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RRH: BICHELL *ET AL.*: PIGMENT PATTERN-BASED IDENTIFICATION

The reliability of pigment pattern-based identification of wild
bottlenose dolphins

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ABSTRACT

Long-term studies often rely on natural markings for individual identification across time. The primary method for identification in small cetaceans relies on dorsal fin shape, scars and other natural markings. However, dorsal fin markings can vary substantially over time and the dorsal fin can become unrecognizable after an encounter with a boat or shark. Although

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dorsal fins have the advantage in that they always break the water surface when the cetacean breathes, other physical features, such as body scars and pigmentation patterns can supplement. The goal of this study was to explore the use of dorso-lateral pigment patterns to identify wild bottlenose dolphins. We employed photographic pigment matching tests to determine if pigmentation patterns showed (1) longitudinal consistency and (2) bilateral symmetry using a 30 yr photographic database of bottlenose dolphins (*Tursiops aduncus*). We compared experienced dolphin researchers and inexperienced undergraduate student subjects in their ability to accurately match images. Both experienced and inexperienced subjects correctly matched dolphin individuals at a rate significantly above chance, even though they only had 10 s to make the match. These results demonstrate that pigment patterns can be used to reliably identify individual wild bottlenose dolphins, and likely other small cetacean species at other sites.

Key words: natural markings, pigment, individual identification, cetacean, bottlenose dolphin, *Tursiops*.

Long-term tracking of individuals in wild populations is crucial in the fields of behavioral ecology, and evolutionary and environmental biology (Kruuk *et al.* 2008, Clutton-Brock and Sheldon 2010). Tracking individuals in the wild plays a large role in wildlife conservation and management of threatened and endangered species by improving models of population change in response to land use change (Caro 1998, McGregor and Peake 1998). The utility and importance of tracking and identifying individuals is undeniable, but a significant challenge in long-term research is to develop a means for reliable identification that is cost-effective and has little impact on the animal's

behavior.

A variety of methods are used to identify and track individuals in the wild. While some have little or no effect on the study organism, others can be invasive (capturing and tagging, branding, or banding) or expensive (Thomas *et al.* 2011, Hurley *et al.* 2013). Such methods can have negative effects on survival, reproduction, and behavior (McFarlane *et al.* 1990, Boitani and Fuller 2000, Wilson and McMahon 2006, Walker *et al.* 2012). Less invasive mark and release techniques include the application of bands, dyes, tattoos (Nietfeld *et al.* 1994), or transponders (Watwood *et al.* 2006).

Though the harmful effects of invasive techniques are widely recognized, even less invasive methods can have negative impacts (Minteer and Collins 2005). For example, among pinnipeds, visual tags attached to the exterior of the animal alter swimming and haul-out behavior, maternal care, and lead to decreased endurance on foraging trips (Walker *et al.* 2012). Similarly, external tags (DTAG2) have been shown to affect swim speeds in cetaceans (van der Hoop *et al.* 2014) and are usually not practical for large numbers of animals. It is therefore highly desirable to use tracking techniques with minimal or no ill effects to meet humane and sustainable goals as well as maintain the integrity of the scientific results.

The use of natural markings (pigment patterns, callosities, scars, and marks) for individual identification is widespread in cetacean research (Table S1), and is minimally invasive. In reviewing the literature on individual identification in cetaceans, we found that individuals of at least 57 species are identifiable over time based on pigment patterns, scars, callosities, dorsal fin, or fluke markings (Table S1). Though

the natural markings used vary by species, the methods of identification are largely the same. Using photographic images, researchers or computer-matching programs scan visible patterns and contours and match patterns across images, within and across years (Würsig and Jefferson 1990, Rugh *et al.* 1992, Kelly 2001, Arzoumanian *et al.* 2005, Beekmans *et al.* 2005). Although the noninvasive benefit of using natural markings is evident, it is not without costs, mainly in terms of researcher effort in both the field (extensive photo-ID effort) and laboratory (ID matching can be tedious and difficult) (Urian *et al.* 2015).

