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# The dolphin FRESH protocol: visual Freshwater-Related Evaluation of Skin Health in free-ranging bottlenose dolphins (*Tursiops* spp.)

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Exposure to freshwater is a pressing health issue for coastal bottlenose dolphins (*Tursiops* spp.). Environmental changes, including increased precipitation events and coastal infrastructure projects, are altering salinity within estuarine systems. Consequently, understanding effects of freshwater exposure on dolphins and developing tools to evaluate related health conditions is urgent. To address this need, a group of veterinarians, pathologists, epidemiologists, natural resource managers, and field biologists convened to create a protocol to visually assess freshwater-related skin lesions in free-ranging bottlenose dolphins. The Dolphin FRESH (Freshwater-Related Evaluation of Skin Health) Protocol guides users without medical backgrounds to screen and evaluate photographs by focusing on the visual identification of three primary indicators of freshwater skin disease: Overgrowth, Target-like Lesions, and Light Discoloration. By determining presence of the primary indicators and scoring associated characteristics, FRESH provides users with a relative assessment of the severity of these skin

anomalies, and metrics to track progressive changes. The Scoring Rubric performed well during systematic testing, with evaluators correctly identifying freshwater cases through recognition of primary indicators and with no significant differences in total severity scores between field biologists and medical experts. FRESH is an important step in advancing knowledge on the effects of salinity fluctuations on dolphin health. When applied to photo datasets over time, this tool will enable researchers and managers to evaluate progression and regression of freshwater skin disease, occurrence and effects of multiple exposures, and the relationship between freshwater exposure skin indicators and health and survival outcomes.

#### KEYWORDS

cetacean - marine mammal, discoloration, freshwater (health/environment), lesions, low salinity, pallor, protocol & guidelines, skin disease

## 1 Introduction

### 1.1 Background

Low salinity exposure due to increasing freshwater inflow, extreme weather events, and altered hydrodynamics has emerged as a critical health issue for bottlenose dolphins (*Tursiops* spp.) residing in coastal habitats. Precipitation intensity and frequency, resulting in nearshore low-salinity events, are increasing due to changing global weather patterns (Easterling et al., 2000; Knutson et al., 2010; National Oceanic and Atmospheric Administration Office of Protected Resources, 2022; Sunkara et al., 2023). Furthermore, planned coastal development and infrastructure projects may alter salinity within estuarine systems where dolphins reside (White et al., 2018; US Army Corps of Engineers, 2021). Consequently, there is an urgent need to better understand the effects of freshwater exposure on dolphins and develop non-invasive tools to evaluate freshwater-associated health conditions.

Coastal bottlenose dolphins have adapted to thrive in water with salinity levels ranging from 18–35 ppt (Ewing et al., 2017; Hayes et al., 2022). Nevertheless, estuarine dolphins are known to utilize low salinity habitats, such as rivers and brackish inlets (Mullin et al., 2015; Hornsby et al., 2017; Mazzoil et al., 2020; Takeshita et al., 2021). Within these estuarine habitats, dolphins have been documented to maintain preferences for salinity levels greater than ~8–11 ppt (Hornsby et al., 2017; Mintzer and Fazioli, 2021). They can survive lower salinity conditions with minimal health effects for short periods of time, but extended exposure will eventually lead to negative health consequences (Ewing et al., 2017; Hornsby et al., 2017; Booth and Thomas, 2021). Moreover, in contrast to gradual seasonal fluctuations in salinity, sudden freshwater input can have detrimental health effects when individuals do not have time to physiologically acclimate

(Carmichael et al., 2012; Duignan et al., 2020). Heavy precipitation events and coastal development/infrastructure projects are an increasing source of these sudden changes to dolphin habitats, reinforcing the need to understand the potential impacts of these types of events.

Physiological responses by dolphins to freshwater, referred to as “freshwater disease” or “freshwater skin disease” (FSD), include altered blood chemistry and electrolyte imbalances; development of skin lesions and subsequent secondary bacterial and fungal infections; and/or algal overgrowth, corneal edema, cerebral edema, and death (Mase-Guthrie et al., 2005; Ridgway and Venn-Watson, 2010; Carmichael et al., 2012; Ewing et al., 2017; Deming et al., 2020; Duignan et al., 2020; McClain et al., 2020). Skin lesions develop due to osmotic stress to the epidermis resulting in hydropic degeneration of epithelial cells progressing to necrosis and ulceration (Duignan et al., 2020). Field observations have demonstrated that some individuals exposed to freshwater can recover if salinity increases, yet time duration and salinity level thresholds for physiological response and recovery have not been defined (Deming et al., 2020; Fazioli and Mintzer, 2020; McClain et al., 2020).

Much of the knowledge regarding physiological response to freshwater has been informed by case studies in which dolphins could be assessed in-hand (e.g., stranding events or dedicated capture-release health assessment efforts) or by out-of-habitat (OOH) case studies of animals found in freshwater or brackish systems, some of which were relocated to suitable habitats (Mase-Guthrie et al., 2005; Rosel and Watts, 2007; Carmichael et al., 2012; Ewing et al., 2017; Deming et al., 2020; Duignan et al., 2020). However, in many cases data were limited or absent for case individuals prior to events, leaving freshwater exposure histories unknown (e.g., salinity levels, exposure duration, and potential co-occurring contaminants or stressors). Consequently, dose-response metrics and links between gross visual characteristics and etiology have been difficult to establish (Booth and Thomas, 2021).

Recognition of these challenges by resource managers led to the 2019 National Oceanic and Atmospheric Administration (NOAA) Freshwater Synthesis Workshop (held at the NOAA Southeast Regional Office, St. Petersburg, FL, USA), which was convened to compile information and pool data across disparate sources in

**Abbreviations:** CBD, Cannot be Determined; FRESH, Freshwater-Related Evaluation of Skin Health; FSD, Freshwater Skin Disease; MMPA, Marine Mammal Protection Act of 1972; NMFS, National Marine Fisheries Service; NOAA, National Oceanic and Atmospheric Administration; OOH, Out of Habitat; Photo-ID, Photographic Identification.

preparation for an expert elicitation aimed at characterizing the effects of low salinity exposure on bottlenose dolphins (Booth and Thomas, 2021). The meeting underscored the paucity of available data and identified numerous data gaps requiring attention. Since then, multiple publications have contributed to defining freshwater disease through detailed characterization of its clinical presentation and underlying pathophysiology (Deming et al., 2020; Duignan et al., 2020; McClain et al., 2020; Townsend and Staggs, 2020). Other efforts have tracked free-ranging animals over time in order to relate behavioral responses and observed skin lesion characteristics to salinity changes and mortality rates (Fazioli and Mintzer, 2020; Taylor et al., 2020; McBride-Kebert and Toms, 2021; Takeshita et al., 2021; Toms et al., 2021; see these and other related studies presented in chronological order in [Supplementary Figure S2](#)).

