Hematologic and Plasma Biochemical Values of Free-Ranging Western Pond Turtles (*Emys marmorata*) with Comparison to a Captive Population

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Abstract: The western pond turtle (*Emys marmorata*), a species of concern in California, is a common wildlife patient in veterinary hospitals and wildlife rehabilitation centers within its geographic range. The objectives of this study were to obtain hematologic and biochemistry values for free-ranging western pond turtles and to compare those values to a captive population. Two populations, free-ranging ($n = 20$) and captive ($n = 10$), were sampled using physical examination, complete blood count, plasma biochemistries, and *Salmonella* spp. culture from cloacal swabs. Heterophil ($P = 0.0001$), azurophil ($P = 0.0001$), eosinophil ($P = 0.0039$), monocyte ($P = 0.0001$), albumin ($P = 0.0001$), aspartate aminotransferase ($P = 0.003$), calcium ($P = 0.001$), glutamate dehydrogenase ($P = 0.0006$), globulin ($P = 0.0082$), sodium ($P = 0.0014$), total protein ($P = 0.0001$), and uric acid ($P = 0.0094$) concentrations were significantly different between the two populations. Creatine kinase ($P = 0.0012$) and phosphorus ($P = 0.0068$) were also significantly different between males and females within the free-ranging population. *Salmonella* spp. cloacal cultures from all turtles were negative. The hematologic and biochemistry intervals reported for this free-ranging population may be used to assess disease for this species; however, differences between the two populations examined highlight how environmental and nutritional factors can induce changes in commonly evaluated hematological and biochemical values.

Key Words: Biochemistry, hematology, *Emys marmorata*, plasma, western pond turtles.

Introduction

The western pond turtle (*Emys marmorata*) is a freshwater turtle in the family Emydidae. This turtle's former geographic range spanned from British Columbia to northern Baja California, although this range is much reduced at this time (Brattstrom, 1988). The U.S. Fish and Wildlife Service and the state of California have listed the western pond turtle as a species of concern (Jennings and Hayes, 1994). Populations throughout the range are in decline secondary to habitat destruction, off-road vehicular use, livestock grazing, contaminant spills, and collection for the pet trade (Brattstrom, 1988; Bury, 1989; Lovich, 1994; Thomson, 2010). The introduction of invasive pest species, most notably the red-eared slider (*Trachemys scripta elegans*), has caused direct competition with the western pond turtle throughout its entire range (Bury, 1989; Lovich *et al.*, 1994; Thomson *et al.*, 2010).

Western pond turtles are common wildlife patients presented to veterinary hospitals secondary to vehicular trauma, lawn mower accidents, or dog attacks. Despite this commonality, normal values for standard blood parameters are lacking. Clinical biochemistry and hematology values are often compared to other freshwater species such as the red-eared slider. The purpose of this study was to report hematology and plasma biochemistry values for a small population of free-ranging western pond turtles. Additionally, the study compares these values to a captive population in northern California with a very different environment and diet. Our hypotheses were that 1) there would be significant differences in hematologic and biochemistry values between captive and free-ranging populations of western pond turtle, and 2) male and female individuals of free-ranging western pond turtle would exhibit differences in specific values associated with reproductive activity.

Materials and Methods

This project was approved by the University of California, Davis Institutional Animal Care and Use Committee (Davis, CA).

Populations: Animals were sampled at two different sites in northern California between September and October 2011. The first population ($n = 20$) was sampled over three visits between 17 and 21 September 2011 at Putah Creek in Davis, California (38.53°N, 121.76°W). Turtles were being captured in buoy-suspended nets as part of a larger research project ongoing at the Bradley Shaffer Laboratory at University of California, Davis. All turtles captured on these dates were included in the study with the exclusion of turtles that were captured more than once. The nets were observed at least once every 24 h; thus, turtles were trapped for less than a 24 h period. This section of Putah Creek is a man-made diversion with concrete embankments over a dried riverbed. Species other than western pond turtle living within this ecosystem include red-eared sliders, softshell turtles (*Apalone* spp.), river cooters (*Pseudemys concinna concinna*), *Kinosterna* spp., common carp (*Cyprinus carpio*), river otters (*Lontra canadensis*), and several species of wild birds.