For bottlenose dolphins (*Tursiops* spp.), individual identification methods based on nicks, scrapes and scars on or near the dorsal fin are well established, a method originally developed in the 1970s by Würsig and Würsig (1977). However, these scrapes and scars are sometimes transient as they come and go with new injuries and healing. Furthermore, dorsal fins can be severely altered or even completely removed by a shark bite (Fig. 1). Unlike scars, pigment patterns tend to endure, evidenced by studies of bowhead whales (*B. mysticetus*) (Rugh *et al.* 1992), sperm whales (*Physeter macrocephalus*) (Beekmans *et al.* 2005), long-finned pilot whales (*Globicephala melas*) (Auger-Méthé and Whitehead 2007), and killer whales (*Orcinus orca*) (Würsig and Jefferson 1990). Bottlenose dolphin pigmentation includes dominant sweep patterns located on the dolphins' medial to dorso-lateral side swooping upward toward their dorsal fin (Fig. 1, 2) as well as on the lateral side of the peduncle. In this study, we examined whether these pigment patterns of wild bottlenose dolphins (*Tursiops aduncus*) can be used for long-term identification of individuals using data from a 32 yr study of a wild cetacean, the Shark Bay Dolphin Research Project

(<http://www.monkeymiadolphins.org>).

Though several of the 15 long-term (10 yr or more) bottlenose dolphin (*T. aduncus* and *T. truncatus*, Mann and Karniski 2017) study sites mention using lateral pigment for individual identification, none have tested its accuracy and reliability across years (Wells et al. 1987, Wilson et al. 1997, Scott et al. 2005). This is likely because pigment is usually a secondary method, with dorsal fin shape, nicks, and scars being the predominant method of identification. Using pigment patterns for identification could be advantageous over dorsal fin or body scars, nicks, and bite marks in several ways. First, they may be more reliable over longer time periods. Second, the patterns on either side of the dolphin's body may be symmetrical, which would enable identification regardless of which side was photographed. Finally, characteristics of the pattern, such as brightness and placement, may be somewhat hereditary, much like the saddle patches of killer whales (Hoekstra 2006, Kaelin et al. 2012), and potentially useful for genealogical and population studies.

Here, we assessed the validity and reliability of using natural medial to dorso-lateral pigment, specifically "sweeps" (Fig. 1), for identifying individual bottlenose dolphins across age classes and from both body sides using a matching test in PowerPoint (2013). We hypothesized that these pigment patterns would be reliable for individual identification across age and symmetrical bilaterally, and that experienced cetacean researchers would be better at matching dolphins based on pigmentation than inexperienced subjects.

METHODS

Shark Bay Dolphin Research Project

The main study site encompasses ~300 km² in the eastern gulf of Shark Bay (25°47'S, 113°43'E) and is home to >1,600 bottlenose dolphins that have been studied since the mid-1980s (Mann *et al.* 2012). Over 80,000 photographs have been taken and evaluated and are maintained in a photographic database and ID catalog.

Experiment 1: Pigmentation Matching Across Age and Human Subject Experience

Photographic images—To select photographs for Experiment 1, we selected high quality images of individuals with known age from the Shark Bay Dolphin Research Project database. Photo quality was determined using a similar method as Auger-Méthé and Whitehead (2007) based on the focus of the image, the size of the dolphin relative to the frame, the percentage of the side visible, the orientation of the side in relation to the frame, and the exposure of the subject.

The identity of dolphins in each photo were known based on nicks, scars, and dorsal fin shape. Birth dates were known for most dolphins born since the early 1980s but were also estimated using size and the degree of ventral speckling (Krzyszczk and Mann 2012). Birth date accuracy varied, but all ages for dolphins in this study were accurate to within 1 yr. Individuals were then classified into age classes defined as: calf (0–4 yr old), juvenile (5–10 yr old), or adult (>10 yr old) based on typical mammalian life history stages (Mann *et al.* 2000, Krzyszczyk and Mann 2012).

