

Salinity Affects Wound Healing in Wild Common Bottlenose Dolphins (*Tursiops truncatus*)

Brianna Hurst* & Dara N. Orbach

Department of Life Sciences, Texas A&M University-Corpus Christi, Corpus Christi, TX

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Student: bburst2@islander.tamucc.edu*

Mentor: dara.orbach@tamucc.edu

ABSTRACT

Dolphins are often individually identified by unique naturally-acquired markings. Identification becomes difficult when markings heal, or new scars appear. As salt accelerates wound healing in many organisms, the diminishment of scars on common bottlenose dolphins (*Tursiops truncatus*) residing in varying natural salinities was determined. South Texas contains the only hypersaline lagoon in the USA, located adjacent to hyposaline waters, with genetically distinct populations of dolphins in the two environments. Photographs of dolphin dorsal fins were collected, and scar stability over time was determined and compared by measuring changes in the relative lengths and surfaces of scars. All scars on dolphins in the hypersaline lagoon completely diminished between three to six years, while scars on dolphins in the hyposaline bay ranged in the amount of fading between three to six years. Data from this case study indicate that high salinity may increase the healing speed of wounds on common bottlenose dolphins compared to low salinity, although a larger sample size is needed for robust statistical comparison. Scar diminishment is an important consideration in determining the temporal reliability of photo identification.

KEYWORDS

Bottlenose dolphin; Corpus Christi Bay; healing; hypersaline; Laguna Madre; photo-identification; salinity; scar

INTRODUCTION

Photo-identification, in which animals are identified by individually distinctive markings, pigmentations, notches, or scars captured by cameras, is the most commonly used approach to determine the population abundance, habitat use, behavior, and impacts of human disturbance on cetaceans (whales, dolphins, and porpoises).¹⁻⁵ Small nicks may be acquired through a natural tattering of the dorsal fin, while larger nicks (termed notches) and wounds may be caused by interactions with predators, boats, fishing gear, or conspecifics, among other sources.³ Nicks/notches on the dorsal fin are considered reliable for individual identification of many species of dolphins because of their visibility from each side of the animal in varied lighting conditions.^{4,5} In contrast, a scar is only present on one side of the animal and may diminish over time. As markings may change naturally, either through healing or acquisition of new markings, consistent identification of individuals may have inherent limitations.¹ For example, pigment spots and bite marks usually persist for six months to one year in common bottlenose dolphins (*Tursiops truncatus*).⁶ Changes in markings could skew data if an individual is misidentified, yet few studies have explored the long-term stability of wounds among cetaceans. Determining how long scars remain visible could provide a metric of the reliability of photo identification in changing environmental conditions (e.g., hurricanes, climate change, freshwater run-off).

Measurements of the distance between notches on the dorsal fins of dolphins are a benchmark in re-sighting individuals using photo-identification⁷ and are the basis of several semi-automated fin-matching software (e.g., finFindR, Finbase, Finscan). However, there is no standardized technique to quantify scar characteristics on the dorsal fins of cetaceans. Research on scars has focused on identifying and categorizing potential sources.^{3,4} Small surface wounds, such as tooth rake marks, generally heal faster than large or deep wounds caused by shark bites,⁶ but the conditions of the water may affect the healing process. Thus, observations collected from free-ranging dolphins are potentially valuable for tracking natural wound healing.

The duration that scars persist may vary with salinity. Saline-treated wounds re-epithelialized (covered with tissue) faster than wounds treated in a dry or moist environment among porcine (pigs)⁸ and showed less necrosis, inflammation, and scar formation.⁹ In humans and mice, cutaneous salt storage boosts the activation of macrophages, facilitates pathogen removal, and aids in combating bacterial infections.¹⁰ Epidermal changes associated with low salinity exposure (0-30 ppt) were recorded in common bottlenose dolphins.¹¹ An uptake of low saline water from the environment suggests low blood sodium, blood chloride

ion, and extracellular plasma sodium concentrations.¹² Of the few studies that have explored wounds of dolphins under extreme salinity conditions, only the effects of low salinities have been assessed.

