Vol. 107: 37–47, 2013
doi: 10.3354/dao02663

DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org

Published November 25

Prevalence of selected pathogens in western pond turtles and sympatric introduced red-eared sliders in California, USA

C. Silbernagel[1,5,*], D. L. Clifford[2,3], J. Bettaso[4], S. Worth[1], J. Foley[1]

[1]Department of Medicine and Epidemiology and [3]Wildlife Health Center, One Health Institute University of California, Davis School of Veterinary Medicine, Davis, California, 95616, USA

[2]Wildlife Investigations Lab, California Department of Fish and Wildlife, Rancho Cordova, California 95670, USA

[4]United States Fish and Wildlife Service, East Lansing, Michigan 48823, USA

[5]*Present address:* Hubbs-SeaWorld Research Institute, San Diego, California 92109, USA

ABSTRACT: Pathogen introduction by invasive species has been speculated to be a cause of declining western pond turtle *Emys marmorata* populations in California, USA. This study determined the prevalence of *Ranavirus* spp., *Herpesvirus* spp., *Mycoplasma* spp. (via polymerase chain reaction of blood and nasal flush contents), and *Salmonella* spp. infection (via fecal culture) in native *E. marmorata* and invasive red-eared sliders *Trachemys scripta elegans* and compared infection prevalence in *E. marmorata* populations sympatric with *T. scripta elegans* to *E. marmorata* populations that were not sympatric by sampling 145 *E. marmorata* and 33 *T. scripta elegans* at 10 study sites throughout California. *Mycoplasma* spp. were detected in both species: prevalence in *E. marmorata* was 7.8% in the northern, 9.8% in the central, and 23.3% in the southern California regions. In *T. scripta elegans, Mycoplasma* spp. were not detected in the northern California region but were detected at 4.5 and 14.3% in the central and southern regions, respectively. All turtles tested negative for *Herpesvirus* spp. and *Ranavirus* spp. Enteric bacteria but not *Salmonella* spp. were isolated from feces. *E. marmorata* populations that were sympatric with *T. scripta elegans* did not have increased risk of *Mycoplasma* spp. infection. For *E. marmorata*, there was a significant association between *Mycoplasma* spp. infection and lower body weight and being located in the southern California region. This study is the first of its kind to document pathogen prevalence in native *E. marmorata* habitats and those sympatric with *T. scripta elegans* in California.

KEY WORDS: Chelonian · Turtle · Pathogen · Introduced species · *Herpesvirus* · *Ranavirus* · *Mycoplasma* · *Salmonella*

INTRODUCTION

Identified as a species of special concern by the California Department of Fish and Wildlife since 1994, the western pond turtle *Emys marmorata* is the only native species of freshwater turtle in California, USA (Jennings & Hayes 1994), its range extending from southern Canada to Baja California, Mexico. Although the last statewide survey was conducted over a decade ago (Wright et al. 2008) and *E. marmorata* population numbers are presently uncertain, declining populations have been documented since the 1980s. Large declines have occurred in *E. marmorata* in southern California and fewer declines in

*Email: csilbernagel@hswri.org

the northern portion of the state (Lovich 1998). Possible causes for declining *E. marmorata* populations include urbanization and habitat destruction with few basking and nesting sites available near pond habitat, poor water quality, and age senescence with poor juvenile recruitment (Rathbun et al. 1992, Lindeman 1999, Spinks et al. 2003, Bury & Germano 2008). Many reptiles, including chelonians, have long life spans and delayed sexual maturity, and effects of habitat alteration or toxins on juvenile recruitment may take generations to realize at the population level (Rowe 2008).

The red-eared slider *Trachemys scripta elegans* is a common introduced turtle in California. This species was imported for the live food market and is popular in the pet industry, often resulting in illegal pet release. *T. scripta elegans* are larger than *Emys marmorata* and can successfully displace them when competing for basking sites, resulting in increased chance of predation for *E. marmorata* (Lindeman 1999). Competition between the 2 species for resources has been further supported in experiments in which European pond turtles *E. orbicularis* experienced weight loss and higher rates of mortality when housed with *T. scripta elegans* than when housed alone (Cadi & Joly 2004). In addition, platyhelminth parasite transmission has been documented from *T. scripta elegans* to captive populations of indigenous *E. orbicularis* in France (Verneau et al. 2011), demonstrating pathogen introduction as a potential threat to native populations from introduced species.

Many pathogens affect wild chelonian populations, some with important impacts on survival; for example, herpesviruses have been documented to cause stomatitis and pneumonia in wild chelonians (Frye et al. 1977, Pettan-Brewer et al. 1996). *Mycoplasma agassizii* is a bacterial pathogen that affects reptiles with clinical lesions of mucopurulent discharge and/or ocular edema (Feldman et al. 2006, Farkas & Gal 2009) and has been confirmed as an upper respiratory pathogen causing disease in the desert tortoise *Gopherus agassizii* (Brown et al. 1994), and major population declines of this species in the Mojave Desert, California, have been implicated with the appearance of upper respiratory tract disease (Rosskopf et al. 1981). Ranaviruses are known to cause mortality in frogs and salamanders with suspected cross-infection to other species (Galli et al. 2006, Ritchie 2006, Johnson et al. 2008). Experimental transmission was achieved utilizing a star tortoise (*Geochelone elegans*) ranavirus isolate that was successfully inoculated into *Trachemys scripta elegans,* and clinical sequelae included lethargy, ocular discharge, and oral plaques (Johnson et al. 2007). Chelonians are also affected by *Salmonella* spp. Reptiles frequently act as reservoirs for the bacteria, which have been linked to outbreak clusters of *S. typhimurium* in children with chelonians as pets (CDC 2010). Although reptile infection rates with *Salmonella* spp. can be high and often subclinical, infection with particularly virulent strains can cause septicemia, pneumonia, and mortality in some chelonians (Johnson-Delaney 2006).

Introduced species may bring new pathogens or virulent strains of pathogens to naïve populations of *Emys marmorata*, but few *E. marmorata* studies have investigated infectious pathogen introduction as a risk factor for species decline. It has been suggested that an introduced turtle species, such as *Trachemys scripta elegans*, may have been responsible for transmission of an unconfirmed upper respiratory pathogen that caused mortality of at least 36 *E. marmorata* in a Washington population in 1990 (Hays et al. 1999), a state where populations of *E. marmorata* are currently state listed as Endangered. Furthermore, in 2006, a die-off of *T. scripta elegans* occurred in Folsom Lake, California. Although the cause of the mortalities was not determined, the event raised concerns about potential pathogen spread to native *E. marmorata* at this lake (S. Torres pers. comm.).

