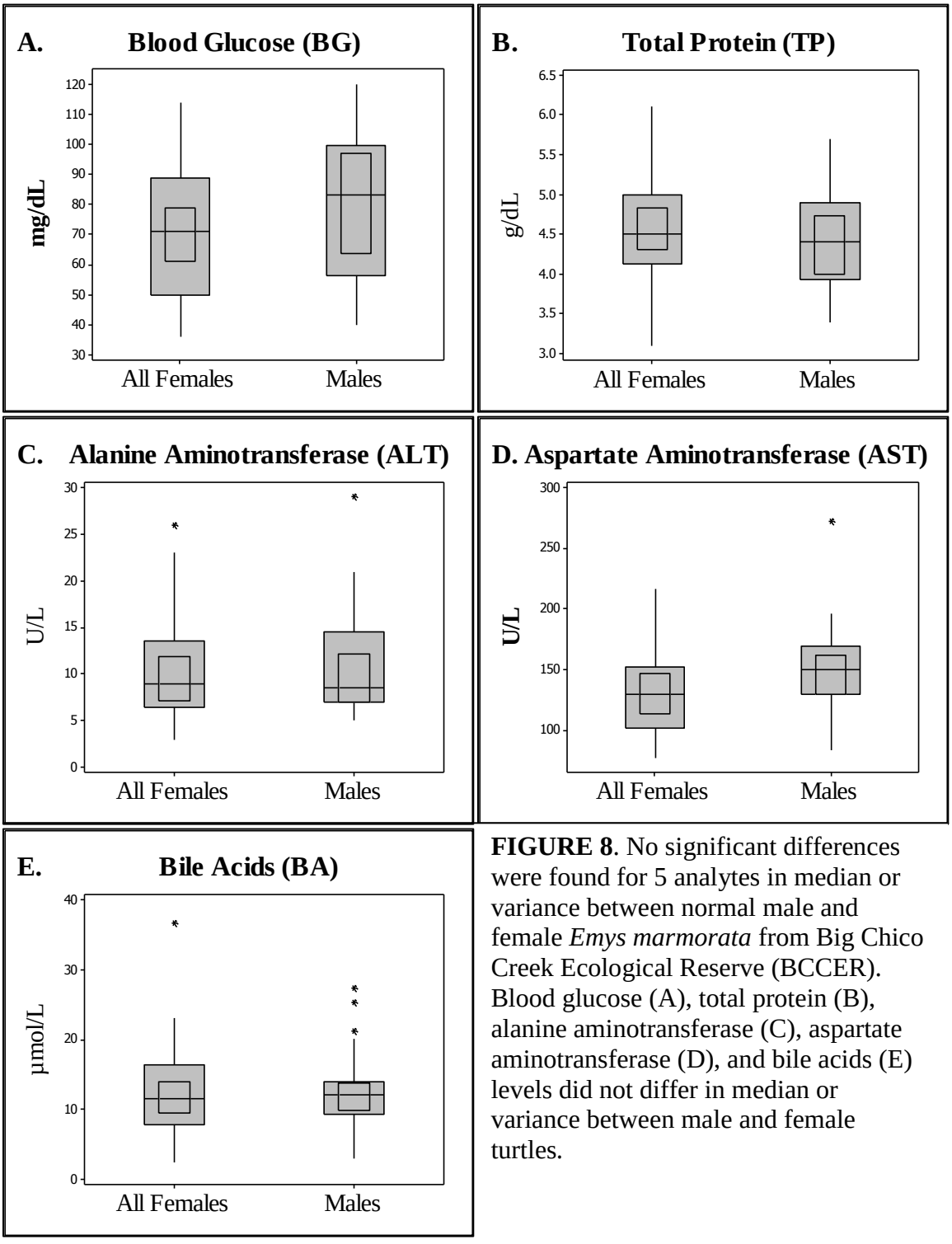

FIGURE 8. No significant differences were found for 5 analytes in median or variance between normal male and female *Emys marmorata* from Big Chico Creek Ecological Reserve (BCCER). Blood glucose (A), total protein (B), alanine aminotransferase (C), aspartate aminotransferase (D), and bile acids (E) levels did not differ in median or variance between male and female turtles.

Comparison of Blood Profiles in Male *Emys marmorata* from a Modified Habitat and a Nature Reserve

Presented here are blood profiles from a sample of 28 male turtles from the BCCER and 26 male turtles from the CWPCP. From the BCCER, this analysis included the 24 clinically healthy male turtles (presented above in baseline data), as well as 4 turtles that were not clinically normal (i.e. they were excluded from the above baseline blood profile calculations due to obvious malaise). Male turtles at the BCCER ranged from 138 to 167 mm (median: 151 mm) in MCL and ranged in mass from 370 to 697 g (median 504 g), while males from the CWPCP ranged from 158 to 195 mm (median: 183 mm) and weighed from 592 to 1072 g (median 927 g; Table 6). When a Mann-Whitney test was performed, male turtles from the CWPCP were significantly larger in MCL (W=887.5; P=0.0029) and mass (W=1075; P<0.0001) than males from the BCCER. Blood profile comparisons for male turtles from the BCCER and CWPCP are shown in

TABLE 6. Summary of morphometric data for *Emys marmorata* sampled for blood profile comparisons from populations at Big Chico Creek Ecological Reserve (BCCER) and the Chico Water Pollution Control Plant (CWPCP) in northern California, USA.

	Minimum Carapace Length (mm)				Mass (g)			
Site	**Median**	**Mean**	**95% CI**	**Range**	**Median**	**Mean**	**95% CI**	**Range**
BCCER Males (n=28)	151	152	148–155	138–167	504	502	473–531	158–195
CWPCP Males (n=26)	183	182	179–186	158–195	927	910	863–958	592–1072
CWPCP Females (n=11)	171	171	163–180	149–191	816	847	732–962	550–1152

TABLE 7. Comparison of blood profiles for male, wild-caught *Emys marmorata* from 2 sites, Big Chico Creek Ecological Reserve (BCCER) and Chico Water Pollution Control Plant (CWPCP), in northern California, USA.

	CWPCP Males				BCCER Males				P-Value		
Blood Analyte	**Median**	**95% CI**	**Range**	**(n)**	**Median**	**95% CI**	**Range**	**(n)**	**Levene's Test**	**T-Test**	**Mann-Whitney U**
PCV (%)	26	25–27	11–34	(24)	29	26–29	19–41	(27)	0.575	0.064	0.0686
BG (mg/dL)	56	47–73	26–211	(26)	80	62–88	40–120	(28)	0.673	0.204	**0.0364**
TP (g/dL)	4.2	3.9–4.6	3.2–5.5	(26)	4.4	4.0–4.8	3.4–5.7	(28)	0.645	0.290	0.3430
ALP (U/L)	69	63–86	34–359	(26)	94	83–113	39–175	(28)	0.436	0.378	**0.0099**
ALT (U/L)	5	4–7	<3–142	(26)	10	7–12	5–29	(28)	0.132	0.226	**0.0118**
AST (U/L)	121	95–180	52–1162	(26)	154	130–166	84–281	(28)	**0.036**	0.226	0.2730
LDH (U/L)	612	463–840	296–1503	(26)	1032	719–1230	217–2496	(28)	**0.042**	**0.004**	**0.0032**
CK (U/L)	347	185–430	83–1573	(26)	306	206–459	77–1847	(28)	0.444	0.628	0.9173
CA (mg/dL)	9.6	8.8–9.7	7.0–15.5	(26)	10.6	10.0–11.4	6.8–14.7	(28)	0.159	**0.023**	**0.0048**
PHOS (mg/dL)	2.6	2.4–2.8	1.4–6.9	(26)	3.5	3.0–3.8	2.6–4.8	(28)	0.387	**0.010**	**0.0000**
C:P (ratio)	3.8	3.4–4.0	1.6–6.3	(26)	3.0	2.8–3.3	2.1–4.2	(28)	0.399	**0.005**	**0.0024**
CHOL (mg/dL)	147	116–172	51–306	(26)	101	93–125	71–207	(28)	**0.006**	**0.007**	**0.0199**
UA (mg/dL)	1.1	0.8–1.3	0.2–3.2	(26)	1.4	1.1–1.9	0.2–5.5	(28)	0.736	0.324	0.1940
CREAT (mg/dL)	<0.2	N.A. ~	N.A. ~	(26)	<0.2	N.A. ~	N.A. ~	(28)	N.A. ~	N.A.~	N.A. ~
BA (µmol/L)	11.6	4.6–20.4	0.0–73.5	(26)	12.5	10.2–14.0	3.1–40.4	(28)	**0.017**	**0.000**	0.5796

Note: Significant P-values are **bolded** (set at a 95% confidence level). The results for Student t-test generally matched those of Mann-Whitney U. T-tests assumed unequal variance. Means, standard deviations, and results from normality and parametric central tendency tests are given in Appendix II.

~ All values for CREAT were below method detection limit of 0.2 mg/dL, so statistical summaries were not possible.

Table 7. For the purposes of evaluating population trends in blood profile parameters, outliers were not excluded from statistical calculations. Results for CREAT were below the method detection limit (0.2mg/dL) and statistical analyses were not performed for this analyte.

Blood profiles were not compared between sites for female turtles, because only 11 female turtles were sampled from the CWPCP; blood profile results for all females that were sampled at the CWPCP were reported separately (Table 8). The mass of females from CWPCP ranged from 550 to 1152 g (median: 816 g; Table 6). The MCL of CWPCP females ranged from 149 to 191 mm (median 171 mm; Table 6). All CREAT values for CWPCP females were below the detection limit of 0.2 mg/dL (Table 8).

TABLE 8. Blood profiles for 11 female, wild-caught *Emys marmorata* from the Chico Water Pollution Control Plant (CWPCP) in northern California.

