NOVEL METHODS FOR ASSESSING AND MITIGATING HANDLING STRESS IN SEA TURTLES

by

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A Thesis

Submitted to the Faculty of Purdue University

In Partial Fulfillment of the Requirements for the degree of

Master of Science

Department of Biological Sciences at Purdue Fort Wayne

Fort Wayne, Indiana

May 2022

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Dedicated to my grandparents Pat & Fred Mills, whose commitment to a lifetime of learning has inspired my thirst for knowledge, and the belief that I can achieve anything

ACKNOWLEDGMENTS

My heartfelt thanks to Dr. Frank Paladino for taking a chance on me at a critical time in my career. Dr. Paladino's unwavering support throughout my degree, coupled with being an inspiring educator has enabled me to reach my true potential.

Dr. Nathan Robinson was my initial inspiration to follow my dream. I thank him for his belief in me, continued mentorship during this journey, and invitations to collaborate on exciting projects, which were the foundations of my motivation to be successful in my future as a marine biologist.

Thank you to Dr. Scott Bergeson for his important sharing of his expertise to assist in the completion of this project and for his in-depth knowledge of animal behavior. I would also like to give a special mention to Dr. Jim Spotila for his advice and edits, and Dr. Jordan Marshall for his assistance and support throughout the graduate program.

I would like to acknowledge and thank the team of graduate students who made moving to the States that much more enjoyable, especially my lab colleagues, Natalia, Trevor and Sheldon, and the ERC team.

The turtle team in the Bahamas helped me at the start of my journey and inspired my love of sea turtles and field research. I would like to particularly acknowledge both Laura St. Andrews and Sebastian Hoefer who both were integral to the collection of this research and the rigors of correct capture of research.

Without the support of The Cape Eleuthera Institute and Dr. Nick Higgs these projects would not have been possible. I must also express my gratitude to Annabelle Brooks and Dr. Andreu Rotger for their data collection, expert advice, and mentorship on using photo-ID.

Western Connecticut State University and the Finding Our Way project provided funding for the Paralenz cameras used in the TurtleCam project. The British Chelonia Group provided the funding for the DJI Phantom 4 Pro drone used for behavioral surveys. Their support has been greatly appreciated and the tools have helped to gain a more in-depth understanding of turtle behavior.

Of course it is important to recognize the hours of dedication from Liberty Boyd, Jack Cuffley, Alex Smith, Candace Fields, Meagan Gary and Ana Miguel Blanco for deploying TurtleCams and drones, and the Island School Fall ’20 students and staff, Sam Tabbutt, Aaron Cogger, Kathryn Waters, Emily Robson, and Erin Parker for their assistance in analyses, data collection and organization of such. Praise goes to the team at The Leatherback Trust for keeping me sane during video analysis and writing.

I could not have achieved this without the unwavering support from my family and my friends, who cheer me on from all corners of the world.

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ABSTRACT

Green turtles (*Chelonia mydas*) perform ocean-crossing migrations, maintain healthy marine ecosystems, generate income through tourism, and are endangered and declining globally. For these reasons, among others, this species has been a focus of numerous research programs worldwide for almost a century. Most of these sea turtle research programs require some form of animal handling to collect the required data (e.g., tagging information or the collection of biological samples). However, this can cause stress, especially for wild animals, and that raises ethical issues. Here, I describe novel methods for assessing and mitigating the effects of handling stress on green turtles. Specifically: (1) I used a combination of animal-borne cameras and drone footage to determine how handling stress altered the post-release behavior of green turtles and (2) I used a photo-ID software to determine whether flipper scales can provide more accurate identifications than the more conventionally used facial scale patterns.

I found that turtles spent more time swimming and had shortened dive intervals in the first 30 mins after capture and attachment of a camera than in the hours that follow. Instances of socializing, foraging and resting increased over the 3-3.5 h after release. Animals recorded by drone and not captured were less likely to rest, which suggests this behavior may be a recovery response to handling and/or stress. The same animals were also more likely to socialize. When determining the accuracy of flipper or facial images for photo-ID, I found that head scales provided correct identifications 80% of the time, whereas the flipper provided correct identifications 100% of the time. This implies that researchers could use the flipper instead of more invasive tagging techniques, such as metal flipper tags or using lights to photograph the face for photo-ID, which can induce stress.

