
THE ROLE OF INCUBATION TEMPERATURE AND CLUTCH EFFECTS IN DEVELOPMENT AND PHENOTYPE OF HEAD-STARTED WESTERN POND TURTLES (*EMYS MARMORATA*)

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Abstract.—As part of a conservation-based head-starting program, we examined nesting behavior, nest site selection, and the factors affecting hatching success, growth, and sex determination in a Northern California population of the Western Pond Turtle, *Emys (Actinemys) marmorata*. Eggs were collected from nests in two consecutive years for constant temperature incubation experiments. A total of 131 hatchlings were raised in a controlled environment (head-started) prior to re-release of juveniles into their natal waters. The relative effects of incubation temperature and maternal/clutch effects on juvenile growth and temperature-dependent sex determination (TSD) were analyzed for this population of *E. marmorata*. Results demonstrate that incubation temperature significantly affects hatching success and incubation duration, while both temperature and clutch have significant effects on juvenile growth. Our data support a 1A pattern (MF) of TSD in this species, but also demonstrate a significant decline in hatching success for eggs incubated above the pivotal temperature. Preliminary field data suggest that cyclical temperature fluctuation in naturally occurring nests of *E. marmorata* may play an important role in sex determination and viability of nests and eggs in wild populations. These data are likely to have important implications for *ex situ* incubation protocols in future conservation programs for *E. marmorata* that include head-starting.

Key Words.—clutch effects; head-starting; juvenile growth; maternal effects; temperature dependent sex determination

INTRODUCTION

The Western Pond Turtle, *Emys* (a.k.a., *Actinemys*) *marmorata* (van Dijk et al. 2011), is the only native aquatic freshwater turtle with a range along the entire Pacific Slope of the United States with populations present from northern Washington to northern Baja California (Storer 1930; Bury 1970). Due to declining populations throughout much of its historical range *E. marmorata* has been listed as Endangered in Washington, Threatened in Oregon, and a Species of Special Concern in California by the respective state departments of Fish and Game (Jennings et al. 1994; Hays et al. 1999; Oregon Department of Fish and Wildlife 2006. Appendix I: Statewide

Summary of Strategy Species).

Population declines of *E. marmorata* in California, in some cases resulting in localized extirpations, have been most severe in wetland habitats that have been altered by extensive urban and agricultural development (Brattstrom and Messer 1988; Spinks et al. 2003; Germano 2010). In response, the California Department of Fish and Wildlife (CDFW) is revising the existing *E. marmorata* conservation strategy (Donald Ashton and Betsy Bolster, pers. comm.). In coordination with these efforts, two pilot conservation programs that incorporate head-starting have recently been sanctioned by CDFW. The program discussed here consists of a collaborative effort in Northern California between Sonoma State Uni-

versity and the conservation departments of the Oakland and San Francisco zoos. There also is collaboration between the conservation department of the San Diego Zoo and the US Geological Survey in Southern California.

Our Northern California program recovered eggs from nesting grounds of a population of *E. marmorata* for *ex situ* incubation, head-starting, and re-release of captive-raised juveniles into the wild. We incubated eggs at five constant temperatures to determine the thermal conditions that maximized hatching success and to assess the role of incubation temperature on development, with a specific focus on finding the pivotal sex-determining temperature range for *E. marmorata*, a species with temperature-dependent sex determination (TSD). We head-started hatchlings from 2008 and 2009 cohorts for 9-10 mo in dedicated quarantine facilities at the Oakland and San Francisco zoos using protocols modified from Washington's established head-starting program for *E. marmorata* at Seattle's Woodland Park Zoo (Frank Slavens, pers. comm.) prior to their release back to natal waters.

Notably, despite the concerns over population declines of *E. marmorata*, many aspects of its ecology, life history, and reproductive biology that are likely to be informative to long-term conservation efforts remain poorly understood (Bury and Germano 2008; Germano and Rathbun 2008). Therefore, we designed the program to tightly integrate investigations into the basic biology of *E. marmorata* with applied head-starting protocols. We recorded data on the reproductive biology of *E. marmorata* in a field setting, including the seasonal and daily timing of nesting forays, as well as data on internal nest temperatures and nest site selection. These field data, while peripheral to the head-starting processes per se, facilitated the collection of eggs in this multiple-year study, and allowed us to gather new information on a range of poorly documented aspects of the ecology and behavior of *E. marmorata*. We used *ex situ* incubation experiments to generate data on the effects of incubation temperature on a range of develop-

mental variables (e.g., incubation duration, hatching success). In addition we analyzed the relative effects of incubation temperature and maternal identity/clutch effects on growth of head-started juveniles. When the juveniles reached appropriate size, we performed endoscopic surgery to determine sex of the hatchlings (Kuchling 2006) from 2008 and 2009 cohorts for calculation of the pivotal sex-determining temperature range for this population of *E. marmorata*.

