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Abnormal Shell Shapes in Northern Map Turtles of the Juniata River, Pennsylvania, USA

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Abstract.—**Northern Map Turtles, *Graptemys geographica*, are a long-lived riverine species of conservation concern. We examined carapace morphology of Northern Map Turtles at Mount Union, a major turtle nesting area and former industrial site along the Juniata River in central Pennsylvania, USA. Among 535 reproductive adult female *G. geographica*, 29% exhibited abnormal shell shape, often in the form of an indentation in one or both sides of the carapace. Older females had a higher incidence of abnormalities than younger females. We quantified variation in shell shape, compared morphology among life history stages, and assessed thermal incubation environments of embryos to determine the magnitude and potential source of shell shape abnormalities. Geometric morphometric analysis showed significant differences among several carapace shape categories of adult turtles. No shell shape abnormalities were observed among 703 hatchlings collected from nests, and no shell shape abnormalities were observed among seven of those marked hatchlings that returned to Mount Union as 11- to 18-yr-old adult females to nest. Historically, most of the nesting substrate at Mount Union consisted of black coal tailings, which exposed developing embryos to high temperatures and potential chemical insults. The high incidence of abnormal carapace shapes of adult female Northern Map Turtles at Mount Union may reflect a delayed morphological response to chemical or thermal conditions encountered in the nesting substrate, direct exposure to contaminants in the Juniata River as subadults, or factors that affected turtles a generation ago but have since abated.**

Map Turtles are highly aquatic river turtles endemic to North America (Lindeman, 2013). Many river turtles are among the most imperiled reptiles due largely to anthropogenic alteration and degradation of their lotic and riparian habitats (Moll and Moll, 2004). Northern Map Turtles, *Graptemys geographica*, are a species of conservation concern in Pennsylvania and a state endangered species in Maryland because of degradation and disturbance of rivers, road mortality, and a very limited distribution (Nagle, 2010; Lindeman, 2013; Richards-Dimitrie et al., 2013; Nagle and Congdon, 2016). The Map Turtle population inhabiting central Pennsylvania and Maryland is geographically isolated from the major area of distribution of the species in the midwestern United States.

Turtles are distinctive among vertebrate taxa in forming protective armor during development. Turtle shells provide protection from predators (Emmons, 1989; Pritchard, 2008a; Magwene and Socha, 2012) as well as camouflage (Pritchard, 2008b) and structural support (Gaunt and Gans, 1969; Landberg et al., 2003), and serve as insolation surfaces (Lindeman, 2013; Jain-Schlaepfer et al., 2016), lactic acid buffers (Jackson, 1997), rain-harvesting devices (Auffenberg, 1963), and foraging instruments (Kaufmann, 1989).

The shells of most turtle species consist of a dense bony substrate overlaid with protective scutes made of keratin that overlap osseous sutures and are arranged symmetrically (Gilbert et al., 2001; Cherepanov, 2014). Shell shape varies from relatively high-domed forms in terrestrial species to lower profile, more hydrodynamic shells in aquatic turtles (Claude et al., 2003; Stayton, 2011). The unique body plan of turtles has promoted high adult survivorship for more than 200 million yr, fostering a broad distribution throughout temperate and tropical habitats (Gibbons, 1987; Shine and Iverson, 1995).

Many factors can affect the symmetry and shape of a turtle's shell (Rothschild et al., 2013), and anomalies in scute patterns and shell shape are relatively easy to detect (Guillermo et al., 2011). Exposure to elevated levels of environmental contaminants including heavy metals, organochlorine pesticides, polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs) in freshwater wetlands are associated with a wide range of developmental abnormalities and anomalous scute patterns in *Chelydra serpentina* (Bishop et al., 1998; Bell et al., 2006; Van Meter et al., 2006; de Solla et al., 2008), *Chrysemys picta* (Bell et al., 2006), and *Emys orbicularis* (Fernandez and Rivera, 2004).

At a regional scale in *E. orbicularis*, low levels of genetic diversity are associated with increased incidence of carapace anomalies (Guillermo et al., 2011). Partial drying during development produces anomalous embryos of *Chelydra serpentina* and *Chrysemys picta* (Lynn and Ullrich, 1950), and exposure to incubation temperatures near the extremes of the range of tolerance produces developmental anomalies in embryos of *Chelydra serpentina* and *Trachemys scripta* (Packard et al., 1977).