The goals of this study were to determine the prevalence of active *Ranavirus* spp., *Herpesvirus* spp., *Mycoplasma* spp., and *Salmonella* spp. infection in *Emys marmorata* and *Trachemys scripta elegans* and to evaluate our hypothesis that infection prevalence is higher in *E. marmorata* populations sympatric with invasive *T. scripta elegans* compared to those that are not sympatric with *T. scripta elegans*. Additionally, we sought to identify risk factors for infection in individual turtles, including species, age, gender, gravidity status, weight, and region. Water quality parameters were compared for differences among regions.

MATERIALS AND METHODS

Field sampling

Emys marmorata and *Trachemys scripta elegans* were sampled from February through September 2011 at 10 pond sites distributed in northern (n = 3), central (n = 3), and southern (n = 4) California (Fig. 1). For each region, study sites were chosen to include at least one pond with sympatric *E. marmorata* and *T. scripta elegans* and one pond that con-

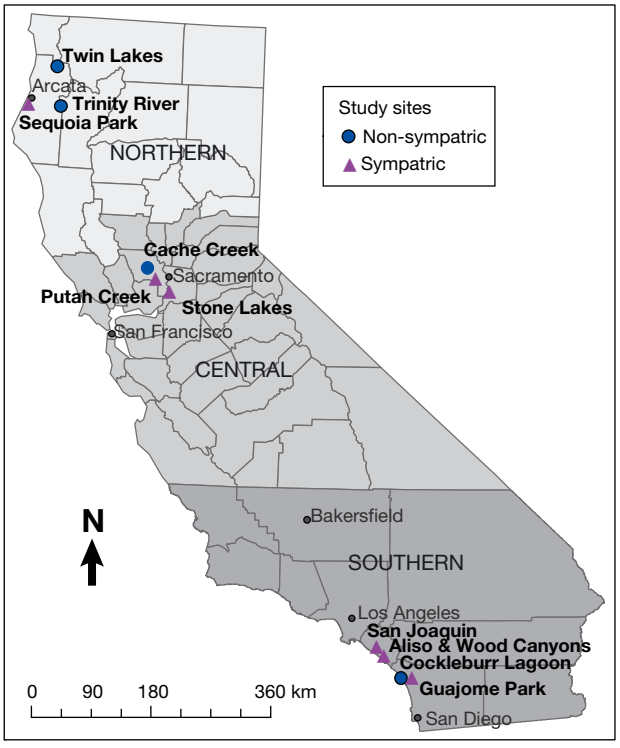

Fig. 1. California, USA, study sites where trapping of *Emys marmorata* and *Trachemys scripta elegans* was performed from February through September 2011. *E. marmorata*-only sites are represented as circles and sympatric sites as triangles. Each sampling region is depicted by varying shades of grey

tained only *E. marmorata*. Inclusion criteria for sympatric ponds were the availability of an experienced ranger or researcher who was familiar with the site and aware of both *E. marmorata* and *T. scripta elegans* presence within the past year. Similarly, for sites where *E. marmorata* was considered the sole chelonian utilizing the pond, an experienced ranger or researcher verified that there was no known history of another species of chelonian in the pond within the past year. A target sample size of 20 turtles per species per site was chosen, so that there would be a 95% probability of detecting a pathogen with a prevalence in the population of 10% or greater (Epi Info StatCalc ver. 3.5.1, CDC). Multiple sampling attempts were made at the study sites in an effort to achieve target sample sizes, but when not achieved after several attempts, additional locations were sought for sampling. For ponds in which sympatric populations were present, achievement of *E. marmorata* target sample sizes was the aim, as this was the species of interest for this study. Recaptured individuals were not re-sampled.

Turtles were caught using a combination of nylon dip traps, hand nets, and snorkeling surveys. Each turtle sampled was uniquely numbered by filing of the marginal scutes. Physical examinations were performed on each individual, and a body condition score (BCS) from 1 to 4 was assigned. A score of 1 (emaciated) was assigned if the carapace or plastron was severely sunken or broken, or if palpated extremities lacked muscle; a score of 2 (poor) was assigned if the shell demonstrated moderate depressions or cracks or if there was minimal muscle palpated; a score of 3 (average) was assigned if the shell contained few mild focal depressions or a moderate amount of muscle palpated; and a score of 4 (good) was assigned if the animal had a large amount of muscle with no associated shell depressions. In addition to obtaining weights for risk factor analysis, we measured maximum and minimum carapace and plastron lengths to estimate age category. Captured individuals were classified as adult or juvenile based on *Emys marmorata* sexual maturity carapace length of 110 mm (Holland 1991a) and *Trachemys scripta elegans* sexual maturity plastron length of 100 mm in males and 150 mm in females (Gibbons & Lovich 1990, Utah Division of Wildlife Resources 2010). An average cutoff of 125 mm plastron length was used for determining sexual maturity in *T. scripta elegans* when gender was unknown, or when measurements were just below the cutoff value and annuli suggested adult status. Secondary sex characteristics of tail and nail lengths and plastron and carapace curvature were used to determine gender (Readel et al. 2008, Vega & Stayton 2011), with gravidity of females assessed via palpation.

Blood samples were collected from the subcarapacial sinus using a 22-gauge needle and tuberculin syringe. Approximately 0.5 ml of blood was obtained from adult turtles weighing ≥250 g, and no more than 3 ml kg^{-1} of body weight was obtained from turtles weighing <250 g (McArthur et al. 2004). Blood was transferred to lithium heparin tubes and chilled on blue ice for transport to the laboratory. To obtain a nasal flush, 0.5 ml of sterile saline were gently flushed and aspirated from the nares using a 1.5 ml fine-tip extended transfer pipette (Samco Scientific) with the tip advanced approximately 5 mm into the nasal cavity. Nasal flush samples were transferred to microcentrifuge tubes and cooled on ice for transport back to the laboratory, with long-term storage of samples at −20°C until laboratory analysis. Cloacal swabs were obtained from each turtle using sterile cotton-tipped applicators (Kendall). Sterile, saline moistened applicators were advanced approximately

1 cm into the cloacal cavity, rolled gently, and removed. The applicator tip was broken off into a tube containing selenite enrichment media, and samples were kept cool during transport to the laboratory.

The location of each sampling site (in UTM, NAD 83) was recorded using a handheld GPS unit (Garmin). Water samples were collected from each study site during at least one visit, with the exception of the Stone Lakes study site (the water quality probe was unavailable during that sampling period). Water samples were tested for dissolved oxygen, pH, conductivity, total dissolved solids, and salinity using a dissolved oxygen probe kit (Global Water Instruments).

DNA extraction and PCR

For active viremia detection of *Ranavirus* spp., DNA was extracted from blood contents per the Qiagen DNeasy™ blood spin-column extraction method using the nucleated protocol. DNA was analyzed using the TaqMan real-time PCR assay as described by Pallister et al. (2007). Each reaction consisted of 12 µl of TaqMan Universal PCR Master Mix (Applied Biosystems), 1.16 µl of CON primer/probe mix, 2 µl of template DNA, and 9.84 µl of water to achieve a final volume of 25 µl. Results were considered positive if the cycle threshold was <40 and there was a characteristic amplification curve. The *Ranavirus* sp. positive control was frog virus 3 in culture.