Information on nesting behavior, including nest site selection, is likely to prove important in assessing appropriate habitats for future reintroduction efforts of captive-raised *E. marmorata*. Data obtained on the effects of temperature on development, pivotal sex determining temperatures and juvenile growth in *E. marmorata* can be used to improve the overall effectiveness of head-starting efforts by improving hatching success, facilitating the manipulation of hatchling sex ratios, and to produce relatively large, robust juveniles for re-release into the wild. It is our hope that similar protocols potentially may be used to inform conservation programs working with *E. marmorata*, as well as other turtle species with TSD.

MATERIALS AND METHODS

Field Protocols.—Field studies took place approximately 150 km north of the San Francisco Bay, at a 62-ha preserve in the California Coastal Range (about 1,000 m elevation) surrounding a vernal lake managed by the Nature Conservancy and CDFW. Oak (*Quercus* spp.), Madrone (*Arbutus menziesii*), Douglas Fir (*Pseudotsuga menziesii*), and Ponderosa Pine (*Pinus ponderosa*) woodlands interspersed with open areas covered by annual grasses characterize the habitat. We selected this site based on evidence of depredated *E. marmorata* nests over multiple years (Matthew Bettelheim, pers. comm.). We found gravid females on nesting forays through visual monitoring of upland areas where depredated nests had been identified in previous years. During the 2008 late May-early July field season, we mon-

itored the site continuously between 800 h and sunset from a hunter's tree blind and/or by hourly walk-throughs. In 2009 and 2010, ground-level patrols were conducted at 0800 h and 1200 h, with continuous tree-blind monitoring and hourly walk-throughs from 0400 h to sunset, to coincide with peak nesting activity. Individual female turtles were palpated to determine gravidity. Mass, carapace and plastron length, and general condition were recorded, and we estimated age by counting plastron scute rings. Each female was marked with a unique identification number with filed notches on marginal scutes (Holland 1991), and gravid females were fitted with radio transmitters (Model R2000, Advanced Telemetry Systems 470 First Avenue North Isanti, Minnesota, USA) epoxied on the anterior vertebral scutes of the carapace for tracking by telemetry, digitally photographed, and then returned to the lake.

Processed gravid females typically resumed nesting forays one to three days after their return to the lake, allowing us to track them to potential nest sites. If a female was tracked to or witnessed in the process of nest excavation, the observer planted a flag marker near the nesting location and moved away from the area, making sure that the observer's exit was obvious enough to be noticed by the turtle. The majority of turtles then quickly resumed nesting. In several cases, the observers were able to position themselves in the tree blind or to retreat 10-20 m from the nesting female to observe nesting behaviors without disturbing the female. When direct observation or telemetry indicated the turtle had moved from the site (and presumably completed egg-laying), the turtle was recaptured to remove the transmitter. Nest GPS coordinates were recorded, and nests were excavated by careful removal of the mud plug that sealed the nest. In 2008 and 2009, eggs were transferred to vermiculite-filled containers for transport to the laboratory at Sonoma State University (SSU) for incubation experiments. We marked each egg lightly with pencil as it was removed from the nest to indicate the top, and a number was added

to identify the nest and mother. In 2009 and 2010, egg dimensions and mass were recorded during nest processing. In 2010, all processed eggs were carefully returned to their original position in the nest, one or two temperature sensors (IButton model DS1921G-2048 \pm 0.5 °C, Thermochron, PO Box 1007, Baulkham Hills, NSW 2153, Australia) were inserted into the nest chamber to record temperature at 30- or 45-min intervals, and the nest plug was restored. After processing, nests were covered with secure wire mesh enclosures to prevent predation and labeled with a marker flag for relocation. After 70 d of natural incubation, the temperature sensors and eggs were collected and returned to the laboratory and all eggs were placed in an incubator at $28 \pm 1^\circ\text{C}$ until they hatched.

Laboratory incubation protocols.—Clutches of eggs recovered in 2008 and 2009 were distributed as evenly as possible among one of five snap-top plastic containers partially filled with moistened vermiculite (e.g., in an average six-egg clutch, one egg was incubated in each container and the remaining egg was randomly assigned a container). The vermiculite substrate was relatively dry (5:1 vermiculite:water by volume) to optimize hatching success (Frank Slavens, pers. comm.). Each container was then placed into an incubator (Model TXR WD, Lyon Technologies, Inc. 1690 Brandywine Avenue Chula Vista, CA. 91911 U.S.A.). The incubators were equipped with liquid-in-glass thermometers (ERTCO Exact Temp Model #1-030_1SR \pm 0.5°C, Thermco Products Inc. 10 Millpond Drive Lafayette, NJ 07848 U.S.A.) to monitor air temperature of the incubator and Thermochron IButton sensors within the snap-top egg containers. Temperature of each incubator was set and adjusted for at least two weeks prior to introduction of eggs to maintain maximal thermal stability throughout the duration of incubation. Incubator temperatures were set at 27, 28, 29, 30, and 31 °C in 2008. Due to the reduced percentage of hatching success at 31

°C in 2008, the temperature set-point of the five incubators was adjusted down by 1 °C in 2009. Eggs were inspected daily after 65 d of incubation to record pipping/hatching events. Hatchlings were marked with a unique number with fingernail polish on the carapace (2008 and 2009), or had marginal scutes clipped for identification (2010) prior to being transferred to facilities at the Oakland or San Francisco zoos.