There are few locations globally with naturally high salinity levels above standard seawater (35 ppt) conducive to assessing long-term healing patterns without manipulating environmental conditions. The Laguna Madre, TX, is the only hypersaline lagoon in the USA, with an average salinity of 36 ppt that has periodically doubled the Gulf of Mexico.¹³ The Laguna Madre extends from Corpus Christi south to Port Isabel, TX, and consists primarily of seagrass beds.¹³ Laguna Madre is inhabited by a stock of common bottlenose dolphins that is genetically distinct from the adjacent Corpus Christi/Redfish Bay population, which are both genetically distinct from dolphins inhabiting the Gulf of Mexico.¹³ Corpus Christi/Redfish Bay is often hypoosmotic (average salinities 22-27 ppt).¹³ The South Texas coastline provides a unique opportunity to compare how varying natural salinity levels affect the temporal stability of wounds on common bottlenose dolphin dorsal fins. We present a case study on bottlenose dolphin wound healing in South Texas. It was hypothesized that wound healing would vary significantly between the two locations, with Laguna Madre dolphins predicted to heal more than Corpus Christi/Redfish Bay dolphins.

METHODS AND PROCEDURES

Photographs of common bottlenose dolphin dorsal fins were captured between 2014 and 2020 during boat-based surveys along the Corpus Christi/Redfish Bay and Laguna Madre, TX, coastline. Data were collected under NOAA NMFS permit numbers 18715, 18881, and 23203. Dolphin group sizes, GPS coordinates, salinity levels, and weather conditions were recorded for each group encountered after photographs were collected with a Nikon D3200 or Nikon D7000 camera with 70-300mm or 80-400mm Nikkor lens. Dolphin groups were defined as individuals swimming within 10m and exhibiting the same behavior state.¹⁴ GPS coordinates were recorded with a Garmin GPS map 540s, Lowrance HDS7, or Lowrance HDS5, and salinity levels were recorded with a YSI Pro 20. Upon visually spotting dolphins, the research boat (17ft Carolina Skiff DLX, 23ft Stoner Supercat, or 18ft Inmar 550R-DR) traveled towards the dolphins in a parallel heading at low speed. Dolphins were observed for under 30 minutes, and follows were terminated if dolphins were evasive. Surveys were only conducted during daylight hours in weather conditions deemed safe for small boats (e.g., low winds, no fog, Beaufort Sea State < 3).

A digital catalog of dolphin dorsal fin photographs was established with metadata associated with each image. Photographs of fins were cropped, quality control-checked (in focus, not obstructed, not submerged, distinguishable features present)⁵ and matched for re-sightings by interns at Texas A&M University-Corpus Christi. Fins were matched using FinFindR,¹⁵ a semi-automated dorsal fin matching software, and verified by two researchers. Fins were matched within and across locations (Laguna Madre and Corpus Christi/Redfish Bay). Data were constrained to include dolphins with a minimum of two photographs collected three to six years apart, in which the same side of the dorsal fin (left or right) was photographed.

Wound healing was determined by measuring scars on dorsal fin photographs in ImageJ¹⁶ using two new techniques:

- 1) *Relative scar length*: Photographs were adjusted in brightness and contrast to highlight scars. To standardize measurements, the length of the dorsal fin was measured using the ‘Straight-line’ tool in ImageJ from the most distal tip down the midline of the fin to its base (**Figure 1**); this fin length was calibrated to a scale of 1. The lengths of each scar were subsequently measured and tallied per photograph to calculate the sum of scars. Measurements of the same scars (visible in ‘old’ photographs) were collected for both ‘old’ photographs (2014-2017) and ‘new’ photographs (2020). The percentage change of scars per individual was derived as

$$\frac{\sum \text{old photo scars} - \sum \text{new photo scars}}{\sum \text{old photo scars}} * 100 \tag{Equation 1.}$$