CHAPTER 1. ASSESSING THE EFFECT OF HANDLING STRESS ON POST-RELEASE BEHAVIOR IN GREEN TURTLES (*CHELONIA MYDAS*)

1.1 Abstract

Researchers often capture and handle sea turtles to collect data. Capture and handling may cause stress in animals, which can lead to behavioral changes. Two useful pieces of equipment to monitor sea turtle populations are attached bio-loggers and drones. Bio-loggers, such as cameras attached to the carapace of sea turtles, require the capture and sustained handling of the individual, whereas drones require no handling or capture. In this study, I compared the behaviors of green turtles using both tools, assuming that the drone would reveal behaviors that are more natural and the attached camera would reveal initial stress responses that decline over the ~3.5 h of recording after release. Video footage from both tools indicated that green turtles swam more persistently whilst taking breaths that are more frequent in the 30 mins immediately after release when compared to animals recorded by drone. Turtles were also more likely to socialize, rest, and forage 3 - 3.5 h after release. The drone revealed less resting than in the bio-logger recording, indicating that resting may be a recovery response from handling.

1.2 Introduction

Sea turtles are symbols of the marine environment which are intertwined into human culture and practices (Campbell, 2003). Furthermore, sea turtles play a role in maintaining healthy marine ecosystems through habitat preservation, food web continuity, and nutrient cycling (Wilson, 2010). To study juvenile sea turtles in their feeding grounds, they can be captured in-water using a variety of methods. Ogren & Ehrhart (1999) describe three of these methods, which all require handling. Up to 90% of sea turtles that experience disturbance begin their flight when the stressor is 3m away (Griffin et al., 2017) indicating a sensitivity to disturbance at a distance. At maximum swimming speed, sea turtles consume 3-4 times more oxygen then when at rest (Prange, 1976). As air-breathing animals (Berkson, 1966), this requires more time, or a higher frequency of, breathing at the surface, with sea turtles hyperventilating during exertion (Prange & Jackson, 1976). Furthermore, the ethical implications of handling wild animals for research purposes can be extreme, with capture and restraint followed by no rewards quoted as one of the most stressful situations for an animal which may then react as in a life or death situation (Wilson & McMahon, 2006). Therefore, studies are required to measure the behavioral impacts of handling.

Monitoring sea turtle behavior can be difficult as they are mobile marine animals that are generally only visible when they surface (Nowacek et al., 2016). Two valuable pieces of technology that address these issues are animal-borne cameras and drones (Wilmers et al., 2015, Schofield et al., 2019). Animal-borne cameras are useful for providing short-term insights into the behavior of individual animals, particularly of enigmatic species (Hays, 2015). Information garnered from such deployments includes insights into diet, dive profiles, and movement (Heithaus et al., 2002, Thomson et al., 2011, Seminoff et al., 2006). An attached device may itself hinder the animal by the added mass or by the shape inhibiting normal functions (Hawkins, 2004). Another potential impact of such devices is increased drag (≤30%, Watson & Granger, 1998), which could have implications for energy use especially during long distance migrations and general overall welfare of the individual. Any attached equipment may also affect an animal's predation evasion ability, which may be exasperated with longer periods of attachment. Furthermore, the attachment of these devices requires the handling of sea turtles, which can in turn result in a behavioral stress response (Gregory et al., 1996). Short-term effects on sea turtle behavior includes persistent swimming away from the site of capture, as well as more time resting after release, which may make them more susceptible to predation (Thomson & Heithaus, 2014).

Another technological tool which has been increasingly used in ecological research in recent years, Unmanned Arial Vehicles (UAV's, drones), have proved useful in monitoring sea turtles and estimating abundance (Robinson et al., 2020). Researchers can use drone footage alongside other tagging methods to produce insights into behaviors of marine animals, such as mating behaviors (Schofield et al., 2017, Schofield et al., 2019). Drones do not require the handling of animals to deploy, however short battery life limits deployments to short intervals (Oleksyn et al., 2021).

