

Healing of Skin Biopsies in Wild Juvenile Green Turtles, *Chelonia mydas*

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ABSTRACT. – Skin biopsies are routinely collected from sea turtles for a variety of molecular analyses; however, almost no information exists on the natural healing rates at the site of the biopsy. Here, we monitored the healing rates of 17 juvenile green turtles (*Chelonia mydas*) for up to 488 d after we took a 6-mm biopsy tissue sample in Eleuthera, The Bahamas. Although scarring persisted for several months, biopsy sampling had a minimal long-term impact—tissue repair and maturation was observed after a year and a half, and there was no evidence of infection at any point during the healing process.

Skin biopsies are routinely collected from sea turtles for a variety of molecular assays, such as stable isotope analysis, heavy metal analysis, or genetic analysis (Dutton and Balazs 1995; Robinson et al. 2016; van der Zee et al. 2019). While these tools can provide valuable ecological insights, skin biopsy collection remains an inherently invasive process. There is also a possibility that the biopsy sites could become infected or leave permanent scarring (Swaim et al. 2001; Gurtner et al. 2015). Nevertheless, there is almost no information available on the healing rates from skin biopsies for sea turtles and especially for individuals in the wild.

Wound healing of cutaneous lesions in reptiles generally involves reconstruction of tissue by collagen synthesis and contraction of cellular matrix deposits, re-epithelization, and the disappearance of inflammatory markers (Smith et al. 1988; Keller et al. 2014; Negrini et al. 2016). Cutaneous healing in turtles is affected by ambient temperatures as well as differences in tissue formation because turtles do not have cutaneous muscles or beds of granulation tissue, and turtle skin tissues are less

mobile (Maderson and Roth 1972; Smith et al. 1988; Keller et al. 2014; Negrini et al. 2016, 2017). Notably, cutaneous healing has been found to be slower in reptiles than in mammals, and fresh water turtle wounds take longer to heal than has been observed for snakes and lizards (Negrini et al. 2016). Indeed, Negrini et al. monitored cutaneous biopsy healing in *Trachemys scripta elegans* and found that re-epithelization was completed 14 d postbiopsy, but new tissue differentiation was not present until 28–42 d after sampling. Inflammation of the biopsy site was recorded up to 28 d after tissue collection and clinical wound healing was completed by 42 d postbiopsy. The data indicate that turtles take longer to heal than other reptiles, so this may also make them more susceptible to infection (Negrini et al. 2017), although this healing time can be reduced at higher temperatures (Brattstrom 1965; Monagas and Gatten 1983; Smith et al. 1988).

We captured juvenile green turtles (*Chelonia mydas*) in southern Eleuthera, The Bahamas, between August and December of 2019. Turtles were captured using a modified “rodeo” technique as described in Ehrhart and Ogren (1999). Upon capture, turtles were tagged, weighed, measured, and visually assessed for injuries or abnormalities. We collected skin samples from 68 juvenile green turtles (42.0–63.5-cm curved carapace length) using a 6-mm-diameter biopsy punch (Integra™ Miltex®). We sterilized the collection site on the left or right shoulder using alcohol wipes before sampling. We then inserted the biopsy punch to a depth of 1–2 mm to remove just the dermal tissue (Fig. 1). To avoid pulling the dermal tissue with force from the underlying base, we held the dermal skin from the biopsy with forceps and used the biopsy punch to cut horizontally through the tissue to extract the sample. Immediately following sample collection, we cleaned the area with Betadine wipes. If bleeding occurred, we applied pressure with sterile gauze until bleeding stopped. The monthly average water temperature during the study period was 26°C–29°C (Weather Atlas).

Of the original 68 turtles, 17 were recaptured opportunistically over the next year and a half. Unfortunately, there was a 9-mo gap in sampling between March 2020 and December 2020 due to fieldwork restrictions associated to COVID-19. Ten of the 17 recaptured turtles were recaptured once, 3 were captured twice, 2 were captured three times, 1 was captured four times, and 1 individual was captured five times. Upon each recapture, we photographed the biopsy site (Fig. 1) and categorized the stage of healing, using six categorical phases, each time an individual was recaptured (Table 1).

