Carapacial Shell Disease Process Revealed by a Long-term Field Study of the Yellow Mud Turtle, *Kinosternon flavescens*, in Texas

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Abstract.—While shell diseases may be often encountered in captive aquatic turtles maintained in less than optimum conditions, cases of nonulcerating shell disease in wild populations are rare. We discovered lesions on the carapace of individual *Kinosternon flavescens* (Yellow Mud Turtle) adapted to a highly aquatic existence in the artificial ponds of a cattle ranch in the Chihuahuan Desert of west Texas. Because the carapacial lesions seemed to represent a continuum of a single process, we followed the gross changes in the lesions on turtles over a 13-yr period, testing the hypothesis that we were seeing a progressive shell disease. We confirmed our working hypothesis as we observed *Arnoldiella chelonum*, a common, filamentous alga, protruding from enlarged pores in newly formed shell and growing under translucent shell lamellae. As the disease advanced, our gross and histological studies revealed that algae were found between more of the shell lamellae, eventually culminating with sloughing of lamellae. Erosion of lamellae led to a localized but complete loss of portions of the scute and exposure of underlying bone. We provide data on the occurrence of this condition among the marked population and progression of the lesions to more-severe forms in individual turtles. Studies of specimens in research collections provided evidence of our observed disease process in *K. flavescens* across time and throughout the distribution of the species in Texas. We suggest that, by capitalizing on permanent artificial water sources, *K. flavescens* has serendipitously allowed *A. chelonum* to invade and damage the nonliving portion of the shell.

Kinosternon flavescens, the Yellow Mud Turtle, is a North American species that overwinters on land but often uses intermittent aquatic habitats for feeding and hydrating. Found across most of the south-central United States, from Nebraska to Texas, additional populations of *K. flavescens* are found in northern Mexico including in the states of Chihuahua, Coahuila, and Nuevo Leon. In the extreme northern part of its range, when conditions allow, it may periodically reside in aquatic habitats for as much as 90 d (Iowa: Christiansen et al., 1985; Nebraska: Iverson, 1991; Illinois: Tuma, 2006); however, in the arid portions of its southern distribution in the Chihuahuan Desert, *K. flavescens* is adapted for xeric habitats, using ephemeral shallow depressions that contain water only during the summer monsoon season (Degenhardt et al., 1996; Legler and Vogt, 2013). The habitat preference for water-filled depressions is observed as far north as Oklahoma, where xeric habitats with temporary pools in grasslands are preferred by this taxon (Mahmoud, 1969).

During an ongoing mark–recapture study of *Kinosternon flavescens* on a 133-km^2 ranch in Jeff Davis and Presidio counties, Texas, we had marked 833 individual turtles in a Chihuahuan Desert grassland. Our trapping effort was focused on four permanent water cattle tanks (unlined, earthen, artificial water, catch basins continually filled by well water), but we had also included temporary tanks and playas that dry between rainfall events. Through our 13-yr study, we had evidence that some turtles remain in the same earthen cattle tank for more than four consecutive months, remaining in the water for an extended period between early May and October. Not all turtles remained in the permanent tanks, but we suspected that some turtles in our population were more aquatic than others, and it is in these more-aquatic turtles that we tracked the development of an undescribed disease process limited to the carapace, hereafter referred to as carapacial shell disease process (CSDP). In the first

year of our study, we observed a range of lesions, the most severe of which were in the oldest turtles. We speculated that CSDP was a progressive process and tested the hypothesis with data collected during the ongoing study which confirmed, through observation of individual turtles, that the process progressed through a total of four recognizable stages. The CSDP ranged from its mildest form of enlarged and presumably natural microscopic pores in the overlying keratinous scutes to its most severe form where the underlying dermal bone of the carapace is exposed. Apparently, CSPD is restricted to the carapace because no such lesions were observed on the plastron. As a result of our study and the data we had recorded for 833 individual turtles, we present evidence that supports the initiation, development, and impact on individual turtles of this previously undescribed shell disease process.

Ulcerative shell disease is often encountered in aquatic turtles, especially those maintained in captivity in less than optimum conditions (Wallach, 1975). A shell disease with obvious inflammation, bone involvement, and loss of scutes was described in *Trachemys scripta* (Pond Slider) and *Pseudemys concinna* (River Cooter) (Lovich et al., 1996; Garner et al., 1997), with the disease more common and more severe on the plastron than on the carapace. A similar shell disease was described in the aquatic kinosternid *Sternotherus depressus* (Flattened Musk Turtle) and was attributed to an impairment of the immune system, ultimately resulting in septicemia (Dodd, 1988). Cutaneous dyskeratosis resulting in systemic decline and death was described in *Gopherus agassizii* (Desert Tortoise) by Jacobson et al. (1994) and necrosis and degradation of scutes was described in *Gopherus berlandieri* (Texas Tortoise) by Rose et al. (2001). None of the symptoms or descriptions of these previously described shell diseases matched the shell conditions we found in our population of *K. flavescens*.

We present data from our 13-yr study, which monitored the novel shell disease process in *K. flavescens*. Through our study, we observed gross morphological changes that are supported by histological evidence of the presence of algae between

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multiple lamellae of the same scale as well as ecological evidence of the conditions that we believed promote the condition (see Proctor, 1958). We assessed whether the observed process is a noninflammatory shell disease process that is limited to the carapace and related to filamentous algae and whether an evolutionary history may have contributed to the susceptibility in *K. flavescens*.