Captive husbandry protocols.—A total of 131 hatchlings from 2008 and 2009 were transferred from the SSU laboratory to be raised in dedicated facilities at the San Francisco Zoo and Oakland Zoo after they had absorbed residual yolk. Air temperatures of the head-starting facilities were maintained at 22 °C. During the first month at the zoo facilities, hatchlings were grouped by clutch in approximately 20 × 30 cm plastic trays with silk foliage hiding areas and basking sites made from longitudinally cut polyvinyl chloride (PVC) pipe. Turtles were provided with 12 h/d of heat and light for basking. Water temperature averaged approximately 27 °C near the basking area in each tub. At 30 d, numbered identification dots (“bee-dots”) were glued to a single scute on the posterior end of the carapace of each hatchling and hatchlings were sorted by size into one of four about 190 L stock trough tanks that were oval and made of plastic (Oakland Zoo) or about 190 L glass aquaria (San Francisco Zoo). Each container was equipped with large artificial hiding places (cut PVC pipe) and silk foliage for cover. Turtles were provided with heated basking sites using a UVB spot lamp and broad-spectrum UV lighting (12 h on/12 h off). All hatchlings were fed a varied high protein diet according to protocols modified from the Washington Pond Turtle Project (Frank Slavens, pers. comm.). Diet included waxworms (replaced with earthworms after the first month in captivity), mealworms, crickets, fish, and diced pinky mice. Food was provided once a day for one hour. After one hour, excess food was removed and weighed, and water in the tanks

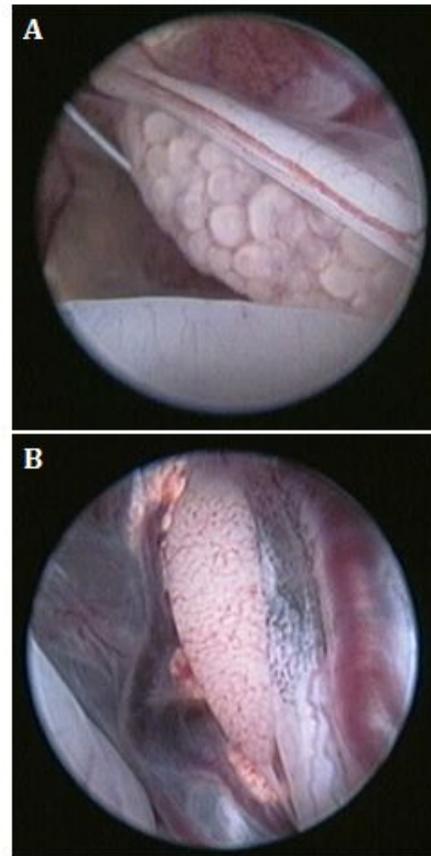


FIGURE 1. Identification of gonads of Western Pond Turtles (*Emys marmorata*) by endoscopy. A. Photograph of an ovary in a 10 mo-old head-started turtle. B. Endoscopic image of testis in a 10 mo-old head-started turtle.

drained and refilled. When all food items were completely consumed each day for one week, the portion was increased by 0.1g/turtle. Tubs were drained and disinfected weekly.

Juvenile growth.—Hatchling morphometrics (e.g., carapace length and width, plastron length and width, height, and mass) were recorded weekly from each turtle using digital calipers and a 100 g capacity scale. As hatchlings grew they were re-sorted by weight and distributed between tubs to minimize effects of competition for food and basking sites on individual growth rates.