We examined carapace morphology of Northern Map Turtles of the Juniata River at Mount Union, the largest riverine turtle nesting area known in central Pennsylvania. More than 200 female *G. geographica* migrate to the site during some years to lay eggs (Nagle and Congdon, 2016). In central Pennsylvania, nesting occurs in late May through July, with females seeking open-canopy, disturbed substrates for oviposition (Nagle, 2010). The habitat at Mount Union is unique in that historically, coal tailings provided much of the nesting substrate, exposing developing embryos to high incubation temperatures and potential chemical insults.

Our objectives were to quantify morphological anomalies in adult female Northern Map Turtles at Mount Union and compare them to hatchling turtles from the same study site and to adult female Northern Map Turtles from a reference population upstream of our study area. We used mark recapture to determine whether female turtles marked and released into the river as hatchlings exhibited carapace anomalies when they returned as adults to nest, and we compared the extreme

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thermal environment of egg incubation in coal tailings to nests in other substrates. We discuss the suite of environmental challenges at Mount Union and potential explanations for the high incidence of shell shape abnormalities in Northern Map Turtles.

Materials and Methods

Study Site and Field Collection.—During most of the 20th century, a large coal tailings pile along the Juniata River provided turtle nesting habitat at Mount Union, although some of the coal was removed in 1999 to construct a new highway. The coal had been mined 30 km south on Broad Top Mountain and the tailings (i.e., coal refuse) were discarded in the early 20th century by a coal-cleaning plant along the river at the junction of the East Broad Top Railroad and mainline of the Pennsylvania Railroad. A remnant coal tailings pile ~500 × 40 m remains in place at Mount Union and thus offered the opportunity to examine characteristics of nests in coal tailings and compare them to other substrates provided to mitigate the loss of nesting habitat because of highway construction.

The coal tailings are uniformly black and sparsely vegetated, and adult female turtles captured on coal tailings have significantly higher body temperatures than females nesting in other substrates (Nagle and Congdon, 2016). During 1999–2000, the Pennsylvania Department of Transportation attempted to mitigate the loss of turtle nesting habitat by depositing >800 m^3 of sand and shale in several long mounds and to mitigate significant turtle road mortality by erecting a 1,150-m-long chain-link fence between the river and the new highway (Nagle and Congdon, 2016).

We captured adult female Northern Map Turtles at Mount Union from 2000 to 2010 during terrestrial nesting forays. We also sampled females from a reference population in the Raystown Branch of the Juniata River, 12–20 km upstream of Mount Union, by dip netting and hand capturing. All turtles were measured using modified tree calipers and weighed on an electronic balance. Notes on injuries, abnormal shell shape, and anomalous scute patterns were recorded.

Map turtles typically have 12 marginal scutes on each side of the carapace as well as 5 vertebral scutes and 2 rows of 4 costal scutes, and we noted deviations from those patterns. Individuals were given permanent, unique identification codes by notching or drilling marginal scutes (Nagle et al., 2017). During 2007, photographs of the carapace of all adult female turtles were taken for analysis of variation in shell shape.

We protected observed turtle nests from predators using wire mesh cages and buried rings of aluminum flashing inside the cages to retain hatchlings as they emerged naturally from the ground (Nagle et al., 2004). At our study site, average clutch size of Northern Map Turtles is 10 eggs, and the nests average 14 cm in depth (Nagle et al., 2004). Hatching occurs in late summer or early autumn, yet 95% of hatchlings remain in their relatively shallow nest cavities throughout winter and emerge during the following spring (Nagle et al., 2004). Hatchlings not emerging of their own accord in the fall were obtained by digging nests with a spoon in the spring after they had overwintered in the nest. We evaluated hatchlings for the same morphological traits as adults, marked them individually using fingernail clippers, and released them into the river.

To determine incubation temperatures, we placed thermistor probes from calibrated temperature data loggers (HOBO loggers, Onset, Inc.) in the center of the egg mass of four to seven nests each year from 2000 to 2005. Nest temperatures during July and August were used to compare thermal conditions of nests in coal tailings to those in other substrates. Telemeco et al. (2013) found that turtle hatchlings from nests exposed to temperatures ≥34°C for >60 h had increased carapace anomalies, so we calculated the number of hours above that thermal threshold for each nest in our study.

Here, we use the term "anomaly" in a broad sense to define any deviation from normal or expected morphological patterns, including those which seem unlikely to have functional significance, such as variation in scute arrangements, as well as abnormalities. "Abnormality" is the term we apply to those deviations in shell shape that may impact function and performance; this term often is used in a medical or physiological context to describe "gross deviation from the normal range in morphological variation" (Johnson et al., 2010). As subsets of the broad category of carapace anomalies, we report both anomalous scutes and shell shape abnormalities.