MATERIALS AND METHODS

Study Area.—The C. E. Miller Ranch is a 133-km^2 cattle ranch that spans portions of Jeff Davis and Presidio counties and is 18 km west of Valentine, Texas. The ranch was the site of an intensive herpetological survey in 1948 (Jameson and Flury, 1949) and we initiated new herpetological survey efforts in 2004, discovering large numbers of *K. flavescens* in multiple earthen cattle tanks across the property (Davis and LaDuc, 2018). Water in each tank consisted mostly of well water supplemented by natural runoff. We began to intensively trap and monitor *K. flavescens* in 2006 and conducted at least one intensive, 4–6-d, mark–recapture survey each year thereafter. Our sampling focused on four permanent earthen cattle tanks, though we opportunistically sampled ephemeral pools and natural depressions when water was present. Turtles were collected with aquatic traps (baited with sardines), seining, and by hand. Upon capture, we determined the sex of each turtle, determined wet mass (to the nearest 1 g), and measured carapace length and width at the widest point, plastron length, shell depth, and posterior carapace–plastron distance using linear or dial calipers (to the nearest 0.1 mm). All turtles were marked by notching marginal scutes (Congdon and Gibbons, 1996). Turtles were immediately released following examination, with some turtles being recaptured multiple times during each sampling period.

Age Estimates.—Ages of turtles were initially estimated by counts of plastral annulae, a method that was demonstrated to be accurate in growing, immature turtles but declined in accuracy after the turtles attained reproductive maturity, particularly in turtles older than 12 yr of age who typically lost annulae as rapidly as new ones formed (Iverson, 1991). In addition, near cessation of growth of old turtles failed to produce visible annulae, resulting in underestimation of turtle ages determined by annular counts. While we provided the best estimates possible based on annular counts, we based our conclusions on turtles with initial capture estimates of age 12 or younger and which were followed by as many as 10 yr of recapture data that rendered a reasonably accurate and oldest estimate of 22 yr. Many of our most advanced disease stage observations may be in turtles that were older than 22 yr because the turtles may have been much older than our estimate at the time of initial capture.

Rating Carapacial Algae Cover.—The extent of algae covering the carapace was rated for each turtle at the time of capture. Assigned ratings ranged from 0–3, with 0 indicating no algae and with 3 indicating long strands of algae covering at least two thirds of the carapace. We assumed that turtles whose algae ratings were lower than the previous rating had recently returned to the water from a terrestrial foray of sufficient duration such that algae dehydrated and detached from the shell surface. These turtles often still had a green cast over the most-dorsal third of the carapace.

Assignment of Disease Process Stages.—We ranked the progressive, deteriorative shell disease process as one of four stages with Stage 1 being the initial (minimal) stage and Stage 4 being the most destructive (severe). Because multiple stages were often found on older turtles, stage assignment was always the most advanced stage we found on that turtle; we describe these stages in detail in the Results section.

Turtle Tissue Biopsies.—We obtained biopsies from two healthy and active individual turtles for the purpose of tissue preparations. Turtle 0L11,11,6R was collected from Wildhorse Draw, an arroyo filled only by periodic rainfall, and was the initial capture of the individual on the ranch. A second turtle (8L8R), which was initially captured 8 yr previously, was collected at Two-Section Tank, a permanent earthen cattle tank and with a Stage 4 lesion ranking at the time of biopsy. We biopsied 8L8R at the Small Animal Clinic, College of Veterinary Medicine, Texas A&M University. Turtles were anesthetized with Propofol (Abbott Laboratories, Lake Bluff, Illinois) and the biopsy sites were cleansed with several applications of 50% ethyl alcohol. Three biopsies were aseptically collected from 0L11,11,6R using an IM-3 high speed drill with a cross-cut fissure bit: one of normal shell, one of a Stage 2 lesion, and one of an Stage 3 lesion. We collected two cylindrical biopsies from 8L8R with a Michele trephine. Biopsies were placed in neutral buffered 10% formalin, and additional scrapings for a fungal analysis were collected from a lesion that was not biopsied. The scrapings were subsequently examined at the Texas A&M University Medical Diagnostic Laboratory. The biopsy sites were filled with Vetspon (Novartis International AG), and sealed with Technovit (Heraeus Kulzer GmbH). Algal scrapings from shell lesions of 8L8R were also taken for identification. Both biopsied turtles were treated with prophylactic antibiotic following surgery and were released at the site of capture.

Histological Techniques.—After fixation in formalin for a minimum of 24 h, we washed biopsies in water, placed them in Cal-Ex™ (Fisher Chemical) with agitation until they could be trimmed with a straight-edged razor blade, then placed them in their uniquely labeled tissue cassette. The samples were washed in running water for 4 h before being processed in a Fisher Excelsior ES™ (Fisher Scientific) tissue processer. For softening of the keratinized scales in the resulting paraffin blocks, the cut surface of the block was placed in a petri dish containing Nair™ (Church and Dwight Co., Inc.) for 5–10 min, cut to test, and repeated if needed. We then cooled the blocks on ice and sectioned them at 6 μm. In addition to hematoxylin and eosin (H&E) stain, sections from the lesions were stained with Brown and Brenn's Gram-stain for detection of spores. The presence of spores is usually the best indicator of the presence of fungus. We also used Brown and Brenn's Gram-stain to distinguish between Gram-positive and Gram-negative bacteria. Gomoris methenamine silver (GMS) and Masson's trichrome stain were used to detect microsporidian spores in histologic sections, and Masson's trichrome stain was also used for tissue differentiation. We used H&E stain to differentiate nuclear material from cytoplasmic components.