Despite progress in the study of FSD, research using visual assessment techniques to characterize skin lesions of unknown etiology in free-ranging dolphins continues to vary in methods of characterization, categorization, and terminology. This variability limits meaningful interpretation and comparability across studies (see [Toms et al., 2020 Supplementary Material](#) for a brief review). A common challenge arises from differences in disciplinary training: investigators without medical backgrounds often lack the clinical vocabulary or diagnostic framework to describe skin lesions in ways that can be consistently interpreted by clinicians, while trained medical professionals may employ terminology that field biologists cannot easily discriminate among or apply reliably. For example, the term *severity* is employed in the medical field to indicate the likelihood of a condition to lead to debilitation or death but is used inconsistently in the literature by field biologists to describe the progression of a variety of visual characteristics that do not necessarily relate to health status and survival of the individual. Studies on free-swimming dolphins are also limited by the fact that they are only partially visible when they surface to breathe, limiting full-body assessment of skin conditions. As a result of these challenges, field investigations of wild populations have been constrained by the lack of standardized, biologically meaningful visual assessment tools that can be used consistently by both medically trained (e.g., veterinarians) and non-medically trained (e.g., field biologists) observers.

## 1.2 Developing a visual assessment protocol

The development of the FRESH protocol was grounded in the thorough description of physiological mechanisms of freshwater response in dolphins provided in the literature (Deming et al., 2020; Duignan et al., 2020; McClain et al., 2020), and in the standardization of terms for the gross description of epidermal breakdown provided by Ewing et al., 2023. These foundational resources provided the context and framework to apply during the process of identifying visual FSD-specific indicators and corresponding biologically relevant descriptors. Therefore, the focused objective of the working group was to create a tool specifically for dolphins exposed to freshwater that could be (1) used to evaluate photographs of free-swimming dolphins for FSD in

the absence of tissue sampling, (2) understood and used reliably by both medically and non-medically trained investigators, and (3) applied efficiently and accurately to large photographic datasets.

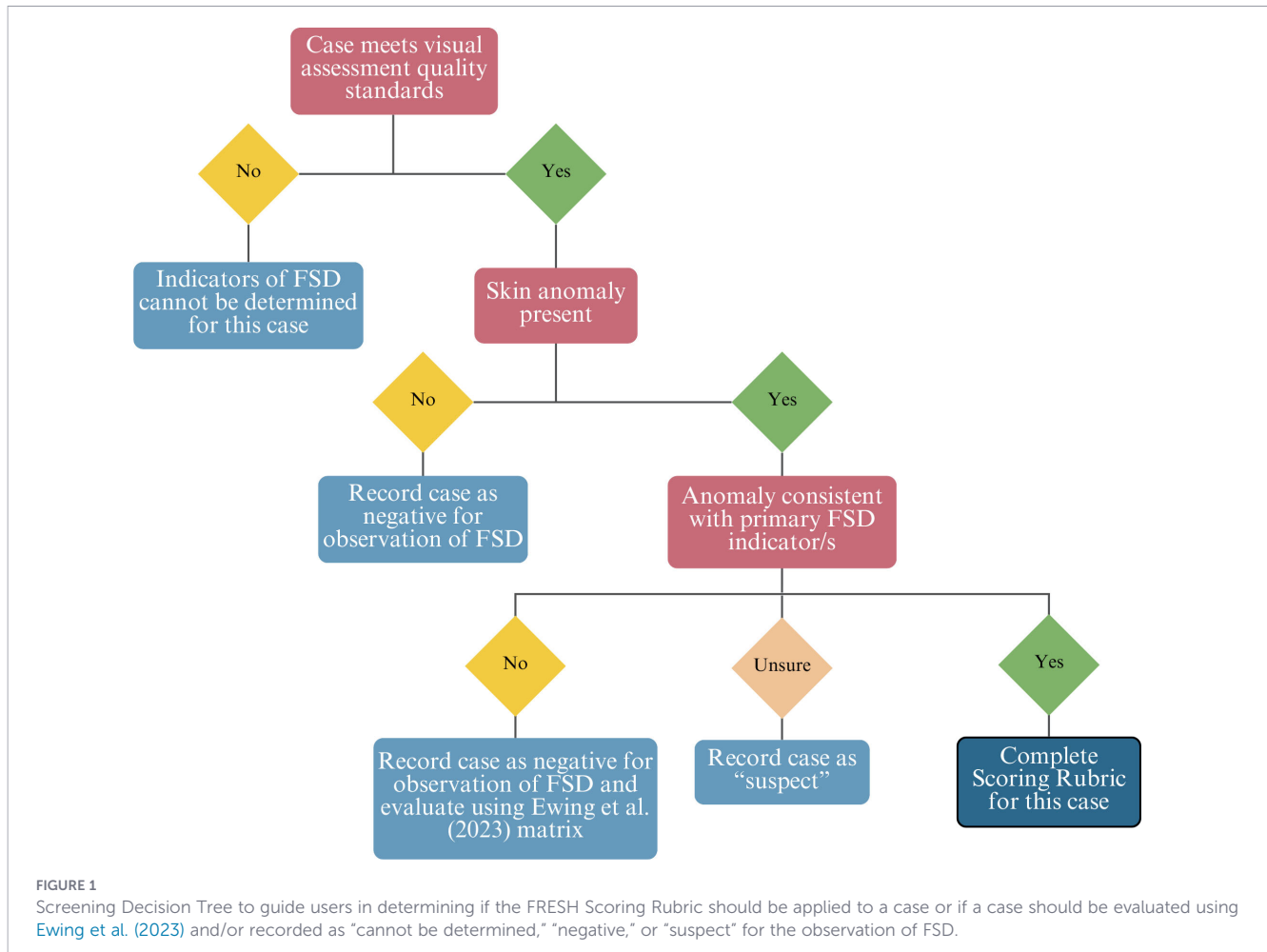
To achieve this goal, input was drawn from 13 marine mammal biologists, 6 medical experts—including veterinarians, pathologists, and epidemiologists—and 5 natural resource managers, all experienced with investigating dolphin health, FSD and other skin lesions. The three major stages of our multi-disciplinary effort included (1) pre-workshop assessments (i.e., Pilot Study), (2) a hybrid 3-day workshop, and (3) post-workshop protocol testing and refinement. Please see [Supplementary Material – Development of the Dolphin FRESH Protocol](#) for details on all project stages.