After compiling 1,622 images, we selected 25 individual dolphins who had the highest quality images of both left and right lateral sides for more than one age class. From these, we selected two images of each individual from different age

classes, excluding any images with other distinguishing characteristics that could be seen near the pigment markings of interest, such as scars or marks on the dolphin's side. Images spanned from calf to juvenile ($n = 10$), calf to adult ($n = 2$), and juvenile to adult ($n = 13$). The average number of years between images was 4.6 ± 3.2 SD (range 1-16 yr). To standardize each image so that the body size and the proportion of the body viewed were similar, we formatted each image by magnifying, cropping off the dorsal fins, rotating for consistent orientation, and marking the anterior side with a green dot and the posterior side with a red dot (Fig. 3). After formatting each image, we placed the image to be matched, designated by a black border, at the top of a Microsoft PowerPoint 2013 slide. The three matching options were then lined up underneath and were each assigned a letter option: A, B, or C. One of these three options was the same individual from a different age class, the "correct match." The other two options were randomly selected, nonrepeating images of other individuals. We created 25 slides, identical in format but with different individuals so that no images were repeated across slides (e.g., Fig. 3). Slides were presented to human subjects in random order to control for possible slide order effects. One image set was removed from analysis after it was determined to be incorrect (the wrong dolphin was selected from an image that had multiple dolphins) and analyses were adjusted accordingly. Only the first author was aware of the identity and age of the dolphins selected, as the remaining authors participated in the study.

Human subjects—Nine individuals with experience identifying cetaceans based on natural markings took part in Experiment 1. These individuals (hereafter experienced subjects) had more than

one year of experience in dolphin research and in matching photographs and at least one Shark Bay field season (~3–6 mo). None of the subjects could identify any of the individual dolphins based solely on pigment patterns, even though most could have identified them based on dorsal fins. In addition, thirty individuals with no previous experience in identifying cetaceans based on natural markings (inexperienced subjects) were also tested. These inexperienced volunteer subjects were undergraduate students enrolled in Marine Biology and Ecology and Evolution courses at Georgetown University, and were asked to participate because of their motivation and interest in wildlife research.

We administered the tests to subjects on a computer in the laboratory. The slides advanced automatically using PowerPoint's built-in function such that subjects had only 10 s to view the test image and the three choices, and then 5 s to record their answer on an answer sheet before the slide advanced. Subjects were monitored and not allowed to stop the test or move forward manually. All subjects completed the experiment.

Analysis—We used a custom permutation test written in R (Appendix S1) to determine whether subjects matched dolphin photographs correctly at levels greater than chance (33.33%). The code produced 1,000 randomly generated answered tests (answers to 24 questions), assuming a one-third chance accuracy for each question. Each answer was coded at 1 for correct, or 0 for incorrect. The results from these permutations, which provided a distribution of expected results based on chance alone, were then compared to our observed results to determine the probability of obtaining our observed results by chance.

Experiment 2: Pigmentation Matching Between Sides and Between

Human Subject Experience

Photographic images—A second matching test was used in Experiment 2 to determine similarity between the left and right sides of an individual based on medial to dorso-lateral pigmentation. Again, inexperienced and experienced subjects were both asked to find the match of an individual to three options. This time, however, the correct answer was the opposite side of the same individual rather than that individual from a different age class. As the results from Experiment 1 indicated that pigment was consistent across age (see Results), left and right side images were not restricted to within a specific age class.

Images for Experiment 2 were selected from those compiled in Experiment 1 based on quality and lateral side shown—one of the left and the other of the right side of the dolphin. The images were formatted according to the protocol in Experiment 1 with the addition that the images were flipped so they appeared to be the same side of the dolphin. Test takers knew they were looking for the opposite side and were asked to select the image that most closely resembled the image to be matched. Two image sets were removed from analysis because the wrong dolphin was selected from an image with multiple dolphins and analysis was adjusted accordingly.