Endoscopic sex determination.—We de-

terminated the sex of turtles when they were approximately 10 mo-old with bodyweights ranging from 18–73 g. Individuals were fasted for 24 h prior to surgery. Each turtle was cleaned with chlorhexidine and alcohol scrubs in the anterior inguinal region and given local anesthesia with subcutaneous injection of lidocaine (1 mg/kg) at the incision site. Turtles were anesthetized with intramuscular (IM) injection of ketamine hydrochloride (15 mg/kg), medetomidine (0.2 mg/kg), and morphine (2.25 mg/kg). The majority of the turtles were relaxed and unresponsive within 20 min of administration and further analgesia was provided in all cases using 1mg/kg lidocaine local anesthesia at the incision site. Turtles were positioned on their side with the hind limb extended caudally and a 9.5Fr 30°rigid endoscope (Karl Storz Veterinary Endoscopy-America, Inc. 175 Cremona Drive Goleta, California, USA.) was inserted into the coelomic cavity through a small stab incision. The coelomic cavity was insufflated with a 0.9% sodium chloride solution. Gonads and accessory ducts were observed and sex identified on the basis of appearance: ovaries were whitish organs with follicles and oviducts clearly visible (Fig. 1A), and testes were smooth gray-pink organs with superficial vasculature visible (Fig. 1B). After the procedures, incisions were sutured and sealed with surgical glue. Following surgery, we implanted sterilized PIT tags (12.50mm × 2.07mm, model TX1411SST Destron Fearing 2980 Commers Drive Suite 500 Eagan, Minnesota, USA.) subcutaneously above the right hind leg and transponder frequencies identified with a Biomark Pocket Reader (Biomark 705 S. 8th Street, Boise, Idaho, USA.). The PIT tag injection site was sealed with surgical glue. Sedation was reversed with atipamezole (1 mg/mg) given intramuscularly after surgeries. Post-operative procedures included monitoring recovering turtles for up to two hours as they regained consciousness and recovered from anesthetic. Turtles were kept dry overnight before being put back in tanks.

Statistical procedures.—All statistical analyses were run in JMP 8.0. All statistics were analyzed using an α value of 0.05. The effect of clutch on hatching success was analyzed by G-test. Incubation duration was analyzed by one-way ANOVA. One clutch from each year (clutch #8 from 2008 and clutch #9 from 2009) was removed from all analyses as these clutches were determined to be infertile (i.e., they did not chalk and showed no development when candled). The effect of incubation temperature on hatching success and sex was analyzed with G-tests for 2009 data only. A one-way ANOVA was used to analyze the effect of incubation temperature on incubation duration for 2008 and 2009 data. Early growth was calculated as total growth of each hatchling (mass at 25 weeks – hatching mass). Preliminary analyses of clutch and incubation temperature on growth were performed using a two-way ANOVA. The interaction of incubation temperature and clutch on growth was found to be insignificant, and then removed from the model ($F_{24,16} = 0.959$, $P = 0.536$).

RESULTS

Nesting behavior.—We observed terrestrial nesting activities by gravid females beginning on 10 June in 2008, 7 June in 2009, and 13 June in 2010. We completed field studies in early July each of the three years, as the frequency of nesting activities decreased dramatically at that time. In 2008, we recorded eight successful nesting attempts; as well as, evidence of 21 depredated nests (depredated nests were identified by the presence of freshly excavated nests chambers accompanied by eggshell fragments). In 2009, we incorporated telemetric tracking of gravid females, and were able to locate 15 nests between 7 June and 3 July. We noted no depredated nests in 2009. In 2010, we documented 24 successful nesting attempts and five depredated nests. Clutch size for all intact nests ranged from 3–10 eggs, with a mean of 6.11 (\pm SE 0.25; Table 1).

TABLE 1. Summary data from 2008, 2009, 2010 including the total number of Western Pond Turtle (*Emys marmorata*) nests found both undisturbed and depredated, total number of eggs recovered, and mean clutch sizes of intact nests.

Year	Total # Nests	Total # Depredated	Total # Intact	# eggs removed	Average Clutch Size \pm
2008	29	21	8	58	7.25 \pm 0.49
2009	15	0	15	97	6.00 \pm 0.53
2010	28	5	23	129	5.76 \pm 0.36
All Years	72	26	46	284	6.34 \pm 0.26

Nesting forays typically began between 1600 and 1800 daily. All recorded nesting attempts occurred in well-drained soils in relatively well-exposed areas covered with annual grasses on the northeast side of the pond. Soils at nest sites ranged from relatively loose and loamy to very hard-packed, rocky substrates. Total time required for gravid females to travel to a nesting site, construct the nest, and return to the pond varied considerably. Successful nesting attempts took place as close to the pond edge as 2–3 m and could be completed in as little as one hour, while forays to nest sites in excess of 300 m from the pond edge often required 1–2 h for the female to reach the nest location.

Nest construction consisted of the female first digging a small hole and voiding her bladder into it. The female then excavated a flask-shaped nest averaging approximately 9–10 cm deep and 20–25 cm wide through a 3–4 cm diameter opening. Time required for nest construction from start to finish varied depending on soil conditions, with several nests being completed in as little as 20–30 min and others taking several hours to complete. After oviposition, females packed a mud nest plug into the opening and the grasses and leaves around the nest site were

rearranged to conceal the nest. Despite the considerable disturbance to the site during nest construction, once the nest was finished, we were usually unable to detect any obvious superficial visual differences between ground cover at the nest and the immediate surroundings. Failed nesting attempts due to the presence of large rocks or roots in the nest chamber were relatively common and caused the female to abandon efforts and return to the pond for one–three days before further attempts. In all cases we observed, if the nesting activities were not completed by dark, activity was curtailed until morning. In cases where the nests were completed just before dark, females typically moved several meters from the site of the completed nest and concealed themselves under bushes, leaves, or dry grass until morning before returning to the pond.