The second population ($n = 10$) was a captive group sampled at the Micke Grove Zoo in Lodi, California (38.07°N, 121.27°W) on 7 September 2011. This population of turtles lived in a mixed species, open-air exhibit with resident ruddy ducks (*Oxyura jamaiciensis*), northern pintails (*Anas acuta*), and a common merganser (*Mergus merganser*). Several migratory waterfowl species, most notably mallard ducks (*Anas platyrhynchos*), are present seasonally. The exhibit is a man-made pond that is drained twice yearly; no filtration is provided and the water is stagnant. The animals are fed chopped, defrosted capelin (*Mallotus villosus*) daily. New animals are subject to a 30-day quarantine with serial fecal examinations and hematology performed before being added to the collection. Previous reference intervals used for this population for interpretation of quarantine hematology were determined by the attending veterinarian. Two of the turtles were captive-born while the other eight were wild-caught. The animals were sampled during their annual physical examination when the pond was drained. This population had been kept indoors and fasted for approximately 24 h in a shallow pool of 3–5 cm of water before examination.

Physical examination: All 30 animals received a physical examination, although an oral examination was only performed if the animal opened its mouth voluntarily. The animals were categorized as male, female, or juvenile based upon secondary sexual characteristics including concavity of the plastron, tail size, and vent location on the tail. Because animals less than 200 g lacked secondary sex characteristics, they were assigned as a juvenile. Body mass and morphometric measurements including the straight carapace length (SCL), straight carapace width (SCW), curved carapace length (CCL), and curved carapace width (CCW) were recorded. The SCL and CCL were defined as the caliper length and tape measure lengths, respectively, on midline from the gular scute (or the median between two gular scutes if applicable) and the midpoint between the twelfth marginal scutes. The SCW and CCW were defined as the caliper and tape measure widths, respectively, from the edge of the left and right marginal scutes at the junction between the second and third vertebral scutes. Each physical examination was less than 10 min in length.

Sample collection: All animals were manually restrained and the tail was extended. Using a dorsal approach to the coccygeal vein, venipuncture was performed using a 25-gauge needle and a 1- or 3-ml syringe. If gross lymph contamination was observed, the sample was discarded and a second sample was obtained proximal to the first site. Blood sample volume was limited to <1% of body weight. The blood sample was divided into dipotassium ethylenediaminetetraacetic acid (K_2EDTA), lithium-heparin, and serum collection pediatric tubes (Microtainer, Becton Dickinson and Company, Franklin Lakes, NJ). A sterile swab (Health Link Transporter Micro Swab, Jacksonville, FL) was introduced into the cloaca approximately 1–2 cm deep for bacteriological sampling. The samples were stored on ice within an insulated cooler after collection and transported to the William R Pritchard Veterinary Medical Teaching Hospital Clinical Pathology Laboratory for analysis within 4 h of collection.

Sample processing: The K_2EDTA blood sample was utilized for automated and manual hematologic analyses. A spun packed cell volume (PCV) was obtained using a microhematocrit tube and a centrifuge. A blood smear was made upon arrival at the laboratory and stained using an automatic stainer (Aerospray® Stat, Wescor, Inc., Logan, UT) then counterstained in Giemsa Stain Solution (EMD Chemicals, Inc., Gibbstown, NJ) for 3–4 min. A 200-cell manual differential count and a hemocytometer white blood cell count (WBC), using the Natt and Herrick's procedure, was performed by the same technologist for all samples. The heterophil:lymphocyte (H:L) ratio was calculated by dividing the mean heterophil count by the mean lymphocyte count for each population. Thrombocytes in the smear were evaluated as being decreased, adequate, or increased in number by subjective assessment.

The lithium heparin plasma samples were analyzed using a Roche Cobas® C311 Chemistry Analyzer (Diamond Diagnostics, Holliston, MA). Values included in the clinical biochemistry profile were albumin, alkaline phosphatase, anion gap, aspartate aminotransferase (AST), bicarbonate, calcium, chloride, cholesterol, creatine kinase (CK), glutamate dehydrogenase (GDH), globulin, glucose, phosphorus, potassium, sodium, total protein, uric acid, and hemolysis index.