- 2) *Relative scar surface*: Photographs were adjusted for brightness and contrast to highlight scars. To standardize measurements, the one-dimensional surface of the dorsal fin was measured with the ‘Freehand Selections’ tool in ImageJ and calibrated to a scale of 1. Surfaces were traced using a Surface Pro 7 tablet. Each scar was subsequently outlined, and its surface was measured. Scar surfaces were tallied per photograph to calculate the sum (**Figure 2**). Measurements were converted to a ratio:

$$\frac{\sum \text{scars}}{\text{surface of dorsal fin}} * \frac{x}{1} \tag{Equation 2.}$$

Measurements of the same scars were collected for both ‘old’ photographs (2014-2017) and ‘new’ photographs (2020). The percentage change of scars per individual was derived using **Equation 1**.

A t-test¹⁷ was performed to determine if the percent wound healing differed significantly between the two locations with varying natural salinity levels. Qualitative patterns were assessed by plotting the straight length scarring ratios and corresponding salinity levels when each photograph was taken.

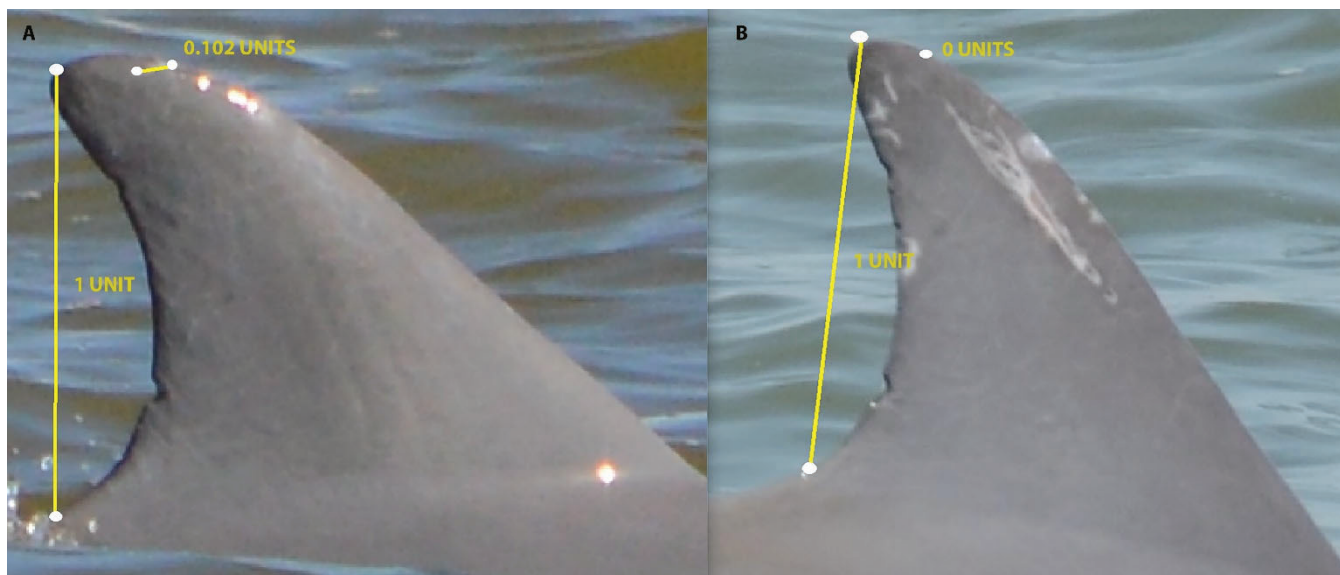


Figure 1: The dorsal fin of one common bottlenose dolphin captured five years apart: A) 'Old' photograph captured December 2015, B) 'New' photograph captured September 2020. White dots indicate the start and endpoints of landmarks. Relative units of measurement are listed. Only scars present in the 'old' photograph were analyzed.