Reference images crucial to the protocol development were collected from case studies that included associated health diagnostic data (in-hand health assessment or necropsy) to corroborate the presence of FSD through clinical diagnosis. Additional images used during the process were of free-ranging individuals taken during documented freshwater exposure events that exhibited lesions identified by experts to be consistent with FSD. These reference cases included images associated with freshwater exposure as described in Mullin et al. (2015); Duignan et al. (2020); Fazioli and Mintzer (2020); Takeshita et al. (2021); Toms et al. (2021); NOAA (2022); and DiVittore-Goodrum and Gibson (2024). During the Pilot Study, medical experts were asked to identify FSD using photos alone and these results were compared to independent conclusions based on available health and environmental records. There was substantial agreement (89%) after excluding low confidence cases (n=6), as indicated by a Watson's kappa value of 0.78 (95% CI 0.58-0.91; values reported following corrections for biases and accounting for chance agreement). The key finding that experts identified FSD from photos alone with acceptable accuracy enabled our team to confidently proceed with the development of the protocol. Herein, we present the Dolphin FRESH protocol (Freshwater-Related Evaluation of Skin Health), referred to as FRESH, that resulted from this highly collaborative effort.

## 2 Method: The dolphin FRESH protocol

### 2.1 Objective and application

FRESH guides researchers in the evaluation of FSD in the absence of physical examination and/or tissue samples based on the presence and severity of specific visual indicators. It is relevant for use in populations of free-ranging dolphins with suspected exposure to low salinity conditions. The FRESH protocol consists of a Screening Decision Tree ([Figure 1](#)), which leads investigators through several decisions to determine if a case meets criteria to be evaluated further, and a Scoring Rubric. The Scoring Rubric focuses on identifying and evaluating key characteristics of three primary FSD indicators: Overgrowth, Target-like Lesions, and Light Discoloration (see Section 2.3). It is designed to be implemented



for a single point in time and repeated for individuals seen on multiple occasions. Importantly, in its current iteration, FRESH should not be used to diagnose or predict individual health outcomes. Although medical experts on our team refined the Scoring Rubric to include visual indicators that likely have the most clinical relevance for health and survival, direct and quantifiable links between FSD indicator severity scores and overall health remain unknown. Therefore, severity in the context of this protocol is a relative classification based on the FSD indicators and used to categorize a case based on its total score (FSD Severity Score). Furthermore, the manifestation of clinical features of FSD is highly variable and can result in various differential diagnoses based on multiple factors, including but not limited to, the individual’s initial health status, environment, and secondary contributors. While visual identification of primary indicators provides strong evidence of FSD, some visual characteristics may overlap with other diseases and lesions of varying etiology. Therefore, presence of visual indicators alone does not necessarily confirm the presence of FSD. Due to this confounding factor, FRESH guides the user, when possible, to flag cases for further evaluation where specific characteristics may indicate the presence of a condition other than FSD.

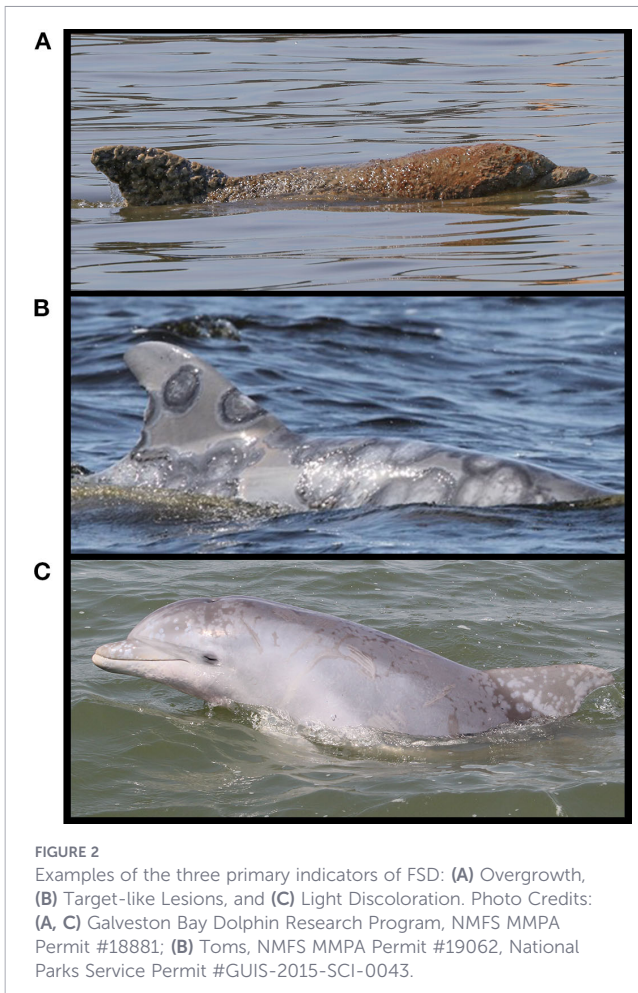
There remains much to learn regarding the relationship between the presence and severity of FSD indicators and risk factors and/or predictive variables, such as environmental factors (e.g., strength and

duration of exposure to varying conditions), demographics (e.g., variability among age/sex classes), and/or movement and habitat use patterns, among others. The output from the FRESH Scoring Rubric offers the first standardized metric of visual FSD indicators, which will enable researchers to explore these relationships and work towards a better understanding of overall health consequences. Additional questions within the Scoring Rubric target documentation of eye condition along with additional indicators that are less understood or may co-occur with FSD. Collecting these complementary data will help address critical knowledge gaps and, over time, allow FRESH to be refined while contributing to broader insights into FSD.

## 2.2 Pre-scoring guidelines

### 2.2.1 User expertise

Users of FRESH should have the expertise and background necessary to discern deviations from “normal” or “typical” when examining dolphin skin for the population in question. This includes having a prior understanding of how skin coloration may vary among individuals unaffected by skin lesions, as well as the ability to confidently discern shadows, glare, water droplets, and other artifacts of photo quality in photographs of free-ranging dolphins. Target users should have moderate to extensive experience with photographic identification (photo-ID) analysis (e.g., Urian et al.,



2015) but are not expected to have a medical background to understand and follow the presented rubric. It is important for users to review and understand [Supplementary Materials](#) and training documents ([galvestonbaydolphin.org/fresh/fresh-protocol](http://galvestonbaydolphin.org/fresh/fresh-protocol)) prior to the practical application of the FRESH protocol.

## 2.2.2 Collecting and selecting photographs for evaluation

An individual dolphin assessed for FSD at a given point in time is considered a “case” for evaluation. Each case is best evaluated using multiple photographs taken at the same point in time of different sections and sides of the body, and from different angles (when possible). When selecting photographs for a case, all photographs of an individual from a time point should be gathered for initial screening. Photographs within a series do not need to show the identifying dorsal fin in all images as long as the user can be certain that the series is of the same identified individual (e.g., a photograph of an individual dolphin’s head taken just before a photograph of the dorsal fin of the same dolphin as it is fully exposed at the surface). When possible, we encourage photographers to use a high-speed continuous shooting mode, focusing on full body photo coverage, rather than only focusing on single dorsal fin photos, as is typical during photo-ID surveys.