DNA was extracted from nasal flush contents per the Qiagen DNeasy™ spin-column extraction method utilizing the non-nucleated protocol and evaluated for *Mycoplasma* spp. and *Herpesvirus* spp. For active detection of *Mycoplasma* spp., conventional PCR methodologies were modified from the protocol described by van Kuppeveld et al. (1992). PCR amplification was performed using a total reaction volume of 25 µl containing 1 µl of template DNA, 2.5 µl each of RNA5 and MGSO primers, 12.5 µl of GoTaq Master Mix (Promega), and 6.5 µl of water. Thermocycler conditions included initial denaturation at 94°C for 4 min, 35 cycles at 94°C for 1 min, annealing of primers at 60°C for 1 min, and extension of primers at 72°C for 2 min. Analysis was performed via electrophoresis on a 1% agarose gel stained with GelStar (Lonza) and examined under UV transillumination. Cultured and sequence-confirmed feline-origin *Mycoplasma* sp. was utilized as a positive control. In order to verify the identification of PCR amplicons, 3 positive samples were randomly selected for DNA sequencing. Samples were tested by PCR as described above, and the products were then resolved on a 1% agarose gel. Products were purified from the gel using a kit (Qiagen) and then submitted for DNA sequencing at a commercial facility (Davis Sequencing, Davis, CA) using the forward PCR primer. Electropherograms were trimmed by eye to remove end-reading errors, and the DNA sequence was then compared to those in the GenBank database using the BLAST algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

For detection of active *Herpesvirus* spp., conventional nested PCR was performed by modifying the protocol as described by Kleiboeker et al. (2002). In the initial round, amplification was achieved using 25 µl total reaction volume including 1 µl of template DNA, 2.5 µl each of DFA, ILK, and KG1 primers, 12.5 µl of GoTaq Master Mix, and 4 µl of water. Thermocycler conditions consisted of 95°C for 12 min, 10 cycles of denaturation at 94°C for 30 s, annealing at 70°C for 30 s, and extension at 72°C for 90 s. Additionally, 40 cycles of denaturation were performed at 94°C for 30 s, with annealing at 48°C for 30 s, and final extension at 72°C for 7 min. In the second round, a total reaction volume of 25 µl was achieved using 2 µl of the first round reaction, 2.5 µl each of TGV and IYG primers, 12.5 µl of GoTaq Master Mix, and 5.5 µl of water. Thermocycler conditions were as described in the initial round. Analysis was performed on a 1% agarose gel stained with Gelstar (Lonza) and examined under UV transillumination. Cultured and sequence-confirmed feline origin *Herpesvirus* sp. was utilized as a positive control.

Salmonella isolation

Cloacal swabs were enriched in selenite and incubated at 37°C for 24 h (WHO 2003). Swabs were then discarded, and the enriched broth was sub-inoculated onto XLT agar plate media (Becton, Dickinson and Co.). Plates were incubated at 37°C for 48 h and then examined for black colony growth. Black colonies were further isolated via sub-inoculation onto XLT plate media at 37°C for 48 h. Isolated colonies were evaluated on triple sugar iron agar slants, and colonies with results consistent with *Salmonella* spp. (black, hydrogen sulfide reaction) were frozen at −20°C. One isolate from each site that had biochemical assay results consistent with *Salmonella* spp. was confirmed and phage-typed at the Microbiology Laboratory at the UC Davis Veterinary Medical Teaching Hospital. Limited project resources precluded phage typing of all positive samples.

Analysis

Data were entered into Microsoft Excel, and statistical analysis was performed in Stata (StataCorp 12.0). For all tests, a cutoff of $p < 0.05$ was used to infer statistical significance. Sex ratios for *Emys marmorata* were assessed with a chi-squared goodness-of-fit test, assuming an even sex distribution of 1:1. Differences in demographic values and weight across sites were assessed as follows: for age (juvenile and adult) and gender (male and female) we used chi-squared contingency tests, for BCS we used a Kruskal-Wallis test, and for weight we used analysis of variance (ANOVA).

The prevalence of infection (proportion of positive animals in the sample) and associated 95% confidence intervals (CI) for each pathogen were calculated for each species at each sampling site. For each pathogen, potential associations between infection and the following variables were evaluated using univariate logistic regressions: site, gender, age class, species, BCS, weight, and region. Univariate logistic regression was used to assess whether infection status in *Emys marmorata* was associated with the presence of *Trachemys scripta elegans* at a site. Odds ratios and 95% exact binomial confidence intervals for the various risk factors were estimated (Mehta et al. 1985).

One-way ANOVAs were used to determine whether weight differed between pathogen-positive and pathogen-negative *Emys marmorata*, grouped by region and separately by site, with statistical significance further evaluated using post hoc Bonferroni testing. A multiple logistic regression model including weight, region, and interaction was created for *E. marmorata Mycoplasma* spp. infection status based on significant univariate results. The best-fitting model was selected using likelihood ratio tests. Mean water pH, dissolved oxygen, salinity, total dissolved solids, and conductivity were compared among study sites using a 1-way ANOVA.

RESULTS

In total, 145 *Emys marmorata* and 33 *Trachemys scripta elegans* were captured, including 51 *E. marmorata* and 4 *T. scripta elegans* in northern California, 51 *E. marmorata* and 22 *T. scripta elegans* in central California, and 43 *E. marmorata* and 7 *T. scripta elegans* in southern California (Table 1). Four non-sympatric and 6 sympatric sites were sampled throughout the state of California, with sites depicted by region in Fig. 1. It was difficult to find sympatric sampling sites in northern California, resulting in a low capture count of 4 *T. scripta elegans* in this region. Southern California sites also had low capture counts of this species, but most populations sampled in this region were small in size, and multiple sampling efforts were required to achieve similar capture counts when compared with northern and central California sampling.