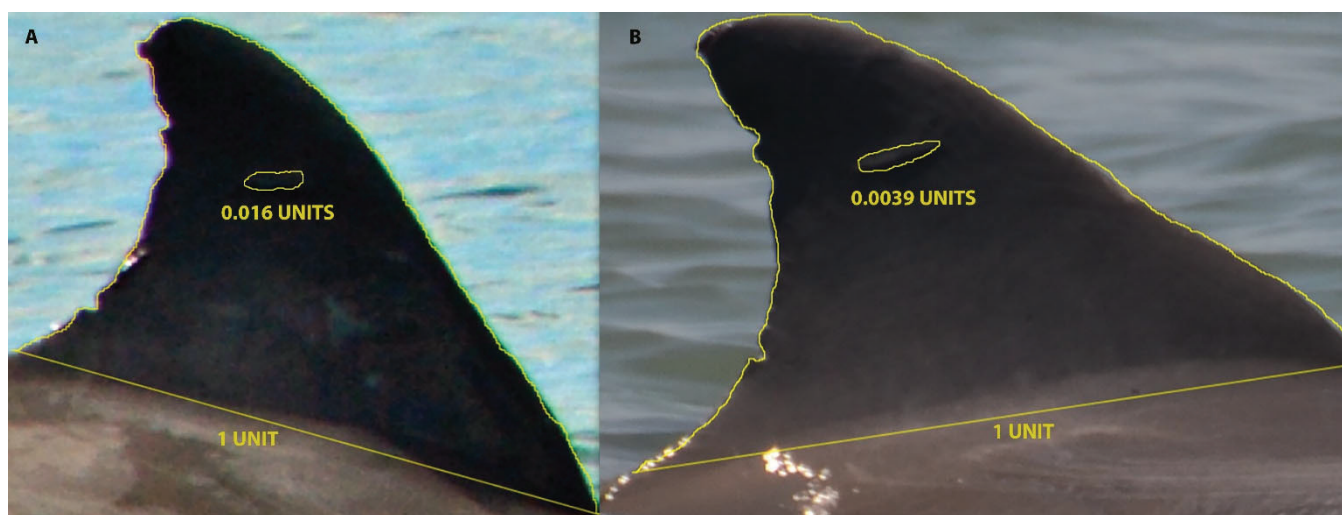


Figure 2: The dorsal fin of one common bottlenose dolphin captured six years apart: A) 'Old' photograph captured October 2014, B) 'New' photograph captured June 2020. Yellow outlines denote surfaces. Relative units of measurement are listed. Only scars present in the 'old' photograph were analyzed.

RESULTS

Between 2014 and 2020, four individuals were re-sighted at least three years apart in Laguna Madre and seven individuals in Corpus Christi/Redfish Bay. Within Corpus Christi/Redfish Bay, one individual was re-sighted on both sides of its dorsal fin during different surveys; each side of the dorsal fin was evaluated independently. LM1 and LM2 (September 2020) and LM3 and LM4 (November 2020) were re-sighted in the same group, respectively, while no other dolphins were initially or subsequently observed in the same group (**Table 1**). There was a 100% change in the length of scars observed within Laguna Madre data; no 'old' photograph scars could be detected in the 'new' photographs (**Figure 3**). In Corpus Christi/Redfish Bay, there was a range of percent changes in relative scar lengths (**Figure 3**). No significant difference was found in the amount of change in relative scar lengths ($t = 1.267$; $p = 0.234$) nor relative scar surfaces ($t = 1.273$; $p = 0.232$) between populations. Both length and surface measurements yielded similar patterns, with neither technique reliably producing higher values (**Table 1**). Within Laguna Madre, but not Corpus Christi/Redfish Bay, salinity levels were consistently higher when dolphins were re-sighted compared to the initial sighting (**Figure 4**; **Table 1**).