The cloacal swab was incubated in selenite broth (Media Services, University of California, Davis, CA) for 18–24 h at 35°C (95°F). At that time it was plated on xylose lysine tergitol-4 (XLT4; Hardy Diagnostics, Santa Maria, CA) and Hektoen enteric (HE) agar (Hardy Diagnostics). Results were interpreted as suspicious for *Salmonella* spp. if they exhibited black-colored colonies on XLT4, HE agar, or both, which is indicative of hydrogen sulfide production. Hydrogen sulfide-producing colonies were subcultured to blood agar for further biochemical testing to confirm their identification as *Salmonella* spp. using spot indole and oxidase tests, triple sugar iron agar, Christensen's urea agar, Simmon's citrate agar, sulfure-indole-motility

semisolid agar, ortho-Nitrophenyl-β-galactoside test, and lysine decarboxylase agar. Biochemical testing consistent with *Salmonella enterica arizonae* was further tested using an API 20E test strip (bioMerieux, Durham, NC).

Statistical analysis: Results from hematology and plasma biochemistry panel were analyzed using descriptive statistics. An exact Wilcoxon–Mann-Whitney test was used to compare the distribution of each value between the two populations described and between sexes within the free-ranging population. Differences between males and females were not evaluated for the captive population because of the small sample size. An exact Pearson chi-square test was used to compare the percentage of males and females in each population (three individuals deemed as juveniles were removed from the analysis). Commercially available software (StatXact, Cytel Software Corporation, Cambridge, MA) was used for the analysis. Statistical significance for all comparisons was set at $P < 0.05$.

Results

Of the 30 animals sampled, 15 (50%) were males, 12 (40%) were females, and 3 (10%) were deemed to be juveniles of unknown sex. At the captive site (zoo) 4/10 (40%) were males, 5/10 (50%) were females, and 1/10 (10%) was a juvenile. At the free-ranging site (Putah Creek) 11/20 (55%) were males, 7/20 (35%) were females, and 2/20 (10%) were juveniles. The mean body weight for all animals sampled was 676.7 g (median: 724.5 g, range: 95.4–1,115 g). The mean SCLs and CCLs were 15.6 cm (median: 16.45 cm, range: 8.2–19.5 cm) and 17.0 cm (median: 17.8 cm, range: 8.7–21.2 cm), respectively. The average SCWs and CCWs were 12.5 cm (median: 13 cm, range: 6.8–16.08 cm) and 16.02 cm (median: 16.9 cm, range: 8.2–19.6 cm), respectively. Within the free-ranging population, the morphometric data is presented in Table 1. There were no significant differences observed in the morphometric measurements between the two populations ($P = 0.21$–0.45); however, there were significant differences between free-ranging male and female morphometric measurements for SCW ($P = 0.04$), SCL ($P = 0.036$), and weight ($P = 0.04$). There was no significant difference between the percentage of male and female animals ($P = 0.68$).

All animals sampled were considered healthy based upon physical examination. Incidental abnormal physical examination findings included a healed carapacial fracture ($n = 1$); scute abnormalities including supernumerary scutes, split scutes, or asymmetric scutes ($n = 4$); missing tail tip ($n = 3$); missing claws ($n = 3$); excessive algae build-up on shell ($n = 2$); radiotransmitter in place ($n = 1$) or epoxy on carapace where a radiotransmitter had been ($n = 2$); scars or hyperemic lesions on the ventral neck ($n = 3$); bruised plastron ($n = 1$); and an abnormally rotated forelimb ($n = 1$). None of these required treatment or hospitalization.

The results of hematologic analysis for the free-ranging and captive populations are presented in Table 2. Values that were significantly different between populations included the absolute numbers of heterophils ($P = 0.001$), azurophils ($P = 0.0001$), eosinophils ($P = 0.0039$), and monocytes ($P = 0.0001$). Total WBC was not significantly different between the two populations but did approach significance ($P = 0.093$). The H:L ratios were significantly different ($P = 0.015$) and were 2.4 and 0.76 in the captive and free-ranging populations, respectively. Thrombocyte counts were estimated as adequate for all turtles. No hemoparasites were observed in any of the blood smears. The results of plasma biochemistry analysis for the two populations are presented in Table 3. Values that were significantly different included albumin ($P < 0.0001$), AST ($P = 0.003$), calcium ($P = 0.001$), GDH ($P = 0.0006$), globulin ($P = 0.0082$), sodium ($P = 0.0014$), total protein ($P = 0.0001$), uric acid ($P = 0.0094$), and hemolysis index ($P = 0.0038$). No *Salmonella* spp. were cultured from any of the turtles sampled.