Blood Analyte	**Median**	**95% CI**	**Mean**	**(SD)**	**Range**
PCV (%)	23	22–26	24	2.0	21–26
BG (mg/dL)	52	38–108	68	40.6	26–138
TP (g/dL)	4.7	4.2–5.2	4.7	0.61	3.8–5.8
ALP (U/L)	82	43–104	78	28.7	34–111
ALT (U/L)	12.0	6–30	20.7	23.6	3–73
AST (U/L)	141	94–228	193	172.1	59–670
LDH (U/L)	332	259–560	383	163.7	217–707
CK (U/L)	134	107–221	168	93.5	44–333
CA (mg/dL)	16.0	12.7–17.7	15.4	3.10	10.0–19.2
CHOL (mg/dL)	165	124 –220	166	64.9	54–289
PHOS (mg/dL)	3.9	3.4–4.8	4.1	0.89	2.7–5.6
C:P (ratio)	3.8	3.2–4.9	3.9	1.18	2.1–5.9
UA (mg/dL)	1.3	1.0–1.5	1.2	0.39	0.5–1.9
CREAT (mg/dL)	<0.2	~	~	~	~
BA (µmol/L)	9.3	5.9–21.5	13.3	11.17	2.7–35.0

Note: ~ All values were below method detection limit (0.2mg/dL).

When comparisons of blood profiles between male turtles from the BCCER and those from CWPCP were made, more than half of the blood analytes showed statistically significant differences between these two sub-populations (Table 7, Figures 9 and 10). Median levels of BG, ALP, ALT, LDH, CA, and PHOS were all higher in male turtles collected from within BCCER, relative to those collected from oxidation ponds at the CWPCP (Table 7; Figure 9). In contrast, median levels of CHOL and C:P were higher in males collected from CWPCP, relative to those collected from BCCER (Table 7; Figure 10). Interestingly, median CHOL levels were roughly 1.5 times higher in male turtles collected at the CWPCP, in comparison to those collected from BCCER (Table 7; Figure 10C). Several blood parameter values also showed higher variation in male turtles collected from CWPCP, relative to those collected from BCCER, including: AST, CHOL, and BA (Table 7, Figure 10). There was higher variation in LDH in the blood serum of male turtles collected in BCCER, relative to male turtles from CWPCP (Table 7; Figure 9D). No significant differences in median or variance were found in PCV, TP, CK, or UA between sites (Table 7; Figure 11).

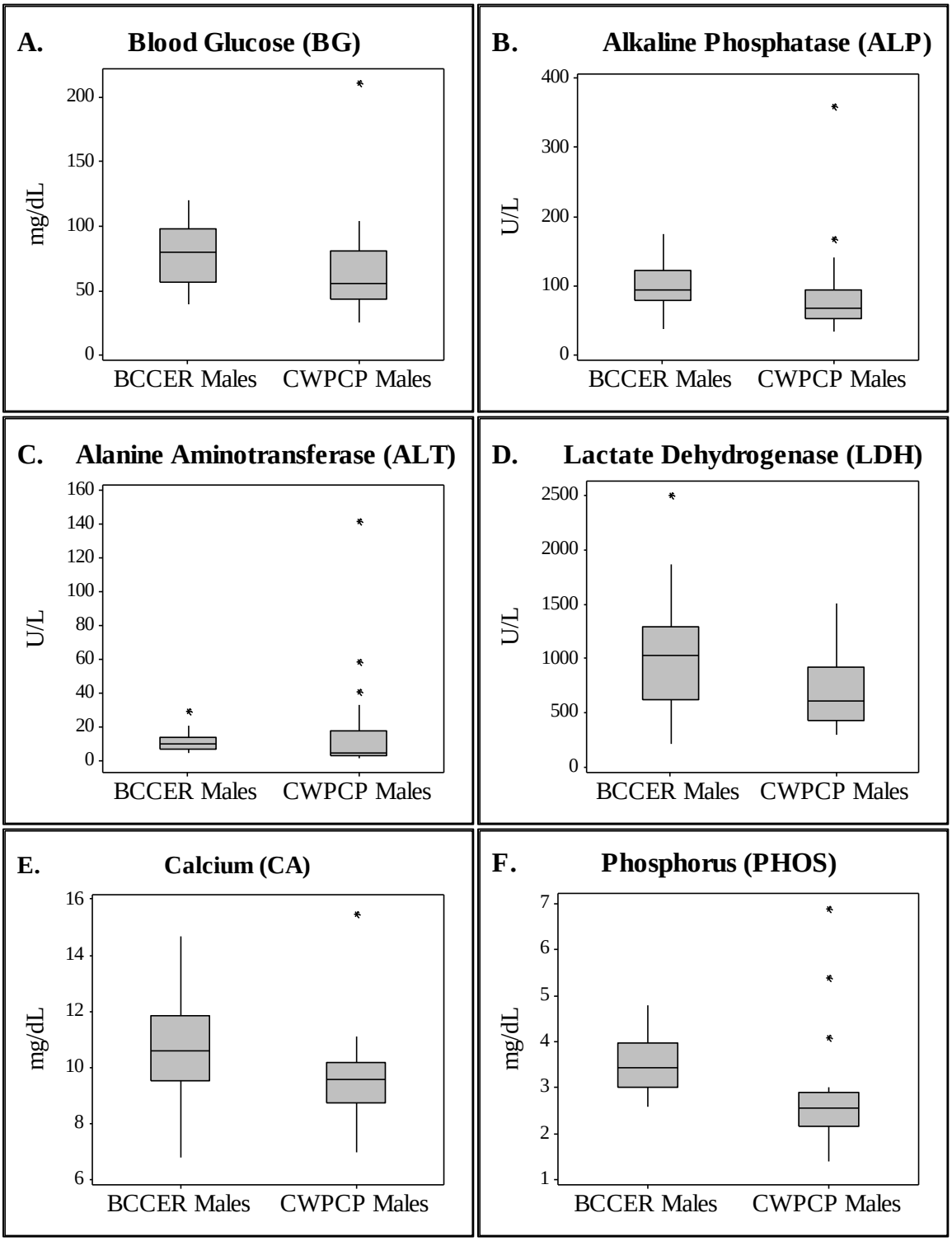

FIGURE 9. Significantly higher median and/or variance in 6 analytes for nature reserve (BCCER), relative to water treatment plant (CWPCP), male *Emys marmorata*. Median BG (A), ALP (B), ALT (C), LDH (D), CA (E), and PHOS (F) were higher in BCCER males, relative to CWPCP males; LDH (D) also had wider variation in BCCER males.

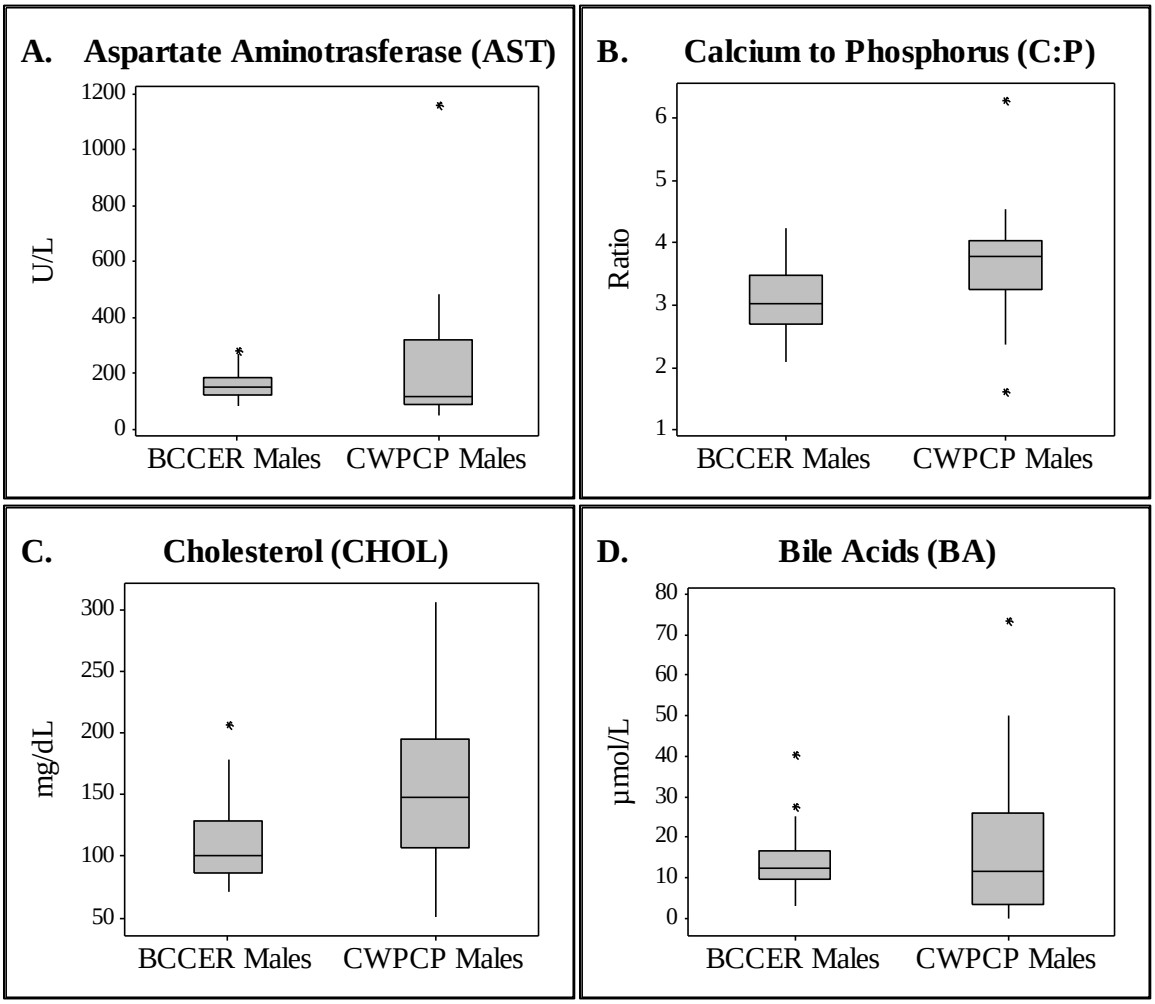

FIGURE 10. Significantly higher median and/or variance in 4 analytes in male *Emys marmorata* from a wastewater treatment plant (CWPCP), when compared to males from a nature reserve (BCCER). Aspartate aminotransferase (A), cholesterol (C), and bile acids (D) levels were significantly more variable in male turtles from CWPCP, as compared with BCCER males. Also, median calcium to phosphorus ratios (B) and cholesterol values (C) were significantly higher in CWPCP males.

