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First Assessment of Skin pH of Blanding's Turtles and Spotted Turtles

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***Abstract.*—Skin pH is important in maintaining skin health, wound healing, and disease processes. Skin pH has been studied in humans and domesticated animals but studies in wildlife are lacking. Freshwater turtles are vulnerable to various dermatological diseases, so understanding turtle skin pH is important for disease management. We measured the skin pH of Blanding's Turtles (*Emydoidea blandingii*) and Spotted Turtles (*Clemmys guttata*) and skin pH did not differ between species, sex, or over time. Across both species, the mean pH was 6.9 ± 0.43 (range of values, 5.2–8.4 pH). Front limbs were more acidic than back limbs, and the ventral foot was more acidic compared to other parts of the limbs. Skin pH was positively correlated with environmental pH, but in acidic environments, turtle skin pH was more alkaline than environmental pH, while in alkaline environments, turtle skin pH was more acidic than environmental pH. Future research could explore how skin pH influences microbial colonization and disease susceptibility, as well as investigate the presence, function, and seasonal activity of skin glands. Additionally, longitudinal studies could examine how skin chemistry varies across sex, species, and seasonal cycles, offering insights into turtle physiology and conservation.**

Key Words.—dermatology; freshwater turtles; reptiles; species at risk; wetland; *Emydoidea blandingii; Clemmys guttata*

Introduction

Anthropogenic threats to aquatic habitats include water pollution, such as acid rain and roadway run-off containing salts, oil, and lead (Likens and Bormann 1974; Mahaney 1994; Karraker 2007; Collins and Russell 2009). These environmental changes can alter the chemistry of freshwater habitats, potentially affecting the physical and chemical properties of the skin of aquatic and semi-aquatic animals. Skin serves as the primary interface between an animal and its environment. Skin pH plays a critical role in maintaining the structural integrity of the epidermis, defense against pathogens, activating enzymes that sustain the skin-water barrier, maintaining lipid function, and promoting wound healing (Elias 2005, 2007). It may also influence disease progression and transmission and how animals respond to treatment (Chikakane and Takahashi 1995; Runeman et al. 2000; Matousek and Campbell 2002; Kuo et al. 2020).

The skin pH of vertebrates is typically 5–8 (where pH below 7.0 are more acidic and pH above 7.0 are more alkaline) and varies with season, age, sex, body part, and species (e.g., Meyer and Neurand 1991; Chikakane and Takahashi 1995; Matousek and Campbell 2002; Vanderwolf et al. 2021; Nkwonta et al. 2025). Surrounding environmental pH can affect skin pH. For instance, Mangrove Killifish (*Rivulus marmoratus*) maintain a skin pH of about 7.2, which is lower than the surrounding water (about 7.9–8.2) through ammonia (NH_3) volatilization at the skin surface (Litwiller et al. 2006). When exposed to air, skin pH increased (about 5.8–7.5), likely due to altered release rates of CO2, $HCO3^-$, H^+, and NH3 (Litwiller et al. 2006). Similarly, Leopard Frogs (*Lithobates pipiens*) may buffer their skin pH against acidification by releasing NaHCO3 from their skin (Friedman et al. 1967). Semi-aquatic species, such as turtles, may also buffer their skin chemistry against environmental effects.

Turtles are susceptible to multiple skin diseases (Norton 2005) and have low survivorship in early life stages (Congdon et al. 1993; Litzgus and Brooks 1998). The most common macroscopic skin lesions in European Pond Turtles (*Emys orbicularis*) are

abscesses and dermatitis, regenerative changes, cutaneous ulcerative disease, keratin layer abruption, blepharoconjunctivitis, and pododermatitis (Aleksić-Kovačević et al. 2013). Shell diseases, which are unique to turtles, vary in prevalence among species (Lovich et al. 1996; Aleksić-Kovačević et al. 2013). Infectious diseases, such as ranavirus (Johnson et al. 2008; Carstairs 2019; McKenzie et al. 2019) and fungal infections (e.g., *Emydomyces testavorans*, Woodburn et al. 2019; Woodburn et al. 2021; Fredrickson et al. 2024), may impact turtles, further complicating conservation efforts.