Within the free-ranging population there was no significant difference between male and female hematologic values. Plasma biochemistry values in male and female individuals within the free-ranging population are presented in Table 4. The values that were significantly different included CK ($P = 0.0012$) and phosphorus ($P = 0.0068$).

Discussion

This is the first study to evaluate the hematologic and plasma biochemical values in the western pond turtle. The small captive population of western pond turtles had

Table 1. Morphometric measurements of free-ranging western pond turtles (*Emmys marmorata*) from Putah Creek, Davis, CA. CCL = curved carapace length, CCW = curved carapace width, SCL = straight carapace length, SCW = straight carapace width. *Denotes a significant difference between male and female turtles.

		Male ($n = 11$)			Female ($n = 7$)		
	Unit	Mean (Median)	SD	Min–max (10th–90th percentile)	Mean (median)	SD	Min–max (10th–90th percentile)
CCL	cm	18.2 (18.6)	2.5	12.2–20.8 (16.1–20.4)	16.0 (15.6)	2.4	12.2–20.3 (14.1–18.4)
CCW	cm	16.9 (17.6)	2.4	11–19.6 (15.5–19.6)	15.7 (15.5)	2.4	11.8 –19.6 (13.42–18.04)
SCL*	cm	16.8 (17.2)	2.2	10.9–19.2 (15.9–18.6)	14.8 (14.7)	2.3	11.1–18.6 (12.72–16.8)
SCW*	cm	13.4 (13.9)	2.1	8.4–16.1 (11.8–15.1)	11.7 (11.4)	1.6	9.2–14.5 (10.4–13.48)
Weight*	grams	768.3 (788)	220.2	213–1091 (654–927)	554.7 (481)	234.3	242–1,007 (371–774.8)

Table 2. Hematology values for captive and free-ranging western pond turtles (*Emmys marmorata*) from northern California. PCV = packed cell volume. *Denotes a significant difference between the two populations.

		Free-ranging population (n = 20)			Captive population (n = 10)		
Parameter	Unit	Mean (median)	SD	Min–max (10th–90th percentile)	Mean (median)	SD	Min–max (10th–90th percentile)
PCV	%	24.98 (25)	3.615	19–32.5 (20.9–31.1)	27.65 (27.25)	3.154	23–33 (23.9–31.65)
White blood cell count	$\mu l \times 10^3$	12.96 (11.9)	4.3	6.8–24 (8.95–20.73)	16.37 (16.35)	5.64	8.2–27 (9.46 –22.5)
Heterophil*	$\mu l \times 10^3$	2.56 (2.41)	1.38	0.66–5.32 (1.13–5.1)	7.58 (7.34)	3.16	3.78–12.76 (4.1–10.85)
Basophil	$\mu l \times 10^3$	2.30 (2.15)	1.33	0.642–6.48 (1.16–4.39)	1.6 (1.24)	1.31	0.150–4.86 (0.63–2.43)
Eosinophil*	$\mu l \times 10^3$	4.1 (3.67)	1.64	1.9–7.92 (2.27–7.27)	2.71 (2.07)	2.18	1.08–8.64 (1.21–3.23)
Lymphocyte	$\mu l \times 10^3$	3.34 (2.72)	3.12	0.11–12.18 (0.6–8.9)	3.11 (1.86)	3.07	0.16–7.83 (0.38–7.59)
Azurophil*	$\mu l \times 10^3$	0.58 (0.50)	0.27	0.21–1.1 (0.291–1.02)	0	0	0
Monocyte*	$\mu l \times 10^3$	0.09 (0)	0.21	0–0.96 (0–0.12)	1.54 (1.11)	1.30	0.15–4.18 (0.48–3.23)

several significant differences in hematologic and plasma biochemistry values when compared to the free-ranging population. Differences have been previously reported between free-ranging and captive populations of turtle species including bog turtles (*Clemmys muhlenbergii*) and Central American river turtles (*Dermatemys mawii*) (Brenner *et al.*, 2002; Rangel-Mendoza *et al.*, 2009). Other studies have shown differences between populations of red-bellied cooters (*Pseudemys rubriventris*) and alligator snapping turtles (*Macrochelys temminckii*) (Innis *et al.*, 2007; Chaffin *et al.*, 2008). Lastly, a previous study in western pond turtles showed differences in limited hematology values in two natural habitats (Polo-Cavia *et al.*, 2010). The findings in previous freshwater turtle studies and the current study indicate that environment, capture methods, and nutrition have a strong impact on several hematologic and biochemical values, and this should be considered when interpreting these values.