Quantitative and Statistical Analyses.—For analysis of shell shape, we used TPSDig2 software to generate homologous landmarks on each digital image (Rohlf, 2001). Twenty-four landmarks were generated for each image at the most distal intersection points of marginal scutes, and scales were set based on field-measured, straight-line carapace lengths. Landmark data were converted to x-y coordinate files by using tps.util.

Coordinate files were uploaded to MorphoJ, where the Procrustes superimposition method was used to reduce bias because of position, orientation, and size for all specimens (Klingenberg, 2011). Canonical variate analysis (CVA) was performed using Procrustes distances grouped by the four types of shell abnormalities and the reference group. Procrustes distances and *P*-values were generated based on the CVA results for 10,000 pairwise distances. Wire frame graphs of average shell shape were generated based on the four shell abnormalities and the reference group.

We used a contingency analysis to compare the number of adult female Northern Map Turtles with carapace anomalies from Mount Union to the number of hatchlings from the same study area. Because of the low number of females from the upstream reference site with observed anomalies, we used Fisher's exact test to compare the proportions of females from Mount Union with anomalies to females from the Raystown Branch of the Juniata. We also used a contingency analysis to compare the number of adult female Northern Map Turtles with shell shape abnormalities from Mount Union between two age categories: females with visible scute annuli whose ages could be estimated (mostly 20 yr of age or younger) and an older group of females without visible scute annuli (mostly older than 20 yr).

We used a two-way analysis of variance (ANOVA) to examine the relationships of nesting substrates and sampling years to both mean and maximum nest temperatures during July and August. We compared the number of hours that nests experienced temperatures ≥34°C between nests in coal and those in other substrates using one-way ANOVA. Values were transformed by square roots before analysis to satisfy assumptions of the model.

Results

Based on field records from 10 yr of observation, 222 (41.5%) of 535 adult female Northern Map Turtles at Mount Union exhibited at least one carapace anomaly (Table 1). The most

TABLE 1. Carapace anomalies recorded for 535 reproductive female Northern Map Turtles (*Graptemys geographica*) at Mount Union, Pennsylvania, USA, from 2000 to 2010. Marginal scute anomalies are atypical numbers of scutes, not atypical shaped scutes (which were coded as abnormal carapace shape).

Anomaly	*N*	Percent
Abnormal carapace shape	156	29.2
Anomalous vertebral scutes	39	7.3
Anomalous costal scutes	26	4.9
Anomalous marginal scutes	20	3.7
Total number of anomalies	241	–
Total individuals with anomalous scutes	66	12.3
Total number of individual turtles	222	41.5

common anomaly was abnormal shell shape, observed in 156 (29%) of 535 adult females, a significantly higher proportion than females from the reference population in the Raystown Branch of the Juniata (2 of 44, 4.5%; Fisher's exact test, $P < 0.0001$). Among the two age categories of adult females at Mount Union, a higher proportion of shell shape abnormalities was observed in the older group of females (55 of 157, 35.0%) compared to younger females with visible scute annuli (101 of 378, 26.7%), although the difference approached significance ($\chi^2 = 3.71$, $P = 0.054$). Examples of carapace shape abnormalities of adult female Northern Map Turtles from Mount Union are shown in Figure 1A–F.

Shape abnormalities to the left side or to both sides of the carapace occurred at the highest frequencies (Fig. 1A,C,D; Table 2). Five turtles with abnormal left side of carapace ($N = 2$) or both sides of carapace ($N = 3$) also exhibited flared posterior marginal scutes (Fig. 1F). Canonical variate analysis of carapace shape abnormalities showed significant differences between turtles in three of the four shape categories and turtles in the reference group (Table 2; Fig. 2). Canonical variates 1 and 2 cumulatively accounted for 71% of the variation in carapace shape.

No carapace shape abnormalities were observed among 703 hatchling turtles from Mount Union collected from natural nests. In addition, no shell shape abnormalities were observed among seven females that were marked as hatchlings, released into the Juniata River, and recaptured during 2017 as returning 11- to 18-yr-old reproductive adults (Fig. 1G,H,I).