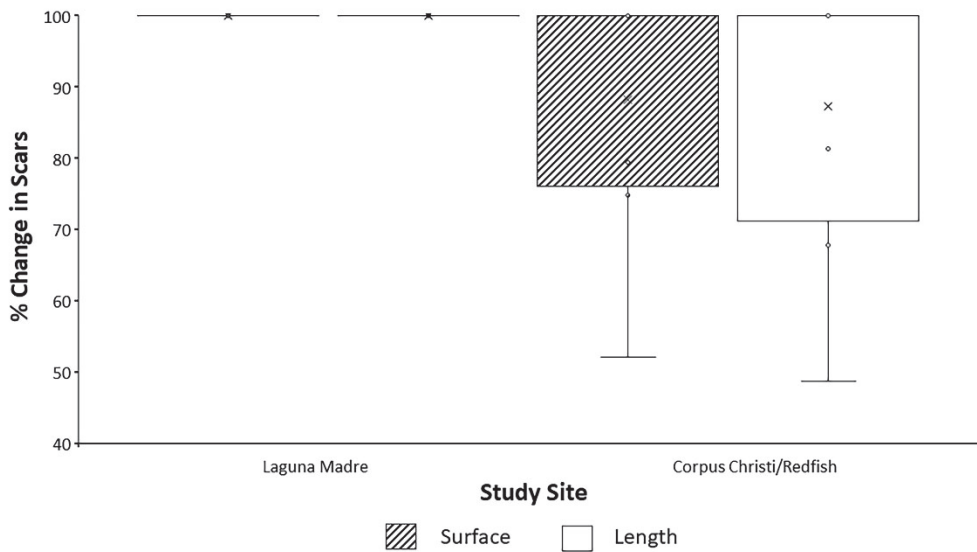


Figure 3: The percent change in cumulative scars for Laguna Madre and Corpus Christi/Redfish Bay populations of dolphins using length and surface measurement techniques. In these box-and-whisker plots, the 'x' represents the mean, and the 'whiskers' depict the maximum and minimum values.