Table 1. *Emys marmorata* and *Trachemys scripta elegans*. Sample sizes, age ratio, sex ratio, number of gravid females, mean weight and median body condition score (BCS) for turtles captured in 2011 at 10 sampling sites in 3 regions of California, USA. Juv: juvenile; M: male; F: female; Unk: unknown; NR: not reported; NA: not applicable. Data without ±SE had only 1 value obtained at the location for the given species

Region and site	*Emys marmorata*						*Trachemys scripta elegans*					
	n	Age (Adult: Juv)	Gender (M:F: Unk)	Gravid	Mean weight ± SE (g)	Median BCS	n	Age (Adult: Juv)	Gender (M:F: Unk)	Gravid	Mean weight ± SE (g)	Median BCS
Northern												
Sequoia Park	8	7:1	6:1:1	0	527.75 ± 61.07	2	4	3:1	0:3:1	0	380.75 ± 73.49	3
Trinity River	22	18:4	9:8:5	1	466.52 ± 52.83	3						
Twin Lakes	21	21:0	10:11:0	2	831.67 ± 32.15	3						
Total	51	46:5	25:20:6	3	626.48 ± 36.82	3	4	3:1	0:3:1	0	380.75 ± 73.49	3
Central												
Cache Creek	21	18:3	9:8:4	2	517.81 ± 50.76	3						
Putah Creek	21	21:0	14:4:3	0	694.56 ± 45.57	3	21	18:3	1:17:3	1	993.76 ± 109.22	3
Stone Lakes	9	7:2	2:5:2	0	615.63 ± 77.70	2	1	1:0	1:0:0	0	495.0	NR
Total	51	46:5	25:17:9	2	602.15 ± 33.04	3	22	19:3	2:17:3	1	971.09 ± 106.58	3
Southern												
Aliso Woods	15	12:3	11:2:2	0	324.19 ± 39.95	3	2	2:0	1:1:0	0	893.35 ± 106.65	3.5
Cockleburr Lagoon	21	15:6	13:8:0	2	227.76 ± 19.22	3						
Guajome Park	1	1:0	1:0:0	0	558.0	NA	5	3:2	3:2:0	0	570.58 ± 226.35	3
San Joaquin Marsh	6	6:0	4:2:0	2	423.83 ± 63.12	3	0					
Total	43	34:9	29:12:2	4	296.44 ± 22.15	3	7	5:2	4:3:0	0	662.80 ± 168.77	3

Roughly 1.6 times as many male *Emys marmorata* were captured than females, although this was not statistically significant (p = 0.08). Differences in sex ratio across sites were also not significant (p = 0.15), although there was a remarkable 14:4 ratio of *E. marmorata* males to females at Putah Creek (Table 1). At all sites (Fig. 1), adults were the primary age category sampled, with few females confirmed to be gravid via palpation (Table 1). Each site was sampled during the breeding season, with the exception of Stone Lakes. Age class did not differ statistically among sites (p = 0.06). BCS did not differ significantly among sites or region (p = 0.4), although southern California turtles had significantly lower weights on average (p < 0.01), most notably at Cockleburr Lagoon and at Aliso and Wood Canyons Wilderness Park, regardless of whether weight was compared by region or site. Most turtles appeared healthy at all sites; a few *E. marmorata* had missing digits, tail injuries, or superficial and deep carapace and plastron erosions, but no purulent nasal discharge or breathing difficulties were observed.

Infections with *Ranavirus* spp. and *Herpesvirus* spp. were not detected in any of the sampled turtles (Table 2). The prevalence of *Mycoplasma* spp. infection in *Emys marmorata* was 7.8% in northern, 9.8% in central, and 23.3% in southern California. *Mycoplasma* spp. infection was not detected in *Trachemys scripta elegans* from northern California but was detected in 4.5 and 14.3% of *T. scripta elegans* sampled from central and southern California, respectively. The subset of *Mycoplasma* spp.-positive PCR samples sequenced demonstrated 100% coverage of the submitted 594 bp fragment with 98% homology to *M. agassizii* (GenBank accession number AF060821.1). *Salmonella* spp. were not specifically isolated from the phage-typed specimens, although *Enterobacteriaceae* (the family that includes *Salmonella* spp.) were detected in *E. marmorata* at a prevalence of 29.4% in northern, 19.6% in central, and 25.6% in southern California. In *T. scripta elegans*, *Enterobacteriaceae* species were detected at a prevalence of 25% in northern, 36.4% in central, and 28.6% in southern California. There were no associations between *Enterobacteriaceae* or *Mycoplasma* spp. infection and age class, gender, or BCS in sampled turtles. Of the gravid females, none tested positive for infection except at Cockleburr Lagoon, where 1 gravid female was positive for *Enterobacteriaceae* and 1 for *Mycoplasma* spp. *E. marmorata* in sympatric populations did not have an increased likelihood of *Mycoplasma* spp. or *Enterobacteriaceae* infection compared with those in isolated populations (p = 0.3).

Table 2. *Mycoplasma* spp. and *Enterobacteriaceae* infecting *Emys marmorata* and *Trachemys scripta elegans*. Pathogen prevalence (%) and 95% confidence intervals (in parentheses) for turtles captured in 2011 at 10 sampling sites in 3 regions of California, USA. NA: not applicable

Region and site	*Emys marmorata*			*Trachemys scripta elegans*			Overall total by region		
	n	*Mycoplasma*	*Enterobacteriaceae*	n	*Mycoplasma*	*Enterobacteriaceae*	n	*Mycoplasma*	*Enterobacteriaceae*
Northern							55	7.3 (0.002, 0.144)	29.1 (0.167, 0.415)
Sequoia Park	8	12.5 (–0.150, 0.400)	25.0 (–0.110, 0.610)	4	0	25.0 (–0.300, 0.800)			
Trinity River	22	9.1 (–0.040, 0.221)	31.2 (0.107, 0.530)						
Twin Lakes	21	4.8 (–0.052, 0.147)	28.6 (0.075, 0.496)						
Total	51	7.8 (0.003, 0.154)	29.4 (0.167, 0.421)	4	0	25.0 (–0.259, 0.759)			
Central							73	8.2 (0.018, 0.147)	24.7 (0.145, 0.348)
Cache Creek	21	14.3 (–0.020, 0.306)	14.3 (–0.020, 0.306)						
Putah Creek	21	4.8 (–0.049, 0.144)	33.3 (0.120, 0.546)	21	4.8 (–0.049, 0.144)	38.1 (0.162, 0.600)			
Stone Lakes	9	11.1 (0.638, 1.140)	0	1	0%	0			
Total	51	9.8 (0.015, 0.181)	19.6 (0.085, 0.307)	22	4.5 (–0.047, 0.138)	36.4 (0.150, 0.577)			
Southern							50	22.0 (0.101, 0.339)	26 (0.134, 0.386)
Aliso Woods	15	13.3 (–0.059, 0.326)	6.7 (–0.075, 0.208)	2	50.0 (–0.560, 1.560)	50.0 (–0.560, 1.560)			
Cockleburr Lagoon	21	33.3 (0.113, 0.553)	33.3 (0.113, 0.553)						
Guajome Park	1	0	0	5	0	20.0 (–0.314, 0.714)			
San Joaquin Marsh	6	16.7 (–0.262, 0.595)	50.0 (–0.075, 1.07)	0	NA	NA			
Total	43	23.3 (0.104, 0.361)	25.6 (0.123, 0.389)	7	14.3 (–0.148, 0.434)	28.6 (–0.090, 0.661)			

ANOVA revealed that *Mycoplasma* spp. infection in *Emys marmorata* was associated with weight; increased *Mycoplasma* spp. prevalence was observed in turtles with lower mean weights in southern California ($p = 0.001$). *Mycoplasma* spp.-infected *E. marmorata* in southern California had an average weight of 184.0 g, compared to their *Mycoplasma* spp.-infected counterparts from northern California (mean = 455.0 g, $p = 0.032$), and central California (mean = 549.2 g, $p = 0.002$). Infection prevalence of *Mycoplasma* spp. was highest, 33.3% (Table 2), at the southern site of Cockleburr Lagoon, where *E. marmorata* had the lowest mean body weight, 227.76 g (Table 1), when compared with all other sites throughout California. Multivariate logistic regression models were created including region, weight, and interactions with the optimal model being 'Myco ~ Region + Weight.' Incorporation of an interaction between region and weight did not improve model fit significantly.