Captive turtles in this study had higher heterophil concentrations and H:L ratios than did free-ranging turtles. Furthermore, captive turtles in this study had higher H:L ratios than those previously reported for this species (Polo-Cavia *et al.*, 2010). Heterophils are influenced by season, inflammation, stress, and neoplastic conditions (Wojtaszek, 1993; Campbell, 2005). In a study that evaluated H:L ratios in two different populations of western pond turtles, turtles from heavily polluted waters had higher H:L ratios than did turtles from a non-polluted ecological reserve (Polo-Cavia *et al.*, 2010). Similar studies in other chelonian species have found increased H:L ratios in relation to heavy metal and organophosphate concentrations (Keller *et al.*, 2004; Yu *et al.*, 2011). The method of restraint prior to venipuncture, which left the turtles without the ability to submerge overnight, may have led to the elevated heterophil concentration and H:L ratios in the captive population. The free-ranging population was trapped using nets that allowed them to stay completely submerged, which may have been less stressful. Other factors to consider in the captive group may include suboptimal water quality, dietary differences, chronic stress associated with display, or an inflammatory condition that was not identified on physical examination. The lack of a toxic change within the heterophil population in this study makes differences due to an inflammatory response less likely (Campbell, 2005).

Free-ranging turtles had greater concentrations of eosinophils and azurophils compared to the captive group. Additionally, eosinophil and azurophil concentrations in the free-ranging population were higher than those reported for other species of freshwater turtles sampled during a similar season (Perpiñán *et al.*, 2008; Chung *et al.*, 2009). The cause of the higher concentration of eosinophils may be associated with parasitic infections (Campbell, 2005; Strik *et al.*, 2007). Free-ranging populations are more likely to suffer from parasitism; however, fecal analysis was not included in this study. Monocytosis can be indicative of chronic inflammation, with azurophils being a subset of monocytes that are thought to be activated within the blood (Campbell, 2005; Strik *et al.*, 2007). The reason for the differences between the two populations may be associated with an inflammatory condition of the free-ranging turtles that was not apparent on physical examination. Corticosterone release secondary to restraint and handling has been documented in several species (Cash *et al.*, 1997; Wojtaszek, 1993; Zachariah *et al.*, 2009), which could be responsible for some of the eosinophil and monocyte concentration differences despite a lack of a lymphopenia.

The captive population of turtles had significantly higher albumin, globulin, and total protein concentrations. Measurement of albumin utilizing the bromcresol green dye-binding method has been associated with inaccurate results when compared to protein electrophoresis (Muller and Brunnberg, 2010). The range for total protein seen in the free-ranging population is similar to that reported for freshwater turtles sampled during a similar season (Perpiñán *et al.*, 2008; Chung *et al.*, 2009). Changes in protein have been associated with differences in health, hydration, sex, hormone concentrations, and season (Bolten and Bjorndal, 1992; Selcer and Palmer, 1995; Chung *et al.*, 2009;

Table 3. Plasma biochemistry values for captive and free-ranging western pond turtles (*Emmys marmorata*) from northern California. ALP = alkaline phosphatase, AST = aspartate aminotransferase, Bicarb = bicarbonate, BUN = blood urea nitrogen, CK = creatine kinase, GDH = glutamate dehydrogenase, TP = total protein. *Denotes significant difference observed between the two populations.