Human subjects—The same experienced subjects that took part in Experiment 1, took part in Experiment 2 but different inexperienced subjects were tested from other undergraduate classes in environmental biology at Georgetown University. For Experiment 2, like Experiment 1, the slides advanced automatically and the matching test was administered on an in-lab computer.

Analysis—Data for Experiment 2 were analyzed using the same

custom permutation methods as that in Experiment 1, adjusting for sample size differences in the R code.

RESULTS

Experiment 1

When matching images across time, inexperienced subjects with no background in dolphin research scored significantly above chance (observed average $67\% \pm 17.8\%$ SD, compared to the mean of the permutations $33.4 \pm 2.1\%$ SD; permutation test, $P < 0.001$), as did experienced subjects (observed average $90.7\% \pm 7.2\%$ SD, compared to the mean of the permutations $33.3\% \pm 3.7\%$ SD; $P = 0.001$).

Experiment 2

When matching images of different sides, inexperienced subjects again scored significantly above chance (observed average $47.6 \pm 10.7\%$ SD, compared to the mean of the permutations $32.1\% \pm 2.2\%$ SD; $P = 0.001$), although with lower accuracy than Experiment 1. Experienced subjects scored significantly above chance, but again, also with lower performance than in Experiment 1 (observed average $58.9\% \pm 9.9\%$ SD accuracy compared to the mean of the permutations $32.2\% \pm 1.8\%$ SD; $P = 0.001$).

DISCUSSION

Over the last 40 yr since Würsig and Würsig's 1977 study showing that bottlenose dolphin dorsal fins could be used for individual identification, few other methods of noninvasive matching have been developed for dolphins. In other cetaceans, pigment, fin shape, fluke shape, scars, and callosities are used to match individual cetaceans across years, and computer software has been developed to aid in matching many of these natural markings (e.g., Kehtarnavaz et al. 2004;)Table S1). This

information has been critical for studying cetaceans. For example, such methods made it possible to document that killer whales can live to be over 100 yr old (Whitehead 2015) and long distance migratory patterns of humpback whales (Kaufman *et al.* 1990). In this study, we examined the long-term reliability of using dolphin lateral pigment for individual identification with a computerized matching test designed to assess the reliability of dorso-lateral pigment patterns. We found that humans, both experienced and inexperienced in dolphin research, were able to match dolphins correctly above chance. Unlike the dorsal fin, from which scars and bite marks are often used to identify individuals, pigment patterns should be usable from early in life to death barring any major dorso-lateral scarring event. Thus, pigment might be a useful identifier when dolphins are young (calf period) and dorsal fin markings are less prevalent. Although many young dolphins have similar dorsal fins, it is unlikely that any two have identical pigment patterns. Though often subtler than a chopped off dorsal fin, the longitudinal consistency of pigment patterns makes them undeniably useful when identifying individuals, particularly after a major alteration of the dorsal fin. For example, an adult male SEV was attacked by a shark in 2007–2008 but was identified from dorso-lateral pigment patterns (sweeps and peduncle pigment; see Fig. 1a, b). Furthermore, the PowerPoint (2013) matching tests used in this study were strictly timed with each slide lasting only 10 s, far less time than is typically devoted to matching identification photographs. Yet, despite the brief exposure to the images, test takers were still able to match individual dolphins—both across age classes spanning 12 yr and between left and right lateral sides—significantly above chance. Accuracy

would likely be higher if photo stimuli were presented for longer than 10 s. A longer duration, paired with the ability to edit the photos (rotate, change contrast, etc.) could both increase accuracy and better mimic field conditions under which this method would be used. Although researchers would have many more dolphins to test against. Such alterations would only further improve accuracy of this method.

In addition to showing consistency over time, our test indicated some degree of symmetry between the pigment patterns on the left and right lateral sides of dolphins. Given this, if one side of an individual is dramatically altered by a shark bite, the other side might still be useful for identification even if historically researchers only have photos from the shark bitten side. While our test indicated left-right symmetry and matching accuracy was significantly above expected, it was still not as high as the same side identification, showing that the left and right sides are likely very similar, but not identical. Thus, bilateral matching based on pigment sweeps can serve as a supplementary identification method to use in conjunction with, rather than in the place of, fin-based identification.