Sea turtle behavior includes breathing, feeding, interacting with other turtles / animals, swimming, and resting. Social interactions are reportedly more frequent in structured settings, such as under rock ledges, than in the open (Thomson et al., 2015). Hawksbills and greens evidently rest most frequently followed by swimming either horizontally or towards the surface, with loggerheads swimming more frequently than any other behavior (Jeantet et al., 2018). Turtles swim excessively when a bio-logger is set to record upon release compared to delaying the start time by a day, which sees more resting and foraging (Thomson & Heithaus, 2014). When recorded by drone, turtles swim most frequently with some individuals spending time foraging, resting or interacting with other turtles (Robinson et al., 2020). The aim of this study was to determine if there were any changes of behavior after handling over time using animal-borne cameras and compare to turtles observed by drones without capture.

1.3 Methods

1.3.1 TurtleCam deployment

I routinely visited four saltwater mangrove creeks located around the coast of Eleuthera, the Bahamas, by boat from the Cape Eleuthera Institute between September 2018 and March 2020. These were Deep Creek (24°46'28.9"N 76°17'00.3"W), Rollins Creek (24°46'11.3"N 76°16'14.9"W), Starved Creek (24°49'07.6"N 76°11'11.7"W), and Half Sound (24°55'36.3"N 76°09'33.3"W) (Fig. 1-1). I captured turtles by hand by means of turtle rodeo, which requires a turtle to be spotted and followed by boat until it exhibits tiredness, then captured by swimmers (Ogren & Ehrhart, 1999). Once I captured a turtle, I recorded the time, checked for flipper tags, and, if none were present, tagged the turtle using metal flipper tags as described by Eckert et al., (1999). I took measurements of the turtle including straight and curved carapace length and width, body depth, and mass. Only those with straight carapace length (SCL) of over 30 cm had an animal-borne camera, hereafter referred to as the TurtleCam, attached to ensure the turtle was large enough to accommodate the equipment.

The TurtleCam consisted of a Paralenz camera (Paralenz, n.d.) with a buoy attached to the battery end. A VHF transmitter attached to the buoy to enables locating the device once detached. On the bottom of the camera, a plastic mount was connected which formed a triangle. Each corner of the plastic mount had a galvanic timed-release attached using plastic zip ties. The other ends of the galvanic timed-releases had a 3 cm x 3 cm square piece of mesh connected (Fig. 1-2). The whole unit was positively buoyant to enable it to float to the surface when detached. I determined a triangle area that was big enough to fit the camera equipment upon on the carapace and dried the area. I applied quick-setting epoxy, 5 mm thick, in a 3 cm x 3 cm square in the corners of the triangle space, and attached the mesh squares of the camera equipment to the carapace using this epoxy. I then released the turtle within 50 m of its original capture location, and recorded the time of release. After 3-4 h, the galvanic timed-releases dissolved and the whole unit, except for the attached mesh, floated to the surface of the water. The attached mesh naturally dislodged from the carapace, or I later removed upon opportunistic recapture. I used radio telemetry to locate the camera. The battery of the camera lasted ~3.5 h for each deployment.