Temperature and clutch/maternal effects.—

For *ex situ* constant temperature incubation of eggs in 2008 and 2009, temperature significantly affected incubation duration, with the minimum mean incubation duration at 28 °C ($F_{5,96} = 2.63$, $P = 0.028$); similarly, hatching success was significantly affected by incubation temperature ($G^2 = 16.12$, $df = 5$, $P = 0.007$) with maximum

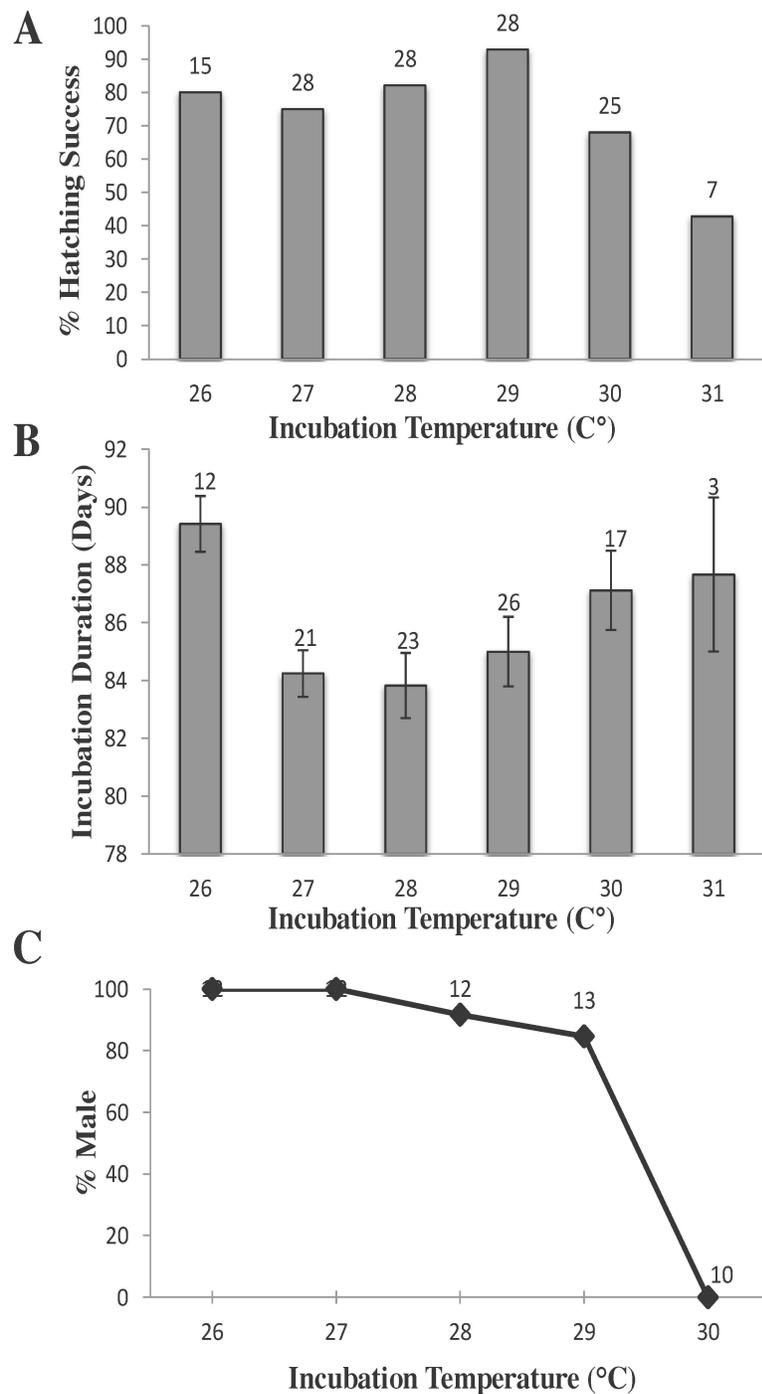


FIGURE 2. Hatching success (A), incubation duration (B), and sex ratio of hatchling Western Pond Turtles (*Emys marmorata*) incubated at different constant temperatures (C). The values above the bars A and B and above the data points in C are sample sizes. Data on incubation duration are given as mean \pm 1 SE. Note that in A and B, data points at 31 °C are from 2008 only, data points at 26 °C are from 2009 only. C includes 2009 data only.