Overall, our study showed that dorso-lateral pigment patterns can be combined with other methods, such as fin-shape, to improve photo-identification accuracy. When photo-identifying individual bottlenose dolphins, any additional tool is useful given the limitations caused by the angle, the lighting, the side, obstructing objects in, and changes in scars in the images that are obtained from boat-based fieldwork. Using a combination of natural marking identification techniques could rival the accuracy of some of the more invasive techniques without the negative side effects of harming or changing the behavior of the

study animal. Furthermore, the results of this study likely apply to any small cetacean with pigment patterns, though the use of pigment patterns in each species requires validation.

One caveat to our study is that it only offered three options from which test takers could choose. Under field conditions, researchers would have to match an image to an entire database of many thousands of images. This could increase the difficulty of pigment-based identification as a researcher would have to be familiar enough with the individuals of study to be able to recognize pigmentation patterns. In other words, this test offered three options from which to choose, forcing the test taker to choose a match. Forcing selection automatically increases accuracy compared to the matching process that actually occurs in the field. What is more, the test asked the test taker to choose the closest match relative to the other options. If a researcher were matching to an entire database, determining whether a match is close enough to stop looking is often the most difficult task. Alternatively, a researcher with less experience would have to search through the entire photo database until they found an image match (if one even existed in the current catalog), which is time consuming and error prone. However, this difficulty could be all but eliminated with the development of matching-aid software such as FinScan (Hillman *et al.* 2002), DARWIN,² or the algorithm used to identify leatherback turtles based on pigment spotting.³ Given these limitations, and those discussed throughout, pigment-based identification should not be used as a primary means of identification. Rather, it can be used as a secondary tool after fin or scar-based identifications to confirm a potential match. Under these circumstances, the matching options would be limited

to a smaller selection group to be matched, better mirroring our experiment here.

Conclusions

Overall, our study shows that bottlenose dolphins show sufficient variation in their pigment patterns that is consistent across age and across both lateral sides, such that it can be used to reliably identify individuals. Pigment-based identification adds to the arsenal of noninvasive identification methods that can be used to supplement dorsal fin identification in the event that two or more dorsal fins are highly similar or when the fin is damaged (Rosel *et al.* 2011). This method is noninvasive and does not require additional equipment or capture of the study animal—ideal for many small cetacean studies that rely on brief and intermittent surfacing opportunities to photograph animals. The method does, however, take considerable researcher time and effort (Urian *et al.* 2015), which could be minimized with the development of pigment matching-aid software like that used for leatherback turtles.⁴ Though this study focuses on bottlenose dolphins, this method can potentially be applied to other small cetacean species.

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SUPPORTING INFORMATION

The following supporting information is available for this article online at <http://>

Table S1. Methods for individual identification of each cetacean species. In one genus, *Neophocaena*, individuals are not identifiable (Morton and Perrin 1997). In 28 out of 89 cetacean species, scientists have not tracked individuals or attempted to do so, typically because individuals are hard to find and

identify (e.g., photo-identification has been used in only 4 of 22 beaked whale species). Species list from Committee on Taxonomy (2016).

Appendix S1. Custom permutation R code summary.

Figure 1. Shark bitten and scarred fin from Shark Bay Dolphin Research Project. (a) SEV seen in 2004, (b) SEV seen in 2008. Shark attacks are a common occurrence in Shark Bay (Heithaus 2001) and can severely alter the dorsal fin, rendering the fin-based identification useless. Sweeps highlighted in yellow box. Green box highlights other areas of pigment that can be used for matching.

Figure 2. Pigment sweep on the dorso-lateral side of a dolphin from the Shark Bay Dolphin Research Project. Sweeps highlighted by yellow box. These pigment patterns are hypothesized to be individually distinctive and potentially reliable for individual identification throughout the lifespan.