The water at southern California sites had higher mean salinity, total dissolved solids, and conductivity, when compared with water from northern and central California sites ($p = 0.0002$ for each respective factor; Table 3). We observed no differences in pH or dissolved oxygen between regions.

DISCUSSION

Populations of the western pond turtle *Emys marmorata*, once abundant throughout much of California, have declined across its range and face ongoing threats from habitat loss and competition with exotic species (Lovich 1998). Infectious pathogens, potentially transmitted by sympatric non-native turtles, could present a serious threat to the survival of this species in California. We evaluated 4 pathogens that could affect turtle populations and identified *Enterobacteriaceae* and *Mycoplasma* spp. infections in *E. marmorata*-only populations and in *E. marmorata* populations sympatric with the introduced red-eared slider *Trachemys scripta elegans*.

While demographic risk factors were not associated with infection risk, we found a significant association between lower body weight and *Mycoplasma* spp. infection in *Emys marmorata*, particularly in the southern California region. The lower weight could be an environmentally or genetically determined outcome associated with lower resistance to infection or could be due to the infection itself. Infection may be associated with low body weight as well as chronic stressors in the environment, which may adversely affect turtle growth. One approach that could be promising in order to evaluate the quality of the response to the environment is to examine heterophil/lymphocyte ratios and corticosterone levels, which have been used as indicators of environmental stress in *Trachemys scripta elegans.* These blood values have been linked to lower body condition in this species (Readel 2009). In birds as well, elevated heterophil/lymphocyte hematological markers were linked with reduced growth (Moreno et al. 2002) and increased infection susceptibility (Al-Murrani et al. 2002). Future research of *E. marmorata* should target clinical chemistry parameters that may give insight as to size in smaller average body weight *E. marmorata* populations in conjunction with pathogen surveys.

Table 3. Water quality parameters at 10 different study sites in California, USA, in 2011, given as means ± SE. Data without SE had only 1 value obtained at the location. DO: dissolved oxygen; TDS: total dissolved solids; NR: not reported (water quality probe unavailable at sampling)

Region and site	pH	DO (ppm)	Salinity (ppm)	TDS (ppm)	Conductivity ($S\ m^{-1}$)
Northern					
Sequoia Park	7.09	6.51	155	214	0.000303
Trinity River	8.05	5.22	146	212	0.000303
Twin Lakes	6.95	4.92	44.4	63.1	0.0000956
Central					
Cache Creek	8.375 ± 0.013	6.360 ± 0.470	306 ± 8.9	431.0 ± 10.0	0.0006217 ± 0.0000191
Putah Creek	8.373 ± 0.303	6.180 ± 0.871	350 ± 70.8	502.8 ± 104.6	0.0007025 ± 0.0001396
Stone Lakes	NR	NR	NR	NR	NR
Southern					
Aliso Woods	8.447 ± 0.201	7.240 ± 0.229	1616.7 ± 40.6	2270.0 ± 66.6	0.003247 ± 0.0001129
Cockleburr Lagoon	7.69	4.45	3420	4720	0.00670
Guajome Park	7.960 ± 0.280	5.915 ± 0.495	1230.0 ± 30.0	1730.0 ± 10.0	0.002470 ± 0.000040
San Joaquin Marsh	7.66	5.97	3180	4600	0.00662

Elevated *Mycoplasma* spp. prevalence in the southern sites was driven primarily by the influence of 1 sampling location, Cockleburr Lagoon. Cockleburr Lagoon is a ca. 2322 m^2 habitat near the Pacific Ocean, located on Camp Pendleton Marine Corps Base. The western pond turtle faces few threats at this location, with limited human access on the nearby beach. Nevertheless, one-third of the 21 turtles tested at this study site were positive for *Mycoplasma* spp. Other than carapace and plastron erosions, we detected no other signs of disease, such as nasal or ocular discharge or swelling (Brown et al. 1999). Additionally, BCS did not reveal a difference between turtles testing positive and negative for pathogens. However, BCS is a subjective measure and is not necessarily a reliable indicator of immune or health status in chelonians (Polo-Cavia et al. 2010).

In general, southern California water bodies had higher levels of salinity, dissolved solids, and conductivity. These differences may be due to closer proximity of the southern sampling site to the ocean, with coastal upwelling affecting water salinity. Specifically, Cockleburr Lagoon is located within meters of the Pacific Ocean, with mixing of lagoon waters frequently observed in periods of high tide. Despite differing physiological adaptations to salinity stressors in other freshwater animals, higher salinities and low temperatures have been linked with reduced growth in juvenile crayfish (Prymaczok et al. 2012). Increasing salinity also has detrimental effects on freshwater invertebrates and aquatic plants in Australia, either directly through toxic physiological challenge or indirectly through habitat modification (Nielsen et al. 2003). Dunson & Seidel (1986) evaluated salinity tolerance in *Trachemys decussata* inhabiting brackish water and found that hatchling growth was impaired at salinities greater than 41% seawater. Studies in other parts of the USA are underway to determine salinity as a stressor in *T. scripta elegans* (Valverde 2012). Our salinity findings were lower than 41% seawater in the southern California study sites, but given the regional differences in salinity documented in this study and literature linking salinity and impaired growth in other chelonians, we recommend future projects with salinity as a risk factor for *Emys marmorata* growth and infection.

Since captive and wild reptiles, including chelonians, are documented reservoirs for *Salmonella* spp. (Hidalgo-Vila et al. 2007, Scheelings et al. 2011), we expected that this pathogen would be found in some of the turtles examined. *Salmonella* spp. prevalence is of particular concern with regard to zoonotic risk of disease for humans, who may come into contact with pet and wild turtles shedding the bacteria, or with contaminated pond habitats (Hidalgo-Vila et al. 2008). Only non-*Salmonella* spp. bacteria in the family *Enterobacteriaceae* could be identified in this study, which is consistent with normal bacterial flora (McArthur et al. 2004, Jacobson 2007). Project financial limitations did not allow for phage testing to be performed on all positive XLT isolates, which may have limited the information obtained from culture techniques. Cloacal swabs were performed on turtles for fecal collection in this study. Prolonged capture times and cloacal flushing methods may have been necessary to increase fecal yield and subsequently the chances of *Salmonella* spp. detection. Molecular methods of *Salmonella* spp. testing could be more sensitive for detection of *Salmonella* spp. in small samples and should be considered in conjunction with culture techniques for future research (Mainar-Jaime et al. 2008). However, studies of wild populations of North American chelonians have shown that free-ranging aquatic turtles are less likely to harbor *Salmonella* spp. than those in captivity (Mitchell & McAvoy 1990, Hidalgo-Vila et al. 2007), even when molecular methods are utilized in conjunction with traditional culture methods (Saelinger et al. 2006).