Selection of Turtles for Study of Disease Process Progression.—Variation in field-recorded disease stage was common and usually averaged to produce the best estimate of actual disease stage in any 1 yr. Averaging disease stage rankings was necessary because the extent of algae covering the carapace would bias our ability to score the disease process. Plastral annular estimated age was corrected to the known number of years elapsed since initial capture. Because some turtles were captured over multiple years, certain individuals may be represented up to 10 times in the database, reflecting a progression through multiple stages in subsequent years. However, each individual is represented only one time in each year's sample, giving a reliable estimate of the
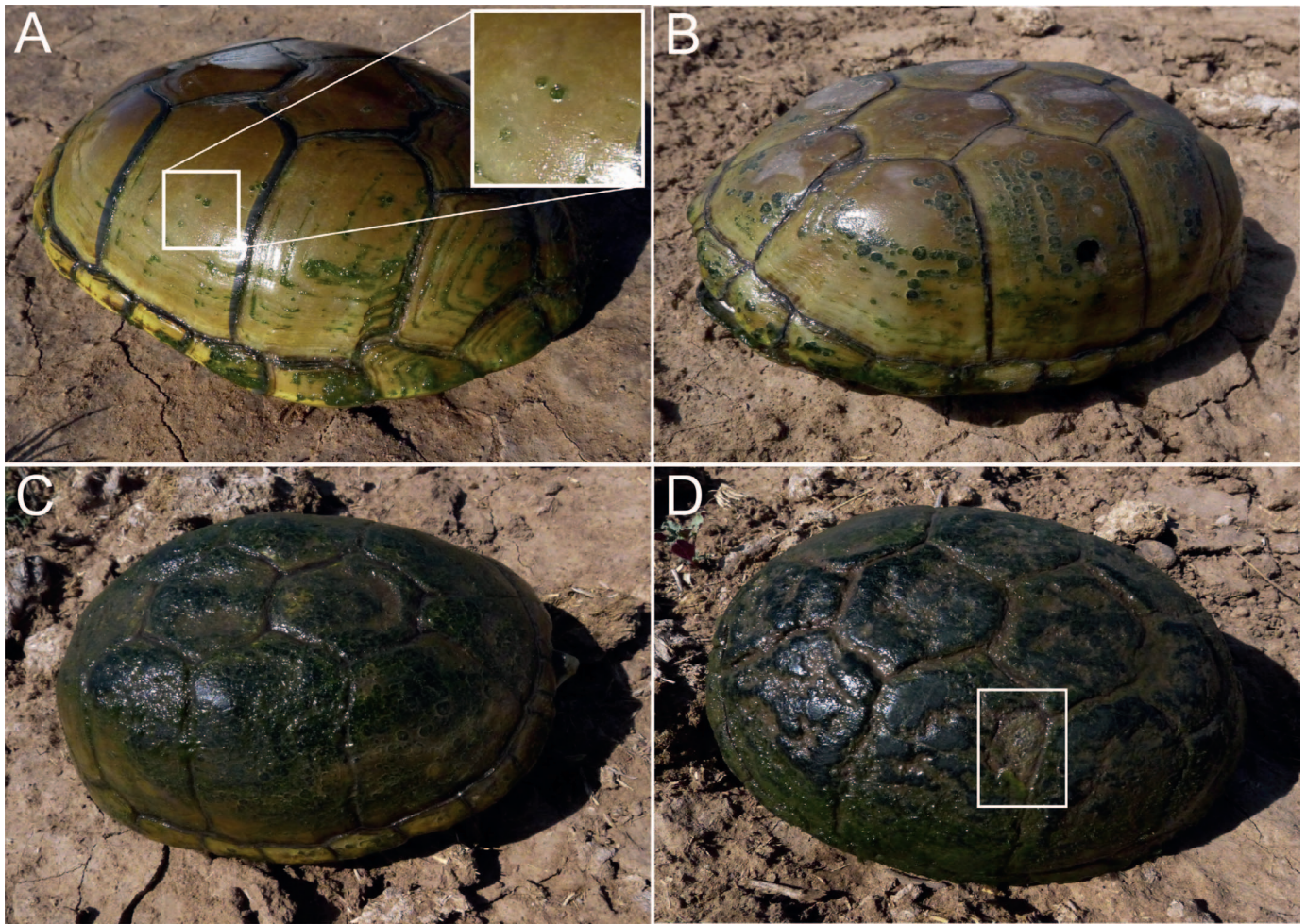
Fig. 1. Stages of algae-associated shell disease. (A) Stage 1: Few enlarged pores, mostly in annular grooves. (B) Stage 2: Enlarged pores more abundant, surrounded with small raised ring of scale, and pores no longer limited to annular grooves. (C) Stage 3: Large raised clusters of scale now concealing many pores, giving shell a rugose texture. The tops of these raised areas are often worn smooth, with extensive algae growth between raised areas on still-normal scale. (D) Stage 4: Similar to Stage 3, but more extreme with at least one area of exposed bone (indicated by white box).

stage for that specific individual. Only turtles of known sex were used in the analysis. We were often uncertain of the sex of young turtles but were able to assign sex to them when recaptured following sexual maturity.