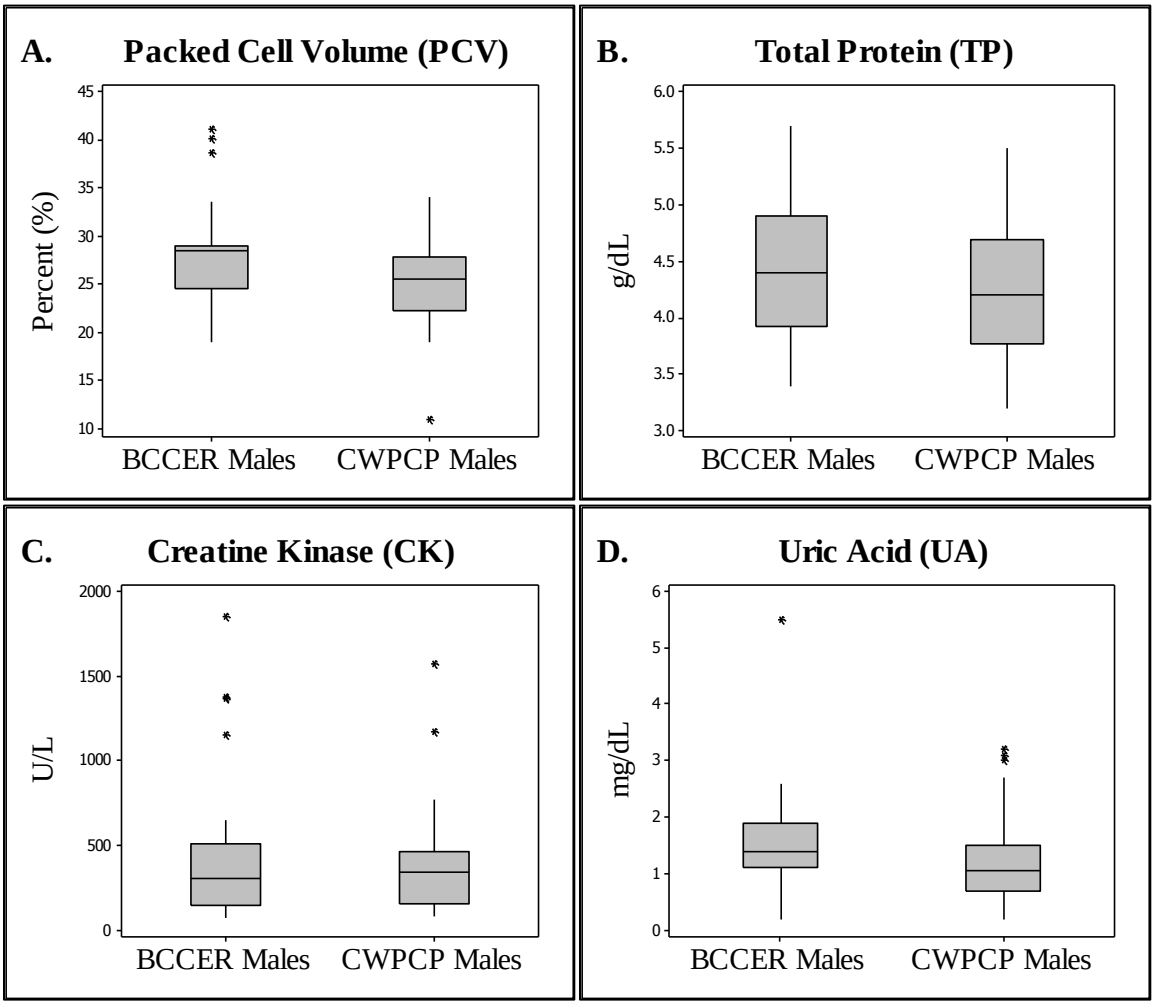

FIGURE 11. No significant differences were found for 4 analytes in median or variance between male *Emys marmorata* from the nature reserve (BCCER) and the wastewater treatment facility (CWPCP) populations. Packed cell volume (A), total protein (B), creatine kinase (C), and uric acid (D) levels did not differ significantly in variance or median between male turtles from BCCER and CWPCP. However, packed cell volume (A) showed a trend towards BCCER males being higher (see table 5).

CHAPTER IV

DISCUSSION

Baseline Blood Profiles for Normal *Emys marmorata* from a Nature Reserve

Hematocrit, or packed cell volume (PCV), levels observed in *Emys marmorata* in this study (Table 3) are within normal ranges for reptiles (Stacy et al. 2011; Campbell 2006; 2014), and are similar to those reported for other freshwater turtle species (Dessauer 1970; Innis et al. 2007; Peripinian et al. 2008; Yilmaz and Tosunoglu 2010; Omonova et al. 2011; Scheelings and Rafferty 2012). Levels of PCV in *E. marmorata* from the BCCER are similar to those reported in another study of this species (Keller et al. 2012), and are similar to PCV levels found in related, wild European Pond Turtles (*Emys orbicularis*; Yilmaz and Tosunoglu 2010). However, we found that PCV levels in *E. marmorata* were higher than those reported in some other Emydid turtles, including the Bog Turtle (*Clemmys muhlenbergii,* Brenner et al. 2002); the Mediterranean Pond Turtle *(Mauremys leprosa*; Hidalgo-Villa et al. 2007), the Yellow-headed Temple Turtle (*Hieremys annandalii;* Chansue et al. 2011) and Map Turtles (*Graptemys spp.;* Perpinian et al. 2008; Hernandez-Divers et al. 2009).

At the Big Chico Creek Ecological Reserve (BCCER), PCV levels were significantly lower in gravid *E. marmorata* females than non-gravid females (Figure 5A; Tables 5). Although few comparisons are available specifically for gravid female turtles, PCV values in gravid *E. marmorata* were similar to those reported for gravid

females in 3 species of wild, freshwater turtle in Australia (Scheelings and Rafferty 2012). In turtles, red blood cell production is affected by reproductive status (Hnizdo 2011), which may explain the observed differences in PCV levels between gravid and non-gravid females. Male and non-gravid females sampled within BCCER did not differ in PCV levels (Figure 5B; Tables 3–4), but in other studies differences in PCV levels between the sexes have occasionally been observed for other turtle species (Anderson et al. 1997; Chung et al. 2009; Yilmaz and Tosunoglu 2010).

In the present study of wild *E. marmorata*, values for total protein (TP) fell within the normal range for reptiles (3–7g/dL in Campbell 2004a; Table 3) and TP levels were similar to other freshwater turtles reported elsewhere (Chaffin et al. 2008; Perpinan et al. 2008; Chung el al. 2009; Chansue et al. 2011; Omonova et al. 2011; Schreelings and Rafferty 2012). When compared to another study of blood chemistries in wild *E. marmorata*, we found TP values to be comparable, but slightly higher in our study (Keller et al. 2012). Values for TP in *E. marmorata* in our study were also higher than values reported for wild Mediterranean Pond Turtles (Hidalgo-Villa et al. 2007) and wild Bog Turtles (Brenner et al. 2002), and were higher than captive Caspian Turtles (*Mauremys caspica*; Metin et al. 2008) and captive Red-bellied Cooters (*Pseudemys rubriventris*; Innis et al. 2007). Furthermore, TP values in our study of *E. marmorata* were higher by almost double the TP levels documented in captive Red-eared Sliders (*Trachemys scripta elegans*; Knotkova et al. 2008), Balkan Terrapins (*Mauremys rivulata*; Metin et al. 2008), European Pond Turtles *(*Metin et al. 2006), and Arrau Turtles (*Podocnemis expansa*; Olivera-Junior et al. 2009). The observed interspecific and intraspecific differences in TP may be explained by variation in dietary preferences, as

carnivory or a high protein diet are expected to increase TP levels (Scheelings and Rafferty 2012; Campbell 2014). We did not find significant differences in TP between male and female *E. marmorata* sampled within BCCER (Figure 8B; Tables 2–3), although some other investigators have reported differences in TP between the sexes in other freshwater turtles (Chung et al. 2009; Yilmaz and Tosunoglu 2010).

Blood glucose (BG) values in *E. marmorata* from the BCCER fell within the normal range for reptiles (60–100 mg/dL; Campbell 2004a; Table 3) and had wide variation. In our study of *E. marmorata*, BG values were similar to those reported for other freshwater turtles (Olivera-Junior et al. 2009) and other Emydid turtles, such as the Bog Turtle (Brenner et al. 2002), the Mediterranean Pond Turtle (Hidalgo-Villa et al. 2007), and the Asian Yellow Pond Turtle (*Ocadia sinensis*; Chung et al. 2009). The BG levels in *E. marmorata* from our study also are comparable to levels in another wild population of *E. marmorata* in northern California (Keller et al. 2012). But, BG values for *E. marmorata* sampled within BCCER were higher than BG levels reported in free-living Alligator Snapping Turtles *(Macrochelys temminckii;* Chaffin et al. 2008), captive Red-eared Sliders (Knotkova et al. 2008), captive European Pond Turtles (Metin et al. 2006), captive Caspian Pond Turtles (Metin et al. 2008), captive Balkan Terrapins (Metin et al. 2008), and wild Yellow-headed Temple Turtles (Chansue et al. 2011). Conversely, BG levels found in *E. marmorata* sampled within BCCER were almost half of BG levels reported in wild, gravid Western Long-necked Turtles (*Chelodina oblonga*), Common Long-necked Turtles (*Chelodina longcollis*), and Murray River Turtles (*Emydura macquarii;* Sheelings and Rafferty 2012). As with TP, there were no significant differences in BG between male and female *E. marmorata* sampled within BCCER

(Figure 8A; Tables 3–4).