		Free-ranging population (n = 20)			Captive population (n = 10)		
Parameter	Unit	Mean (median)	SD	Min–max (10th–90th percentiles)	Mean (median)	SD	Min–max (10th–90th percentiles)
Albumin*	g/dl	1.345 (1.35)	0.398	0.7–2 (0.8–1.81)	2.34 (2.5)	0.46	1.6–2.9 (1.78–2.72)
ALP	IU/L	204.7 (154)	131.4	73–635 (87.3–323.5)	234.3 (211)	75.75	148–390 (163.3–313.5)
Anion gap		12.4 (10.5)	8.84	4–42 (4.9–18)	15.4 (16)	4.222	9–22 (9.9–21.1)
AST*	IU/L	138.3 (86.5)	140	45–616 (54.8–223.3)	301.6 (216)	286	98–1,055 (112.4–510.5)
Bicarb	mmol/L	30.93 (32.5)	7.872	26–38 (27.3–37.1)	32.2 (32.5)	4.158	26–41 (26.9–34.7)
BUN	mg/dl	25.35 (22.5)	16.78	6–63 (7.9–46.6)	37.9 (33)	19.68	15–76 (18.6–60.7)
Calcium*	mg/dl	10.91 (10.5)	2.392	8.7–16.9 (8.79–15.2)	15.08 (12.55)	4.966	10.3–25.2 (11.11–21.87)
Chloride	mmol/L	96.45 (97)	3.145	89–100 (90.9–100)	95.4 (95.5)	4.695	88–104 (89.8–99.5)
Cholesterol	mg/dl	156 (124)	97.58	61–414 (65.9–300.5)	253.8 (206.5)	123.2	119–458 (135.2–389.6)
CK	IU/L	339.8 (234)	317.8	39–1,392 (86.1–638.8)	672.5 (420.5)	704.3	149–2,554 (202.1–1070.8)
GDH*	IU/L	24.25 (6)	55.67	2–240 (2–32.2)	54.3 (42.5)	40.24	12–123 (15.6–107.7)
Globulin*	g/dl	2.37 (2.4)	0.4366	1.7–3.3 (1.7–2.91)	3.09 (2.75)	0.8185	2.2–4.9 (2.38–3.91)
Glucose	mg/dl	68.15 (60)	35.82	28–167 (32.6–97.3)	56.2 (53.5)	20.5	29–103 (38.9–72.4)
Phosphorus	mg/dl	3.165 (3)	0.808	2.1–4.9 (2.38–4.18)	3.72 (3.8)	0.939	2.1–5.2 (2.55–4.93)
Potassium	mmol/L	3.755 (3.7)	0.5671	2.9–5.2 (3.09–4.43)	3.59 (3.7)	0.3635	2.8–4 (3.07–3.82)
Sodium*	mmol/L	136 (135.5)	2.026	132–140 (133.9–138.1)	139.2 (139)	2.394	135–143 (136.8–142.1)
TP*	g/dl	3.725 (3.65)	0.6942	2.6–5 (2.96–4.53)	5.43 (5)	1.137	4.4–7.8 (4.4–6.45)
Uric acid	mg/dl	0.67 (0.6)	0.2364	0.3–1.3 (0.39–0.91)	0.49 (0.5)	0.0994	0.3–0.6 (0.39–0.6)
Hemolysis* index		8.7 (7.5)	5.78	2.0–22 (2.9–17.5)	3.4 (2.5)	2.88	0–8 (0.9–7.1)

Deem *et al.*, 2009), and a nutritional role has been implicated as a cause for elevation in some piscivorous species (Lee *et al.*, 2007; Knotkova *et al.*, 2008; Anderson *et al.*, 2011). Because the captive group sampled here was fasted overnight, the higher protein concentrations may have been a result of dehydration secondary to their overnight holding without the ability to swim and submerge in water. This is further supported by the significantly higher sodium concentrations seen in this population, although one would expect a concomitant increase in chloride concentrations, which was not appreciated. Due to the inaccuracies reported using the bromcresol green dye-binding method, further evaluation of the differences between these two populations is warranted.

The free-ranging population had significantly higher uric acid concentrations with no significant differences in blood urea nitrogen levels. Aquatic turtles are ureotelic and excrete urea as a primary breakdown product of protein catabolism (Campbell, 2005). The uric acid concentrations of the free-ranging turtles in this study are similar or lower to that reported for red-eared sliders and Central American river turtles (Knotkova *et al.*, 2008; Rangel-Mendoza *et al.*, 2009). Thus, despite a difference between the two populations, there may be no clinical significance of these findings.