The number of adult females with scute anomalies (66 of 535, 12.3%) differed significantly from hatchling turtles from Mount Union (43 of 703, 4.7%; $\chi^2 = 14.80$, $P = 0.0001$), but not from adult females in the reference population in the Raystown Branch of the Juniata River (4 of 44, 9.1%; Fisher's exact test, $P = 0.64$). The occurrence of hatchlings with scute anomalies from nests in coal tailings (3 of 97, 3.0%) did not differ from nests in other substrates (30 of 606, 4.9%; $\chi^2 = 0.645$, $P = 0.42$). The number of adult females with both scute and shell shape anomalies was low (3.7%).

Incubation temperatures were obtained for 30 nests at Mount Union: 14 in coal tailings and 16 in other substrates including various mixtures of sand, soil, clay, shale, and gravel. Mean temperatures during July and August of nests in coal tailings (27.6°C) significantly exceeded those in other substrates (25.9°C; $F_{1,20} = 8.09$, $P = 0.01$). Effects of sampling year ($F_{4,20} = 2.70$, $P = 0.06$) and the interaction of sampling year and nesting substrate on mean nest temperatures during July and August ($F_{4,20} = 0.38$, $P = 0.82$) were not significant.

Maximum temperatures in nests typically occurred during the first 2 wk of August, during the latter half of the incubation period. Maximum temperatures during July and August of nests in coal tailings (44.2°C) significantly exceeded those in other substrates (37.3°C; $F_{1,20} = 16.82$, $P = 0.001$). Effects of sampling year ($F_{4,20} = 2.08$, $P = 0.12$) and the interaction of sampling year and nesting substrate on maximum nest temperatures during July and August ($F_{4,20} = 0.91$, $P = 0.47$) were not significant.

Six nests in coal tailings exceeded 45°C and two nests exceeded 50°C during their incubation periods. No live hatchlings were produced in nests that exceeded 50°C, but live hatchlings were found in nests that reached 45.9°C (3 hatchlings; clutch size = 8), 48.0°C (13 hatchlings; clutch size = 16), and 49.0°C (9 hatchlings, clutch size = 9); these three nests that produced viable hatchlings exceeded 45°C for periods of 1–2 h on three, five, and seven different days, respectively.

Nine of 14 nests in coal tailings and three of 16 nests in other substrates exceeded 60 h at temperatures ≥34°C. The mean ± SD number of hours ≥34°C for nests in coal (96.2 ± 57.1; minimum–maximum = 6–174 h) significantly exceeded that in other substrates (30.6 ± 32.0; minimum–maximum = 0–103 h; $F_{1,28} = 14.65$, $P = 0.001$).

DISCUSSION

More than 150 adult female Northern Map Turtles at Mount Union had abnormally shaped shells, often in the form of an indentation in one or both sides of the carapace (Fig. 1A–F). The number of turtles with abnormalities on both sides of the carapace was roughly equivalent to the number with abnormalities on one side only. Although their appearance might suggest that these abnormalities resulted from a physical constriction around the eggshell (Fig. 1C,D), our results indicate that they did not occur during embryonic development.

The complete absence of carapace shape abnormalities in more than 700 hatchling turtles collected from nests at Mount Union suggests that the incubation environment did not proximately influence turtle shell shape. We infer that the abnormal shell shapes of adult Northern Map Turtles at Mount Union reflect a delayed morphological response to chemical or thermal conditions encountered in the nesting substrate, direct exposure to contaminants in the Juniata River as subadults, or factors that affected turtles a generation ago but have since abated.

Scute Anomalies.—Although carapace scute anomalies in turtles seem superficial and unlikely to directly affect function or performance, they can indicate underlying developmental problems and correlate negatively with fitness (Telemeco et al., 2013). The incidence of carapace scute anomalies in Northern Map Turtles from Mount Union (13%) was similar to the levels reported for Painted Turtles (*Chrysemys picta*) from unpolluted sites: 13% (of >900 examined) from the White Oak Sanctuary in Pennsylvania (Ernst, 1971) and 14% (of >4,000) from the Edwin S. George Reserve in Michigan (Bell et al., 2006). These rates of scute anomalies were slightly higher than that of Northern Map Turtles from our reference site, the Raystown Branch of the Juniata River (9%). The proportion of hatchling turtles in our study with anomalous scutes was significantly lower than the proportion of adult females with anomalous scutes, which contrasts with results from other studies that found elevated levels of scute anomalies in hatchlings (Fernandez and Rivera, 2004; Telemeco et al., 2013).