Alkaline phosphatase enzyme (ALP) activity in healthy *E. marmorata* from the BCCER (Table 3) generally fell within normal levels for turtles (20–150 U/L; Divers 2000), with levels that were similar to those reported for other freshwater Emydid turtles, including the Asian Yellow Pond Turtle (Chung et al. 2009) and the Yellow-headed Temple Turtle (Chansue et al. 2011). When compared to a study sampling another population of wild *E. marmorata* in northern California (Keller et al. 2012) and a study of captive Red-eared Sliders (Knotkova et al. 2008), *E. marmorata* sampled at the BCCER had substantially lower ALP activity. However, activity of ALP enzymes were about two times higher in *E. marmorata* from the BCCER than in wild caught Mediterranean Pond Turtles (Hidalgo-Villa et al. 2007).

We found ALP values were lower in female *E. marmorata* than in males at the BCCER (Figure 7A; Tables 3–4). Similarly, captive male Yellow Pond Turtles were found to have higher ALP values when compared to females (Chansue et al. 2011). Conversely, wild-caught female Mediterranean Pond Turtles had higher ALP levels than males (Hidalgo-Villa et al. 2007). However other studies have reported no differences between male and female turtles in ALP activities (Chansue et al. 2011). Given that increased ALP can suggest osteoblastic activity (Campbell 1996, 2014) and that we sampled females during the nesting season, it is possible that we observed decreased ALP in females due to the down-regulation of bone formation, as females deposit calcium resources into eggshell development (Perrault et al. 2012).

For wild, healthy, *E. marmorata* sampled from within BCCER, alanine aminotransferase enzyme (ALT) activity was within the levels that are considered normal

guidelines for reptiles and turtles (<20 U/L; Campbell 2006; Table 3). For *E. marmorata* sampled within BCCER, ALT levels were similar to those reported for captive European Pond Turtles (Metin et al. 2006), Red-eared Sliders (Knotkova et al. 2008), and Yellow-headed Temple Turtles (Chansue et al. 2011). However, we found ALT levels in wild *E. marmorata* sampled from the BCCER were lower, by roughly half, than the levels found in wild Alligator Snapping Turtles (Chaffin et al. 2008). Conversely, ALT levels in wild *E. marmorata* sampled from the BCCER were about twice as high as those reported for captive Balkan Terrapins and ALT levels in female *E. marmorata* were twice as high as those reported for female captive Caspian Turtles (Metin et al. 2008). While this study of *E. marmorata* and other works (Chaffin et al. 2008) did not find differences in ALT between male and female turtles (Figure 8C; Tables 3–4), some studies do show differences in ALT between the sexes (Dickinson et al. 2002; Metin et al. 2006; Chung et al. 2009; Chansue et al 2011).

Emys marmorata sampled from within BCCER had aspartate animotransferase (AST, also known as SGOT) levels that were generally within the levels reported as normal for reptiles (<250 IU/L; Campbell 2006, 2014; Table 3) and were comparable to values reported for other freshwater turtle species such as captive Red-eared Sliders (Knotkova et al. 2008), captive European Pond Turtles (Metin et al. 2006), captive Caspian Turtles and Balkan Terrapins (Metin et al. 2008), wild Alligator Snapping Turtles (Chaffin et al. 2008), and wild Mediterranean Pond Turtles (Hidalgo-Villa et al. 2007). AST levels found in *E. marmorata* sampled within BCCER were similar to those found in another wild population of *E. marmorata* (Keller et al. 2012). However, AST levels were higher in *E. marmorata* from the BCCER than for wild,

freshwater Bog Turtles (Brenner et al. 2002), African Side-necked Turtles(*Pelusis sinatus*; Omonova et al. 2011), Common Long-necked Turtles and Murray River Turtles (Scheelings and Rafferty 2012), as well as Yellow-headed Temple Turtles (Chansue et al. 2011). Conversely, AST levels in wild Western Long-necked Turtles (Scheelings and Rafferty 2012) were more than twice as high as those found in *E. marmorata* from the BCCER.

Although not statistically significant, AST levels in *E. marmorata* from the BCCER illustrated a trend that suggests median levels in males may be higher than levels in females (P=0.0715; Figure 8D; Tables 3–4). Similar findings have been reported in other studies of turtles and tortoises, with males showing higher AST levels than females in another Emydid turtle, the Yellow Pond Turtle (Chung et al. 2009) and in the Desert Tortoise (*Goperus agassizi;* Dickinson et al. 2002). However, in Mediterranean Pond Turtles, levels were reportedly higher in females than in males (Hidalgo-Villa et al. 2007) and in other studies where AST levels were examined between sexes, no differences in AST were found (Chaffin et al 2008; Chansue et al. 2011; Omonova et al. 2011).

Generally, creatine kinase (hereafter referred to as CK; also known as creatine phosphokinase or CPK) levels in wild, normal *E. marmorata* from the BCCER had comparable CK values to another wild *E. marmorata* population in northern California (Keller et al. 2012), as well as to wild Bog Turtles (Brenner et al. 2002) and Alligator snapping turtles (Chaffin et al. 2008). In *E. marmorata* sampled from within BCCER, CK was more than two times lower than values recorded for captive Massachusetts Red-bellied Cooters (Innis 2007) and more than four times lower than wild Mediterranean Pond Turtles (Hidalgo-Villa et al. 2007).

We found that male turtles sampled from within BCCER had significantly more variable CK levels than female turtles (Tables 3–4), with male turtles having an increased number of elevated values (Figure 7C). CK enzymes are specific to muscle tissue and are released during muscle cell damage (Campbell 2004a). Muscle injury, over exertion, venipuncture, and systemic infections can all increase CK (Campbell 2004a). It is possible that male turtles may naturally be subjected to increased fatigue or injurious activities, such as stresses caused by male-male intraspecific competition during the mating season, which may also explain increased variation in CK activity in males. We observed heel scarring and minor abrasions on the rear hind feet of some males, which could suggest competitive encounters with other males while mating. Alternatively, we incidentally noticed that male turtles tended to struggle more than females during handling and restraint, which could increase their likelihood for fatigue or increased trauma from the blood draw procedure, and may explain increased CK level variation in male turtles. Similarly, a study of Hermann's Tortoise (*Testudo hermanni*) showed higher CK levels in males and authors hypothesized that the increased CK levels in males were due to increased activity (Scope et al. 2013). Other studies reported no significant differences in CK between males and females (Brenner et al. 2002; Chaffin et al. 2008).

Lactate dehydrogenase (LDH) levels in wild, normal *E. marmorata* from the BCCER approached or exceeded values considered normal in reptiles (<1000 U/L; Campbell 2004a, 2006, 2014; Table 3). LDH levels in wild Alligator Snapping Turtles were more than 5 times higher than those from *E. marmorata* in this study (Chaffin et al. 2008), and levels in captive Red-eared sliders were about 1.5 times higher (Knotkova et

al. 2008). In our study of *E. marmorata* sampled from within BCCER, male turtles had significantly higher and more variable LDH values than females (Figure 7B; Tables 3–4). Median LDH levels in females fell within ranges that are deemed normal in reptiles (<1000 U/L; Campbell 2004a, 2006, 2014), but LDH in males (using the 95% CI of the median) approached or exceeded these values (Table 3). Levels in *E. marmorata* male turtles sampled within BCCER were similar to levels reported for male Mediterranean Pond Turtles (Hidalgo-Villa et al. 2007) and European Pond Turtles (Metin et al. 2006). In female *E. marmorata* sampled within BCCER, we found that levels of LDH were lower than levels reported for female Mediterranean Pond Turtles (Hidalgo-Villa et al. 2007) and European Pond Turtles (Metin et al. 2006). Other studies reported no significant differences in LDH between the sexes (Hidalgo-Villa et al. 2007; Chaffin et al. 2008).

Generally, calcium (CA) values for *E. marmorata* sampled from within BCCER (Table 3) fell within normal ranges reported for reptiles (Campbell 2004b). Calcium values for male turtles fell at the high end of what is considered to be normal in reptiles (Campbell 2004a, 2006, 2014) and were comparable to levels found in wild, male Mediterranean Pond Turtles (Hidalgo-Villa et al. 2007) and captive European Pond Turtles (Metin et al. 2006). We found that CA levels in male *E. marmorata* collected within BCCER were similar to those reported from another wild population of the species (Keller et al. 2012), but this study did not report analytes by sex and pooled male and female turtles. However, CA levels in wild, male *E. marmorata* from BCCER were slightly higher than levels in wild, male Yellow-headed Temple Turtles (Chansue et al. 2011), wild Bog Turtles (Brenner et al. 2002), captive Balkan Terrapins and captive

Caspian Turtles (Metin et al. 2008). Levels in female *E. marmorata* sampled within BCCER were similar to wild, female Mediterranean Pond Turtles (Hidalgo-Villa et al. 2007), but higher than CA levels reported in wild Bog Turtles (Brenner et al. 2002), wild Alligator Snapping Turtles (Chaffin et al. 2008), wild Yellow-headed Temple Turtles (Chansue et al. 2011), captive European Pond Turtles (Metin et al. 2006), captive Balkan Terrapins, and captive Caspian Turtles (Metin et al. 2008).

We found that male and female turtles differed in CA, P, and C:P ratio (Tables 3–4). Female turtles had higher CA values than male turtles in our study of wild *E. marmorata* at BCCER (Figure 6A). The observed differences in CA levels between the sexes are likely to due physiological differences caused by reproductive processes (Campbell 2004a; Selleri and Divers 2006), and have been documented in other, freshwater turtles (Brenner et al. 2002; Hidalgo-Villa et al. 2007; Chaffin et al. 2008; Metin et al. 2008), tortoises (Dickinson et al. 2002; Scope et al. 2013) and reptiles (Knotkova et al. 2005b). Elevated calcium levels indicate vitellogenesis and follicular development in female turtles (and other vertebrates) (Campbell 2006; Selleri and Divers 2006). When estrogen stimulates the liver to produce vitellogenins, blood calcium levels can substantially increase because calcium is often bound to vitellogenin proteins (Gibbons 2001), and CA and P increase during egg production in females (Irizarry-Rovira 2004). Turtles for this study were sampled during the breeding season; 8 of 37 females sampled had calcified eggs on palpation (Table 2) and it is likely that many of the other non-gravid females had follicles developing during this time. Elevated CA has also been shown in other female freshwater turtles, such as Bog turtles (Brenner et al. 2002), Mediterranean Pond Turtles (Hidalgo-Villa et al. 2007), Alligator snapping turtles

(Chaffin et al. 2008), and Caspian Turtles (Metin et al. 2008).