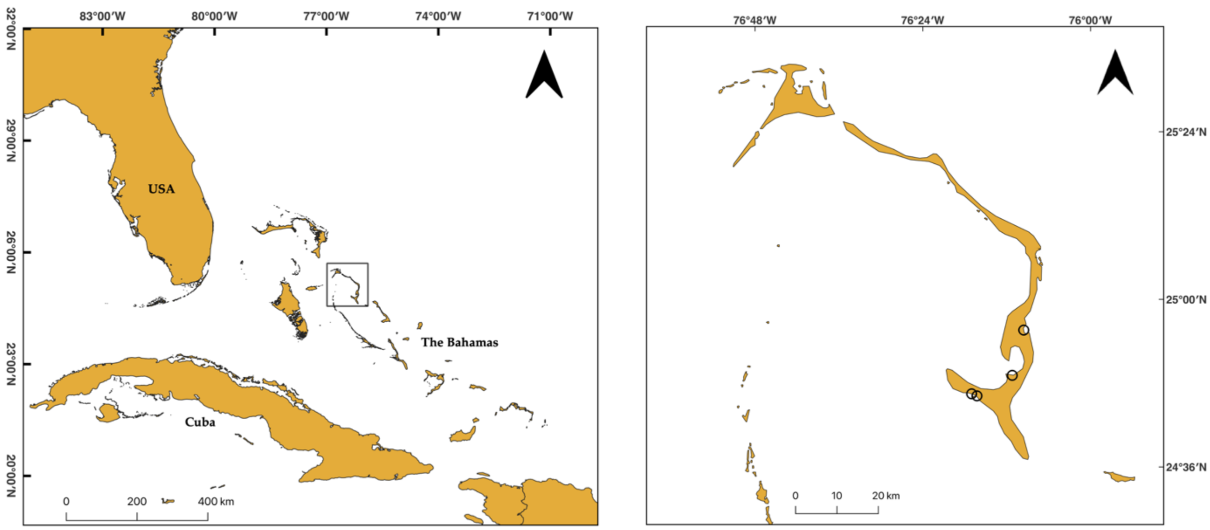

Figure 1-1 Outline map of the Bahamas and the island of Eleuthera (insert) with sampled creeks denoted by black circles

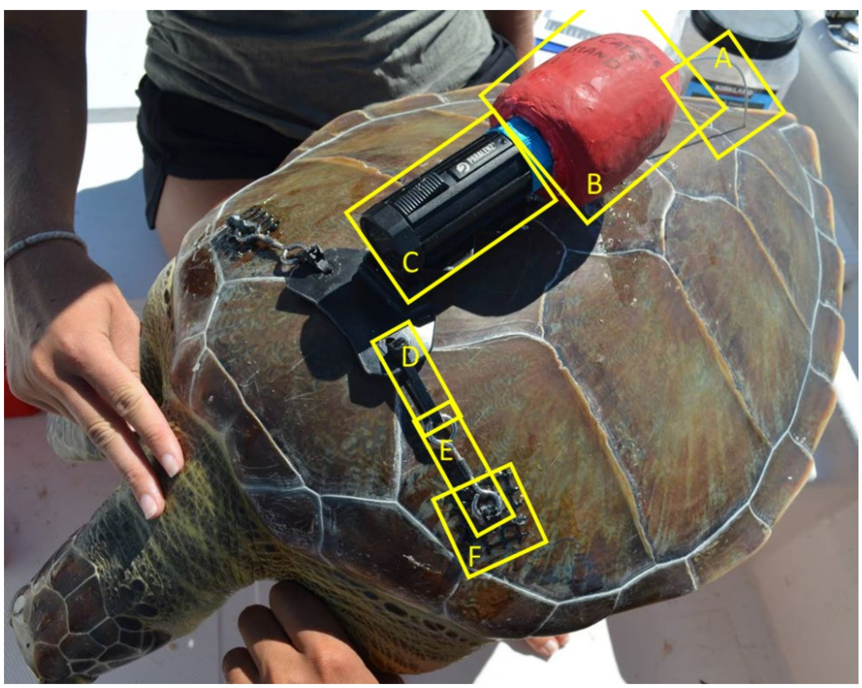

Figure 1-2 Camera unit, referred to as TurtleCam, attached to a green turtle carapace consisting of A) radio transmitter B) floatation device C) Paralenz camera D) cable tie E) galvanic timed-release F) mesh attached to carapace with epoxy. Image credit: Dr. Nathan Robinson

1.3.2 Drone deployment

I visited the same creeks by road on an opportunistic basis on different days to those when I visited by boat and deployed TurtleCams. This was to ensure I had not captured and handled the turtles recorded by drone in the 12 h prior. I operated a drone (DJI, n.d.), and searched the creek at a height of 30 m until a turtle was spotted, at which point I lowered the drone to 15 m above the turtle, and started the video recording. I followed the turtle for as long as the drone battery allowed, ~20 mins, and then I directed the drone back to land. If the turtle was lost during recording, I stopped the recording and brought the drone back to land. The drone recorded up to three turtles per day.

1.3.3 TurtleCam behavior analysis

I only recorded behavior upon release of the sea turtle, after which I conducted focal sampling methods to record the behavior of the sea turtle in each video (Altmann, 1974). Specifically, I recorded the time (s) the turtle spent in each state described in Table 1-2 and visually represented in Fig. 1-3. In some instances, I was aware of potential other behaviors but did not include them as I could not confidently code them. For example, when a turtle was resting under substrate (e.g. rock) it occasionally looked to be scratching itself against the rock, however I could not say with certainty whether this was purposeful or due to moving current, and thus this was coded as resting under substrate. Substrate was determined as a natural or man-made structure that sits on top of, or protrudes out of, the sea bed, which a turtle can use to sit under or next to, such as coral, rock, fishing cages, or seaweed.