Geographic and Temporal Distribution of Disease Process.—To determine the question of whether the carapacial shell disease process was unique to our study site, we examined dry- and fluid-preserved turtles collected in Texas from six museum collections (Biodiversity Collections, The University of Texas at Austin; Biodiversity Research and Teaching Collections, Texas A&M University; UTEP Biodiversity Collections, The University of Texas at El Paso; The James Scudday Vertebrate Collections, Sul Ross State University; Museum of Southwestern Biology, University of New Mexico; and the National Museum of Natural History, Smithsonian Institution). Assignment of the disease process stage for preserved specimens was identical to the protocol for live turtles.

Results

Gross Identification and Development of the Condition.—In all turtles captured during the course of the study, no CSDP lesions displayed visible redness, inflammation, or necrosis of the plastron or soft tissues and all turtles were vigorously active,

showing no signs of lethargy or systemic disease. Damage to the plastron from CSDP was absent and, when damage was present on the plastron, it likely had a different etiology (e.g., mechanical puncture from mammalian predator). The progressive stages of the disease process as we have refined them through the study are as follows:

Stage 1.—Pores visible with dark green, algae-filled centers scattered linearly along annular grooves of the carapace. The pores range from 3 to as many as 20 on a single costal or marginal scute (Fig. 1A).

Stage 2.—Pores enlarged, dark green with associated algae, usually in rows in the annular grooves of the carapace. The pores are surrounded by rings of slightly elevated scale with a green cast because of algae below the surface, and these ringed pores are frequently in contact with each other (Fig. 1B).

Stage 3.—Some pores are no longer visible but the areas of elevated, distorted scale surrounding the pores are grouped, forming clusters. Raised areas are darker than the surrounding shell, possibly as a result of deeply imbedded algae. Increased number of enlarged pores and Stage 2 pores no longer associated only with annulae. Development is most severe on the most-dorsal portions of the costal scutes and on vertebral scutes (Fig. 1C).

TABLE 1. Capture history with growth and associated progression of the CSDP stage for biopsied male *Kinosternon flavescens* (8L8R). CL: carapace length; Adj Age: adjusted age based on plastral annulae at first capture plus number of years captured after first capture, likely an underestimate because many annular counts after age 12 showed little consistent increase, so age at initial capture could be greater; Algae: average amount of carapacial algae present, defined by qualitative estimates (0–3) over course of a field season; CSDP range: range of estimates of CSDP stages (0–4) over course of a field season; Avg CSDP: average of CSDP stages (0–4) assigned over field season.

Year	No. captures	Date range	Weight (g)	CL (mm)	Adj age	Algae	CSDP range	Avg CSDP
2007	10	6 June–4 September	372.5	123.4	14	1.7	2–3	2.4
2008	7	30 June–2 September	389.8	124.0	15	2.3	2–3	2.7
2009	7	9 July–4 September	366.4	123.9	16	1.3	3	3.0
2010	1	9 June	380.9	123.7	17	2.0	3	3.0
2011	1	1 July	408.9	125.0	18	3.0	3	3.0
2012	3	23 June–19 July	385.4	124.6	19	3.0	3	3.0
2013	3	7 June–6 July	385.7	124.5	20	2.5	3–3.5	3.2
2014	4	5 July–15 August	413.3	124.6	21	1.5	4	4.0
2015	1	11 August	405	125.1	22	3.0	4	4.0
2017	1	4 August	408	125.7	24	3.0	4	4.0
2018	4	13–16 August	391.4	124.3	25	3.0	4	4.0

Stage 4.—Elevated scale clusters are extensive and severe on the most-dorsal two thirds of the carapace, severely distorting the normal contours of a typical kinosternid shell. Elevated areas of the shell are softer than normal, easily penetrated with a sharp probe. Loss of this damaged scale in at least one area, exposing bone, is necessary for classification as Stage 4 (Fig. 1D).

Cytological Studies.—Using light microscopy, analysis of shell scrapings from the first biopsied turtle (0L11,11,6R) revealed two morphologically distinct filamentous fungi: one *Curvularia* sp. and an unidentified species, but we considered neither to be significant, and fungi may represent normal epibionts. Suspecting that algae were heavily associated with this condition, we removed scrapings from the lesions of the second biopsied turtle (8L8R) for examination. Prominent throughout these samples was the filamentous alga *Arnoldiella chelonum* (formerly *Basicladia chelonum* [Boedeker et al., 2012]). Additionally, at least one unidentified cyanobacterium was found. Our tissue sections revealed only filamentous algae in the lesions.