We found that phosphorous (P) levels in *E. marmorata* from the BCCER (Table 3) were similar to levels reported in other wild, freshwater turtles (Chaffin et al. 2008; Chansue et al. 2011) and captive Emydid turtles (Metin et al. 2006; Metin et al. 2008). Levels of P in normal *E. marmorata* were generally higher than those reported in Bog Turtles (Brenner et al. 2002) and almost two times higher than those reported in wild Mediterranean Pond Turtles (Hidalgo-Villa et al. 2007). Male *E. marmorata* in our study had P levels that were within the normal range expected in reptiles (1–5 mg/dL; Campbell 2006), but levels in females were on the high end of normal or exceeded this range (Table 3). In our study of *E. marmorata* at the BCCER, P levels were significantly higher in females than in males (Figure 6B; Tables 3–4). Elevated P in females is consistent with other studies of wild caught, freshwater turtles (Brenner et al. 2002; Hidalgo-Villa et al. 2007; Chaffin et al. 2008), and is related to increased estrogen and reproduction (Clark 1965). However, some research has documented higher P in male turtles (Metin et al. 2008). Levels of P in *E. marmorata* males and females sampled within BCCER were slightly higher than those reported for Mediterranean Pond Turtles (Hidalgo-Villa et al. 2007). While levels of P in male *E. marmorata* sampled from BCCER were comparable to male captive Caspian turtles, levels in female *E. marmorata* from BCCER were higher than levels reported for female Caspian Turtles (Metin et al. 2008).

Few previous studies have reported calcium to phosphorus ratios (C:P). The C:P ratios found in clinically normal, wild *E. marmorata* from the BCCER were higher than levels reported for other reptiles by about 1.5 times (Selleri and Divers 2006). But,

C:P ratios in male *E. marmorata* at the BCCER were similar to those documented for juvenile, captive Massachusetts Red-bellied Cooters, whereas female *E. marmorata* had higher C:P ratios (Innis et al. 2007), presumably due to physiological processes during reproduction in mature female turtles. As with C and P, C:P ratios were significantly higher, on average, for female, relative to male, turtles sampled within BCCER (Figure 6C; Tables 3–4).

Cholesterol (CHOL) in normal *E. marmorata* sampled within BCCER (Table 3) was similar to levels reported in other Emydid turtles, including the Red-eared Slider (Knotkova et al. 2008), Mediterranean Pond Turtle (Hidalgo-Villa et al. 2007) and Asian Yellow Pond Turtle (Chung et al. 2009). Levels of CHOL were 1.5 to 2 times higher in *E. marmorata* than in wild Alligator Snapping Turtles (Chaffin et al. 2008), captive European Pond Turtles (Metin et al. 2006), captive Balkan Terrapins, captive Caspian Turtles (Metin et al. 2008) and farmed Arrau Turtles (Olivera-Junior et al. 2009). Our results indicate that CHOL was elevated in female *E. marmorata* relative to males (Figure 6D; Tables 3–4). These results are supported by the finding in another study of this same population that indicated female turtles had higher body condition than male turtles (Polo-Cavia et al. 2010). Similarly, other authors have reported elevated CHOL in female freshwater turtles (Anderson et al. 1997; Chung 2009), tortoises (Dickinson et al. 2002; Scope et al. 2013), sea turtles (Deem et al. 2009), and other reptiles (Knotkova et al. 2005b). Female Mediterranean Pond Turtles (Hidalgo-Villa et al. 2007), Asian Yellow Pond Turtles (Chung et al. 2007), and Alligator Snapping Turtles (Chaffin et al. 2008) had higher CHOL levels than males. Elevated CHOL in female testudines has been associated with increased estrogen (Clark 1965), vitellogenesis, and egg laying

(Dessauer 1970; Dickinson et al. 2002). Thus, the observed differences in CHOL between sexes in our study of normal *E. marmorata* are likely due to variation in reproductive physiology.

We found that uric acid (UA) levels in wild, healthy *E. marmorata* from the BCCER (Table 3) were consistent with expected values in reptiles (Campbell 2006) and were generally similar to those reported for other freshwater, Emydid turtles (Brenner et al. 2002; Chung et al. 2009; Chansue et al. 2011; Knotkova et al. 2008). UA levels in male *E. marmorata* were similar to those reported in wild, male Mediterranean Pond Turtles, but levels in *E. marmorata* females were slightly lower than those reported in female Mediterranean Pond Turtles (Hidalgo-Villa et al. 2007). However, UA levels in *E. marmorata* sampled within BCCER were almost twice as high as those reported for Alligator Snapping Turtles (Chaffin et al. 2008). UA values in wild turtles are expected to vary widely because nutritional status is not controlled for and, in carnivorous animals, UA levels rise substantially post-prandially (following feeding); (Anderson et al. 1997; Irizarry-Rovira 2004). Male *E. marmorata* from the BCCER had significantly wider variation in UA when compared with females, and illustrated a non-significant trend toward higher levels of UA, relative to females (Figure 7D; Tables 3–4). Similarly, male Yellow Pond Turtles had higher UA levels than females (Chung et al. 2009). However, other studies have found no significant differences in UA between the sexes (Brenner et al. 2002; Chaffin et al. 2008).

Creatinine (CREAT) values in *E. marmorata* sampled from the BCCER were consistent with levels typically seen in reptiles (Campbell 2006) as well as other aquatic, freshwater turtles (Chaffin et al. 2008; Chung et al. 2009; Chansue et al. 2011; Knotkova

et al. 2008). However, specific comparisons were limited because CREAT results were below the method detection limit for both male and female turtles (Table 3). We found CREAT in *E. marmorata* to be lower than Mediterranean Pond Turtles (Hidalgo-Villa et al. 2007), and more than three times lower than CREAT in captive European Pond Turtles (Metin et al. 2006) and Caspian Turtles (Metin et al. 2008).

Little is known about the levels of bile acids (BA) in reptiles, in general (Divers and Cooper 2000; Campbell 2004a; Irizarry-Rovira 2004; Hnizdo 2011), and few researchers have documented BA levels in freshwater turtles (Anderson et al. 1997; Chaffin et al. 2008; Knotkova et al. 2008). BA levels in *E. marmorata* sampled within BCCER (Table 3) were similar to those documented in captive Red-eared Sliders (*Trachemys scripta elegans*; Knotkova et al. 2008), a related freshwater turtle and were about half of levels found in New Guinea Snapping Turtles (*Elseya novaeguineae*; Anderson et al. 1997). However, levels in turtles from BCCER were approximately 50 times higher than those found in Alligator Snapping Turtles (*Macrochelys temminckii*; Chaffin et al. 2008). We found that bile acids (BA) levels in *E. marmorata* from the BCCER did not differ between the sexes (Figure 8E; Tables 3–4), although male Hermann's Tortoises have been shown to have higher BA seasonally when compared to females (Scope et al. 2013).

Overall, there was notable variation between clinically normal male and female turtles sampled from the *E. marmorata* population at BCCER, with significant differences in more than half of the blood profile analytes between the sexes at this site (Figures 5–7, Tables 3–4). Previous comparisons of these baseline results from the BCCER *E. marmorata* population to other works should be interpreted cautiously

because blood profiles in reptiles can vary widely between species (Campbell et al. 2014). In addition, even comparisons of studies in the same species have indicated that there is wide variation in results (Metin et al. 2006), which may be due to differences in season, methodology, age, or other factors that varied between study populations. In addition, there has been documented variation in the blood profiles of captive and wild populations within the same species (Swimmer 2000; Brenner et al. 2002; Keller et al. 2012), and many of the works we compared to our study of *E. marmorata* used captive turtles. Further contributing to difficulty in comparison, the reviewed studies of blood profiles in other turtle species varied widely in both sample sizes and methodology. Comparisons between species are confounded by variation in venipuncture site between studies (among other disparities), making it difficult to disentangle differences in blood analyte results due to variation in study methodology from true interspecific variation. For example, differences in PCV, TP, UA, CA, P, AST, ALT, and LDH have been seen from samples taken from the brachial vein versus dorsal coccygeal vein (Lopez-Olvera et al. 2003). Additionally, differences in blood profile analytes have been noted when plasma versus serum samples are analyzed (Fazio et al. 2011). Nonetheless, the baseline values from normal *E. marmorata* represent a relatively large sample size of non-gravid female, gravid female, and male turtles (Table 2) sampled from a population from a natural habitat at a nature reserve that will be of use for comparisons with future studies.

Comparison of Blood Profiles in Male *Emys marmorata* from a Nature Reserve and a Modified Habitat

Comparisons between blood profiles of male *E. marmorata* sampled within Big Chico Creek Ecological Reserve (BCCER) and Chico Water Pollution Control Plant (CWPCP) allowed a coarse examination of possible differences between nearby populations that reside in a relatively natural habitat, versus a highly modified site, respectively. Given the substantial variation observed between male and female turtles sampled from within BCCER (Tables 2–5), and the low numbers of females sampled at CWPCP (Table 6), comparisons between sites were made for male turtles only (Table 7) and values for female turtles sampled from CWPCP were reported separately (Table 8).