It is surprising that *Herpesvirus* spp. were not identified in any of the turtles sampled. Herpesviruses have been well documented in both captive and wild chelonians, including terrestrial and aquatic species (Origgi & Jacobson 2000). Our sampling and testing methods may have limited detection of herpesviruses given that molecular methods targeted only active viral particles in nasal contents. We targeted nasal contents because herpesviruses have been documented to be highly transmissible horizontally from nares exudate, and while the exact route of transmission is unknown in the wild, it is likely that direct contact is the primary route (Muro et al. 1998, Origgi & Jacobson 2000). During disease outbreak in land tortoises with the presence of rhinitis and stomatitis, more herpes-positive animals were identified from pharyngeal swab samples when compared with blood or cloacal swab sampling (Marschang et al. 1997). Viremia, turtles infected with *Herpesvirus* spp. not undergoing viral production (latent infection), or those that may have succumbed to infection were not identified in our study. While ranaviruses have been identified in reptiles, including box turtles *Terrapene ornata* (De Voe et al. 2004, Johnson et al. 2008), they are more commonly reported in amphibians (Hoverman et al. 2012). However, host shifts have been documented across poikilothermic species (Jancovich et al. 2010), and with common habitats among them, these viruses

can be devastating if active disease is detected. A potential limitation of our diagnostics is that pathogen detection was only successful if active viremia was present. Blood was chosen to assess active infection in the absence of clinical signs (Allender et al. 2013). Our methods would fail to detect viral particles in tissues or bodily excretions, and given the scarcity of literature on ranaviruses in chelonians, it may be likely that more advanced or new diagnostic techniques are needed for pathogen detection. Therefore, it is important to continue to monitor and document these ranaviruses and their clinical effects in habitats containing multiple reptilian and amphibian species.

Diminished habitat quality and failure of juvenile recruitment are important causes for declining indigenous *Emys marmorata* populations in the western USA (Spinks et al. 2003, Bury & Germano 2008), but prior work has not been reported examining the risk of pathogen introduction to *E. marmorata* from introduced species such as *Trachemys scripta elegans*. In most populations at our study sites, regardless of sympatric or *E. marmorata*-only pond status, the gender and age distributions of captured *E. marmorata* were disproportionately skewed towards adult males. Seasonal differences in time of sampling across sites as well as nesting behavior of gravid females may have affected the number of female turtles and juveniles captured. Previous studies have documented females leaving the water in the late afternoon or early evening hours to explore nesting areas (Holland 1994, Crump 2001). This would coincide with the timing of trap placement, occurring primarily in the early evening hours. The traps used may have allowed small juveniles to more readily escape, or bait size may not coincide with juvenile feeding behavior, with foraging differences observed among males, females, and juveniles (Bury 1986). Alternatively, the adult male-predominant captures may be indications of unhealthy gender and age distributions. An exception to this pattern was identified at Putah Creek, a sympatric study site, where the predominant turtle was *T. scripta elegans* females even though this site was sampled during the early breeding season. *T. scripta elegans* is almost twice the size of *E. marmorata*, and it therefore may outcompete smaller *E. marmorata* for the few basking resources available at this site (Spinks et al. 2003). Furthermore, basking availability has been correlated with juvenile growth in other chelonian species (Koper & Brooks 2000), and lack of basking sites may affect juvenile survival of *E. marmorata.* Study site population counts and individual turtle basking behaviors were not the focus of this experimental design, but future studies could target these to determine basking behavior and availability as risk factors for infection. Few gravid females were captured, and this may be explained by our sampling period extending past mid-July, the end of the *E. marmorata* nesting season (Scott et al. 2008). Females captured later in the sampling period may have been gravid earlier in the season, or early gestational females may have been missed because of lack of sophisticated diagnostic techniques, i.e. radiography.

Our results represent a small portion of California pond turtle habitats. Extensive, longer-term research is needed to better quantify the risk of pathogens in native and invasive turtle species. Additionally, we tested for only 4 pathogens; other possible pathogens may be harbored in pond habitats. The prevalence of infection specifically in peri-urban areas should also be addressed in additional studies because we demonstrated the highest *Mycoplasma* spp. prevalence in *Emys marmorata* populations of urban southern California, and a subset of our PCR-positive samples were all confirmed to be *M. agassizii*, a species documented to cause upper respiratory tract disease in desert tortoises (Brown et al. 1994, 1999). Further studies are needed to determine infection incidence, onset of clinical signs, if any, and temporal trends, with initial focus on southern California populations of *E. marmorata*.

This study is the first to document pathogen prevalence in California native *Emys marmorata* populations living with and without invasive *Trachemys scripta elegans* and thus provides the first insight into California *E. marmorata* population health, providing a reference to which future outbreak investigations can be compared. Based on our findings, wild native *E. marmorata* populations living in the same habitats as introduced *T. scripta elegans* did not have a higher prevalence of infection with *Ranavirus* spp., *Herpesvirus* spp., *Mycoplasma* spp., or *Salmonella* spp. Despite repeated sampling attempts at pond locations, target sample sizes for western pond turtles were not always achievable, and this may have limited the power of our conclusions. Furthermore, given the diagnostic and sampling methods used, pathogen-positive chelonians that may have succumbed to or recovered from disease would go undetected. Accordingly, given continued concerns about the long-term persistence of *E. marmorata* populations in California, we recommend that any mortality events in *E. marmorata* populations or sympatric *T. scripta elegans* populations be investigated to determine the cause of death and that more extensive pathogen testing be conducted as part of longer-term ecological studies.

Acknowledgements. We thank S. Schuster, B. Nerhus, N. Stephenson, N. Dotson, the Foley family, the UC Davis herpetology students, and S. DiRocco for their assistance with trapping efforts and data collection, and D. Rejmanek, K. Azarvand, J. Truong, and J. Dinstell for their assistance with laboratory work. Additionally, we thank the biologists at USGS San Diego; the Schaffer lab at UC Davis; B. Bolster, California Department of Fish and Wildlife; L. Patterson; the Fish & Wildlife biologists at Stone Lakes National Wildlife Refuge; staff at Sequoia Park in Eureka, the Cache Creek Conservancy, and UC Davis and UC Irvine arboretums; rangers at Guajome Regional Park and Aliso and Wood Canyons Wilderness Park; and R. Sosa of the Camp Pendleton Marine Corps Base for helping to facilitate this project. Funding was provided by the California Department of Fish and Wildlife and a UC Davis Wildlife Health Center Fellowship awarded to C.S.