The skin pH of freshwater turtles has not been studied, and understanding the role skin chemistry plays in disease susceptibility, treatment response, and general dermatological health could be important to the health of turtles (Kuo et al. 2020). Antimicrobial peptides, part of the defense system of skin, are present in turtles (Lorenzo 2013) and are pH dependent in other species. For example, in Tiger Salamanders (*Ambystoma tigrinum*), antimicrobial activity against the fungal pathogen *Batrachochytrium dendrobatidis* was significantly reduced above pH 6.4 and nearly absent at pH 6.7, indicating optimal activity at more acidic conditions (Sheafor et al. 2008). This pattern also occurred in North American Bullfrogs (*Rana catesbeiana*), but results varied by population (Krynak et al. 2015). Water pH also influences the skin microbial community structure of frogs, which may affect disease susceptibility (Krynak et al. 2015). Therefore, investigating turtle skin chemistry may yield valuable epidemiological insights for managing endangered species.

Our primary objective was to conduct the first quantitative assessment of skin pH in Blanding's Turtles (*Emydoidea blandingii*) and Spotted Turtles (*Clemmys guttata*), both of which are listed as Endangered in Canada under the Species at Risk Act (https://laws-lois.justice.gc.ca/eng/acts/S-15.3/.). We hypothesized that skin pH would differ by species, sex, body part, and over time, consistent with patterns observed in other vertebrates. We predicted that turtle skin pH would be positively correlated with environmental pH over time, and that this impact would not differ by species, sex, or body part.

Materials and Methods

We conducted fieldwork in the summer of 2024 in the Georgian Bay Mnidoo Gamii Biosphere in central Ontario, Canada. The Georgian Bay Biosphere is along the eastern shoreline of Georgian Bay, Lake Huron, and contains a unique variety of habitats such as mixed-wood forests, rivers, exposed bedrock, wetlands (e.g., bogs, fens), and lakes (Crins et al. 2009; Wester et al. 2018).

We radio tracked *E. blandingii* and *C. guttata* as part of a separate study, which allowed us to monitor temporal variability of skin pH among individuals. We radio tracked six adult *E. blandingii* (two males, four females) and five adult *C. guttata* (two males, three female) between 15 July and 21 August 2024. We tracked turtles up to three times across the six-week period (recaptured at least 4 d apart) to measure skin and environmental pH.

We measured skin pH using a non-invasive pH meter (PH905, 12 mm measuring surface; Courage and Khazaka Electronic GmbH, Köln, Germany) attached to a multiprobe adapter system (MPA2; Courage and Khazaka Electronic). We quantified dorsal and ventral skin pH of each individual by collecting triplicate measurements (subsequently averaged) at three locations on both front and rear limbs (Fig. 1). Upon capture, if the skin was covered in sediment, we gently placed the turtles under the water to ensure the skin was clear. The probe was very sensitive, and variation in the position of the probe relative to the skin produced variation in the measured values. We remeasured pH if triplicate measurements differed by > 0.2 pH. We stored the end of the skin pH probe in KOH and washed it in distilled water between each set of measurements, as recommended by the manufacturer. We calibrated the pH probe once a week with 4.0 and 7.0 pH buffers, exceeding the pH meter recommendation of calibration every three weeks by the manufacturer. We also measured the pH of the environment (i.e., water or *Sphangum* moss) in which we found each turtle using a pH meter (ExStik EC500 Meter; Extech, Teledyne FLIR Commercial Systems Inc., Wilsonville, Oregon, USA, and 3500 pH Meter; Reed Instruments, Newmarket, Ontario, Canada). If we captured a turtle in an aquatic habitat (i.e., peatland or marsh) we measured the pH of the water the turtle was in. If we captured a turtle in a peatland with no nearby patches of open water, we measured the pH of the *Sphagnum* moss. If we found a turtle on land (i.e., road), we did not take an associated environmental pH measurement. We also calibrated the environmental pH meter weekly with 4.0 and 7.0 pH buffers, following the instructions of the manufacturer.