Once I had inputted behaviors, I separated the TurtleCam data into half-hour segments and determined the time spent in each behavioral state within each 30 min time segment. I also determined the frequency of breaths in a separate column. I then created independent segments using footage from 0 to 20 mins and from 180 to 200 mins, to compare to the drone footage of maximum 20 mins. Finally, I analyzed the behaviors against the size of the turtle using the SCL measurement. Once I had analyzed all videos, I randomly selected 10-min videos from 11 different TurtleCams and analyzed them again to create a percent agreement.

1.3.4 Drone analysis

I coded behaviors recorded with the drone using the same descriptors as those used for the TurtleCam (Fig. 1-3, Table 1-2). I recorded the time (s) spent in each behavioral state within the full timeframe along with the frequency of breathing events to compare to the TurtleCam results. After analyzing all videos, I randomly selected ~5-min videos from 11 different drones and analyzed them again to determine a percent agreement.

1.3.5 Statistical analyses

I first calculated proportions within each behavior before performing an Arcsine Transformation. I then performed a nested ANOVA to first test whether there was a variation between the TurtleCam and drone results, and then to test where the significance is within the independent time segments (0 to 20 mins of the TurtleCam, 180 to 200 mins of the TurtleCam, total drone footage) (Krzywinski et al., 2013). For the length of breathing interval, I performed a one-way ANOVA to analyze the means between the independent time segments (as above) (Ross & Wilson, 2017). I then determined where the significance could be found using Tukey HSD as a post-hoc test (Abdi & Williams, 2010).