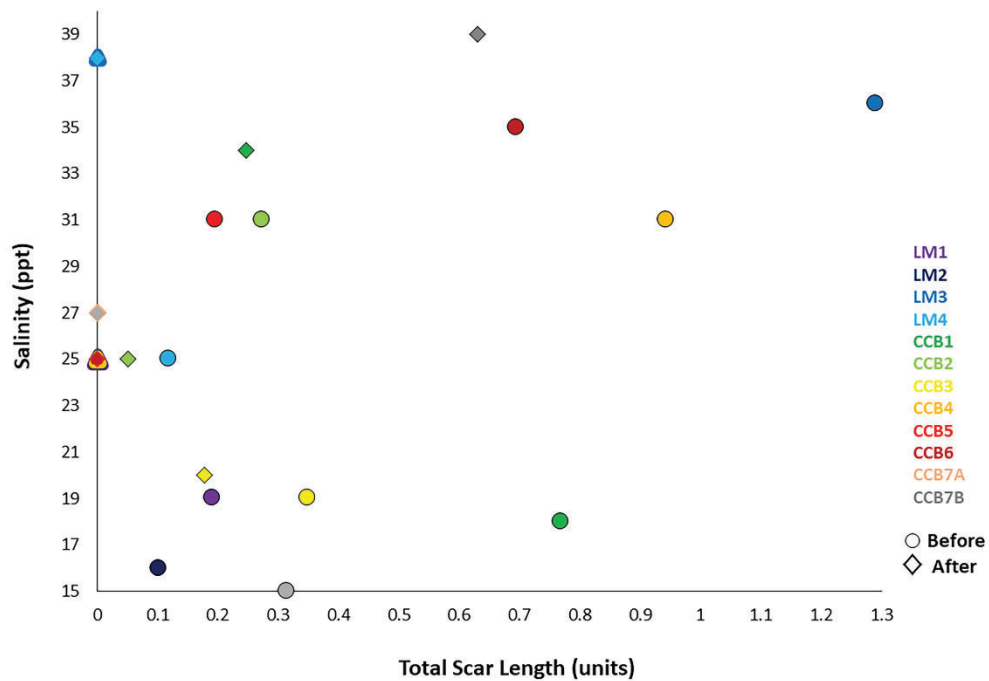


Figure 4: The total relative length of scars on common bottlenose dolphins at different salinities with the 'old' photograph delineated with a circle and the 'new' photograph delineated with a diamond. Each dolphin is designated by a different color. Symbols with multiple colors indicate more than one identical data point.

Individual	Site	Date-Old Photo	Salinity-Old Photo	Date-New Photo	Salinity-New Photo	% Change Linear Method	% Change Surface Area Method
LM1	Laguna Madre	December 2015	19	September 2020	25	100	100
LM2	Laguna Madre	December 2015	16	September 2020	25	100	100
LM3	Laguna Madre	November 2015	36	November 2020	38	100	100
LM4	Laguna Madre	December 2015	25	November 2020	38	100	100
CCB1	Corpus Christi Bay	April 2017	18	June 2020	34	67.88	52.18
CCB2	Corpus Christi Bay	April 2014	31	June 2020	25	81.32	79.46
CCB3	Corpus Christi Bay	October 2014	19	June 2020	20	48.85	74.89
CCB4	Corpus Christi Bay	April 2015	31	August 2020	25	100	100
CCB5	Corpus Christi Bay	April 2015	31	June 2020	25	100	100
CCB6	Corpus Christi Bay	April 2015	35	June 2020	25	100	100
CCB7A	Corpus Christi Bay	February 2017	39	September 2020	27	100	100
CCB7B	Corpus Christi Bay	February 2017	15	September 2020	27	100	100

Table 1. The site location, date, salinity, and percent change in scarring for each common bottlenose dolphin.

DISCUSSION

Our case study provides preliminary data suggesting that high salinities may diminish the scars of common bottlenose dolphins faster than low salinities. Dolphins residing in a hypersaline environment (Laguna Madre) showed a greater amount of scar reduction compared to dolphins in a hyposaline environment (Corpus Christi/Redfish Bay) over three to six years, as predicted, although this pattern was not statistically significant. All dolphins analyzed from Laguna Madre experienced 100% diminishment in scars over time, unlike dolphins from Corpus Christi/Redfish Bay. Dolphins in Laguna Madre were consistently re-sighted in elevated salinities (although initial salinities could sometimes not be hypersaline), whereas dolphins in Corpus Christi/Redfish Bay were re-sighted in variable salinities. As dolphins in Laguna Madre were generally not sighted in the same groups, and as dolphins in Corpus Christi/Redfish Bay were never observed in the same groups, both populations may have fission-fusion societies where individuals inhabit different parts of their ecosystem and are exposed to different salinities at any given time. It is unclear why patterns differed for CCB 1-3 compared to CCB 4-7, although the individuals in Corpus Christi that retained scars for at least three years (CCB1-3) appeared to have comparatively deep wounds that take longer to heal than shallow wounds in a low saline environment. This pattern may explain the range of error bars observed. Accordingly, it appears that salinity may be important in wound healing as found in other species.⁸⁻¹⁰ As Laguna Madre experiences little water turnover due to its shallow depth and connection to few contributing sources of water, fluctuations in the salinity observed in the Laguna Madre may reflect recent rain events that increased freshwater inflow and lowered salinity.¹⁸ A larger sample size is needed to determine if the observed trends are robust as statistics may not be biologically meaningful for a small sample size. Additional environmental parameters like water temperature, contaminate load, and pH could be tested as confounding variables in future studies.