The captive population in this study had higher concentrations of AST and GDH in comparison to the free-ranging group. Elevations in AST have been associated with hepatocellular damage from septicemia, toxemia, excessive glucocorticoid production (stress), post prandial status, hepatic lipidosis, and hepatitis (Wojtaszek, 1993; Divers, 2000; Campbell, 2005; Hernandez-Divers, 2005; Anderson *et al.*, 2011). In general, synchronous evaluation of CK allows assessment of whether elevations are from muscle or liver tissue (Campbell, 2005). In this study there was no difference in CK concentrations and the captive group had been fasted overnight; therefore, the elevation in AST was most likely from liver tissue. Glutamate dehydrogenase is not well studied in reptiles; however, elevations have

Table 4. Plasma biochemistry values for male and female western pond turtles (*Emmys marmorata*) from northern California. ALP = alkaline phosphatase, AST = aspartate aminotransferase, Bicarb = bicarbonate, BUN = blood urea nitrogen, CK = creatine kinase, GDH = glutamate dehydrogenase, TP = total protein. *Denotes a difference between the two populations.

		Male (n = 11)			Female (n = 7)		
Parameter	Unit	Mean (median)	SD	Min–max (10th–90th percentiles)	Mean (median)	SD	Min–max (10th–90th percentiles)
Albumin	g/dl	1.25 (1.2)	0.44	0.7–1.9 (0.7–1.8)	1.41 (1.5)	0.3	0.9–1.7 (1.08–1.7)
ALP	IU/L	169 (141)	85012	81–319 (88–294)	176.4 (157)	65.32	73–268 (118.6–253)
Anion gap		12.45 (10)	10.38	4–42 (6–16)	12.71 (11)	8.01	4–27 (4.6–21)
AST	IU/L	117 (81)	110.6	45–424 (53–201)	175.4 (99)	197.9	62–616 (67.4–351.4)
Bicarb	mmol/L	30.69 (33)	9.70	2.6–38 (30–37)	31.29 (31)	6.16	21–38 (25.2–37.4)
BUN	mg/dl	23.91 (20)	17.77	7–63 (8–45)	22 (22)	11.59	6–42 (11.4–34.8)
Calcium	mg/dl	9.70 (9.5)	0.95	8.7–11.3 (8.7–10.9)	12.77 (10.9)	3.13	9.5–16.9 (9.8–16.4)
Chloride	mmol/L	96.91 (97)	2.8	90–100 (95–100)	95.57 (97)	4.07	89–100 (90.2–99.4)
Cholesterol	mg/dl	128.8 (113)	67.44	65–298 (66–175)	144.7 (129)	87.05	61–323 (68.2–228.8)
CK*	IU/L	478.3 (420)	361.9	87–1392 (227–799)	116.9 (107)	52.52	39–187 (62.4–172)
GDH	IU/L	29.09 (6)	70.3	2–240 (2–24)	21.14 (4)	37.93	2–106 (2.6–54.4)
Globulin	g/dl	2.45 (2.4)	0.37	1.7–3 (2–2.9)	2.36 (2.4)	0.54	1.7–3.3 (1.82–2.94)
Glucose	mg/dl	54.18 (49)	22.56	28–94 (29–88)	78.71 (70)	45.57	33–167 (34.8–123.2)
Phosphorus*	mg/dl	2.72 (2.6)	0.47	2.1–3.8 (2.2–3)	3.71 (3.6)	0.91	2.7–4.9 (2.76–4.9)
Potassium	mmol/L	3.67 (3.7)	0.60	3–5.2 (3.1–4.0)	3.8 (3.8)	0.58	2.9–4.7 (3.2–4.4)
Sodium	mmol/L	136.4 (136)	2.11	133–140 (135–139)	135.7 (136)	2.22	132–138 (133.2–138)
TP	g/dl	3.7 (3.5)	0.71	2.6–4.8 (3–4.5)	3.8 (3.7)	0.72	2.6–5 (3.2–4.5)
Uric acid	mg/dl	0.7 (0.6)	0.28	0.3–1.3 (0.4–1)	0.64 (0.6)	0.21	0.3–0.9 (0.42–0.84)
Hemolysis index		8.73 (8)	3.64	4–17 (6–12)	7 (4)	7	2–22 (2–14)

been associated with hepatic lipidosis (Divers, 2000; Hernandez-Divers, 2005). It is possible that the diet fed to the captive animals caused a mild hepatic lipidosis. Free-ranging western pond turtles are considered omnivorous and have a more varied diet than just the exclusive capelin diet fed to the captive group (Bury, 1986). The captive diet may have also led to a thiamine deficiency due to excess thiaminase. Low level, chronic myopathy and neuropathy have been reported in humans with thiamine deficiencies (Sato *et al.*, 2000; Koike *et al.*, 2006), which could cause elevations in AST and GDH values. Further thiamine analysis for this population would be required to determine if this was a potential cause of increased AST and GDH concentrations.