Hematocrit or packed cell volume (PCV) measures the percent red blood cells per volume of whole blood. Although we found no differences in PCV between male *E. marmorata* from BCCER and CWPCP (Table 7; Figure 11A), PCV levels can be useful in evaluating health in individuals and between populations. Low PCV levels can indicate anemia, which can arise from blood loss or hemolytic diseases, while high PCV levels can indicate dehydration. Anemia can result from traumatic blood loss, decreased erythrocyte production, or hemolysis from viral infections or bacterial septicemia (Hnizdo 2011). Non-regenerative anemia can result from chronic infections, metabolic problems, kidney diseases, liver diseases, and hyperthyroidism (Campbell and Ellis 2007; Hnizdo 2011), but low PCV may also result from lymph contamination. Low PCV was linked to clinical illness in Green Sea Turtles, *(Chelonia mydas*; Whiting et al. 2007) and stranded Loggerhead Sea Turtles (*Caretta. caretta*; Deem et al. 2009), and was reported in wild chelonians with chronic infectious diseases (Campbell and Ellis 2007), such as

sea turtles infected with fibropapillomastosis (Work and Balazs 1999) and tortoises with chronic upper respiratory disease (Muro et al. 1998; Jacobson 1991). PCV values under 15% indicate anemia (Campbell and Ellis 2007). One male turtle from the CWPCP presented with clinical anemia (PCV=11%); we assume that the low PCV was due to hemorrhage, as his tail was previously amputated to the cloaca. Although we found no difference in PCV between male *E. marmorata* from CWPCP and BCCER (Figure 11A; Table 7), PCV differed between study sites in Sonoran Desert Tortoises (Dickinson et al. 2002) and varied seasonally in Alligator Snapping Turtles (Chaffin et al 2008) and Yellow Pond Turtles (Chung et al. 2009). PCV can also vary in reptiles with temperature and season (Dessauer 1970; Campbell and Ellis 2007; Hnizdo 2011), as well as with physiological status and body condition, diet, and habitat (Campbell and Ellis 2007).

Total Protein (TP) can be useful in diagnosing chronic inflammatory diseases, malnutrition, and many other diseases (Campbell 2004, 2014). Lower levels of TP can indicate problems with intestinal uptake of proteins, loss of proteins in the kidneys, liver insufficiency, and chronic malnutrition, whereas high levels of TP may indicate dehydration, and chronic antigenic stimulation or intestinal parasitism (Irizarry-Rovira 2004; Campbell 2014). Although, we did not find differences in TP between males in *E. marmorata* from the CWPCP and BCCER (Figure 11B; Table 7), declines in TP previously have been linked to illnesses and stranding in the Loggerhead Sea Turtle, *Caretta caretta* (Deem et al. 2009; Fazio et al. 2012).

Blood glucose (BG) was higher in male *E. marmorata* sampled from the BCCER in comparison to male turtles sampled from CWPCP (Figure 9A; Table 7). BG is known to vary by species, nutritional status, temperature and other environmental

conditions (Campbell 2004a). Increased BG can be due to glucocorticoid release from stress responses or infectious disease, and BG can also vary with age, diet, nutritional status, and environment (Irizarry-Rovira 2004; Campbell 2006). Decreased BG can occur due to starvation, liver disease, septicemia, or hormone problems (Irizarry-Rovira 2004). The differences in BG that we observed between *E. marmorata* from the BCCER and CWPCP may be attributed to differences in environmental temperature between the sites, diving behavior between populations, or possibly differences in capture method. The water temperature at the BCCER is cooler and temperature is known to inversely affect BG levels in other Emydid turtles (Dessauer 1970; Campbell 2006). Although we regrettably did not measure water temperature regularly at our sites, the BCCER water was considerably colder (not tolerable without a 3–7mm wetsuit for more than 10 minutes at a time) than the water at the CWPCP (which could generally be described as tepid). Furthermore, diving behavior affects increases in BG because freshwater turtles undergoing extended dives have been shown to utilize anaerobic glycolysis for energy (Dessauer et al. 1970). It is possible that turtles at the BCCER may regularly have increased diving behavior, as compared to turtles at the CWPCP, due to natural differences in the ecology between the two habitats. It is also conceivable that capture by snorkeling increased BG levels in BCCER turtles by forcing them to hide (dive) for extended periods of time or elicited capture stress and a subsequent corticosteroid response that resulted in an increased BG (Dessauer 1970; Snoddy et al. 2009). Seasonal differences in BG have been observed in some freshwater turtles (Dessauer 1970) as well. Alternatively, organochlorine contaminants have been inversely correlated with BG levels in the Loggerhead Sea Turtle (Keller at al. 2004) and we cannot rule out the

possibility that contaminants may differ between our study sites and contribute to the observed differences in BG levels.

Levels of alkaline phosphatase (ALP) were significantly lower in male turtles sampled from CWPCP when compared to *E. marmorata* male turtles sampled from BCCER (Figure 9B; Table 7). ALP is not found in turtle liver tissues (Hnizdo 2011) and is distributed widely in many tissues (Divers 2000; Campbell 2004a). Perturbations ALP levels may be linked to pathogenic processes (Swimmer 2000; Knotek et al. 2002; Keller 2004; Flint et al. 2010) or can indicate osteoblastic activity (Campbell 2004a). Lower ALP activity in *E. marmorata* from CWPCP could potentially be attributed to pathogens or toxins in this habitat: For example, decreased ALP was found in 35% of unhealthy Green Sea Turtles (Flint et al. 2010) and ALP activity was decreased in iguanas with kidney disease (Knotek 2002). Increased ALP levels were associated with fibropapillimatosis, a tumor disease, in captive Green Sea Turtles (Swimmer 2000) and ALP levels were negatively associated with organochlorine contaminant exposure in Loggerhead Sea Turtles (Keller et al. 2004). Alternatively, as increased ALP may suggest bone growth, ALP levels may have been higher in males from the BCCER due to differences in size and growth. Males at the BCCER were smaller in carapace length and mass than males from the CWPCP (Table 6). Increased ALP levels in BCCER males could indicate that they were actively undergoing bone growth at the time of sampling, while most males at the CWPCP may not have been. A study in Hermann's Tortoises found ALP activity peaked mid-summer, and authors hypothesized that this may be attributed to increased bone growth at this time (Scope et al. 2013). Similarly, a seasonal

component associated with elevation differences may be driving differences in ALP levels between BCCER and CWPCP males.

Alanine aminotransferase (ALT) activities were significantly lower for male *E. marmorata* sampled from CWPCP, relative to those sampled from BCCER, with median ALT values from CWPCP approximately one half of the values at BCCER (Figure 9C; Table 7). Differences in median ALT levels between sites is likely a product of differences in habitat, as site and seasonal conditions are known to cause differences in ALT levels in Alligator Snapping Turtles (Chaffin et al. 2008) and different ALT values were found in healthy Green Sea Turtle populations sampled from different habitats (Aguirre and Balazs 2000). ALT is not organ specific in reptiles, but increased ALT activity may indicate acute liver damage (Divers and Cooper 2000; Knotkova et al. 2008). Although ALT activity is high in kidney tissues in reptiles, high levels in the blood are not usually associated with renal disease because, in this case, the enzymes released are excreted in the urine (Campbell 2006). The difference in median ALT between populations most likely represents natural intraspecific variation between populations. There is insufficient evidence that the increased median ALT in BCCER males is of pathogenic origin because the levels are within the accepted intervals for reptiles (Campbell 2006) and because it is not accompanied by increases in AST (see below), which can be a better indicator of liver disease (Campbell 2006). Interestingly, although median ALT levels were higher in BCCER males, 23% [6/26] of male *E. marmorata* from the CWPCP had ALT values at or above the normal decision level (20 U/L) in reptiles (Campbell 2006), while only 11% [3/28] of BCCER males had ALT

activity above the decision level. Observing ALT levels at or above published decision levels in reptiles raises concerns about the health of these particular individuals.

Aspartate aminotransferase (AST) levels were significantly more variable in *E. marmorata* males from CWPCP than in BCCER males, with more males at CWPCP exhibiting elevated AST (Table 7, Figure 10A). Although median AST did not differ significantly between sites (Table 7), 27% [7/26] of male *E. marmorata* sampled from CWPCP exhibited AST levels above the normal decision levels for reptiles (<250 U/L; Campbell 2006), whereas only 7% [2/23] of male turtles sampled from BCCER exceeded this level. Notably, all 6 of the turtles with elevated ALT levels reported above also exceeded the decision level for AST levels. The observed differences in AST variance could have been due to differences in the environment, as significant differences in AST were found between Alligator Snapping Turtles from different locations and AST levels also were found to be higher in the summer than in early spring (Chaffin et al. 2008). Alternatively, the observed differences in AST variance could potentially reflect differences in breeding behavior in the male turtles: For example, other research with captive turtles and wild tortoises has found that AST levels increased seasonally, as males began to fight and copulate, suggesting that increased AST may indicate injuries from male-male aggression (Dickinson et al. 2002; Chung 2009). Sex ratios at the CWPCP are male-biased, while sex ratios at the BCCER are not, which may increase male-male aggression at the CWPCP. However, although increased male-male aggression may occur as a result of the male biased sex ratio at the CWPCP, one would expect the injuries related to male-male aggression to increase LDH and CK values as well, and these were not consistently co-elevated in individuals with high AST, which increases

suspicions of disease, rather than trauma. Differences in the variance of AST levels also may be due to variation in pathogenic processes between sites. AST enzymes are found in kidney (Selleri and Divers 2006), liver, and skeletal muscle tissues (Irizarry-Rovira 2004), and elevated AST can indicate kidney, liver, or muscle damage (Divers and Cooper 2000; Campbell 2004a, 2006; Selleri and Divers 2006). Elevated AST can also be the result of septicemia or toxic diseases that damage these tissues (Campbell 2004a) or may indicate renal damage (Selleri and Divers 2006; Knotek at al. 2002). Increased AST was found in clinically ill Green Sea Turtles (*C. mydas*) (Whiting et al. 2007; Flint et al. 2010) and a study of over 100 Loggerhead Sea Turtles (*C. caretta*) clinically ill turtles had increased AST activity (Fazio et al. 2012), while another study found that organochlorine contaminants were correlated with AST (Keller et al. 2004). While the high AST levels in more than a quarter of the male turtles sampled from the CWPCP cannot be definitively attributed to pathogenic causes, additional research is warranted.