We encountered 5 individuals in Phase 1 (Open, Healing). Turtles in Phase 1 were encountered between 7 and 9 d postbiopsy sampling, and all exhibited rapid healing, already possessing formed granulation tissue

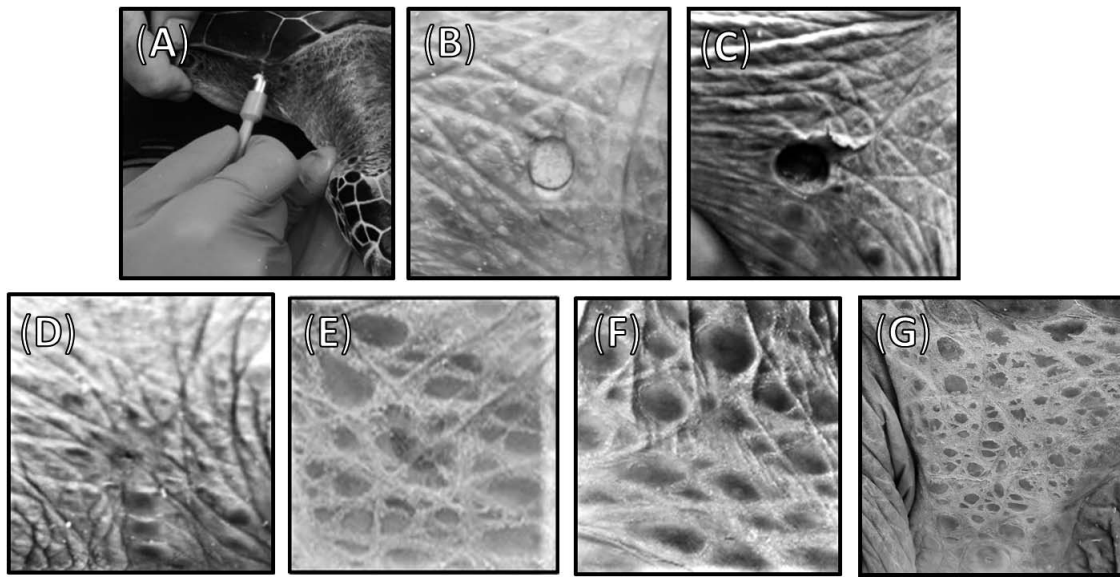


Figure 1. (A) Tissue collection site on shoulder of juvenile green turtle. (B) Biopsy site immediately after tissue collection. (C–G) Representative photos of days since biopsy sampling and the phase of healing: (C) Phase 1, 9 d; (D) Phase 2, 28 d; (E) Phase 3, 50 d; (F) Phase 4, 91 d; (G) Phase 5, 488 d. Photos taken by Laura St. Andrews (A–F) and Liberty Boyd (G).

(Fig. 2). Two individuals were encountered in Phase 2 (Closed, Indented). Turtles in Phase 2 were recaptured between 19 and 28 d. We observed Phase 3 (Closed, Discolored) in 5 turtles between 29 and 77 d postbiopsy. Phase 4 (Healed, Visible) was observed for 8 individuals between 67 and 176 d after tissue collection. Thus, even though superficial damage caused by biopsies is healed within a few weeks, external disfiguration can last for many months. After 176 d, no turtles had reached Phase 5; however, sampling was not possible for 9 mo. When COVID-19 field restrictions lifted, Phase 5 (Healed, Indistinguishable) was observed in 5 individuals that were captured between 455 and 488 d postbiopsy. No discernible scars were observed after 455, 468, or 488 d postbiopsy in these individuals. However, our sample size was limited and the days in between sampling do not indicate when Phase 5 is likely to occur, other than between 176 and 455 d. In addition, these are likely overestimations of the time required to reach each phase

because individuals may have reached different phases before they were sampled. Variation in the time to reach each healing phase could be explained by individual differences in age and body composition, nutrient availability, microbial colonies, depth of biopsy, and water temperature (Bennett 1996; Bjorndal 1997; Cailouet et al. 1997).

The impact of biopsy sampling from our finding supports the results of previous studies (Brattstrom 1965; Monagas and Gatten 1983; Smith et al. 1988; Negrini et al. 2016) and provides preliminary data to suggest that this is a relatively safe technique. In our findings, initial healing was rapid, there were no noticeable detrimental effects observed in any turtles, and long-term wounding did not appear. No detectable signs of infection at the biopsy scar site were observed in recaptured individuals at any phase.

Acknowledgments. — Special thanks to The Cape Eleuthera Institute (CEI), Center for Marine Conservation

Table 1. Description of phases of healing in green turtle (*Chelonia mydas*) skin after tissue biopsy collection.