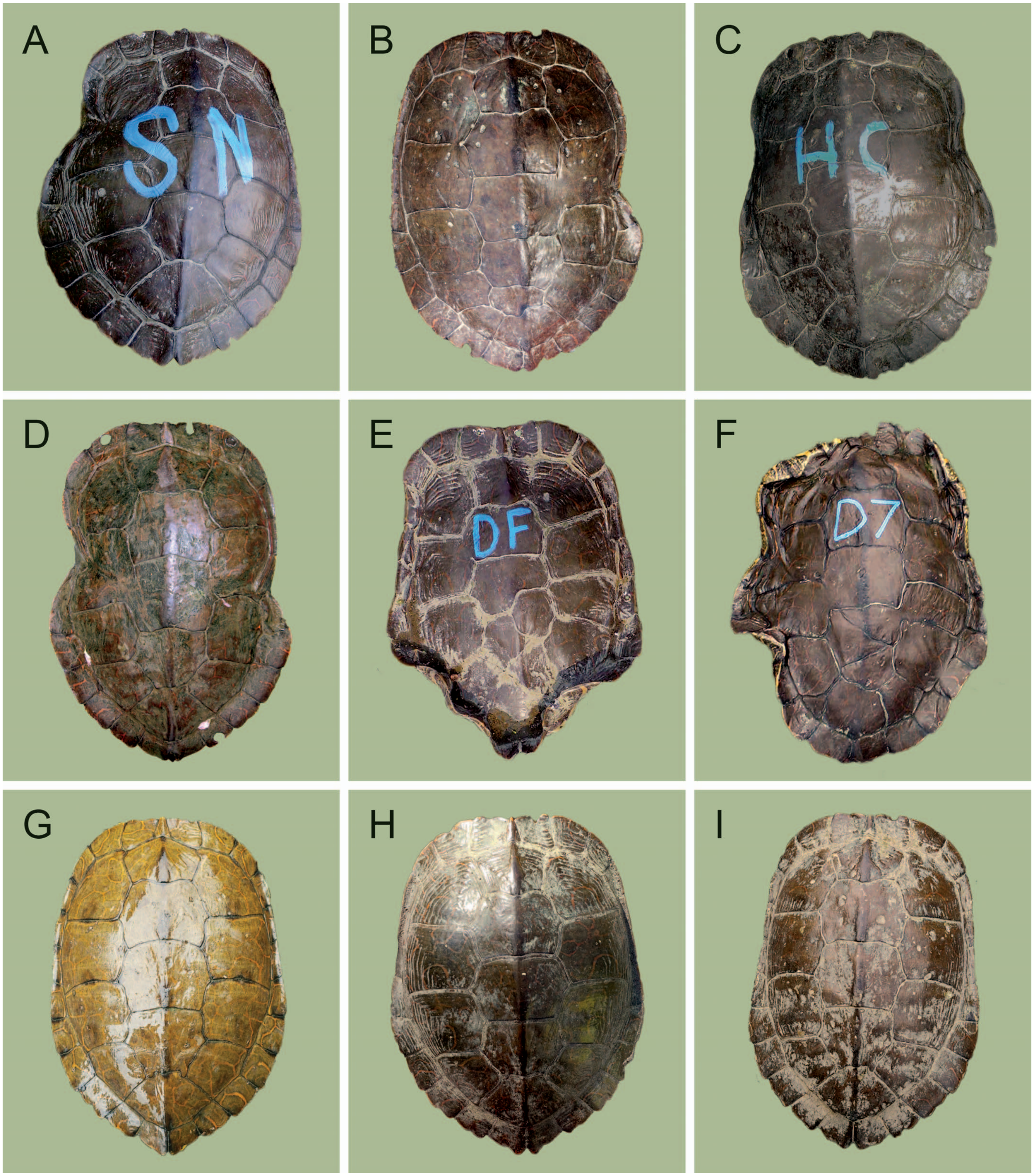

FIG. 1. Carapaces of Northern Map Turtles (*Graptemys geographica*) from Mount Union, Pennsylvania, USA. Shell shape abnormalities include (A) left side, (B) right side, (C and D) both sides, (E) flared posterior marginal scutes, and (F) both sides as well as flared posterior marginal scutes. In addition, (B), (E), and (F) have anomalous vertebral and costal scutes. Blue paint codes on turtles allow identification of individual nesting females from a distance. Turtles in (G), (H), and (I) with normal carapace shapes were marked as hatchlings, released into the Juniata River, and recaptured during 2017 as 11- to 18-yr-old reproductive adult females.