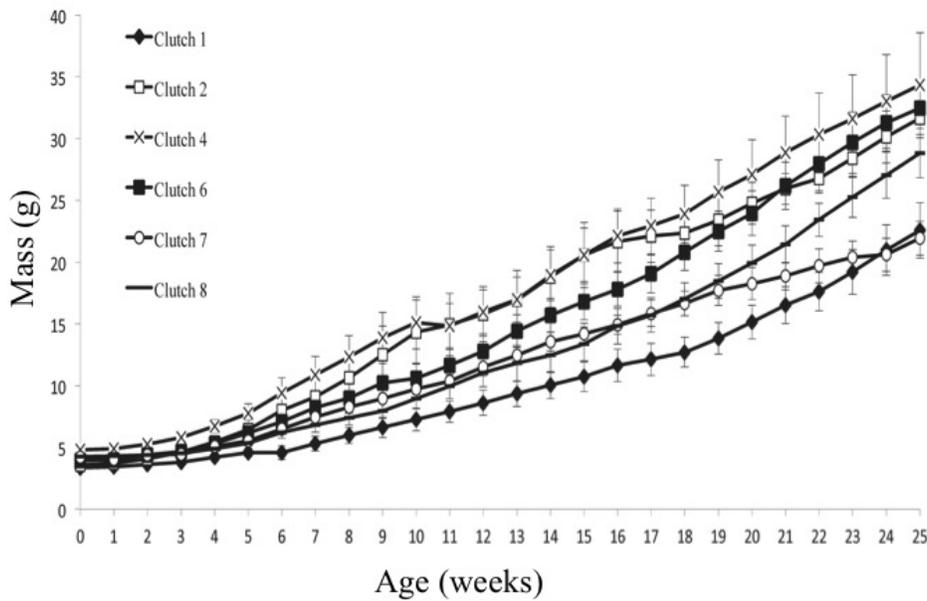


FIGURE 3. Post-hatching captive growth of hatchling Western Pond Turtles (*Emys marmorata*) over the first 25 weeks of captivity. Values are expressed as mean \pm 1 SE. Includes data from five randomly selected clutches from 2008.

percentage hatching success at intermediate temperatures (Fig. 2A). Incubation duration increased markedly below 27 °C, while hatching success decreased sharply above 29 °C (Fig. 2B). Clutch/maternal identity had significant effects on incubation duration ($F_{21,97} = 60.65$, $P = 0.001$) but not hatching success ($G^2 = 25.72$, $df = 22$, $P = 0.264$).

Temperature-dependent sex determination.—The pivotal temperature (i.e., the temperature that gives a 1:1 sex ratio) for the 59 turtles from the 2009 cohort that were endoscopically examined was calculated to be 29.4 °C. A three-parameter sigmoid nonlinear equation, $y = -5.769x^3 + 39.927x^2 - 85.714x + 152.82$, $r^2 = 0.985$, modified from Du et al (2007), was developed to estimate the pivotal temperature using constant temperatures incubation (Fig. 2C). Incubation temperatures significantly affected the sex ($G^2 = 44.18$, $df = 4$, $P < 0.001$): temperatures of 27 °C

and lower yielded 100% males, and 30 °C and above produced 100% females, with 27 °C and 28 °C giving a predominantly male sex ratio.

Juvenile growth.—Mean growth (increases in mass) of all individuals in this study was recorded weekly for the first 25 weeks after hatching. Preliminary analyses of early juvenile growth indicate that clutch had significant effects ($F_{6,6} = 2.63$, $P = 0.036$; Fig. 3), while incubation temperature did not have significant effects ($F_{4,4} = 0.50$, $P = 0.735$; Fig. 4).

DISCUSSION

Observations of nesting behavior indicate that gravid females display considerable variation in nest site selection. While virtually all the females we tracked used fairly well exposed, well-drained locations covered with annual grasses, nesting occurred as close as 1–2 m from the pond or as far