Histological Studies.—Our first biopsy of a living turtle was a Stage 3 specimen (0L11,11,6R) for which we had no previous capture history. Light-microscopic observation of tissue sections
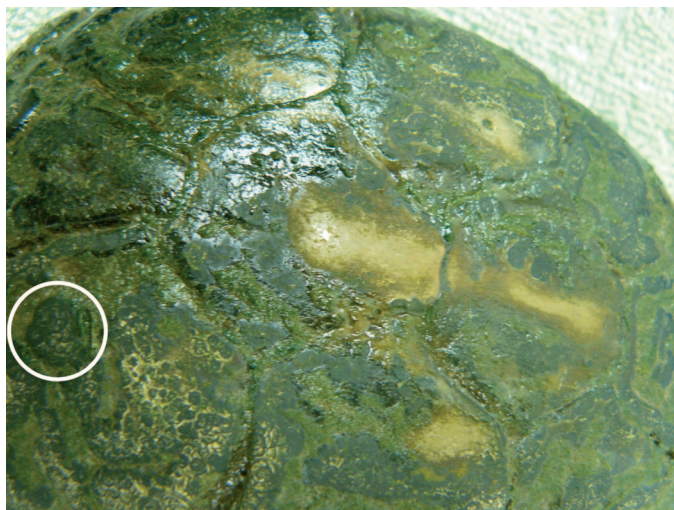

FIG. 2. Biopsied adult male *Kinosternon flavescens* (8L8R) showing grossly disfigured shell and areas of exposed bone. White circle indicates location of biopsy producing the sections shown in this paper (Fig. 3). Note green cast to shell despite removal of superficial algae, demonstrating algae below the surface of the shell.

from the biopsy revealed algae between laminae of the carapace scales. To confirm these findings, we collected and biopsied a second turtle (8L8R) with a 12-yr history of recaptures (Table 1). This turtle, a male, was recaptured multiple times most years throughout the study, and we averaged estimated disease process stages within and sometimes between years to smooth out variation in different observer field assessments and factors artificially masking the stage (e.g., increased/thick algal growth). Over the 13 yr, these averages showed a steadily advancing condition from Stage 2 to Stage 4, the latter with considerable exposed bone (Fig. 2). Even though the turtle was mature and had the advanced condition, its shell continued to grow and the turtle's weight increased each year except one. Additional evidence that the condition does not become systemic in this species is provided by the recapture of the first biopsied turtle in a survey the following year, which had increased in weight from 220.8 g to 247.5 g.

Filamentous algae (Fig. 3) were readily observed histologically and were positioned horizontally between adjacent laminae, separating the laminae and resulting in softer, thicker areas of shell (appearing dark green in Fig. 1). Further, deep separations of laminae adjacent to a pore with edges of algal cells were visible (Fig. 3B). There were no fungal mycelia or abundant spores, which would be expected if this condition involved fungi. Examination of a shell section with deeply penetrating algae, a Stage 3 lesion (Fig. 3B), showed no increase in dermal or epidermal melanomacrophage distribution and concentration (pigmented macrophages, e.g., Christiansen et al., 1996) or smaller melanocytes in the dermis, suggesting no inflammatory response. This is a condition of the carapace, with the plastron remaining unaffected even in the turtles that have demonstrated advancement of the condition over many years.

Sexual Difference, Timing of Stages.—Our program of annually monitoring this turtle population, and our survey of the stages, provided us a picture of the age distribution of turtles with each stage of the process. Our data suggested a slight difference in extent of disease process in the two sexes, with females tending to attain disease process stages a year earlier than did males (Fig. 4). The oldest turtle with no recognizable stage was 12 yr; all turtles older than 12 yr collected in earthen cattle tanks had at least Stage 1 but often a more advanced stage. While the age of these turtles could be underestimated, it is apparent that advanced stages are associated with older turtles, consistent with progression of this disease process with age (Fig. 4). There were no data in our 13 yr
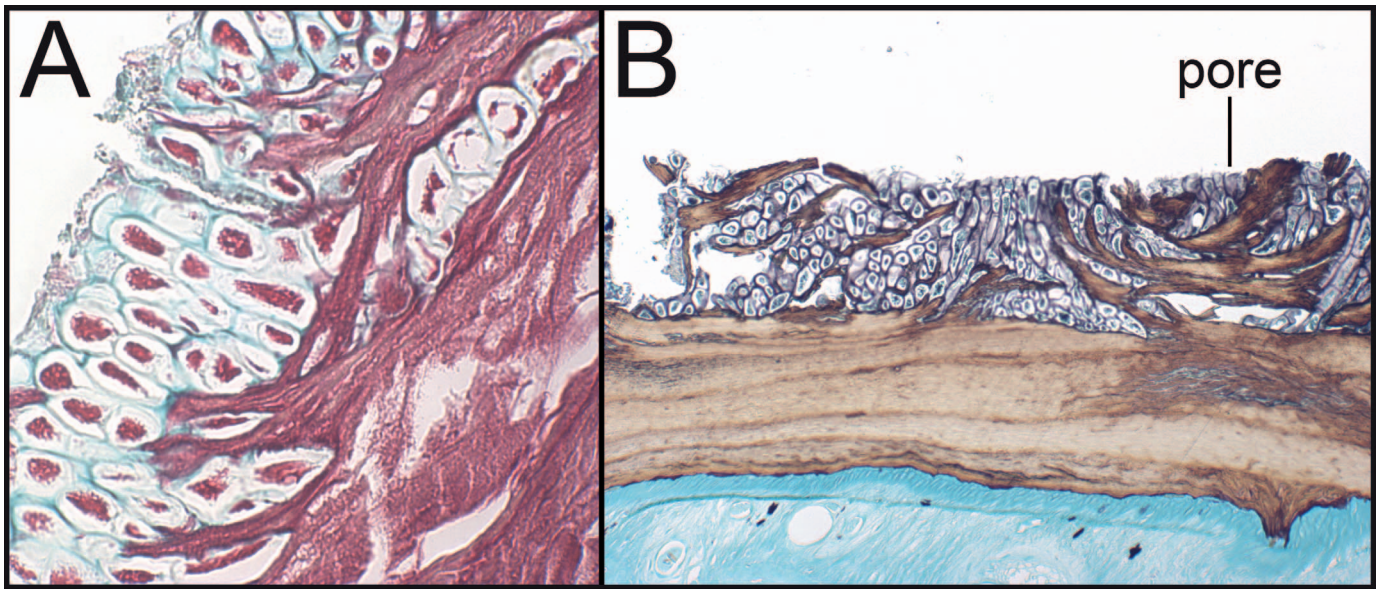
FIG. 3. (A) Sections through carapace of biopsied adult male *Kinosternon flavescens* (8L8R) showing filamentous algae invading shell between lamellae. Dorsal is upper right corner of image. Image magnification × 40. (B) Shell lamellae (brown) and superficial underlying bone adjacent to a Stage 4 pore showing deep invasion of algae (nucleated cells) from the pore and lack of melanomacrophages (pigmented monocytes) in the dermis (blue), consistent with a lack of inflammatory response. Image magnification × 10.