The proportion of adult female turtles from Mount Union observed with both scute anomalies and shell shape abnormalities was low (<4%) relative to the proportions with only scute anomalies or shell shape abnormalities. The infrequent coincidence of these two morphological categories suggests that they are rarely expressed together and may be influenced independently.

Shell Shape Abnormalities.—Twenty-nine percent of adult female Northern Map Turtles at Mount Union had shell shape

TABLE 2. Carapace shape abnormalities of 124 reproductive female Northern Map Turtles (*Graptemys geographica*) at Mount Union, Pennsylvania, USA, photographed in 2007. Proportions are calculated based on a total of 124 females. Procrustes distances and *P*-values from 10,000 permutation rounds for each shape abnormality versus the reference group from canonical variate analysis are also included. Asterisks indicate significant *P*-values (<0.05).

Abnormality	*N*	Percent	Procrustes distance	*P*-value
Left side of carapace abnormal	18	14.5	0.0242	0.0319*
Right side of carapace abnormal	9	7.3	0.0281	0.0850
Both sides of carapace abnormal	28	22.6	0.0226	0.0408*
Flared posterior marginal scutes	8	6.5	0.0519	0.0288*
Total number of individuals	57	46.0		

abnormalities, and significant differences in shape were observed between Mount Union Map Turtles and turtles from the reference site in the Raystown Branch of the Juniata. The high percentage of abnormalities in adult females at Mount Union relative to sites where there are no apparent exposures to coal or other known contaminants is of concern. Mount Union is the largest nesting site for Northern Map Turtles in central Pennsylvania and likely serves as a source population for the Juniata and Susquehanna rivers (Nagle and Congdon, 2016).

The high incidence of carapace shape abnormalities in female turtles at Mount Union may indicate exposure to environmental insults and may negatively affect turtles at both the individual and population level. Map turtles are fast swimmers and navigate strong currents, especially during early summer (Pluto and Bellis, 1986, 1988). Individuals of both sexes make substantial seasonal migrations for feeding, mating, and other activities (Pluto and Bellis, 1988). Shell shape abnormalities in a hydrodynamic turtle impact performance traits such as swimming speed (Rivera and Claude, 2008) and could ultimately affect survivorship.

Asymmetry in Northern Map Turtle shells significantly weakens their ability to tolerate stresses from externally applied forces, increasing their vulnerability to predation (Rivera and Stayton, 2013). Because shell shape abnormalities compromise both hydrodynamic efficiency and shell strength, aquatic turtles in general and riverine map turtles in particular should experience intense selective pressure against asymmetry (Rivera and Stayton, 2013).

Adult female Northern Map Turtles are highly molluscivorous (Lindeman, 2006; Bulté et al., 2008; Lindeman, 2013; Richards-Dimitrie et al., 2013), specializing on hard-shelled mussels and other filter-feeding benthic invertebrates that can accumulate high levels of environmental contaminants because of their foraging mode and direct contact with sediments (e.g., Peltier et al., 2009). Long-lived turtles that feed on contaminated prey can accumulate high levels of contaminants in their tissues and transfer contaminants to eggs and offspring (Russell et al., 1999; Nagle et al., 2001; Ashpole et al., 2004; Rowe, 2008).

The combination of longevity, foraging strategies, habitat use, and oviparity make aquatic turtles and their eggs excellent bioindicators of environmental contamination (Bishop et al., 1995, 1996, 1998; de Solla and Fernie, 2004; de Solla et al., 2007, 2008). Turtles with morphological abnormalities may be living environmental indicators and serve as historical records of developmental threats and injuries to other organisms.

Sources of Environmental Contamination.—Several environmental factors at the Mount Union nesting area could potentially influence the health and morphology of turtles. The use of coal as a nesting substrate by Northern Map Turtles is of conservation concern because of the potential problems of thermal stress from exposure to high incubation temperatures and the exposure of developing embryos to low pH and elevated levels of heavy metals (Nagle and Congdon, 2016). However, in an experimental study that incubated eggs of another emydid turtle species (*T. scripta*) in metal-rich coal fly ash, the eggshells and eggshell membranes seemed to protect embryos from accumulating heavy metals in the contaminated substrate (Nagle et al., 2001).