Photographs should be filtered for quality according to specific research program guidelines, ensuring that focus and lighting are suitable for individual identification (see [Urian et al., 2015](#)) and for the confident evaluation of skin details (e.g., fine details discernable, not backlit, free from excessive glare and water spray). Next, users should group the best photos from each angle or part of the body together as a case. Considering all photos together, at least 10% of the dolphin’s body should be visible above the water for evaluation (as recommended by [Bearzi et al., 2009](#); visual references provided by [Fazioli and Mintzer, 2020](#); [Toms et al., 2021](#)). Medical experts solicited for this tool development support this 10% requirement to be sufficient for evaluation of FSD since the entire body is subject to exposure, and areas of skin lesion development are typically diffuse or random.

The specific criteria for pre-screening a collection of images to ensure a case meets visual assessment quality standards to include for evaluation with FRESH may vary by the research questions and program goals for each investigator; however, it is imperative to establish criteria (e.g., based on confidence, number of images available of the skin at a given level of image clarity) that is explicit and applied consistently within a program (and across programs wherever possible).

## 2.2.3 The FRESH Screening Decision Tree

For all cases that meet visual assessment quality standards, users should review the condition of the skin to determine if a case should be scored using the Scoring Rubric. Users should be familiar with the three FSD primary indicators and need to determine if at least one indicator is present in a case prior to applying the Scoring Rubric. When uncertain, users should err on the side of being more conservative in their decision. The scenarios listed below are possible, and the corresponding Screening Decision Tree ([Figure 1](#)) should be followed:

- There are no skin anomalies present → record this case as negative for the observation of FSD.
- There are skin anomalies present, but none are consistent with a primary FSD indicator → record this case as negative for the observation of FSD and consider evaluating for other skin anomalies as appropriate using the matrix provided by [Ewing et al. \(2023\)](#).
- There is a skin anomaly that could be one of the primary indicators, but the user has low confidence (“unsure”) → record this case as “suspect” (these could be mild or early stage FSD and may be used in examining progression for individuals that are scored for FSD in the future).
- There is a skin anomaly in which the user has medium-to-high confidence in identifying the presence of at least one FSD primary indicator → complete the Scoring Rubric for this case.

## 2.3 The FRESH Scoring Rubric

Questions that comprise the FRESH Scoring Rubric, answered in the specified order, produce a descriptive and semi-quantitative analysis of a case. The complete rubric questionnaire is provided in

**Supplementary Material - FRESH Scoring Rubric Questionnaire** and can also be accessed directly as an interactive sample ArcGIS Survey123 form here: <https://arcg.is/0jX8yr>. The Microsoft Excel template to re-create this form using ArcGIS Survey123 Connect software is available online at: [galvestonbaydolphin.org/fresh/fresh-protocol](http://galvestonbaydolphin.org/fresh/fresh-protocol).

The Scoring Rubric is comprised of questions designed to (1) evaluate specific characteristics deemed by medical experts to be most clinically relevant for determining the severity of visual indicators in a given case, and (2) capture features that demonstrate progressive stages of FSD (e.g., counts of the number and size of a given indicator). Chosen metrics are assigned weighted scores which, when summed, reflect the overall severity of visual indicators at the time that photographs were taken. Other metrics are not given numeric scores but instead provide users with specific criteria for evaluating progressive changes over time within

individuals. Question order and weighting of scores were designed considering that the presence of some indicators may interfere with the evaluation of others (e.g., indicators can layer on top of each other).

Most of the Scoring Rubric focuses on identifying, describing, and scoring the three primary FSD indicators: Overgrowth, Target-like Lesions, and Light Discoloration (Figure 2; see **Supplementary Material** for additional photographs of each indicator):

- Overgrowth (Figure 2A) = mats or films (e.g., algal, fungal, bacterial, epibiota) of brown, tan, green, yellow, orange, red color (or similar). Films are thin, flat layers of growth that may be translucent, while mats are thick, raised, and not translucent.
- Target-like Lesions (Figure 2B) = consisting of a centralized area of discoloration that is surrounded by one or more

TABLE 1 Standardized metrics included in the FRESH Scoring Rubric.

Metric	Choices	Possible scores	Associated flag
<b>Visual freshwater skin disease primary indicators</b>			
<b>Overgrowth (mats and films)</b>			
Counts of each size class (<2 cm, 2–10 cm, >10 cm) of mats and films	None, Few (1-10), Many (>10)	n/a	No
Rough Film	No, Yes, CBD	0, 1	No
Overgrowth limited to an injury	No, Yes, CBD	n/a	Yes
Mat coverage	1-10%, 11-49%, 50-89%, 90-100%	1, 3, 6, 13	No
Film coverage	1-10%, 11-49%, 50-100%	1, 2, 4	No
<b>Target-like Lesions</b>			
Rough, eroded, or ulcerated	None, Few (1-10), Many (>10)	0, 2, 4	No
Ulcerated target-like lesions	No, Yes (indicate confidence)	n/a	Yes
Target-like lesions limited to an injury	No, Yes, CBD	n/a	Yes
Target-like coverage	1-10%, 11-49%, 50-100%	1, 3, 6	No
<b>Light Discoloration</b>			
Uniform Light Discoloration with indistinct margins	No, Yes, CBD	n/a	No
Count of each size class (<2 cm, 2–10 cm, >10 cm) of distinct multifocal, multifocal to coalescing, or coalescing light discoloration	None, Single, Few (<10), Moderate (10-30), Many (>30)	n/a	No
Rough Light Discoloration	No, Yes, CBD	0, 1	No
Light Discoloration limited to an injury	No, Yes, CBD	n/a	Yes
Light Discoloration coverage	1-10%, 11-49%, 50-100%	1, 3, 6	No
<b>Additional context</b>			
Patch(es) of rough/irregular texture skin (not associated with primary indicators)	No, Yes, CBD	0, 1	No
Patch(es) of dark discolored skin (not associated with primary indicators)	No, Yes, CBD	n/a	No
Cloudy white eyes	No, Yes, CBD	n/a	No
Squinting eyes	No, Yes, CBD	n/a	No
Nodules on the skin	No, Yes, CBD	n/a	Yes
Ulcerated areas on the skin (non-target-like)	No, Yes, CBD	n/a	Yes
Tattoo-like lesions present	No, Yes, CBD	n/a	Yes

Choices for each metric are listed, as well as possible numerical scores or if it has a flag associated with it. Metrics that are not scored or flagged offer specific criteria for evaluating progressive changes. See **Supplementary Material - FRESH Scoring Rubric Questionnaire** for detailed definitions and questions.

concentric areas (e.g., rings, ovals, scallop-edged irregular shapes, etc.); targets may coalesce and may appear smooth, rough, eroded, or ulcerated.