Data analysis.—We used R v4.4.2 to conduct all statistical analyses (R Core Team 2024) and

Figure 1. The location of three skin pH measurements on ventral rear limb (left; Spotted Turtle, *Clemmys guttata*) and three skin pH measurements on dorsal rear limb (right; Blanding's Turtle, *Emydoidea blandingii*), as indicated by the circled numbers. We measured the skin pH of all four limbs with six measurements per limb (three per view). (Left photograph by Rachel Fallas and right by Serina Tourangeau).

constructed all graphs using ggplot2 (Wickham 2016). We used Linear Mixed-effects Models with package lme4 (Bates et al. 2015) to determine which variables were related to skin pH. We set individual identification as a random effect in each model. We inspected the residuals of each model to check that they met assumptions of normality and homoskedasticity, and we confirmed the data met all parametric assumptions. We checked for multicollinearity among variables within models also by examining paired plots. The first model tested whether skin pH varied with body part. In this model, fixed effects comprised view (dorsal/ventral), body side (right/left), limb position (front or back of turtle), limb part (close to shell, middle, and foot), and the interaction of view and limb part. The second model tested whether skin pH varied with species, sex, day of year, environmental pH, and whether there was an interaction between species and sex, or between species and environmental pH. We used the drop1 function in R to determine the effect of each variable and we performed Tukey's *post hoc* tests on significant variables.

Results

We measured the skin pH of 11 adult turtles (combining species), nine of which we measured three times. Of these nine turtles, five were *E. blandingii* (three females, two males) and four were *C. guttata* (two females, two males). We measured the remaining two turtles (one female *C. guttata* and one female *E. blandingii*) once each. In total, we measured skin pH 16 times for *E. blandingii* (six unique individuals) and 13 times for *C. guttata* (five unique individuals). We primarily found turtles in wetland habitats such as peatlands (i.e., bog or fen) and marshes. We encountered one turtle on the road.

Across both species, mean turtle skin pH was 6.9 ± 0.43 (± standard deviation; range of values, 5.2–8.4 pH), with a mean skin pH of 7.0 ± 0.39 for Blanding's turtles (range 5.7–8.4 pH) and 6.8 ± 0.46 for Spotted turtles (range of values, 5.2–8.1 pH). Mean pH of *Sphagnum* moss was 5.1 ± 0.12 (range of values, 5.0–5.2 pH), peatland water was 6.6 ± 0.67 (range of values, 5.2–7.7 pH, n = 30 measurements), and marsh water was 6.1 ± 0.40 (range of values, 5.5–6.9 pH, n = 30 measurements), with an overall mean environmental pH of 6.3 ± 0.68 (range of values, 5.0–7.7 pH).