In addition to the coal tailings, a 6.3-ha area that was occupied by a creosote treatment plant lies within 100 m of the Juniata River and the northern end of the Mount Union turtle nesting area. The Pennsylvania Railroad treated up to 500,000 railroad ties per year at the plant from 1908 to 1960. Two creosote sludge ponds were adjacent to the river upstream of the turtle nesting area, and the entire area was inundated during

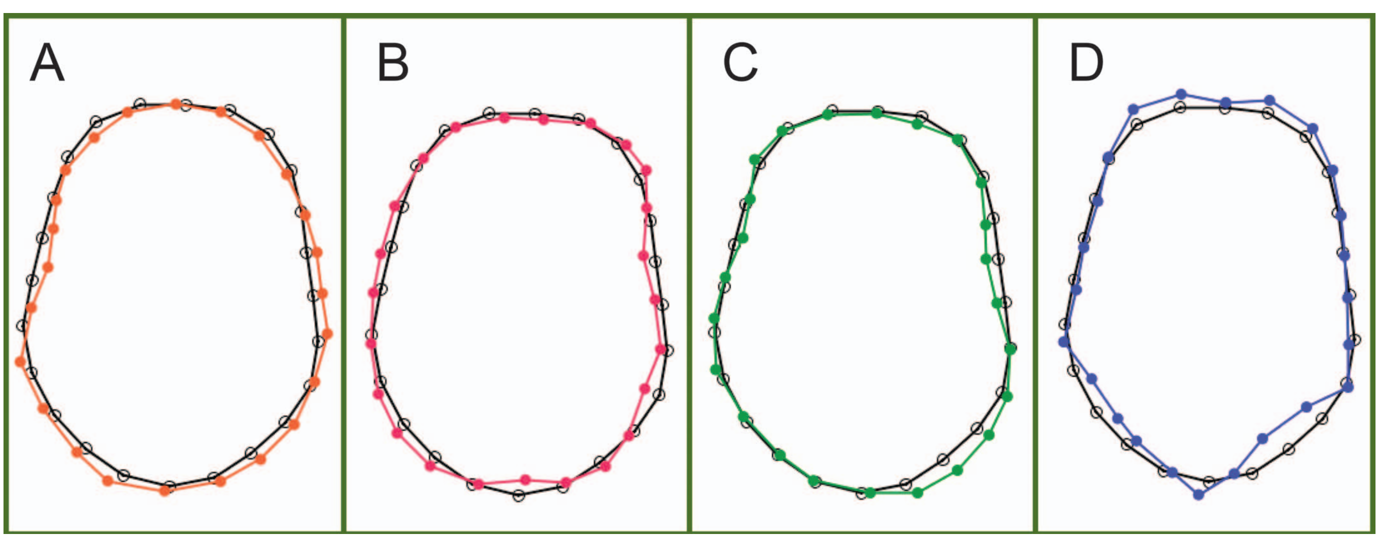

FIG. 2. Wire frame graphs of four carapace shape abnormalities in Northern Map Turtles (*Graptemys geographica*) at Mount Union, Pennsylvania, USA. The four colored lines show shell abnormalities on (A) left side, (B) right side, (C) both sides, and (D) flared posterior marginal scutes in relation to the black lines showing carapace shapes of the reference group. See Table 2 for numbers in each category from a total of 124 turtle carapace photographs.

flooding events. Later, in 1999, some of the contaminated surface soil was excavated and disposed of (to build the new highway), but most of the tie yard area used for soaking and drying creosote impregnated railroad ties was covered with fill materials and soil, and high school athletic fields were constructed over it (Pennsylvania Bulletin, 2002).

Creosote contains toxic compounds, including numerous PAHs; those PAHs above regulatory standards at Mount Union in shallow groundwater were benz[*a*]anthracene, benzo[*a*]pyrene, and benzo[*b*]fluoranthene (Pennsylvania Bulletin, 2002). In *Chelydra serpentina* embryos and hatchlings, elevated levels of PAHs from contaminated natural environments (Bishop et al., 1998) and experimental exposures (Van Meter et al., 2006) produced developmental abnormalities including deformed tails; missing limbs; and misshapen, missing, or extra scutes. In another habitat contaminated by PAHs, Bell et al. (2006) observed a variety of developmental deformities in 45–71% of *Chrysemys picta* and 13–19% of *Chelydra serpentina*.

Studies describing correlations between environmental contaminants (including PAHs) and morphological abnormalities in turtles have shown them to be expressed in hatchlings, not only in adults, as we observed. Abnormalities of the head, toes, tails, and scutes of hatchling *Chelydra serpentina* were associated with elevated levels of PCBs and organochlorine pesticides in the Great Lakes Basin (de Solla et al., 2008). Research on an Iberian population of *E. orbicularis* inhabiting a heavily industrialized watershed polluted with heavy metals and organochlorine pesticides showed that 75% of turtles had anomalous carapace scutes, and the highest proportion of anomalies was found in hatchlings (Fernandez and Rivera, 2004).