LITERATURE CITED

Al-Murrani WK, Al-Rawi IK, Raof NM (2002) Genetic resistance to *Salmonella typhimurium* in two lines of chickens selected as resistant and sensitive on the basis of heterophil/lymphocyte ratio. Br Poult Sci 43:501–507

Allender MC, Bunick D, Mitchell MA (2013) Development and validation of TaqMan quantitative PCR for detection of frog virus 3-like virus in eastern box turtles (*Terrapene carolina carolina*). J Virol Methods 188:121–125

Brown MB, Schumacher I, Klein P, Harris K, Correll T, Jacobson E (1994) *Mycoplasma agassizii* causes upper respiratory tract disease in the desert tortoise. Infect Immun 62:4580–4586

Brown MB, McLaughlin GS, Klein PA, Crenshaw BC, Schumacher IM, Brown DR, Jacobson ER (1999) Upper respiratory tract disease in the gopher tortoise is caused by *Mycoplasma agassizii.* J Clin Microbiol 37:2262–2269

Bury RB (1986) Feeding ecology of the turtle, *Clemmys marmorata.* J Herpetol 20:515–521

Bury RB, Germano DJ (2008) *Actinemys marmorata* (Baird and Girard 1852) — western pond turtle, Pacific pond turtle. In: Rhodin AGJ, Pritchard PCH, van Dijk PP, Saumure RA, Buhlmann KA, Iverson JB (eds) Conservation biology of freshwater turtles and tortoises: a compilation project of the IUCN/SSC Tortoise and Freshwater Turtle Specialist Group. Chelonian Res Monogr No. 5, p 001.1–001.9. www.iucn-tftsg.org/cbftt (accessed May 2012)

Cadi A, Joly P (2004) Impact of the introduction of the red-eared slider (*Trachemys scripta elegans*) on survival rates of the European pond turtle (*Emys orbicularis*). Biodivers Conserv 13:2511–2518

CDC (United States Centers for Disease Control and Prevention) (2010) CDC: turtles associated with large *Salmonella* outbreak. J Am Vet Med Assoc 236:810

Crump D (2001) Western pond turtle (*Clemmys marmorata pallida*) nesting behavior and habitat use. MS thesis, San Jose State University, San Jose, CA

De Voe R, Geissler K, Elmore S, Rotstein D, Lewbart G, Guy J (2004) Ranavirus-associated morbidity and mortality in a group of captive eastern box turtles (*Terrapene carolina carolina*). J Zoo Wildl Med 35:534–543

Dunson W, Seidel ME (1986) Salinity tolerance of estuarine and insular emydid turtles (*Pseudemys nelsoni* and *Trachemys decussata*). J Herpetol 20:237–245

Farkas SL, Gal J (2009) Adenovirus and *Mycoplasma* infection in an ornate box turtle (*Terrapene ornata ornata*) in Hungary. Vet Microbiol 138:169–173

Feldman SH, Wimsatt J, Marchang RE, Johnson AJ, Brown W, Mitchell JC, Sleeman JM (2006) A novel *Mycoplasma* detected in association with upper respiratory disease syndrome in free-ranging eastern box turtles (*Terrapene carolina carolina*) in Virginia. J Wildl Dis 42:279–289

Frye FL, Oshiro LS, Dutra FR, Carney JD (1977) Herpesvirus-like infection in two Pacific pond turtles. J Am Vet Med Assoc 171:882–884

Galli L, Pereira A, Marquez A, Mazzoni R (2006) Ranavirus detection by PCR in cultured tadpoles (*Rana catesbeiana* Shaw, 1802) from South America. Aquaculture 257: 78–82

Gibbons J, Lovich J (1990) Sexual dimorphism in turtles with emphasis on the slider turtle (*Trachemys scripta*). Herpetol Monogr 4:1–29

Hays DW, McAllister KR, Richardson SA, Stinson DW (1999) Washington State recovery plan for the western pond turtle. Washington Department of Fish and Wildlife, Olympia, WA

Hidalgo-Vila J, Diaz-Paniagua C, De Frutos-Escobar C, Jiminez-Martinez C, Perez-Santigosa N (2007) *Salmonella* in free living terrestrial and aquatic turtles. Vet Microbiol 119:311–315

Hidalgo-Vila J, Diaz-Paniagua C, Perez-Santigosa N, De Frutos-Escobar C, Herrero-Herrero A (2008) *Salmonella* in free-living exotic and native turtles and in pet exotic turtles from SW Spain. Res Vet Sci 85:449–452

Holland DC (1991a) A synopsis of the ecology and status of the western pond turtle *Clemmys marmorata* in 1991. Report to the National Ecological Research Center. United States Fish and Wildlife Service, San Simeon, CA

Holland DC (1994) The western pond turtle: habitat and history. US Department of Energy, Bonneville Power Administration, Portland, OR

Hoverman JT, Mihaljevic JR, Richgels KL, Kerby JL, Johnson PT (2012) Widespread co-occurrence of virulent pathogens within California amphibian communities. EcoHealth 9:288–292

Jacobson ER (2007) Infectious diseases and pathology of reptiles: color atlas and text. CRC Press, Boca Raton, FL

Jancovich JK, Bremont M, Touchman JW, Jacobs BL (2010) Evidence for multiple recent host species shifts among the ranaviruses (family *Iridoviridae*). J Virol 84:2636–2647

Jennings M, Hayes M (1994) Amphibian and reptile species of special concern in California. California Department of Fish and Game, Rancho Cordova, CA

Johnson AJ, Pessier AP, Jacobson ER (2007) Experimental transmission and induction of ranaviral disease in western ornate box turtles (*Terrapene ornata ornata*) and red-eared sliders (*Trachemys scripta elegans*). Vet Pathol 44: 285–297

Johnson AJ, Pessier AP, Wellehan JFX, Childress A and others (2008) Ranavirus infection of free-ranging and captive box turtles and tortoises in the United States. J Wildl Dis 44:851–863

Johnson-Delaney CA (2006) Reptile zoonoses and threats to public health. In: Mader D (ed) Reptile medicine and surgery. Elsevier, St. Louis, MO, p 1018–1022

Kleiboeker SB, Miller MA, Schommer SK, Ramos-Vara JA, Boucher M, Turnquist SE (2002) Detection and multigenic characterization of a herpesvirus associated with malignant catarrhal fever in white-tailed deer (*Odo-*

coileus virginianus) from Missouri. J Clin Microbiol 40: 1311–1318

Koper N, Brooks RJ (2000) Environmental constraints on growth of painted turtles (*Chrysemys picta*) in northern climates. Herpetologica 56:421–432