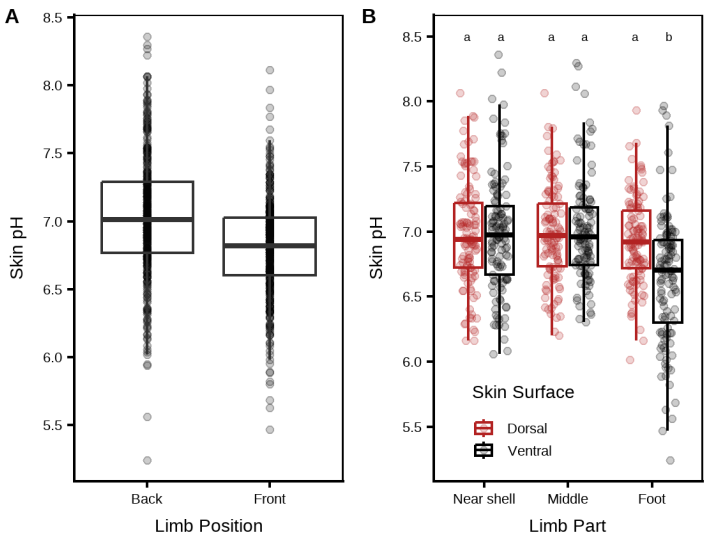

Figure 2. The skin pH of four Spotted Turtles (*Clemmys guttata*) and five Blanding's Turtles (*Emydoidea blandingii*) on different parts of the limbs measured in Ontario, Canada. Skin pH on (A) back and front limbs and (B) the interaction of the dorsal and ventral surfaces with limb part. Box plots in (B) with different letters above them indicate a significant difference at $\alpha = 0.05$, which shows the ventral foot was more acidic than other body parts. Points represent the raw data. The horizontal line inside the box represents the middle value of the dataset. The rectangle spans the middle 50% of the dataset (interquartile range). The lines extending outside the box show the spread of the remaining data (1.5 times the interquartile range).

The skin pH of the left limbs did not differ from the right limbs ($F_{1,669.1} = 0.02$, $P = 0.882$), but the front limbs were more acidic than the back limbs ($F_{1,670.1} = 91.3$, $P < 0.001$; Fig. 2). There was an interaction between limb part (shell, middle, foot) and view (dorsal/ventral; Fig. 1), as the ventral foot was more acidic than other body parts ($F_{2,670.0} = 16.6$, $P < 0.001$; Fig. 2). Overall, skin pH increased by 0.17 for every 1 unit increase in environmental pH ($F_{1,516.6} = 43.9$, $P < 0.001$; Fig. 3), however, in acidic environments, turtle skin pH was more alkaline than environmental pH, while in alkaline environments, turtle skin pH was more acidic than environmental pH (Fig. 3). There was no interaction between species and sex ($F_{1,5.9} = 0.43$, $P = 0.537$), no effect of sex ($F_{1,6.8} = 2.89$, $P = 0.134$), no effect of day of year ($F_{1,513.2} = 2.56$, $P = 0.110$), no interaction between species and environmental pH ($F_{1,504.8} = 1.53$, $P = 0.217$), and no effect of species on skin pH ($F_{1,7.8} = 0.33$, $P = 0.581$). Some individual turtles did not have consistent skin pH at each time point (Fig. 4).

Discussion

Turtle skin pH overlapped with the skin pH range of humans and both domestic and wild animals, although it was generally more alkaline (Meyer and Neurand 1991; Chikakane and Takahashi 1995; Matousek and Campbell 2002; Vanderwolf et al. 2021). The variation in skin pH we documented among individuals is similar to other taxa (e.g., bats; Vanderwolf et al. 2021). Unlike findings in other taxa, we observed no differences in skin pH between sexes or across the study period, although we note our sample size was small. The absence of temporal variation is likely due to the short sampling window of 38 d and the fact that sampling occurred during the post-nesting season, when turtle movements typically decrease (Rasmussen and Litzgus 2010; Millar and Blouin-Demers 2011). Differences in skin pH between sexes is not consistent across taxa (e.g., mammals) as previous studies found all possible outcomes: no difference, males are more acidic than females, and females are more acidic than males (e.g., Meyer and Neurand 1991; Chikakane and Takahashi 1995; Matousek and Campbell 2002; Vanderwolf et al. 2021). It is unclear what behavioral or physiological differences drive sex differences in skin pH and how these patterns change across taxa. *Emydoidea blandingii* and *C. guttata* are mainly active from March to October/November, exhibiting seasonally variable habitat use and movement patterns (Rasmussen and Litzgus 2010; Millar and Blouin-Demers 2011; Markle and Chow-Fraser 2014), which may influence exposure to diverse habitats and, in turn, affect skin pH.