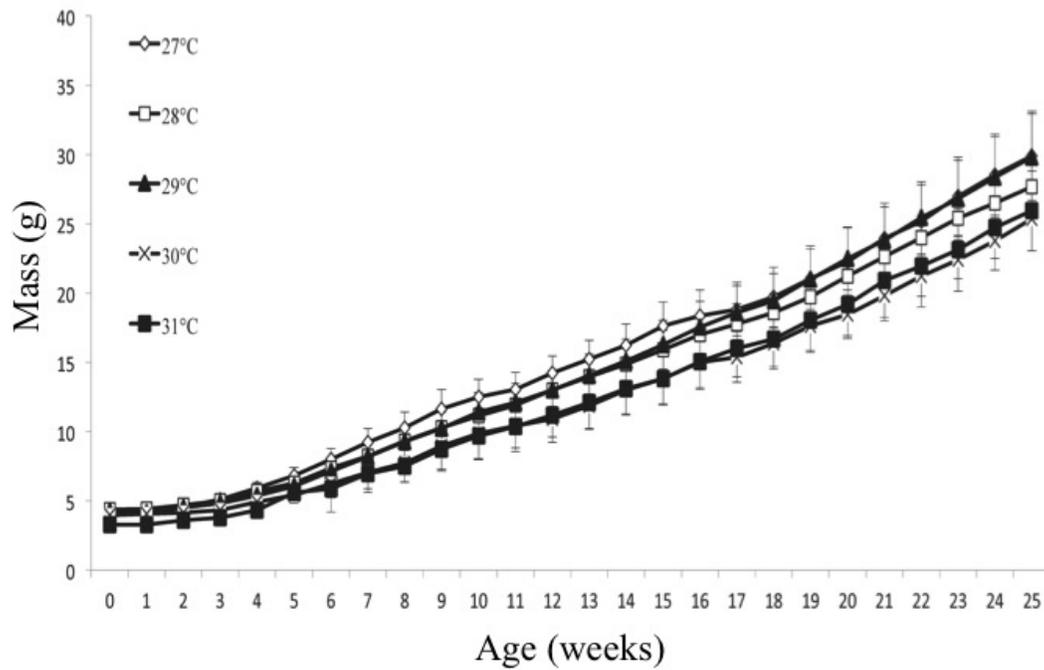


FIGURE 4. Post-hatching captive growth of head-started juvenile Western Pond Turtles (*Emys marmorata*) from different constant incubation temperature regimes over the first 25 weeks of captivity. Values are expressed as mean \pm 1 SE. Includes data from 2008 head-starts only.

as 300+ m in several cases. The entire process of nesting, including travel time, nest construction, and return to the pond could be completed in as little as one hour for nests close to the shoreline, or last as long as 12+ hours in the case of the more distant nests. In situations with nests at considerable distance from the pond, all activities ceased at nightfall and resumed after sunrise. Nesting sites were limited to south-facing sides of the lake, an observation consistent with previous reports of nesting behavior in *E. marmorata* (Rathbun et al 1992). Several nesting behaviors provide intriguing hints of philopatry, with multiple females returning 1–3 d after failed nesting attempts to dig new nests within a few meters of the abandoned attempt. In one case, a female struck a rock while digging her nest and abandoned the attempt, to return two days later to nest successfully within 5 cm of the failed attempt. These behaviors suggest that multi-year studies of nest site selection at this site may reveal data of nesting philopatry in *E. marmorata* similar to reports for other freshwater turtles (Valenzuela and Janzen 2001; Morjan 2003).

The constant temperature incubation experiments in 2008 and 2009 have significantly increased the minimal database on the effects of incubation temperature on hatchling success, incubation duration, and sex determination for *E. marmorata* (c.f., Feldman 1982; Ewert et al 1994). Our results support a IA pattern of sex determination (i.e., males produced at lower temperatures and females at higher temperatures) for *E. marmorata* (Ewert et al.1994). Notably, our results demonstrate that egg incubation at constant temperatures that consistently yield female offspring (i.e., 30°C and above) potentially dramatically reduce hatchling success. Furthermore, these data present a bit of a conundrum. It seems unlikely that *E. marmorata* would have evolved under naturally occurring temperature conditions that would produce exceedingly narrow thermal maxima (or minima) so limiting to the viability of either sex. In response to this puzzling scenario, we have begun to examine temperature

profiles within naturally incubated nests at our field site. Preliminary data from temperature sensors within the nests demonstrate high levels of daily temperature fluctuation, with the majority of nests varying more than 20°C in a consistent diurnal pattern, with extreme temperatures in the field commonly dropping below 20°C at night and often exceeding 40°C in the afternoon (Fig. 5). Additionally, our data indicate a temperature gradient within nests, with eggs at the bottom of the nest experiencing lower temperature fluctuations than those near the top (Fig. 5). Early results from 2010 indicate that cyclical variation of temperature, with maximum temperatures often well above the lethal threshold in constant temperature incubation, does not necessarily reduce hatching success dramatically, and provides hints that temperature fluctuation is likely to play a critical role in sex determination and sex ratios of wild populations of *E. marmorata*. These observations are particularly significant in regard to future potential conservation efforts that include ex situ incubation procedures to increase recruitment in wild populations. Any such efforts to manipulate hatchling sex ratios in favor of females under constant temperature incubation will likely experience low viability rates. These data suggest that the role of temperature fluctuation in influencing hatching success and sex determination during incubation needs more examination, not only for *E. marmorata* but possibly for a number of other turtle species as well.