Figure 3. Example slide from the matching test. Human subjects were presented with a 25-slide, randomized matching test where they were asked to match one of the options (A, B, or C) to the black-bordered image at the top of the frame. The correct match was the same individual from a different age class or from a different side. Images were rotated and dorsal fins were cropped off to avoid fin-based identification.

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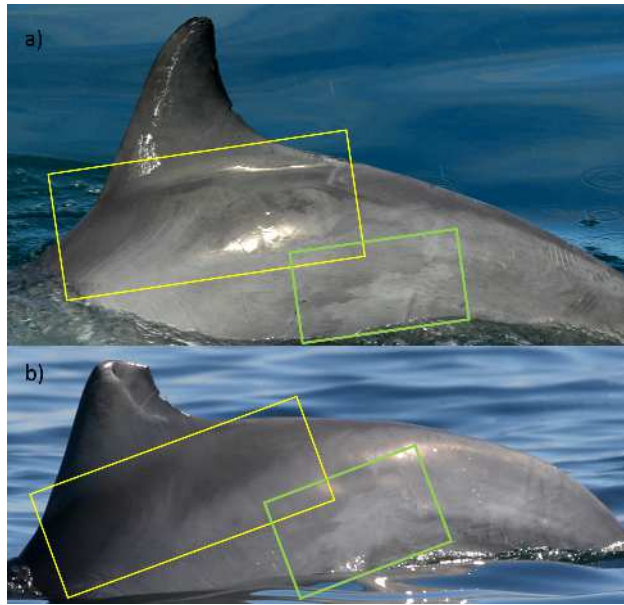
² Scott Hale, May 2008,
<http://darwin.eckerd.edu/publications/thesis-hale.pdf>.

³ Danielle Buonantony, May 2008,
http://dukespace.lib.duke.edu/dspace/bitstream/handle/10161/467/MP_dmb28_200805.pdf%3Fsequence%3D1.

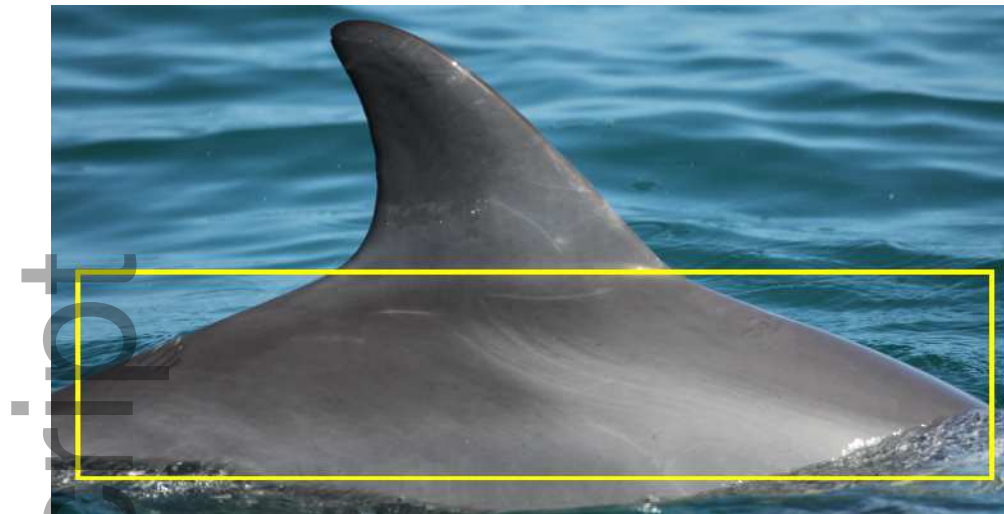
⁴ Danielle Buonantony, May 2008,

[http://dukespace.lib.duke.edu/dspace/bitstream/handle/10161/467/
MP_dmb28_200805.pdf%3Fsequence%3D1](http://dukespace.lib.duke.edu/dspace/bitstream/handle/10161/467/MP_dmb28_200805.pdf%3Fsequence%3D1).