Although our results show turtle skin pH is influenced by environmental pH, turtles can maintain a skin pH that differs from environmental pH. All individuals in this study were located within the same

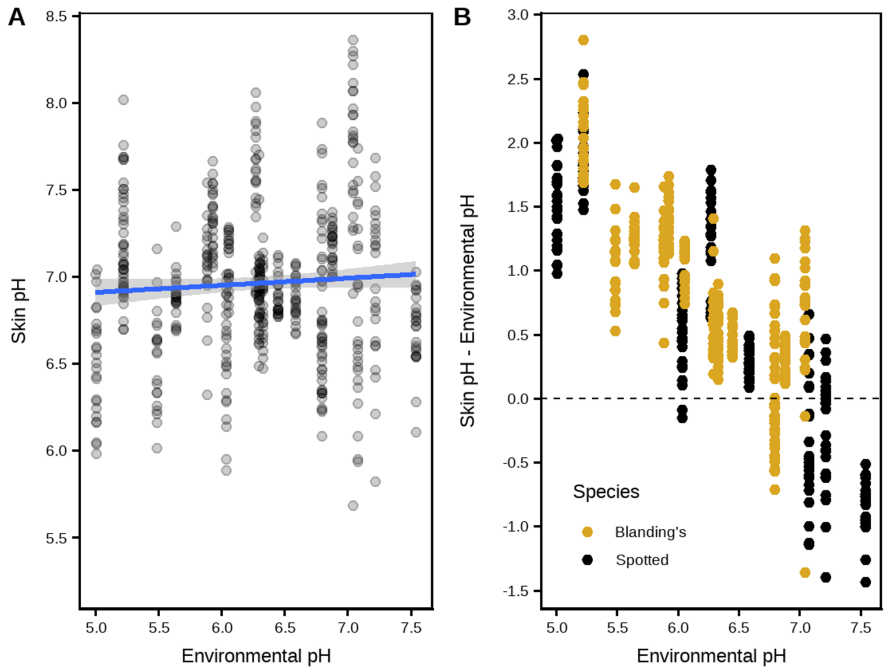

Figure 3. (A) Turtle skin pH of four Spotted Turtles (*Clemmys guttata*) and five Blanding's Turtles (*Emydoidea blandingii*) and paired environmental pH measured in Ontario, Canada. The blue line is the fitted trend line and the grey shading shows the 95% confidence interval. (B) The difference between skin pH and environmental pH by environmental pH. The dashed line indicates where skin and environmental pH are identical.