Head-starting is a controversial conservation tool for long-lived animals such as turtles (see Russell Burke, this volume). The effectiveness of head-starting programs is likely to be limited when it is used as a primary or sole means of slowing or reversing population declines in small remnant wild populations (Heppell et al 1996), as it does not address the chief causes of the declines (e.g., habitat loss, high mortality rates in wild populations, etc.). However, head-starting may be a valuable component of integrated conservation strategies that include habitat restoration, measures to reduce mortality rates of adults

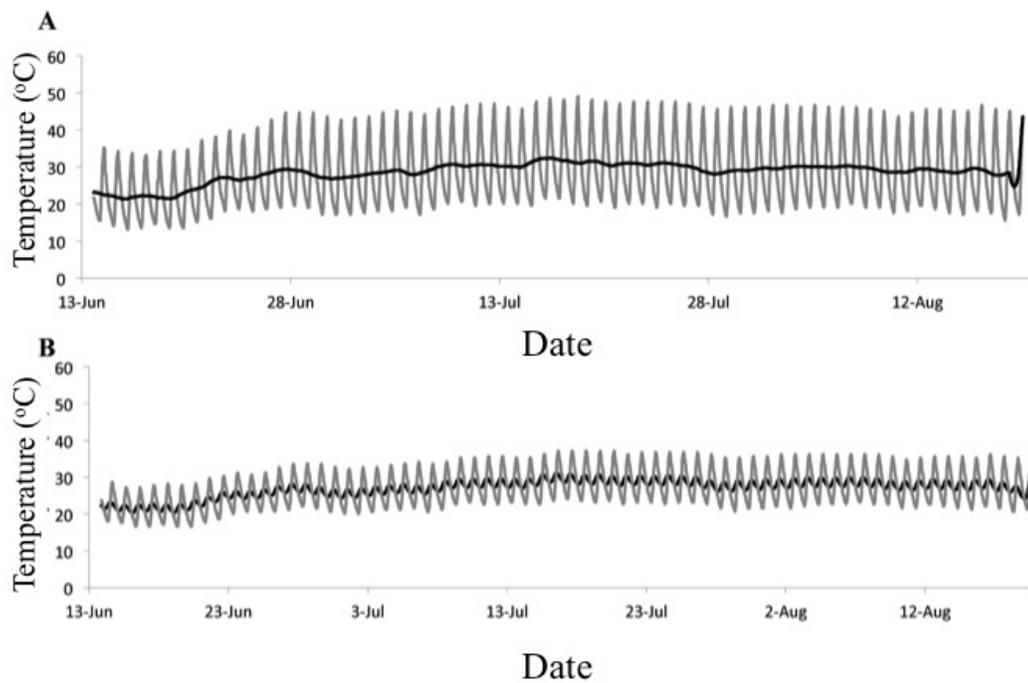


FIGURE 5. Temperature fluctuations of nest of Western Pond Turtles (*Emys marmorata*): A. Temperatures recorded approximately at the level of top layer of eggs, 55.6 mm from ground surface. B. Temperatures recorded approximately at the level of lowest layer of eggs, 94.2 mm from surface. Bold lines are daily running mean temperatures.

in wild populations, basic research, and public education and outreach.

We have taken a proactive approach to conservation of *E. marmorata* by working with a robust and relatively stable Northern California population, and head-starting has allowed us to investigate and address a number of specific gaps in our knowledge of its biology. Moreover, data acquired on reproductive behavior and development in this project can potentially be applied to conservation efforts for more severely threatened *E. marmorata* populations elsewhere in its range. Other turtle conservation-based head-starting programs dealing with critically low wild populations may be limited in the latitude they can take in performing experimental manipulations of eggs and juveniles that might reduce the total number of head-started juveniles returned to the wild. For example, the population of *E. marmorata* in Washington had dropped to such low numbers by the 1990s, with estimations of less than 200 individuals remaining in western Washington (Frank and Kate Slavens, pers. comm.), that conservation efforts were narrowly focused on the urgent need to increase recruitment of females in wild populations to forestall local extinction. This situation constrained the program from performing a number of experimental manipulations (e.g., incubation experiments at a relatively wide temperature range, surgical sex determination, etc.) we performed because such practices could potentially limit the number of viable female offspring. In contrast, our pilot *E. marmorata* conservation program has incorporated head-starting primarily as a way to acquire data on a range of poorly documented or unknown behavioral, developmental, and phenotypic characteristics in a large and relatively stable Northern California population, and secondarily as a means to directly bolster wild *E. marmorata* populations. The benefits of our model include allowing us the freedom to focus our efforts on gathering novel data on nesting behavior, thermal effects on development and TSD, and captive growth of head-started hatchlings. It is our

hope that the data gathered during this study will inform and potentially improve the efficacy of programs such as the Washington program.

To summarize, we see value in chelonian conservation programs that incorporate head-starting as part of an integrated strategy. Without addressing the myriad of underlying causes of population declines in turtles, head-starting provides, at best, a hit-or-miss solution to such problems. While we acknowledge that the inclusive approach we have taken is often not feasible for a number of turtle species experiencing catastrophic population declines and critically low wild population numbers, we suggest that when possible a proactive strategy like the one described here can provide tangible benefits beyond the controversial objective of simply increasing juvenile recruitment levels in threatened wild populations of turtles.

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