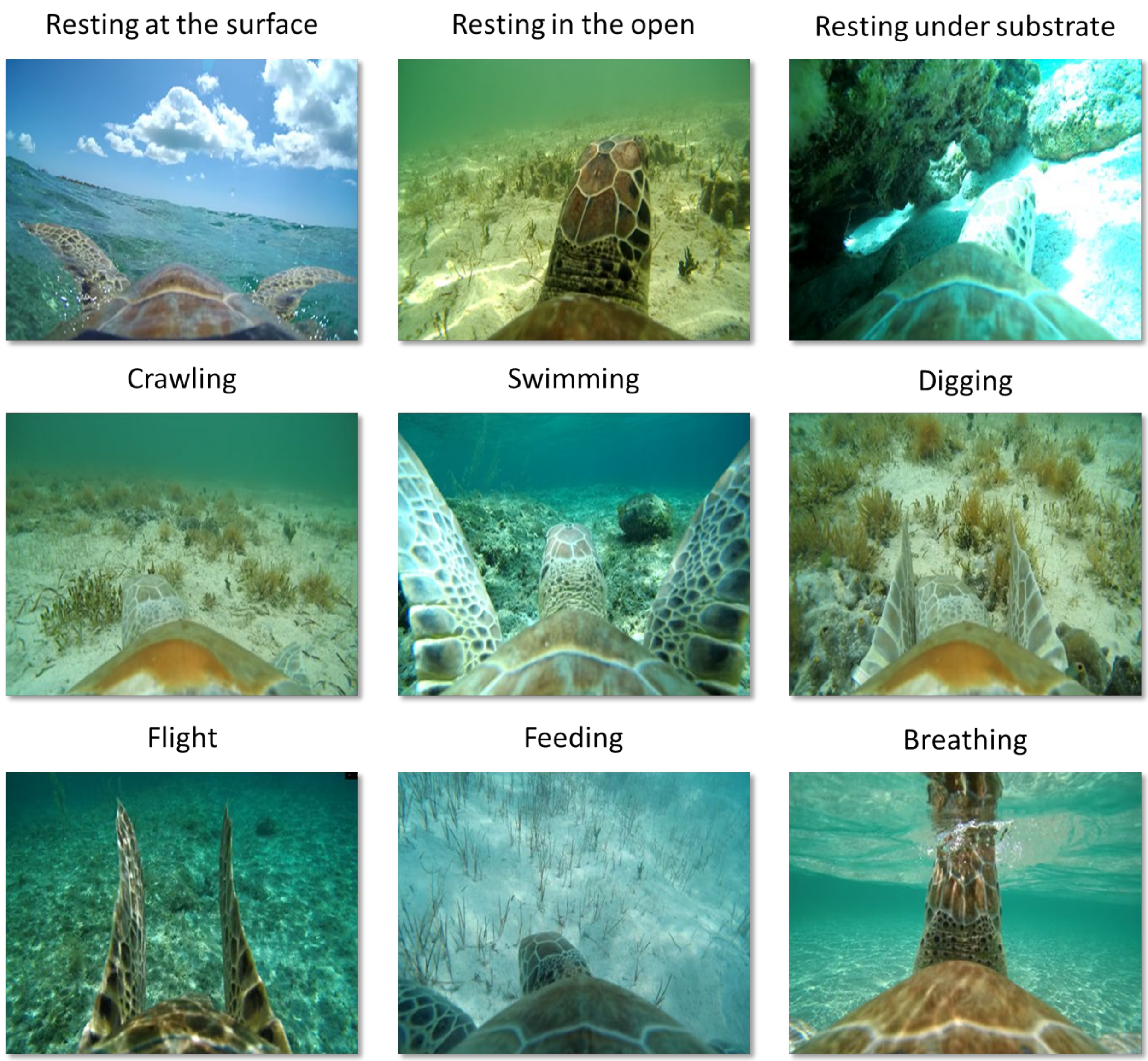

Figure 1-3 Visual ethogram of behaviors green turtles exhibited in the Bahamas, 2018 - 2020

Table 1-1 Written ethogram of behaviors green turtles exhibited in the Bahamas, 2018 - 2020

Type of Behavior	**Behavior**	**Code**	**Description of Behavior**
Resting	At the surface	RS	Turtle is at the water surface but is not actively swimming (e.g. no flipper movement) or in the middle of a breath. This includes the time between breaths if the turtle remains inactive.
	Seafloor in the open	RO	Turtle is on the sea floor but is not actively swimming or crawling. The tidal currents will often make the turtle sway and so the best way to confirm resting is by a lack of movements from the front flippers. Determined as sand being directly beneath the animal, without any part of the animal being underneath substrate, such as coral or rock.
	Under substrate	RU	Turtle is on the sea floor but is not actively swimming or crawling. The tidal currents will often make the turtle sway and so the best way to confirm resting is by a lack of movements from the front flippers. Determined by animal not moving whilst underneath or directly next to substrate, such as rock or coral, as seen by the animal positioning itself with its carapace (shell) underneath or against substrate.
Locomotion	Crawling	CR	Front flippers are moving independently whilst the animal is on the sea floor, allowing movement along the sand.
	Swimming	SW	Turtle is in motion and using its flippers for propulsion. Front flippers can be seen moving either simultaneously or independently and not touching the sea floor, allowing the animal to move through the water.
Basic functions	Digging	DI	Digging starts when the front flippers move forward, and is determined by the throwing of sand backwards towards the turtle. This behavior will often throw a lot of sand on the turtle's carapace. If the turtle starts resting (the flippers are no longer digging) with sediment on its carapace, then this will be recorded as 'Resting – seafloor in the open'.
	Feeding	FE	Feeding behavior seen largely through the head movements of the turtle, which may include biting, chewing, or swallowing food. Animal seen pointing head downwards and grasping sea grass within the beak. Animal seen moving the jaw to chew.
	Breathing	BR	Animal at surface of the water with head above the water line and opening mouth to take in air. Only recorded for the seconds the animal has its head above water. Breathing will start the instant that the turtle's nostrils raise above the surface of the water and will end as soon as the turtle's nostrils submerge again. Time when the turtle is swimming to and from the surface coded as swimming (S).
Other	Unlisted	UL	Describe the other unlisted behavior in detail in the notes column of the spreadsheet.
Social	Social	SOC	Any instance when two or more turtles are interacting. When a turtle is on screen and the target individual does not interact or react, the prevailing behavior is recorded

1.4 Results

1.4.1 Data collected

I collected TurtleCam footage from 50 green turtles, consisting of 7 from Deep Creek, 3 from Half Sound, 14 from Rollins Creek, and 26 from Starved Creek. Drone footage totaled 32 recordings, consisting of 10 from Deep Creek, 11 from Half Sound, 6 from Rollins Creek, and 5 from Starved Creek. The length of footage from the 50 TurtleCams ranged from 123 mins to 208 mins (M = 181.78, SD = 2.46) (Fig. 1-4A). The 32 individual drone videos ranged from 4 mins to 20 mins (M = 14.13, SD = 0.68) (Fig. 1-4B). I assigned a behavior to a total of 545,363 secs (151 h) of TurtleCam footage and 27,061 secs (8 h) of drone footage. When I analyzed randomly selected videos a second time, I agreed with my original behavior input 95% of the time for the TurtleCam and 97% of the time for the drone footage.