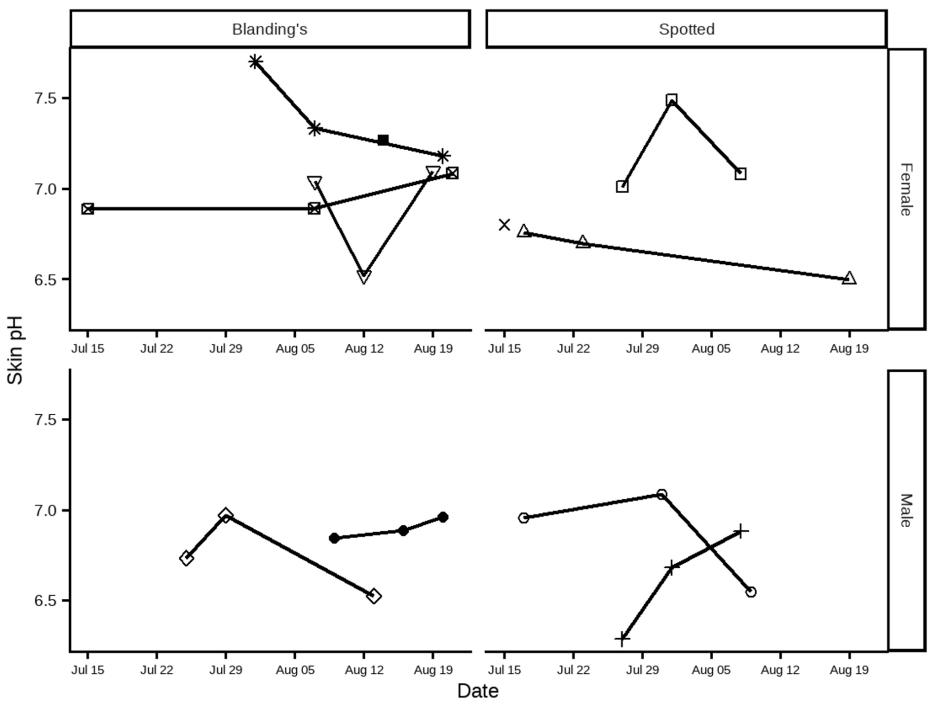

Figure 4. Temporal variation in female and male turtle skin pH over three different time points for nine individuals (represented by lines connecting points), and once for two individuals (represented by the single point). Each shape represents an individual Blanding's Turtle (*Emydoidea blandingii*) or Spotted Turtle (*Clemmys guttata*). Points represent the mean of all measurements taken per turtle (as depicted in Figure 1).

broader study area; although *E. blandingii* and *C. guttata* did not occupy the same wetlands, they did use similar wetland types (i.e., marsh, peatland) and microhabitats (e.g., moss hummock, sedge hummock, open water). Individuals of both species that used more acidic microhabitats, such as *Sphagnum* moss hummocks (pH range of values, 5.0–5.2), had more alkaline skin pH than the environment, whereas turtles that used more alkaline microhabitats, such as open water patches in marshes (pH range of values, 5.5–6.9), maintained more acidic skin pH than the environment. Turtles appear less affected by environmental pH (0.17 unit increase in skin pH per 1-unit increase in environmental pH) compared to *Lithobates pipiens* (0.37 unit increase), but more affected than *L. clamitans* (0.12 increase; Nkwonta et al. 2025). In frogs, diffusion of CO2 via the epidermis leads to acidification, and glandular secretions leads to alkalinization (Friedman et al. 1967). The presence of similar regulatory mechanisms in turtles has not yet been described.

Consistent with patterns observed in other vertebrates, turtle skin pH varied among body parts (Meyer and Neurand 1991; Chikakane and Takahashi 1995; Matousek and Campbell 2002; Vanderwolf et al. 2021). Differences in skin pH across turtle limb parts may reflect variable environmental exposure or intrinsic factors such as skin structure and gland distribution. We found the ventral foot was most acidic, but the foot pads of mammals are usually neutral to slightly alkaline, potentially due to the presence of eccrine glands (Meyer and Neurand 1991). Given that freshwater turtles have fewer glands in their skin compared to mammals (McArthur et al. 2004), the mechanisms driving pH differences may differ. The ventral foot is also the body part in contact with the ground during terrestrial movement, which could affect skin pH. Although we focused on skin pH, the pH of the shell may also be of importance to diseases but the biology and epidemiology of pathogens such as *Emydomyces testavorans* are largely unknown (Adamovicz et al. 2020).

Freshwater turtles are vulnerable to various skin diseases (Norton 2005) and some, such as those caused by *Salmonella* spp. and ranavirus, can be transmitted via direct skin contact or contaminated water (Pees et al. 2013; Brenes et al. 2014; Carstairs 2019). The role of skin chemistry, including pH, in pathogen defense and maintenance of health remains unstudied in turtles. Limited information exists on the biodiversity and functionality of turtle skin microbiomes (McKnight et al. 2020). Future research could explore how skin pH influences skin microbiomes and disease susceptibility, as well as investigate the presence, function, and seasonal activity of skin glands. Additionally, longitudinal studies could examine how skin chemistry varies across sex, species, and seasonal cycles, offering insights into turtle physiology and conservation.

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