- Light Discoloration (Figure 2C) = skin that is lighter than the normal range of what would be considered a “typical” shade of gray for the population being assessed. May be lighter gray to white in color. May appear as a uniform change in the shade of the skin with indistinct margins (i.e., uniform light discoloration, “pallor”), or as distinct focal, multifocal, or coalescing patches.

Additional questions beyond the three primary indicators provide supplementary information to either support and expand the freshwater exposure analysis or to flag a case for further review (Table 1; Supplementary Material – FRESH Scoring Rubric Questionnaire). Each of these additional questions target specific data gaps or challenges identified by the workshop participants. Included are other skin changes such as patches of dark discoloration or rough/irregular texture that are not associated with a primary indicator but may be associated with FSD;

however, more information is necessary to understand these anomalies. Collecting data on these skin changes will help researchers learn more about their association with FSD and potentially add them as indicators in the future. When available, evaluation of the dolphin’s eyes allows users the opportunity to gather more information about changes associated with freshwater exposure including the development of corneal edema or displays of ocular pain (i.e., expressed as squinting) alongside skin changes.

Since other, non-freshwater-related skin anomalies can have similar gross lesion characteristics to FSD, the working group identified the most likely differential diagnoses and then designed questions in the rubric to flag a case for additional review to aid in either ruling out non-FSD cases or identifying co-occurring lesions. Top differential diagnoses for FSD include poxvirus infection, bacterial or fungal infection, and trauma (see Townsend and Staggs (2020) and Souter et al. (2026) for visual references and detailed clinical presentation of these differentials). Target-like lesions associated with FSD have the potential to co-occur or resemble lesions caused by cetacean poxvirus (Duignan et al., 2020; compare Supplementary Figures S4 and S7). Poxvirus

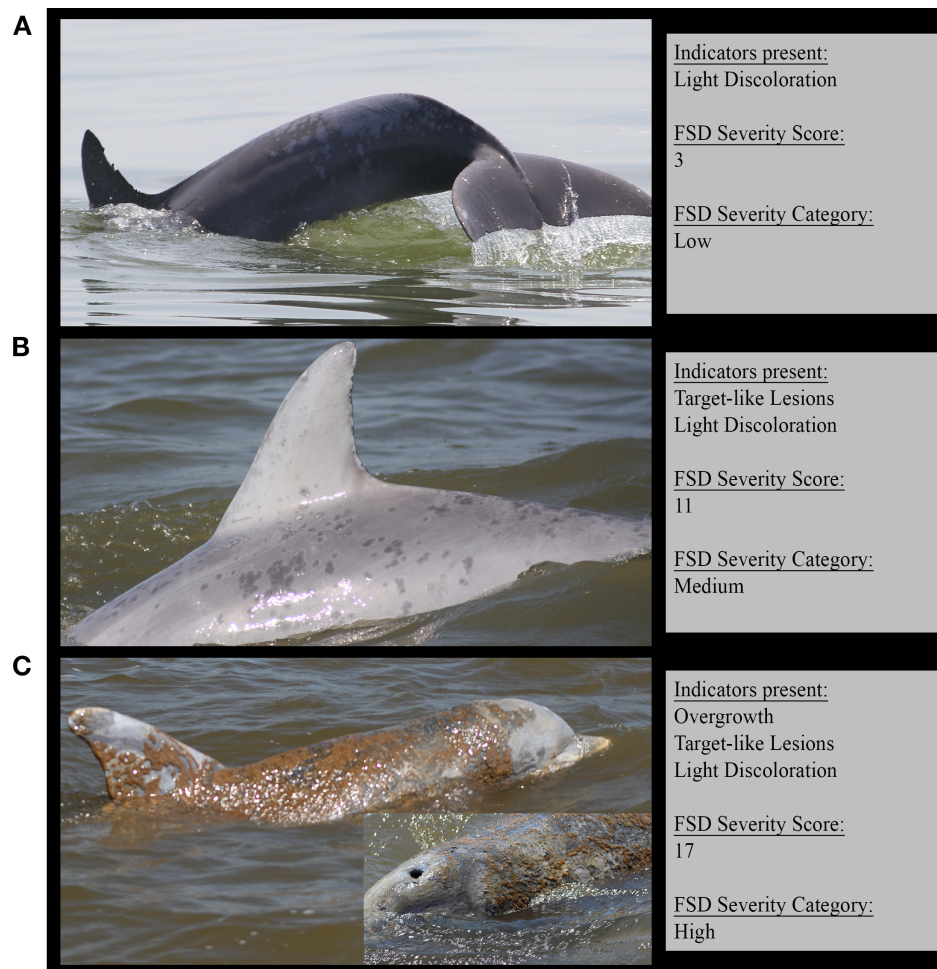


FIGURE 3

Examples of three cases evaluated by field biologists using the FRESH Scoring Rubric. FSD Severity Scores categorized these cases as (A) low (1-5), (B) medium (6-12), and (C) high (>12) severity. Photo Credits: (A) Galveston Bay Dolphin Research Program, NMFS MMPA Permit #18881; (B) NOAA Southeast Fisheries Science Center, NMFS MMPA Permit # 779-1633; (C) Dauphin Island Sea Lab, NMFS stranding agreement under the authority of the MMPA.

lesions, however, are more often characterized by dark, hyperpigmented, or stippled borders hence can be referred to as “tattoo-like”. Discrete or coalescing nodular lesions in the skin may be ulcerated or covered by intact epidermis and can be indicative of bacterial or fungal infection or scar formation after trauma. Thus, the presence of nodules will flag a case for additional review. Skin ulcerations, with loss of the epidermis and exposure of the dermis, can occur due to other etiologies such as trauma or infection. Though ulcerated regions of FSD lesions may occur, the presence of skin ulcers without other primary FSD lesions will flag a case for additional review.

## 3 Results

### 3.1 Expected outcomes

The main outcomes of FRESH are standardized descriptive and semi-quantitative metrics that correspond to each individual case (see [Table 1](#)). Each metric may be used individually to compare and evaluate different aspects of skin condition within or between individuals or populations. Numeric summary scores, including a sub-score for each indicator, and a total score for each case (FSD Severity Score) provide a relative measure of severity based on characteristics of the FSD indicators. FSD Severity Scores are binned into categories of low (1-5), medium (6-12), and high (>12) severity ([Figure 3](#)). As noted previously, this is a level of severity for the indicators and does not imply an expected health and/or survival outcome. The Scoring Rubric allows for users to choose “Cannot be Determined” (CBD) for some metrics when they are unable to discern a feature or are unsure of what they are seeing. Choosing CBD allows users to evaluate characteristics but does not affect the resulting scores.