of field work that suggested turtles are able to heal themselves from this shell condition, but we continue to monitor the marked population annually.

Geographic and Temporal Distribution of Disease Process.—To understand whether this disease process was unique to our study site or had a broader distribution across the state, we examined 475 dry- and fluid-preserved turtles collected in Texas from six museum collections (dataset available from the authors). Out of

the 121 counties represented in our dataset, specimens from 73 counties (60.3%) demonstrated presence of this disease process (Fig. 5), confirming that the disease process we described is present across the distribution of *K. flavescens* in Texas. Unfortunately, most specimens lack associated ecological data, but the presence of algae on nearly all turtle specimens with lesions suggests an aquatic history. Absence of the disease process from 48 counties examined may be related to low sample
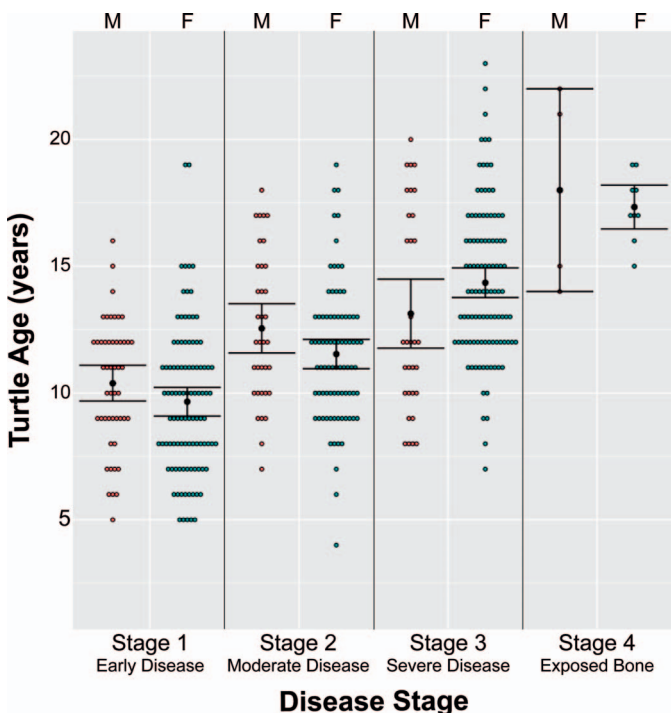

FIG. 4. Disease stages by age and sex in *Kinosternon flavescens* from the Chihuahuan Desert of west Texas. Means are large dots and 95% confidence limits are transverse bars. Males (M) are indicated by orange and females (F) are indicated with teal dots.
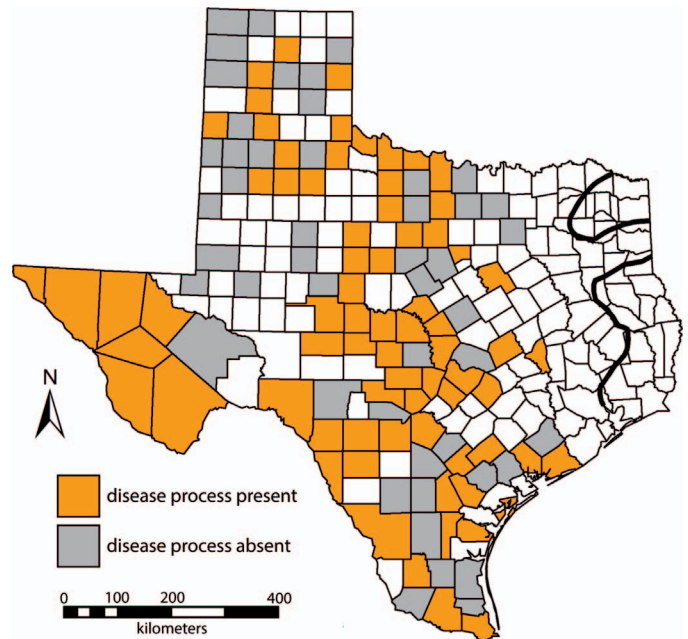