There are limited studies dedicated to dietary manipulations in chelonian species (Zhou *et al.*, 2003; Zhou *et al.*, 2004; Fledelius *et al.*, 2005). Additionally, there are only a few reports on the effect of malnutrition on clinicopathologic parameters in chelonian species (Samour *et al.*, 1986; Christopher, 1999; Zhou *et al.*, 2003; Tavares-Dias *et al.*, 2009). For example, there is a lack of information on the long-term effects of over-supplementation of protein and fat, as seen in the captive population of turtles. Further studies are needed to elucidate the complex relationships between nutrients fed and common hematologic and biochemistry parameters in chelonian species.

Free-ranging male turtles in this study had higher CK values than did females. Creatine kinase is primarily an enzyme released during muscle damage and can be associated with handling, exertion, or traumatic venipuncture (Campbell, 2005). Free-ranging male turtles were larger than females, and this increased muscle mass may lead to larger CK concentrations as seen in humans (Wolf *et al.*, 2012). Although behavioral observations were not a part of this study, it is possible that males exhibited more exertional behavior during restraint or in the traps, causing elevation of CK. Free-ranging female turtles in this study had higher phosphorus concentrations in comparison to their male counterparts. The elevated phosphorus concentration in females is likely secondary to folliculogenesis and not to

in vitro hemolysis, based upon the lack of a difference in hemolysis indices (Campbell, 2005; Chaffin *et al.*, 2008).

The dorsal coccygeal vein was used to collect blood samples from all turtles in this study. Gross lymph contamination was observed in three turtles, and repeat venipuncture was successful in these three turtles without gross lymph contamination. Venipuncture sites in chelonian species include the jugular vein, occipital sinus, subcarapacial sinus, brachial plexus, femoral vein, and the dorsal coccygeal vein (Campbell, 2005; Martinez-Silvestre *et al.*, 2002). Several studies in other species have evaluated the likelihood of lymph contamination or differences in hematologic and biochemical values at several venipuncture sites (Gottdenker and Jacobson, 1995; Werner and Linley, 2005; Perpiñán et al., 2010). One study has shown that the dorsal coccygeal vein has higher occurrences of lymph contamination than other sites in the Diamondback terrapin (*Malaclemys terrapin*) (Werner and Linley, 2005). In the authors' experience with western pond turtles, the dorsal coccygeal vein allows for easy access with minimal restraint required. Although no gross lymph contamination was present in most samples, possible contamination cannot be eliminated.

Lithium heparin is commonly cited as the anticoagulant of choice for use with blood samples from reptiles, especially chelonians (Muro *et al.*, 1998; Campbell, 2005). However, K_2EDTA has been described as a superior anticoagulant for hematology when not associated with hemolysis (Muro *et al.*, 1998; Campbell, 2005; Martinez-Jimenez *et al.*, 2007). Our findings indicate that western pond turtles do not exhibit *in vitro* cell lysis in K_2EDTA and that this anticoagulant may be utilized for future hematologic sampling in this species.

Cloacal *Salmonella* spp. cultures were pursued to allow for a further health assessment; however, other diagnostics such as fecal analysis, plasma protein electrophoresis, micronutrient concentrations, concentrations of heavy metals, pesticides, testosterone, and corticosterone concentrations were not pursued as they had been done in other studies in chelonians (Bolten and Bjorndal, 1992; Selcer and Palmer, 1995; Chaffin *et al.*, 2008). These additional diagnostics may have allowed for further evaluation of the health of the animals examined here, and the lack of these diagnostics may represent a limitation to this study.

In conclusion, the free-ranging and captive populations examined here had several differences in hematologic and plasma biochemistry values. The captive population had biochemistry values suggestive of dehydration and low-grade hepatopathy, which we postulate is due to the diet fed and the method of overnight restraint. The values for hematologic and plasma biochemistry parameters presented in this research can be used to assess individual wild or captive western pond turtles but should be interpreted with other variables such as anticoagulant used, laboratory technique, season, sex, postprandial status, diet, environmental factors, and venipuncture sites.

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