### 3.2 Challenges

#### 3.2.1 Testing and improvements

A preliminary version of the Scoring Rubric was systematically tested on 16 cases to identify challenges, limitations, and pitfalls. The test evaluations were carried out by small cetacean experts, including 16 stakeholder field biologists with dolphin photo analysis experience and three medical experts involved in the protocol development process for comparison (one veterinary pathologist and two veterinarians). The field biologists had at least five years of experience in photo-ID or prior background in assessing dolphin skin anomalies, along with varying levels of experience in stranding response and/or live-animal health assessments.

Results of this preliminary testing were reviewed by the broader team of experts to discuss revisions to the protocol. Details about the testing stage are available in [Supplementary Material – Development of the Dolphin FRESH Protocol](#). A training document with an introduction to the Scoring Rubric, detailed definitions, instructions, and sample photographs (similar to finalized training materials: [galvestonbaydolphin.org/fresh/fresh-protocol](http://galvestonbaydolphin.org/fresh/fresh-protocol)), was provided to the evaluators to read prior to

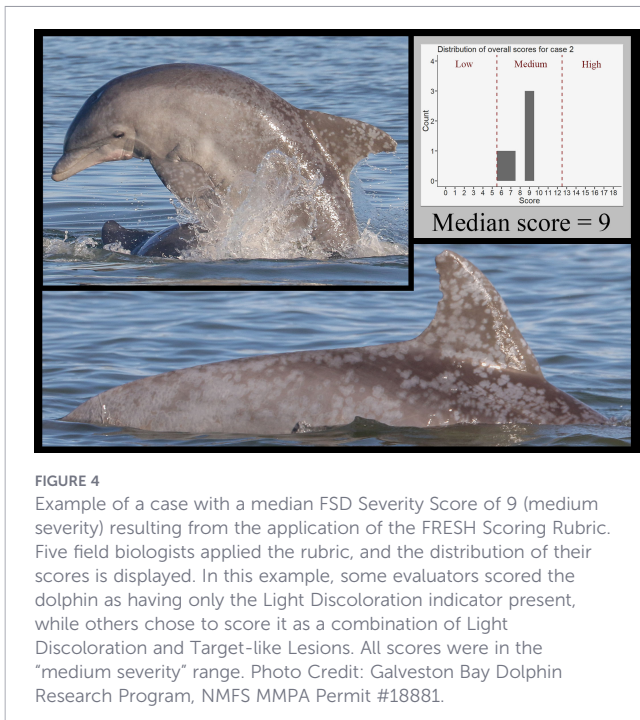
conducting test evaluations. All test cases had supporting evidence (i.e., pathology reports, environmental data, health assessment data) for the presence or absence of FSD. This supporting information was withheld from evaluators, who were required to test the rubric using only photographs. Evaluators first determined whether any primary indicators were present in a case, which then dictated whether the case would be evaluated using the Scoring Rubric.

The Scoring Rubric performed well in testing: field biologists and medical experts recognized the primary indicators and chose to apply the Scoring Rubric in all cases of FSD (i.e., no freshwater cases were missed). There was no significant difference in resulting FSD Severity Scores between the two groups of evaluators (Wilcoxon Signed-Rank Test:  $n = 16$ ,  $V = 40$ ,  $p = 0.159$ ). Furthermore, the level of experience of a field biologist in photo-ID, health assessments, and stranding response did not predict whether they correctly identified FSD indicators (Logistic Regressions,  $0.239 < p < 1$ ) (see [Supplementary Material – Development of the Dolphin FRESH Protocol](#) for details).

Testing revealed that field biologists had trouble differentiating between characteristics of Target-like Lesions, particularly when identifying “eroded” versus “ulcerated,” and this affected the final scores considerably. Consequently, in the final Scoring Rubric, the case score does not vary according to this differentiation, but rather only reflects if Target-like Lesions are not smooth (appearing “rough,” “eroded,” or “ulcerated”). It also flags a case for further review if the evaluator confidently indicates any lesions to be “ulcerated” (as medical experts noted that ulceration indicates a more severe case of FSD; see [Table 1](#)).

Another difficulty was the ability to estimate percent coverage of the primary indicators, especially in scenarios with overlapping indicators. To address this challenge, key changes were made to the order and presentation of coverage-related questions (see [Supplementary Material – Development of the Dolphin FRESH Protocol](#) for details). It was also evident from the testing results that some evaluators scored certain lesions as Light Discoloration, while others scored them as Target-like Lesions (and vice versa). This was unsurprising considering that the two indicators can share similar characteristics at some stages. This prompted us to ensure that scores were weighted in a way that the total score was similar for these cases regardless of which indicator was chosen (see [Figure 4](#) as an example). This highlights the importance of considering the total FSD Severity Score for a case and putting less emphasis on the individual indicator sub-scores.

Based on the lessons learned from the preliminary testing, we updated the FRESH Scoring Rubric and conducted a second, smaller evaluation. This initial test of the final rubric by five biologists did not reveal any further challenges or limitations. [Figure 4](#) displays an example of one case that was evaluated by five biologists with a resulting median FSD Severity Score of 9 (medium severity). Only one of the original 16 test cases was deemed to be particularly difficult to evaluate, with FSD Severity Scores ranging from 7 (medium) to 16 (high). When possible, we highly recommend that multiple evaluators apply FRESH to the same case to identify challenging cases and enable discussions about the resulting scores. Moreover, we expect that as FRESH is further



priorities, the challenge in the top position was “How to determine when to evaluate a lesion with the FSD assessment protocol.” The additional challenges that scored the most votes in all of the top three positions were, “How do we train people to reliably use the protocol?”; “What criteria need to be developed for photo quality, tagging, sorting and management?”; and “How do we maintain a database of scores for each individual linked to sighting and environmental data?” Based on further discussion of these topics, we developed a stakeholder “wish list” for addressing top priorities (see Appendix 1 – Stakeholder Wishlist).

## 4 Discussion

The purpose of FRESH is to guide researchers in the evaluation of FSD based on the presence and severity of specific visual indicators without in-hand physical examination or tissue samples. The intent is to apply FRESH to photographs of free-ranging bottlenose dolphins with suspected exposure to low salinity conditions and provide standard metrics for comparative analysis within datasets. Links between the severity scores for specific indicators and overall health outcomes remain unknown. FRESH provides common vocabulary and training tools for researchers across the field, but with additional testing, analysis, and updates from a broad scope of experts, it could provide a standardized methodology to generate datasets for meta-analysis across study sites, groups, and time.

tested and applied by diverse users and to various dolphin populations, future challenges may arise. We encourage mindful review and discussion of final scores within the context of the study system and population.