In male *E. marmorata* sampled from the BCCER, lactate dehydrogenase (LDH) was both higher and more variable than LDH levels of male turtles sampled from CWPCP (Figure 9D; Table 7). LDH can be a nonspecific indicator of tissue injury (Irizarry-Rovira 2004; Hnizdo 2011) and elevated LDH levels can result from damage to the liver, kidney, or muscle tissues (Divers and Cooper 2000; Campbell 2004a; Selleri and Divers 2006) or poor venipuncture technique (Hznido and Pantcev 2011). Although LDH can be elevated as a result of damage tissues, it is doubtful that a traumatic or pathogenic origin resulted in the observed elevated median and wide variation in LDH levels in male turtles from the BCCER because the vast majority individuals with high LDH (>1000 U/L) did not have concurrent AST elevations [1/14]. Although increased

LDH can result from poor venipuncture technique, it is unlikely that this is the origin of the increased median and variation in LDH in males from BCCER because the same two technicians performed venipuncture at both the BCCER and the CWPCP and male turtles did not seem to struggle more intensely at either site. Furthermore, CK also can be elevated due to poor venipuncture technique (Hnizdo 2011) and CK was not significantly different between the two populations. We assert that the increased LDH and wider variation in BCCER male turtles is likely due to differences in natural diving behavior between the populations, because similar trends were observed for female turtles (from both sites). Alternatively, observed differences may have been an artifact of the different capture methods used between sites (snorkel hand captures at BCCER versus trapping at CWPCP). When turtles are captured by hand while snorkeling, they may be forced to hide underwater for longer periods prior to capture and they may also be subjected to forced 'dives', as the surveyor carries the turtle while swimming to the processing site. Lactate is released when surfacing after diving, when the blood vessels in muscles dilate (Dessauer 1970). As a result, increased LDH levels could indicate recent diving behavior in BCCER turtles and may be an artifact of sampling method from being captured by snorkeling rather than by trap.

Creatine kinase activity (CK) was not significantly different in median or variance between male *E. marmorata* sampled from BCCER and CWPCP (Figure 11C; Table 7). CK is important to measure because it is specific to muscle tissue, while other enzymes (e.g., ALP, ALT, AST, and LDH) are not tissue specific in their distribution (Divers and Cooper 2000; Irizarry-Rovira 2004; Campbell 2014). CK was positively associated with blood mercury concentrations (Day et al. 2007) and was elevated in

stranded Loggerhead Sea Turtles (Deem et al. 2009). Elevated CK can indicate muscle damage, can occur from excessive struggling, or result from venipuncture trauma (Campbell 2004b). As male *E. marmorata* from the BCCER and CWPCP do not differ in CK, we have increased confidence that our different capture methods between sites (hand capture while snorkeling at BCCER versus trapping at CWPCP) did not result in differences in muscle fatigue between sites that might confound the interpretation of other enzyme analytes. Furthermore, the lack of difference in CK between sites also bolsters our confidence that the observed differences in median or variance in ALP, ALT, AST, and LDH between sites reflect differences in site-specific processes on physiology, and are not confounded by differences in muscle damage, nor variation in venipuncture trauma, between sites.

Cholesterol (CHOL) was significantly higher and more variable in CWPCP male *E. marmorata* than male turtles sampled from within BCCER (Figure 10C; Table 7). Cholesterol is a lipid that is a necessary component of cellular structure of animals and can be ingested from the diet or fabricated by the liver. Diet affects CHOL, with CHOL levels often reportedly lower for herbivorous turtles relative to carnivorous species (Anderson et al. 1997). Previous research indicated that turtles at the CWPCP have higher body condition than turtles at the BCCER (Polo-Cavia et al. 2010), which corroborates the observed higher levels of CHOL in CWPCP males during this study (Figure 10C; Table 7). Differences in CHOL between male *E. marmorata* at the sites is likely due to differences in diet, temperature, prey availability, thermoregulatory behavior, and growth rates between populations. Turtles at the CWPCP can be active all year because water temperatures and ambient temperature are higher at this site in

comparison with the BCCER. Due to the cooler water temperatures, narrow canyon topography, and increased canopy cover, turtles at the BCCER are expected to spend more time basking (Lubke and Wilson 2007) to thermoregulate than turtles at the CWPCP. Research suggests that when *E. marmorata* occupy lower elevation habitats, with organic muddy substrates and a more open canopy structure, the combined effects of increased prey availability and reduced need for thermoregulatory behaviors facilitates valley populations growing larger, on average, relative to foothill populations (Lubke and Wilson 2007), as was observed in this study (Table 6). Cholesterol levels can elevate after ingestion of a meal (Irizarry-Rovira 2004; Anderson et al. 2011), and it is possible that potential differences between the timing in foraging between populations may also have contributed to elevated CHOL levels at the CWPCP. We have evidence that *E. marmorata* at the CWPCP forage at night because we have captured them in traps very early in the morning after baiting the traps the previous evening.

Although higher body condition or variation in feeding phenology likely both contributed to the observed higher levels of CHOL in CWPCP males, high CHOL levels have also been linked to disease processes as well. Elevated CHOL has been associated with liver problems (Divers 2000; Campbell 2014), such as hepatic lipidosis (Divers 2000; Irizarry-Rovira 2004). Furthermore, high maternal cholesterol has been linked to reduced hatching and emergence success in Leatherback Sea Turtles (*C. caretta;* Perrault et al. 2012). It has been suggested that 230 md/dL is the upper limit of normal CHOL levels in reptiles (Divers 2000); four males sampled from CWPCP (15%; n=26) were equal to or exceeded this limit, while all males (n=28) collected from BCCER had CHOL levels below this limit. While the high CHOL levels in these four individuals from the

CWPCP could be due to disease processes, examination of other blood analyte levels did not reveal any obvious abnormalities, lending further support to the hypothesis that the increased CHOL levels are due to another factor such as feeding phenology, diet, or body condition.

Calcium (CA) and phosphorous (PHOS) levels were lower in CWPCP male *E. marmorata* than in BCCER males (Figure 9E-F; Table 7), but CWPCP males had higher calcium to phosphorus (C:P) ratios (Figure 10B; Table 7). Calcium facilitates critical life functions such as, the formation of bone and eggshell, coagulation in blood, muscle contractions, nerve impulse transmission, and secretion from glands (Gibbons 2001). The observed differences in PHOS and CA between populations likely reflect dietary differences between the populations: For example, a more herbivorous diet can increase level of PHOS (Campbell 2004b) and increased carnivory can increase CA levels (Campbell 2014), particularly when diets include species with calcium carbonate shells or exoskeletons. Lower levels of CA and PHOS in male turtles sampled at CWPCP may also be linked to differences basking habits between populations at the CWPCP and BCCER. Exposure to sunlight produces the active form of vitamin D3, which stimulates absorption of CA and PHOS from the gut (Campbell 2014). However, it is possible that variation in disease processes may have also contributed to observed differences in CA and PHOS levels between male turtles sampled from CWPCP and BCCER. Previous research suggests that problems with CA metabolism and elevated PHOS may indicate kidney disease in reptiles (Rosenthal et al. 2000; Knotek et al. 2002; Hnizdo 2011). In other research, lower CA levels and PHOS levels were found in Green Sea Turtles with lesions, wounds, or parasites (Labrada-Martagon 2010). Similarly, lower CA levels were

observed in Iguanas with renal disease (Boyer et al. 1996), and elevated P was observed in iguanas with renal disease (Knotek et al. 2002).

Higher calcium to phosphorus ratios (C:P) were observed in male *E. marmorata* sampled at the CWPCP, relative to male turtles sampled within BCCER (Figure 10B; Table 7). Examination of C:P ratios can help identify metabolic disorders (Campbell 2006; Hnizdo 2011). C:P ratios also have been reportedly altered with renal disease in some reptiles, with renal disease suspected when CA levels dip below PHOS levels in blood plasma (Rosenthal et al. 2000; Knotek et al. 2002). Similarly, alteration in C:P ratios have been linked to renal disease in Iguanas and are thought to be an earlier indicator of kidney disease than UA levels (Knotek et al. 2002). Elevated PHOS can indicate kidney disease (Hnizdo 2011) when the C:P ratio is less than one or, in male reptiles, when the product of CA multiplied by PHOS exceeds 55 mg/dL (Irizarry-Rovira 2004; Selleri and Divers 2006). No individual male turtle in the present study had a C:P ratio below one and there were only two male turtles from CWPCP, and one male turtle from BCCER, where the product of CA and PHOS levels exceeded 55mg/dL. Collectively, this suggests that disease processes are unlikely to be underlying the observed differences in median C:P ratios among populations and that the differences may be due to intrinsic differences between the populations, such as diet or basking habits.

Uric acid (UA) levels did not differ significantly in median or variance between male *E. marmorata* sampled from the CWPCP and BCCER (Figure 11D; Table 7). Uric acid (UA), urea, and ammonia are the products of protein catabolism (Dessauer 1970). Increased UA can indicate a recent ingestion of a protein rich diet item (Divers

2000; Hnizdo 2011). In terrestrial reptiles, increased UA levels can also indicate aminoglycoside toxicity (antibiotics), septicemia, and calcification of nephrons in the kidney (Knotek 2002), although, UA may only become elevated at the advanced stages of kidney disease (Divers 2000). However, increased UA has been reported to be a better indicator of kidney disease in terrestrial turtles than in aquatic turtles (Hnizdo 2011) and the clinical relevance of differences in UA levels in aquatic turtles is uncertain (Keller et al. 2012).