Lindeman PV (1999) Aggressive interactions during basking among four species of emydid turtles. J Herpetol 33: 214–219

Lovich J (1998) Western pond turtle *Clemmys marmorata*. United States Geological Survey, Western Ecological Research Center, Riverside, CA

Mainar-Jaime RC, Atashparvar N, Chirino-Trejo M (2008) Estimation of the diagnostic accuracy of the *invA*-gene-based PCR technique and a bacteriological culture for the detection of *Salmonella* spp. in caecal content from slaughtered pigs using Bayesian analysis. Zoonoses Public Health 55:112–118

Marschang RE, Gravendyck M, Kaleta EF (1997) Herpesviruses in tortoises: investigations into virus isolation and the treatment of viral stomatitis in *Testudo hermanni* and *T. graeca.* J Vet Med Ser B 44:385–394

McArthur S, Wilkinson R, Meyer J (2004) Medicine and surgery of tortoises and turtles. Blackwell Publishing, Malden, MA

Mehta CR, Patel NR, Gray R (1985) On computing an exact confidence interval for the common odds ratio in several 2 × 2 contingency tables. J Am Stat Assoc 80:969–973

Mitchell JC, McAvoy BV (1990) Enteric bacteria in natural populations of freshwater turtles in Virginia. Va J Sci 41: 233–242

Moreno J, Merino S, Sanz J, Arriero E (2002) An indicator of maternal stress is correlated with nestling growth in pied flycatchers *Ficedula hypoleuca.* Avian Sci 2:1–9

Muro J, Ramis A, Pastor J, Velarde R, Tarres J, Lavin S (1998) Chronic rhinitis associated with herpesviral infection in captive spur-thighed tortoises from Spain. J Wildl Dis 34:487–495

Nielsen DL, Brock MA, Rees GN, Baldwin DS (2003) Effects of increasing salinity on freshwater ecosystems in Australia. Aust J Bot 51:655–665

Origgi FC, Jacobson ER (2000) Diseases of the respiratory tract of chelonians. Vet Clin North Am Exot Anim Pract 3: 537–549

Pallister J, Gould A, Harrison D, Hyatt A, Jancovich J, Heine H (2007) Development of real-time PCR assays for the detection and differentiation of Australian and European ranaviruses. J Fish Dis 30:427–438

Pettan-Brewer KCB, Drew ML, Ramsay E, Mohr FC, Lowenstine LJ (1996) Herpesvirus particles associated with oral and respiratory lesions in a California desert tortoise (*Gopherus agassizii*). J Wildl Dis 32:521–526

Polo-Cavia N, Engstrom T, Lopez P, Martin J (2010) Body condition does not predict immunocompetence of western pond turtles in altered versus natural habitats. Anim Conserv 13:256–264

Prymaczok NC, Chaulet A, Medesani DA, Rodriguez EM (2012) Survival, growth, and physiological responses of advanced juvenile freshwater crayfish (*Cherax quadricarinatus*), reared at low temperature and high salinities. Aquaculture 334–337:176–181

Rathbun GB, Siepel N, Holland D (1992) Nesting behavior and movements of western pond turtles, *Clemmys marmorata.* Southwest Nat 37:319–324

Readel AM (2009) Effects of habitat on physiology and infection in aquatic turtles. PhD dissertation, University of Illinois at Urbana-Champaign, Champaign, IL

Readel AM, Dreslik MJ, Warner JK, Banning WJ, Phillips CA (2008) A quantitative method for sex identification in emydid turtles using secondary sexual characters. Copeia 2008:643–647

Ritchie B (2006) Virology. In: Mader D (ed) Reptile medicine and surgery. Elsevier, St. Louis, MO, p 391–417

Rosskopf WJ, Howard E, Gendron AP, Walder E, Britt JO (1981) Mortality studies on *Gopherus agassizii* and *Gopherus berlandieri* tortoises. In: Hashagan KA (ed) Proc Desert Tortoise Council Symposium. The Desert Tortoise Council, Palm Springs, CA, p 108–112

Rowe C (2008) 'The calamity of so long life': life histories, contaminants, and potential emerging threats to long-lived vertebrates. Bioscience 58:623–631

Saelinger CA, Lewbart GA, Christian LS, Lemons CL (2006) Prevalence of *Salmonella* spp in cloacal, fecal, and gastrointestinal mucosal samples from wild North American turtles. J Am Vet Med Assoc 229:266–268

Scheelings TF, Lightfoot D, Holz P (2011) Prevalence of *Salmonella* in Australian reptiles. J Wildl Dis 47:1–11

Scott N, Rathbun G, Murphey T, Harker M (2008) Reproduction of Pacific pond turtles (*Actinemys marmorata*) in coastal streams of central California. Herpetol Conserv Biol 3:143–148

Spinks PQ, Pauly GB, Crayon JJ, Shaffer HB (2003) Survival of the western pond turtle (*Emys marmorata*) in an urban California environment. Biol Conserv 113:257–267

Utah Division of Wildlife Resources (ed) (2010) Red-eared slider *Trachemys scripta elegans*. http://wildlife.utah.gov/pdf/AIS_plans_2010/AIS_12tRed-earedSlider-Crystal-final.pdf (accessed November 2011)

Valverde R (2012) Salinity as a stressor of the freshwater turtle, *Trachemys scripta* in the Lake Pontchartrain Basin. Lake Pontchartrain Basin Research Program, Project Overview. Southeastern Louisiana University, Hammond, LA. http://www2.southeastern.edu/orgs/pbrp/studyPages/valverde.html (accessed April 2012)

van Kuppeveld FJM, Logt JTM, Angulo AF, Zoest MJ and others (1992) Genus- and species-specific identification of *Mycoplasmas* by 16S rRNA amplification. Appl Environ Microbiol 58:2606–2615

Vega C, Stayton CT (2011) Dimorphism in shell shape and strength in two species of emydid turtle. Herpetologica 67:397–405

Verneau O, Palacios C, Platt T, Alday M and others (2011) Invasive species threat: parasite phylogenetics reveals patterns of processes of host-switching between non-native and native captive freshwater turtles. Parasitology 138:1778–1792

WHO (World Health Organization) (2003) Global Salm-Surv. A Global *Salmonella* Surveillance and Laboratory Support Project of the World Health Organization. www.antimicrobialresistance.dk/data/images/salmonella2_pdf.pdf (accessed April 2012)

Wright D, Nguyen C, Ball C (2008) Western pond turtle (*Actinemys marmorata*) telemetry study. Resource Assessment, Region 2. California Department of Fish and Game, North-Central Region, Rancho Cordova, CA

Editorial responsibility: Lee Skerratt, Townsville, Queensland, Australia

Submitted: December 17, 2012; Accepted: August 27, 2013
Proofs received from author(s): October 27, 2013