### 3.2.2 Stakeholder feedback

During the workshop phase of protocol development (see Supplementary Material –Development of the Dolphin FRESH Protocol), we hosted a “Stakeholder Engagement Session” to identify challenges and priorities for real-world implementation of FRESH. A total of 14 field biologists and natural resource managers, representing 10 organizations from the U.S. Gulf and Atlantic coasts, participated in the session. At the start of the session, we introduced FRESH. Then, we presented a list of expected challenges related to application of the protocol and used interactive online polling to determine the stakeholder group’s priorities. These priorities became the focus of discussion (Table 2). Upon voting for their top three

### 4.1 Conservation and management applications

Based on the feedback collected during the Stakeholder Engagement Session (see Section 3.2.2) and additional comments received during the presentation of FRESH at the Society for Marine Mammalogy’s biennial conference (Fazioli et al., 2024), we expect that the protocol will be applied widely by research programs throughout the world where freshwater exposure has been identified as an ongoing and/or increasing threat for dolphins. FRESH is a robust tool for researchers and managers to track these affected populations.

TABLE 2 Expected challenges of real-world implementation of FRESH and the stakeholders’ voting results.

Challenge	Votes in first priority spot	Total votes in top three
<b>How do we train people to reliably use the protocol?</b>	3	12
<b>What criteria need to be developed for photo quality, tagging, sorting, and management?</b>	2	11
<b>How do we maintain a database of scores for each individual linked to sighting and environmental data?</b>	2	11
<b>*How to determine when to evaluate a lesion with the FSD assessment protocol.</b>	8	10
How do we need to adapt field photo and data collection?	3	9
How do we deal with variability in the available visible skin among individuals in analyses?	3	7
How could we use AI and what can it do for us?	0	6
How much of the dolphin needs to be available for evaluation to have confidence in the skin assessment?	2	3

The top challenge chosen by the stakeholders is identified with an asterisk and in bold. The other three top challenges are in bold.

Efforts are already underway to apply FRESH to data collected between 2013–2020 by the Galveston Bay Dolphin Research Program in Galveston Bay, Texas. Individuals of this population have exhibited FSD indicators over the last decade (Fazioli and Mintzer, 2020; Mintzer and Fazioli, 2021), and these dolphins are expected to become increasingly vulnerable to FSD as the frequency and intensity of freshwater events increase due to changing global weather patterns (Sunkara et al., 2023). Currently, lesion characterization of dolphins with high site fidelity to Galveston Bay (Mintzer et al., 2023) is being paired with environmental data from their estimated core ranges to further examine the effects of freshwater on dolphins as they are exposed to varying salinity over time. This work will further characterize the visual indicators of FSD stages and how they are affected by environmental and demographic variables and repeated freshwater exposure.

Monitoring skin lesions in dolphins in the Southeast region of the United States has been identified as a priority by the Marine Mammal Commission (2024). Data on the timing and progression of FSD will provide key information for understanding the health of dolphin populations experiencing periodic freshwater exposures. Furthermore, it will help inform planning and management decisions related to proposed future infrastructure projects (e.g. coastal protection barrier; Army Corps of Engineers, 2021), that could lead to temporary and/or permanent changes to salinity in estuaries with resident dolphin populations.

FRESH complements current efforts to standardize sampling and response procedures for cases of acute freshwater exposure that may require human intervention. Scores for a given case could facilitate decisions about triage, intervention/response, and release vs. rehabilitation. Additionally, FRESH could be incorporated into response efforts involving dead, stranded, or OOH dolphins exposed to freshwater. Comparisons of scores between individuals may inform cause of stranding and death diagnoses related to freshwater exposure. This will facilitate the continued refinement and understanding of FSD progression and severity. As FRESH is applied to more cases with known outcomes and synchronous health data, we will begin to enhance our understanding of relationships between FSD Severity Scores and specific health and survival outcomes. The application of this tool is particularly timely and important considering the 2019 northern Gulf coast freshwater unusual mortality event (UME; National Oceanic and Atmospheric Administration Office of Protected Resources, 2022; see Supplementary Figure S2). Establishing baseline prevalence of FSD in dolphin populations is a goal to help prepare for potential future UMEs and the development of large infrastructure projects that could impact salinity.

## 4.2 Recommendations and next steps

### 4.2.1 Evaluator experience and training

Use of FRESH is dependent on the assumption that evaluators have the skills to discern deviations from “normal” or “typical” when examining dolphin skin for their research population (See Section 2.2.1). It is worth noting that all field biologists who

participated in the testing of the FRESH Scoring Rubric had either a minimum of five years of photo-ID experience or specific experience in assessing cetacean skin anomalies, whereas some of them had no stranding response or health assessment experience. We recommend that researchers have moderate to extensive photo analysis experience (i.e., photo-ID), ideally with the population they are evaluating, prior to applying FRESH. However, FRESH users do not need to have a medical background.

In agreement with the stakeholder feedback (see Section 3.2.2), we recognize that an immediate and important need exists to improve training resources associated with the application of FRESH. Currently, training materials are limited to one document available online: [galvestonbaydolphin.org/fresh/fresh-protocol](https://galvestonbaydolphin.org/fresh/fresh-protocol). This resource contains guidelines to identify the primary indicators, with additional photographs, and it addresses some of the more challenging aspects of applying the protocol (e.g., determining percent coverage). While this will assist researchers who have photo-ID experience with learning to apply FRESH, it does not provide a means for potential users to evaluate their own ability to discern FSD indicators and associated characteristics/metrics. Hence, we highly recommend the development of an interactive and accessible training tool that will allow potential users to test and improve their skills before they apply FRESH to their dataset.

### 4.2.2 Facilitating use and accessibility

Given that FRESH can be applied widely by research programs collecting dolphin photo-ID data, an ideal future step is to develop an interface for easy application of the assessment tool within a photo-ID monitoring database. For example, FRESH could be added as an optional tool in the FinBase Photo-Identification Database System (Adams et al., 2006) that would allow for visual assessment data to be associated directly with dolphin sighting, environmental, and photo-ID data.