For male turtles samples from CWPCP, bile acids (BA) levels had significantly wider variation when compared with BA levels in male turtles from the BCCER (Figure 10D; Table 7). Little is known about BA levels in reptiles (Divers and Cooper 2000; Campbell 2004a; Irizarry-Rovira 2004; Hnizdo 2011), but previous research suggests that BA can be a sensitive indicator of liver function in birds (Campbell 2004a) and elevated BA may indicate liver disease in reptiles (Divers and Cooper 2000). It is thought that measurement of BA may indicate liver function, while measurement of ALT and AST enzymes can indicate the damage to hepatic tissue, but provide no insight as to liver function (Divers and Cooper 2000; Knotkova et al. 2008). Interpreting the meaning of the differences in BA variance between male *E. marmorata* populations is difficult as BA levels in mammals and some reptiles can elevate post prandially (McBride et al. 2007) and the time since the most recently ingested diet item was unknown for wild turtles in the present study. In an experiment with iguanas, post-prandial (after feeding) BA levels rose about 4 times higher than pre-prandial BA levels at 3 and 7.5 hours following a meal (McBride et al. 2007), but post-prandial elevation in BA levels were not found in Red-eared Slider Turtles (Knotkova et al. 2008). Further,

elevated post-prandial BA levels, following a brief period of fasting, may be associated with liver diseases in reptiles (Divers and Cooper 2000). Some studies in reptiles indicate that the upper limit of normal BA levels could be 60 μmol/L (Divers and Cooper 2000). However, a recent study in Red-eared Slider Turtles suggests that the upper normal limit for BA is likely much lower in Emydid turtles, as the highest level observed among 10 healthy captive turtles was 19.1 μmol/L (Knotkova et al. 2008) and the highest level reported in Hermann's Tortoises was 11 μmol/L (Scope et al. 2013). In the present study, 8/26 (31%) males from the CWPCP and 5/28 (18%) from BCCER fell above the highest level reported in Red-eared Slider Turtles by Knotkova et al. (2008).

Overall, there were significant differences found in median or variance in 2/3 of blood profile analytes tested in male *E. marmorata* sampled between CWPCP and BCCER sites. Environmental conditions between the CWPCP and the BCCER vary widely, and these differences in habitat, and associated changes in behavior (e.g., diving and basking), could likely be responsible for the observed differences in blood profiles between the sites. Alternatively, it is also possible that altered blood profiles between sites may be due to differences in pathogenic exposure between the two habitats. A prior study of *E. marmorata* at these same two sites found that turtles from the CWPCP had increased heterocyte to lymphocyte ratios, in addition to decreased immune responses to a novel immune challenge, which suggested increased immune challenges from infectious agents in the water (Polo-Cavia et al. 2010). Furthermore, the possible influences of agricultural chemicals and pharmaceutical metabolites that are more likely to be present in CWPCP water are unknown and may possibly contribute to observed differences in blood profile analytes. Furthermore, it is impossible to rule out differences

in capture method (trap versus snorkeling) as a possible cause for some of the observed variation in blood profile analytes between male *E. marmorata* at the two sites. Additional research, including a test of the effect of capture method on blood profile results within a given population, is warranted to tease apart the important variation that was captured within these initial comparisons of blood profiles. Repeated sampling of *E. marmorata* at the BCCER and CWPCP over time could help determine contribution of the elevational differences between sites and associated differences in environmental conditions throughout the season to variation in blood analytes between populations. Study of blood profiles in *E. marmorata* populations from higher elevation, lentic impacted sites in the foothills and highly natural, lotic sites in lowland valley would be ideal for comparison to the present study. Further, a spatiotemporally explicit approach, including the collection of both habitat and water quality data at the time / site of capture, may help to explain some of the observed variation, both within and between populations.

Conclusions

This study characterized baseline blood profiles for a robust sample of clinically normal, wild *E. marmorata* from a highly natural habitat (BCCER) and examined intraspecific variation between adult male and female turtles in this subpopulation. Significant differences between male and female *E. marmorata* were found in over half of blood analytes, underscoring differences in physiology between the sexes and highlighting the importance of reporting blood profiles separately by gender. This study also compared blood profiles of all male *E. marmorata* captured from the

nature reserve population (BCCER) to all males captured from a highly modified habitat (CWPCP) at a wastewater treatment facility. Two thirds of analytes differed between males in either median or variance when blood profiles of all males captured from the BCCER were compared with all males captured from CWPCP. These results indicate differing physiologies between turtles living at a highly modified site and a highly natural site. While some of these observed differences may be due to pathogenic or disease processes, many of these differences may be attributed to differences in ecology, resource availability, or intrinsic habitat characteristics between populations. Future studies replicating this work at an increased number of and variety of natural and highly modified sites would elucidate physiological changes that occur due to habitat modification versus habitat type. Our understanding of the ecophysiology of *E. marmorata* would be strengthened by investigating blood profiles at unaltered, natural valley sites, with lentic conditions and at highly modified habitats in lotic foothill habitats. This work indicates that blood profiles may be a useful tool in determining the physiology of turtles in altered environments, and with further study, could guide our understanding of the suitability of altered landscapes to support healthy, fit populations in the future.

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APPENDIX A

Appendix A. Summary of blood profiles with means (± standard deviation), normality tests, and parametric central tendency tests for wild-caught, clinically normal *Emys marmorata* from Big Chico Creek Ecological Reserve (BCCER) in northern California, USA.

	Males						Females						T-test	
					Anderson-Darling test						Anderson-Darling test			
Blood Analyte	**Mean**	**±**	**SD**	**(n)**	**A²**	**P**	**Mean**	**±**	**SD**	**(n)**	**A²**	**P**	**T**	**P**
PCV (%)	28	±	5	(24)	0.97	**0.012**	28*	±	4*	(28)*	0.74	**0.047**	-0.31	0.757
TP (g/dL)	4.4	±	0.6	(24)	0.22	0.825	4.5	±	0.7	(37)	0.19	0.889	0.46	0.644
BG (mg/dL)	80	±	25	(24)	0.33	0.490	72	±	23	(36)	0.43	0.287	-1.28	0.208
ALP (U/L)	97	±	28	(24)	0.15	0.949	82	±	23	(36)	0.79	**0.037**	-2.16	**0.036**
ALT (U/L)	11	±	6	(24)	1.38	**<0.005**	10	±	5	(37)	0.83	**0.028**	-0.64	0.527
AST (U/L)	151	±	40	(23)	0.45	0.257	133	±	38	(37)	0.50	0.191	-1.71	0.095
LDH (U/L)	990	±	436	(24)	0.29	0.569	726	±	268	(37)	0.43	0.289	-2.65	**0.012**
CK (U/L)	448	±	374	(23)	1.62	**<0.005**	253	±	180	(34)	1.91	**<0.005**	2.33	**0.027**
CA (mg/dL)	10.7	±	1.8	(24)	0.30	0.564	17.0	±	4.0	(37)	0.24	0.755	8.18	**<0.001**
PHOS (mg/dL)	3.5	±	0.6	(24)	0.32	0.508	4.7	±	0.8	(37)	0.38	0.390	6.49	**<0.001**
C:P (ratio)	3.1	±	0.6	(24)	0.75	**0.044**	3.7	±	0.7	(37)	0.34	0.488	3.27	**0.002**
CHOL (mg/dL)	113	±	36	(24)	0.73	**0.050**	132	±	41	(37)	0.61	0.102	1.88	0.065
UA (mg/dL)	1.5	±	0.6	(22)	0.37	0.396	1.2	±	0.3	(33)	0.69	0.064	-1.73	0.094
CREAT (mg/dL)	<0.2		~	(24)	~	~	<0.2		~	(37)	~	~	~	~
BA (µmol/L)	12.8	±	6.0	(23)	0.67	0.068	12.4	±	6.5	(37)	0.52	0.174	-0.23	0.821

Note: T-test was run without assuming equality of variance. *PCV includes females that were NOT palpably gravid only; palpably gravid females (n=8) were significantly different and therefore were not pooled (Table 5).
~ All values for CREAT were below method detection limit of 0.2 mg/dL, so statistical summaries were not possible

APPENDIX B

Appendix B. Summary of blood profiles with means (± standard deviation), normality tests, and parametric central tendency tests for wild-caught, male *Emys marmorata* from Big Chico Creek Ecological Reserve (BCCER) and Chico Water Pollution Control Plant (CWPCP) in northern California, USA.

	CWPCP			**BCCER**			**P-Value**		
Blood Analyte	**Mean**	**SD**	**Range**	**Mean**	**SD**	**Range**	**Anderson - Darling CWPCP**	**Anderson - Darling BCCER**	**T-Test**
PCV (%)	25	5	11–34	28	6	19–41	0.089	**0.014**	0.064
TP (g/dL)	4.3	0.6	3.2–5.5	4.4	0.6	3.4–5.7	0.942	0.716	0.290
BG (mg/dL)	66	38	26–211	78	25	40–120	**<0.005**	0.548	0.204
ALP (U/L)	87	64	34–359	99	30	39–175	**<0.005**	0.770	0.378
ALT (U/L)	16	29	<3–142	11	6	5–29	**<0.005**	**<0.005**	0.226
AST (U/L)	213	230	52–1162	156	46	84–281	**<0.005**	0.089	0.226
LDH (U/L)	693	317	296–1503	1042	503	217–2496	0.100	0.261	**0.004**
CK (U/L)	404	339	83–1573	457	447	77–1847	**<0.005**	**<0.005**	0.628
CA (mg/dL)	9.7	1.5	7.0–15.5	10.6	1.8	6.8–14.7	**<0.005**	0.950	**0.023**
PHOS (mg/dL)	2.8	1.1	1.4–6.9	3.5	0.6	2.6–4.8	**<0.005**	0.146	**0.010**
C:P (ratio)	3.7	0.9	1.6–6.3	3.1	0.6	2.1–4.2	0.057	0.112	**0.005**
CHOL (mg/dL)	153	67	51–306	112	34	71–207	0.550	**0.040**	**0.007**
UA (mg/dL)	1.3	0.8	0.2–3.2	1.6	1.0	0.2–5.5	**<0.005**	**0.007**	0.324
CREAT (mg/dL)	<0.2~	~	n/a ~	~	~	n/a ~	n/a	n/a	n/a
BA (µmol/L)	16.4	17.5	0.0–73.5	14.0	7.7	3.1–40.4	**<0.005**	**<0.005**	**0.000**

Note: T-test was run without assuming equality of variance.
~ All values for CREAT were below method detection limit of 0.2 mg/dL, so statistical summaries were not possible