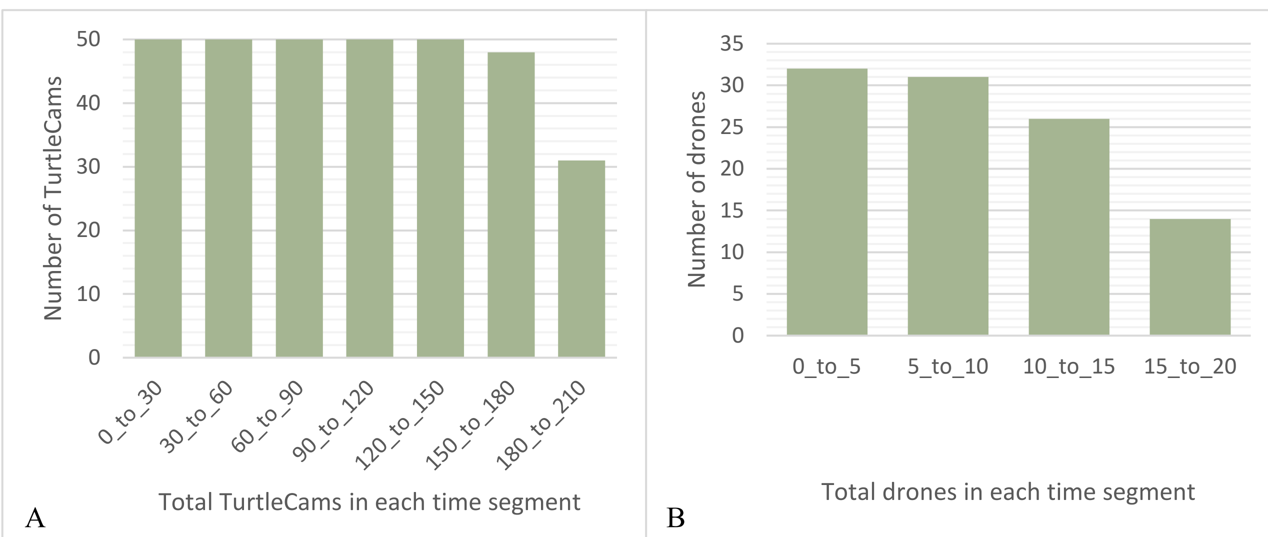

Figure 1-4 Total number of attached cameras and drones in each time segment, from turtles recorded in the Bahamas, 2018 - 2020

1.4.2 Behavior comparison

Locomotion, including swimming and crawling, generally decreased over time after attachment of the TurtleCam, with only a slight rise during 150-180 min. Locomotion decreased overall from 86% of the first 30 min segment (0 to 30 min) to 48% of the last 30 min segment (180 to 210 min) (Fig. 1-5). The drone footage recorded locomotion 80% of the total time. Mins 0 to 20, 180 to 200, and the drone footage were all significantly different to one another (0 to 20 – 180 to 200 P<.001; 0 to 20 – drone P= .006; 180 to 200 – drone P<.001) (Fig. 1-6). Swimming was the most common form of locomotion throughout all the footage, with the highest percentage occurring in the first 30 mins of the TurtleCam (98%) and the lowest occurring in the 60-90 min segment (89%). The drone footage revealed swimming 97% of the time.

Feeding recorded with the TurtleCam generally increased over the time segments, from <1% during the first 30 min to 16% at 180-210 min (Fig. 1-5). The drone recorded feeding for 5% of the total time. Feeding during mins 0 to 20 was significantly different to 180 to 200 (P= .001). There was no significant difference between the TurtleCam and drone footage (Fig. 1-6).

Social interactions on the TurtleCam increased over time, from <1% in the first 30 min, to 2% in the last 30 min. The drone footage recorded social interactions 3% of the total time (Fig. 1-5). There were no significant differences in social interactions between the TurtleCam and drone footage, nor over time (Fig. 1-6).