FRESH could benefit from the integration of computer learning and Artificial Intelligence (AI). Currently, the application of clinical AI algorithms in human dermatopathology is rapidly developing. However, challenges encountered during algorithm training due to data source limitations plague the process, limiting reliability and generating biased results (Daneshjou et al., 2021). For example, in their study, Daneshjou et al. (2021) identified three key issues hampering clinical implementation, including a reliance on data sets that included nonstandard and unverified disease labels for algorithm development and testing. In this context, FRESH (with the clinician-recommended terms and definitions) could greatly assist in the development of an algorithm training database, which could be used to help researchers detect and monitor dolphin populations for FSD as a component of photo-ID and visual health assessment studies. Murphy et al. (2025) developed a machine learning model to classify skin lesions in Tamanend's bottlenose dolphins (*Tursiops erebennus*). They found that model performance relies heavily on image quality, sample size, and the ability to define distinct lesion types. Their work supports the potential for future projects to incorporate FRESH indicators into skin lesion detection algorithms.

### 4.2.3 Individual context

FRESH should be applied within the context of the specific study population and its individuals, and with consideration that many estuarine dolphin populations experience multiple and ongoing exposures to low salinity (Mullin et al., 2015; Taylor et al., 2020; McBride-Kebert and Toms, 2021; Mintzer and Fazioli, 2021; Takeshita et al., 2021; Toms et al., 2021). While FRESH is applied to a point in time, using it on the same individual at different time points will help to develop a more comprehensive understanding of the individual's exposures, FSD progression, regression, and potential recovery. Knowing an individual's freshwater exposure and life history will give more meaning to the application of FRESH and allow researchers and managers to make informed decisions regarding an individual and/or study population. For example, over time, researchers may see that certain individuals repeatedly exposed to low salinity develop FSD indicators, while other individuals do not. Elucidating these trends and patterns will aid researchers and managers in developing informed questions and focusing resources where they are most needed. FRESH is intended to be applied and interpreted contextually, considering additional factors that may fall outside the scope of the protocol.

### 4.2.4 Beyond FRESH

The work presented herein demonstrates that the standardized use of terms can enable researchers to categorize a lesion/disease pattern. The routine collection of images and corresponding skin lesion descriptions during photo-ID surveys, visual health assessments, and post-mortem examinations can lead to the identification of characteristics that can be used to develop differential diagnosis listings. Combining health diagnostic data (e.g., from in-hand health assessments, stranding responses, or post-mortem examinations) with synchronous FSD indicator data and freshwater exposure histories will help establish the direct correlations between visual FSD indicator progression and health/survival outcomes. Refinement of FSD indicator score weightings may be necessary during the exploration of these relationships.

When cases are flagged for review within the FRESH Scoring Rubric, or when skin anomalies are observed during the screening process that do not visually correspond to FSD primary indicators, we recommend conducting a standardized descriptive assessment of the skin changes as presented in Ewing et al. (2023). Similar to how FRESH was developed, there may be opportunities to identify visual primary indicators for other diseases. Creating tools like FRESH that bridge the knowledge, skills, and talents of field researchers and medical investigators, allows for clear communication of findings at important intersections where their work comes together. These collaborations are essential to study cetacean health in our rapidly changing environment.

## Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: Photographs and data evaluated for the

development of this protocol were contributed by multiple organizations operating under NOAA Marine Mammal Protection Act permits and stranding agreements. Data was contributed under agreement with the co-authors for limited use intended for workshop and training materials only. Requests to access these datasets should be directed to KF, [kfazioli@chartedmarine.com](mailto:kfazioli@chartedmarine.com).

## Ethics statement

Ethical approval was not required for the study involving animals in accordance with the local legislation and institutional requirements because the development of this method did not require the direct collection of data, but rather the use of data collected for prior unrelated studies. All efforts for contributed materials were conducted under the following authorizations, and according to protocols approved by Institutional Animal Care and Use Committees (IACUC) across the multiple institutions and organizations: NMFS MMPA Permits #18182, #19403, #23782, #18881, #23203, #18786, #19062, #779-1633, #932-1905/MA-009526; NOAA NMFS General Authorization, Letter of Confirmation #23796; NOAA NMFS Stranding Agreements under the authority of the Marine Mammal Protection Act; National Park Service permit GUI5-2015-SCI-0043; Victorian Department of Environment and Primary Industries (Australia), Wildlife Act 1975 Research Permit #10003250.

## Author contributions

KF: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft. CT: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Writing – original draft. RT: Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing – review & editing, Resources. AQ: Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. DG: Conceptualization, Investigation, Methodology, Writing – review & editing. TR: Conceptualization, Methodology, Resources, Supervision, Writing – review & editing. LS: Conceptualization, Resources, Supervision, Writing – review & editing. KC: Conceptualization, Investigation, Methodology, Validation, Writing – review & editing. RE: Conceptualization, Investigation, Methodology, Resources, Writing – review & editing. DF: Conceptualization, Investigation, Methodology, Validation, Writing – review & editing. DB: Conceptualization, Methodology, Writing – review & editing. AB: Data curation, Investigation, Writing – review & editing. PD: Conceptualization, Investigation, Methodology, Writing – review & editing. EF: Conceptualization, Methodology, Writing – review & editing. LH: Conceptualization, Methodology, Writing – review & editing. SM: Conceptualization, Data curation, Investigation, Methodology, Writing – review & editing. TM: Conceptualization, Investigation, Methodology, Writing – review & editing. CS: Conceptualization, Methodology, Writing – review & editing. TS: Conceptualization,

Methodology, Writing – review & editing. MS: Conceptualization, Investigation, Methodology, Resources, Writing – review & editing. HW: Conceptualization, Methodology, Resources, Writing – review & editing. VM: Conceptualization, Data curation, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – original draft.

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## Conflict of interest

KF was employed by company Charted Marine Consulting. DG was employed by company Ocean Associates, Inc.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

## Generative AI statement

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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2026.1743541/full#supplementary-material>

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## Appendix 1 – Stakeholder Wishlist

- Best practice recommendations of when to use the FSD assessment tool and when to further investigate an unknown skin lesion using other means.
- A pathology “contact” that programs can send questionable cases to for further evaluation.
- Clear training materials/system for programs to independently implement use of the tool, potentially including:
  - Written manual with clear definitions and example images.
  - Accessible, standardized form with integrated instructions/tips/tools/example photos.
  - Training video and practice case training dataset associated with reliability testing protocol to demonstrate scoring comparability with other programs.
  - “Train the trainer” workshop.
- Integration into “FinBase” for tracking individual lesion photos and scores alongside sighting data, starting with an initial checkbox indicating that an individual within a sighting should be evaluated further for skin lesions upon entering it into FinBase (will require FinBase updates and support moving forward).
- A centralized repository with a single curator to standardize assessment and facilitate comparative studies (i.e., Gulf of Mexico Dolphin Identification System for skin lesions).
- Pursue AI technology for multiple steps of the workflow process, including:
  - Photo quality metrics.
  - Measurement of lesion coverage.
  - Lesion detection.
  - Combination of multiple images from a sighting to create a more complete, single 3D photo of an individual for evaluation.