New methods were implemented to measure scars. Both linear and surface measurements yielded similar scar diminishing patterns, suggesting comparable accuracy. A suite of measurements is recommended to standardize assessments of scar patterns on dolphins, including alternative metrics. Affine-invariant grids have been proposed to measure identifiable markings on humpback whales (*Megaptera novaeangliae*).¹⁹ Melanistic leopards (*Panthera pardus*) have been reliably identified using infrared wavelengths to illuminate pigmentations of rosettes.²⁰ In humans, scar healing has been monitored using stimulated infrared thermography, where scars appear colder than peri-scar areas.²¹ Stimulated infrared thermography presents a novel way to follow the progress of treatment for a scar²² and may be applicable for marine mammals if limitations imposed by temperature, water opacity, and environmental factors can be overcome. Dorsal fins can act as thermal windows in infrared thermography.²³ The ability to re-sight a dolphin will vary with wound, scar, and other identifying marks that may change over time. Studies using photo-identification may have varying reliability. Understanding the prevalence, longevity, reliability, and rate of change in markings used to identify individuals are critical as cetacean population studies primarily use long-term photo-identification data.²⁴ Epithelium repair occurs rapidly in bottlenose dolphins because of the exposed dermal papillae of the wound and extensive folding of the germinal layer.⁶ Epidermal sloughing (shedding) is estimated to be 8.5x faster than in humans, occurring 12x daily in bottlenose dolphins;²⁵ however, rates of sloughing may fluctuate with hormonal influence, diurnal effects, temperature, salinity, and response to trauma.²⁵ Constant and gentle irrigation of a wound in water may accelerate tissue repair.⁶

The duration between re-sighting dolphins likely greatly impacts scar diminishment. Three to six years between sightings appears to be too long to reliably assess the speed of wound healing. Instead, one year between sightings is recommended for common bottlenose dolphins, as small wounds such as bite marks and pigment spots usually heal annually.⁶ Recommended durations are species-specific. For example, protruding fat marks can persist for six years, and scrapes can last several decades in Risso's dolphins (*Grampus griseus*).²⁶ Scars were unreliable for individual identification across years in northern bottlenose whales

(*Hyperoodon ampullatus*) compared to notches and back indents that had reduced rates of loss.²⁴ Additional research on small, closed populations of dolphins in which frequent re-sightings are possible can expand the scope of this case study and allow for fine-tuned quantification of the duration for scars to diminish entirely.

CONCLUSION

Data from this case study provide preliminary evidence that salinity may affect the diminishment of scars in bottlenose dolphins, although a larger sample size is needed for robust statistical analysis. As dolphins do not typically reside in hypersaline waters, Laguna Madre provided a unique environment to address questions about natural wound healing. We developed two novel metrics to quantify scars that may be widely applied to other cetacean species or marine fauna. Future studies may be able to apply these metrics in determining sources of scars. The development of additional metrics of scar size and rates of change are warranted for cetaceans as markings are prevalently used for population estimates despite evidence that scars do not appear to be stable over long durations.²⁵

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ABOUT STUDENT AUTHOR

Brianna Hurst graduated in May 2021 from Texas A&M University-Corpus Christi with a Bachelor's in Biology. Brianna completed the research presented as part of her National Science Foundation McNair Scholarship.

PRESS SUMMARY

A case study was conducted on the wound healing of wild common bottlenose dolphins. Two genetically and geographically distinct populations of dolphins reside in South Texas; one population occurs in the only hypersaline lagoon in the USA, while the other occurs in a hyposaline environment, providing a unique opportunity to explore how scars heal under varying natural salinities over six years. Non-invasive photography techniques were used to identify individual dolphins, and novel techniques were implemented to monitor changes in scarring, which can be widely applied to other taxa. All scars diminished among dolphins residing in the hypersaline lagoon while healing was varied in the hyposaline environment, potentially suggesting that elevated salinity augments wound healing. Scars do not appear to be stable over long durations, which has important implications for their prevalent use in photo-identification research on dolphins.