Abnormal shell shapes in adult female Northern Map Turtles at Mount Union might reflect a historical contaminant problem that has abated over time. No carapace shape abnormalities were observed among more than 700 hatchling turtles, and no carapace shape abnormalities were observed among seven females marked as hatchlings and recaptured as 11- to 18-yr-old reproductive adults (Fig. 1G,H,I). Moreover, 27% of younger females had carapace shape abnormalities compared to 35% of older females.

Of the 535 adult female turtles captured at Mount Union from 2000 to 2010, ages of 386 individuals could be estimated based upon visible scute annuli. These individuals ranged in age from 9 to 21 yr, with a median age of 14 yr (Nagle and Congdon, 2016). More than a quarter of adult females were too old to accurately age because of worn scute annuli; however, these females are likely to be at least 21 yr old. This older group of turtles seemed to be most impacted at Mount Union. Northern Map Turtles are a relatively long-lived species (Nagle and Congdon, 2016), and those at Mount Union may have been impacted decades ago, affecting the current adult population, but not embryos and hatchlings produced more recently, during the course of our study.

Differences in adult turtles may also be the result of differential gene expression, as other research showed that exposure to creosote and PAHs at superfund sites caused differential gene expression in mRNA in the livers of fish (Roling et al., 2004). Because of the long lifespans of turtles, environmental influences such as contaminants can lead to effects that are latently expressed or that persist in populations for very long periods (Shelby and Mendonca, 2001; Rowe, 2008).

Thermal Influences on Shell Abnormalities.—Although maximum temperatures tolerated by developing embryos of freshwater turtles have been reported to be about 40°C (Ewert, 1979), Northern Map Turtle embryos in our study incubated in coal tailings tolerated acute exposure to much higher temperatures. Burger (1976) reported that temperatures of some Northern Diamondback Terrapin (*Malaclemys terrapin*) nests that approached 45°C for about 1 h per day produced viable hatchlings. Three Northern Map Turtle nests in coal tailings in our study exceeded 45°C for 1–2 h on three to seven different days.

Incubation temperatures exceeding 45°C might have produced elevated levels of heat shock proteins; such proteins can affect the assembly and structure of keratin intermediate filament networks (Kayser et al., 2013). Substantial disruption in keratin formation might explain the indentations at the bridge, typical of shell shape abnormalities in adult female Northern Map Turtles at Mount Union. Perhaps abnormalities in one side of the turtle or the other are explained by the position of embryos in nests in coal tailings, with the sides of embryos near the top of the nest, which likely experience the highest temperature spikes being differentially affected compared with those elsewhere.

In a study of *C. picta* in Illinois, Telemeco et al. (2013) reported a high incidence of carapace scute anomalies in nests that experienced temperatures $\geq$34°C for 60 h or more during incubation. In our study, most nests in coal tailings exceeded that thermal threshold; the median time nests in coal exceeded 34°C was 107 h and the maximum time was 174 h, almost triple the number of hours observed by Telemeco et al. (2013) to be the threshold for development of scute anomalies in *C. picta*. Yet in our study, the sustained high temperatures in coal tailings did not result in a high incidence of scute anomalies in hatchlings. Northern Map Turtle nests in coal tailings at Mount Union regularly experienced prolonged and acute exposure to very high incubation temperatures, however, and the relationships between thermal incubation environment, shell shape abnormalities, and physiological ecology merit further investigation.

Conclusion.—The environmental pressures on Northern Map Turtles in the Juniata River basin are multifaceted, and precaution must be taken to ensure the long-term viability of this unique population. Shell shape abnormalities in riverine map turtles could be indicative of thermal stress; a localized contaminant problem; or broader challenges affecting the Juniata River (Blazer et al., 2014), the second largest tributary of the Susquehanna River.

We observed a high incidence of shell shape abnormalities in adult female Northern Map Turtles at Mount Union, whereas abnormalities were absent in hatchlings at Mount Union and rare in adults collected at an upstream reference site. We suggest several environmental explanations that may be responsible for the abnormal shell shapes of adult female turtles. The role that incubation substrate plays on morphology is a particularly relevant question because of the historical and continued use of coal tailings for nesting habitat at Mount Union. The Mount Union population of Northern Map Turtles could be a model of the early effects of climate warming on reproduction in a reptile species of concern, because nests placed in coal tailings already experience extreme incubation temperatures.

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