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mms_12440_f1.tif



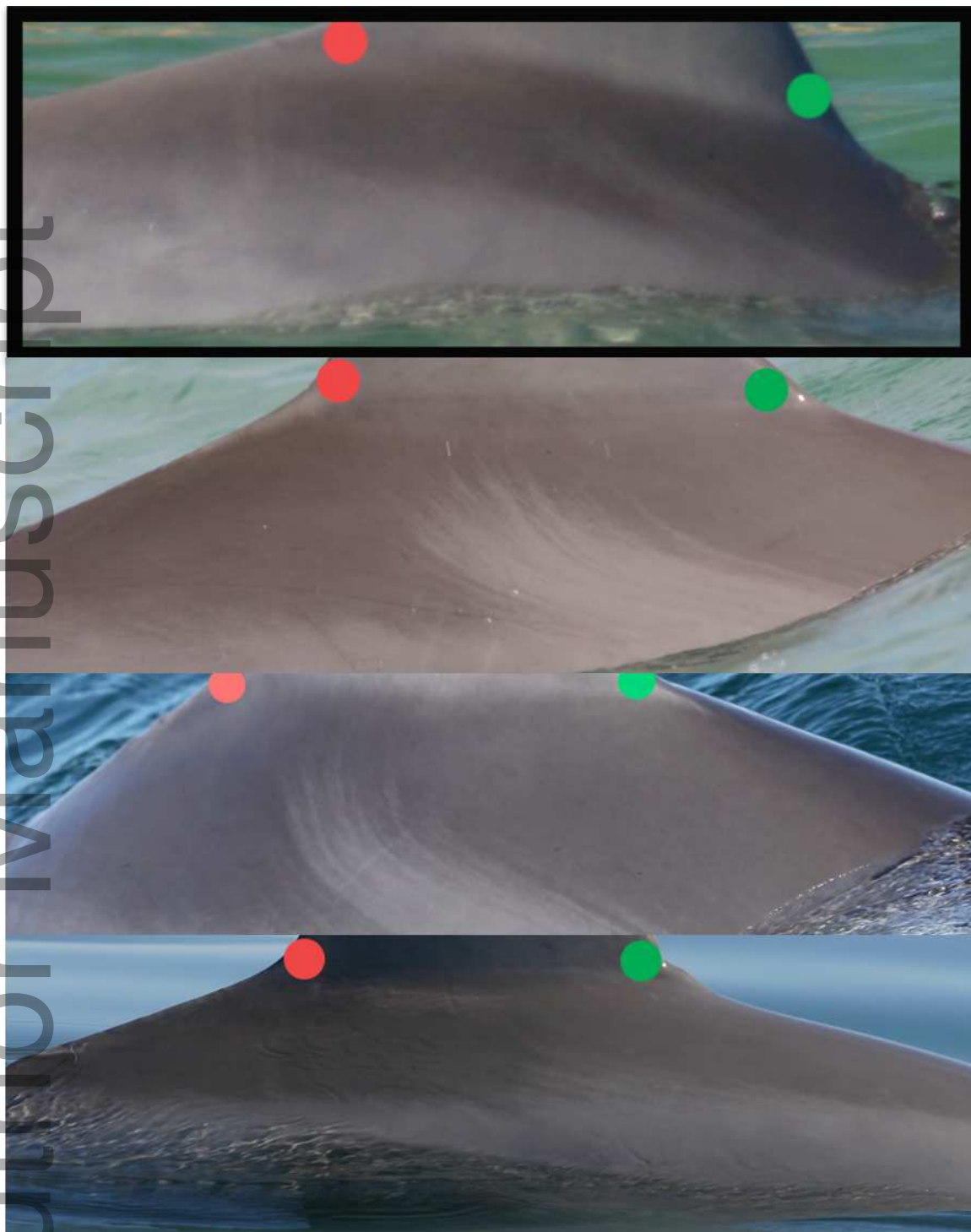
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#24

A

B

C



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