Phase	Biopsy identification	Description
0	Open, Fresh	The wound was open, potentially bleeding, and indiscernible from the initial moment of sampling.
1	Open, Healing	The wound remained open but was partially filled with fibrin or granulation tissue and swelling or redness of the surrounding area was present.
2	Closed, Indented	The wound was closed and covered by a thin layer of regenerated epidermis (re-epithelialized), but a depression from the biopsy site was still visible. The regenerated skin was hypopigmented and lacked the scalation of surrounding area. There was no swelling or redness.
3	Closed, Discolored	The wound was closed, no indentation was visible, and the healed epidermis over the biopsy site was more mature and beginning to resemble the surrounding skin; however, pigmentation remained abnormal.
4	Healed, Visible	The biopsy site was reduced in size and remained visible as focal hypopigmented scar.
5	Healed, Indistinguishable	The biopsy site was no longer distinguishable in color or texture from the neighboring skin.

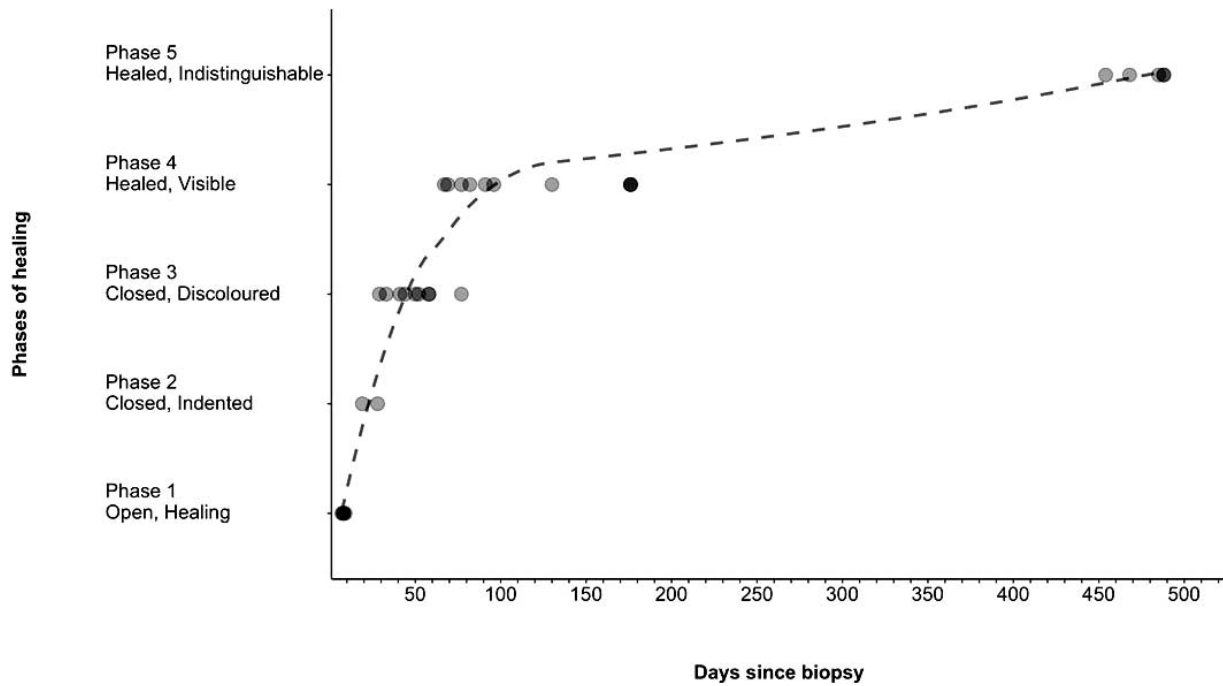


Figure 2. Phases of healing postbiopsy sampling. The grey dots represent all recaptured turtles combined. Darker dots symbolize multiple turtles overlapping. The dashed line shows a loess regression for the number of days to reach each healing phase.

at Purdue University Fort Wayne, and the Schrey Distinguished Professorship for providing the funding and resources for this research. A heartfelt thank you to the CEI fall 2019 interns, Sophie Mills, Sam Tabbutt, Aron Cogger, and Kathryn Waters; CEI adjunct scientist, Annabelle Brooks; and the Island School 2019 research class for the countless hours of fieldwork that helped make this research possible. Research was conducted under Cape Eleuthera Institute permit number MA&MR/FIS/9 and under the Institutional Animal Care and Use Committee and Purdue Animal Care and Use Committee ethics for animal treatment qualification no. STA-1434.

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Received: 2 October 2020

Revised and Accepted: 15 March 2021

Published Online: 8 November 2021

Handling Editor: Jeffrey A. Seminoff