FIG. 5. Map of Texas, USA showing counties where this algae-mediated disease process was present (orange) and absent (gray) in *Kinosternon flavescens* museum specimens ($n = 475$). Most of the counties where the disease process was not observed were represented by fewer than three examined specimens. Bold lines indicate the eastern edge of the range of *K. flavescens* in Texas (modified from Hibbitts and Hibbitts, 2016).

sizes of specimens available from these six collections (45 counties were represented by three or fewer specimens). Additionally, the temporal range of these specimens spanned over a century of collections in Texas from 1891 to 2012. Initial examination of photos submitted to iNaturalist (https://www. inaturalist.org/), a citizen science observation database, revealed the presence of this shell condition at multiple localities in Texas as well (LaDuc et al., unpubl. data), and two figures published by Legler and Vogt (2013:figs. 15.1, 15.2) serendipitously illustrated Stage 2 of this disease in Mexican *K. flavescens*.

DISCUSSION

The algae *Arnoldiella chelonum*, originally described from the carapaces of *Chrysemys picta* (Painted Turtle) and *Sternotherus odoratus* (Common Musk Turtle) (Collins, 1907), has been reported on a variety of turtles from Nova Scotia to Mexico (Edgren et al., 1953; Vinyard, 1955; Dixon, 1960; Garbary et al., 2007; Legler and Vogt, 2013). However, no authors to date, including Proctor (1958) and Rothschild et al. (2013), described any algal-associated shell disease process. We have described stages of a disease process whereby algae and potentially other organisms enter microscopic pores in growing carapacial scutes with lateral, subcutaneous invasion, causing elevation and eventually sloughing of the scute. The four stages of the algal-mediated CSDP we describe herein are easily recognizable without magnification in our west Texas population of *K. flavescens*. Stage 3 and Stage 4 carapace lesions appear to decrease in size following prolonged dehydration of the shell, such as would occur with extensive terrestrial forays, nesting, or hibernation, but appear to swell to their previously elevated size following a period of rehydration. Some turtles assigned as Stage 3 or Stage 4 the previous year were assigned a lower stage upon their initial capture for a calendar year (TJL, unpubl. data). It is our opinion that a substantial decrease in carapace algae can be an indication of a recent terrestrial foray, as this appears to correlate with lower stage ratings in the field resulting from flattening of the normally lumpy carapace scutes that have been invaded by algae. In addition, mud or heavy algae growth on the shell can obscure lesions, making Stage 3 hard to detect in some turtles. More than 50 yr in fluid preservative appears to have the same effect on Stage 3 or Stage 4 turtles as do extended periods of terrestrial activity or terrestrial hibernation, in that the raised algae-impregnated parts of the shell had returned nearly to the original contours of the shell, making this disease condition in these turtles more difficult to detect.

Because these turtles overwinter terrestrially in kangaroo rat (*Dipodomys* sp.) burrows in our study area (TJL, unpubl. data), they may spend up to half the year with little exposure to light or water. Therefore, the algae *A. chelonum* must endure dehydration and desiccation but also be able to regenerate and renew growth soon after entering the water (TJL, pers. obs.). Work by Proctor (1958) supported the hypothesis that *A. chelonum* is resistant to prolonged periods of darkness and dehydration and demonstrated the presence of rhizoids of *Arnoldiella* between the laminal layers of three species of turtles: *Chelydra serpentina* (Common Snapping Turtle), *Kinosternon subrubrum* (Eastern Mud Turtle), and *Trachemys scripta* (Pond Slider). The rhizoids are holdfasts for the algae, usually consisting of more than 50 cells in contact with the lamellae, with the rhizoidal mass often exceeding the mass of the erect, green filaments found on the carapace (Proctor, 1958). The algae produce free-swimming gametes with flagella that can swim

several minutes after fusion (Hamilton, 1948), allowing them to move through an aquatic environment and onto the shell of a turtle. Proctor (1958:640) surmised "...[save *Clemmys* and *Apalone*], it is probable that *Basicladia* [now *Arnoldiella*] occurs beneath the laminal layers, free from the competition of most other algae, on nearly all mature North American freshwater turtles."

The presence of the algae *A. chelonum* on turtle carapaces in both arid and mesic habitats is indicative of its widespread distribution. We postulate that *K. flavescens* in the Chihuahuan Desert has evolved toward a terrestrial, arid land lifestyle, taking advantage of temporary pools for hydration and nutrition. We further suggest the presence of artificial, long-term water impoundments, coupled with the ability of *A. chelonum* to 1) adhere and penetrate between carapacial laminae of kinosternids, and 2) resist prolonged desiccation and darkness (Proctor, 1958), make *K. flavescens* a vulnerable host to *A. chelonum*. What is unclear is the cause of the seemingly unique progression of this undescribed degenerative shell sloughing condition to complete loss of the scute in *K. flavescens*.

Our evidence supports the concept that when some Chihuahuan Desert *K. flavescens* assume long-term residence (more than 2 mo for multiple years) in earthen cattle tanks with no natural basking structures, *A. chelonum* attaches to the sun-exposed portions of the soft, newly forming, growing dorsolateral scutes of the carapace and enters normal microscopic pores. We propose that replicating algae enlarge those pores and, after 2–5 yr, begin lateral invasion between the earlier deposited annual lamellae of the scale. Most turtles in the present study were associated with permanent earthen cattle tanks, and we suspect that *K. flavescens* that depend solely on ephemeral water source would not acquire the CSDP because they would lack the persistent exposure to water required for the colonization of algae.

Biopsies from live turtles demonstrated the absence of an inflammatory response that would otherwise involve melano-macrophages forming focal centers in the hepatic sinusoids and spleen of *K. flavescens* (Christiansen et al., 1996) as well as secondary aggregations around infections and some parasitic infestations (Rund et al., 1998; Johnson et al., 1999; Christiansen et al., 2005). The lack of an inflammatory response is consistent with a lack of invasion of living tissue by the algae, or any other organisms, and distinguishes the CSDP from other turtle shell diseases with the possible exceptions of the conditions described by Jacobson et al. (1994) and Hernandez-Divers et al. (2009). Unlike most of the known turtle shell diseases, the process described herein does not involve inflammation, pigment changes, ulceration, or any damage to the plastron. Algae-associated pits on the carapace and plastron of *C. picta* in Pennsylvania were described and photographed by Ernst (1971), although the illustrated pits appear to be isolated, deeply penetrating pits with the surrounding shell lacking the soft, lumpy disruption typical of the condition described herein. The carapacial pitting reported by Carpenter (1956) in *Terrapene carolina triungis* (Three-Toed Box Turtle) was primarily restricted posterior to and along the suture between the second and third costal scutes.

Our gross morphological observations showed the algae projecting from the pores in Stage 1 and as the green tint under the scale of Stage 2 turtles. The association of the algae with Stage 1 pores and between the laminae adjacent to the raised edges of Stage 2 pores was confirmed in a biopsy passing adjacent to a pore. The biopsy displayed algae growing laterally

deep within the shell, seemingly having gained access to the layers of scale through enlarged pores. While some scale fragments remained around the pore at the surface, most of the superficial scale was lost along the edge of the pore, with only masses of filamentous algae remaining. At this point on the shell, algae do not appear to have penetrated as far as the living epidermis, and there is no resulting inflammatory response. The normal weights, activity, and response to stimuli of turtles with all stages of this disease suggest that the algae are not producing systemic disease, even when bone has become exposed. We have not studied the exposed bone surface to determine whether any vascular dermis remains or whether a periosteum remains. The loss of the scale portion of the shell will subject the turtle to less protection from predators and to increased desiccation, both critical to its long-term survival.

Males tend to attain disease process stages ∼1 yr later than do females. Turtles under the age of 5 yr rarely have clearly defined stages of the disease process, although they may have an occasional enlarged pore or two which may be a precursor of this process. There was a 100% prevalence of identifiable disease processes in earthen cattle tank-dwelling turtles that were older than 13 yr. Because new annulae on plastral scutes of turtles over age of 12 yr are often not visible, many of the turtles with the most advanced stages of the disease process are likely much older than indicated.

A condition similar to our observed CSDP was reported by Hernandez-Divers et al. (2009) for *Graptemys* spp. (Map Turtles) where they found a noninflammatory disease associated with algae and scute erosion. Diatoms and filamentous algae were found only on the shell, with mats of fungal spores and presumably bacteria located between the lamellae (Hernandez-Divers et al., 2009). In our study, the green filamentous algae, *A. chelonum*, was present in pores in the earliest stages of the disease process and between the lamellae (under layers of scale) adjacent to the pores as the disease process progressed. Ultimately, invading *A. chelonum* produced softened, raised, dark green lesions in later stages, but we found few fungal spores among the algae invading the scute lamellae. While we found no pathologies of the plastron in *K. flavescens*, Hernandez-Divers et al. (2009) observed lesions on the plastron as well as on the marginal, costal, and vertebral scutes of the carapace in all four *Graptemys* species. As would be expected from lesions that are strongly associated with green algae, lesions were absent on the shell regions of *K. flavescens* that were not exposed to sunlight in our study, including the entire plastron. In our study, the lesions in all stages were on the more sun-exposed costal, vertebral, and marginal scutes of the carapace. We suspect that the carapace lesions found in Hernandez-Divers et al. (2009), which appear to show enlarged pores with slightly raised pore edges, may have an etiology similar to ours. However, we are unclear as to whether these lesions were biopsied or whether their biopsies were focused on the seemingly more erosive lesions.

We found *K. flavescens* that showed CSDP in specimens that were collected throughout its range in Texas and in specimens that were collected as early as 1891. While biopsies obtained from fluid-preserved specimens were inadequate for study, patterns of gross morphological damage to the shell enabled us to clearly diagnose and characterize CSDP in museum specimens. To determine the distribution of CSDP and its potential environmental causes will require, in part, the examination of various kinosternid species that are maintained in various research collections, although we recognize that there are likely to be many aquatic conditions and pathogens that contribute to CSDP in turtles. We encourage other investigators to assess the diseases associated with CSDP in other species of turtles.

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