In the TurtleCam footage, resting increased from the first 30 min (8%) to 90 to 120 min (39%), after which it decreased at 180 to 210 min (to 33%) (Fig. 1-5). The drone footage recorded resting 11% of the total time. There was a significant different in resting between 0 to 20 mins and 180 to 200 mins (P<.001) (Fig. 1-6). TurtleCam individuals rested at the surface 47% of the total resting time in the first 30 mins and 7% of the total resting time in the last 30 mins (Fig. 1-7A). The drone recorded minimal resting at the surface (<1%) (Fig. 1-7B). Resting in the open by TurtleCam turtles initially increased from 32% in the first 30 mins to 63% during the 90-120 mins, but decreased after that point to 53% during the 180-210 mins. Turtles recorded by drone rested in the open 86% of the total resting time. Resting under substrate such as rock or coral generally increased over time, from 21% in the first 30 mins, to 40% in the last 30 mins. The drone footage recorded resting in the open for 13% of all resting instances.

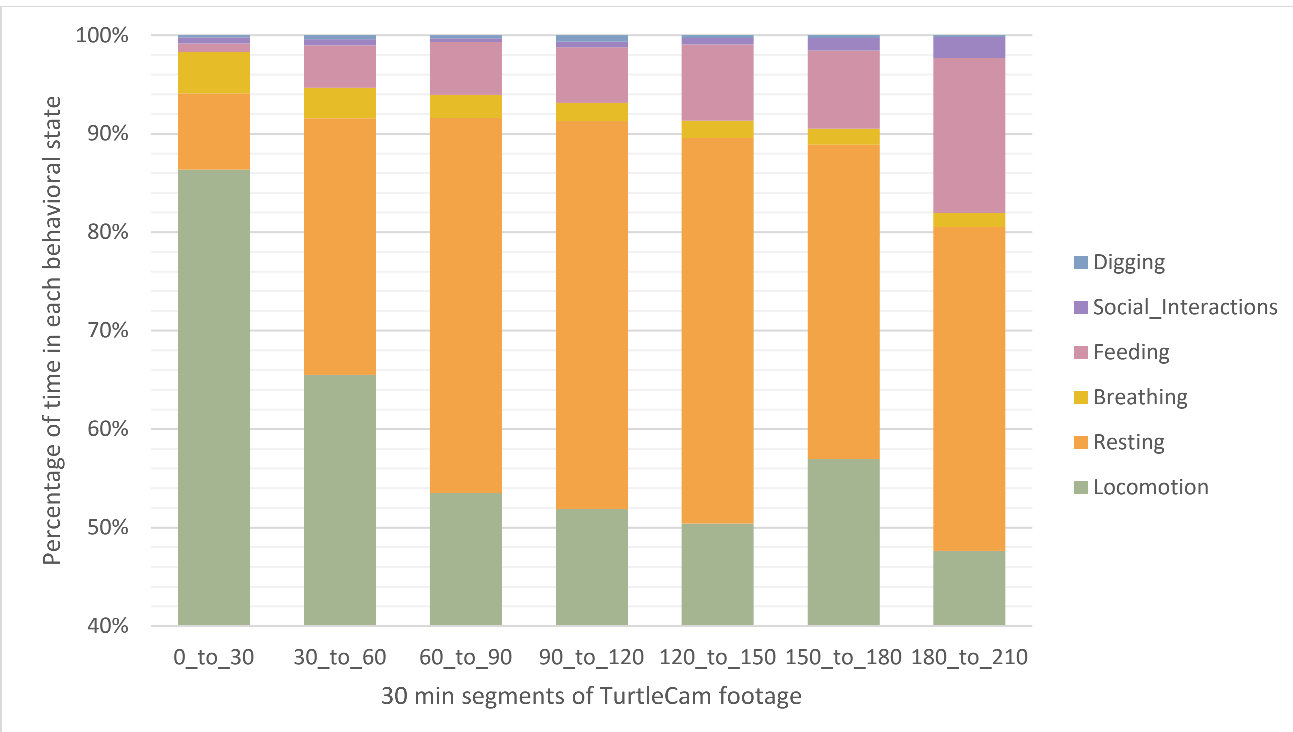

Figure 1-5 Percentage of time green turtles spent in each behavioral state at half hour intervals following deployment of an attached camera (TurtleCam) in the Bahamas, 2018-2020

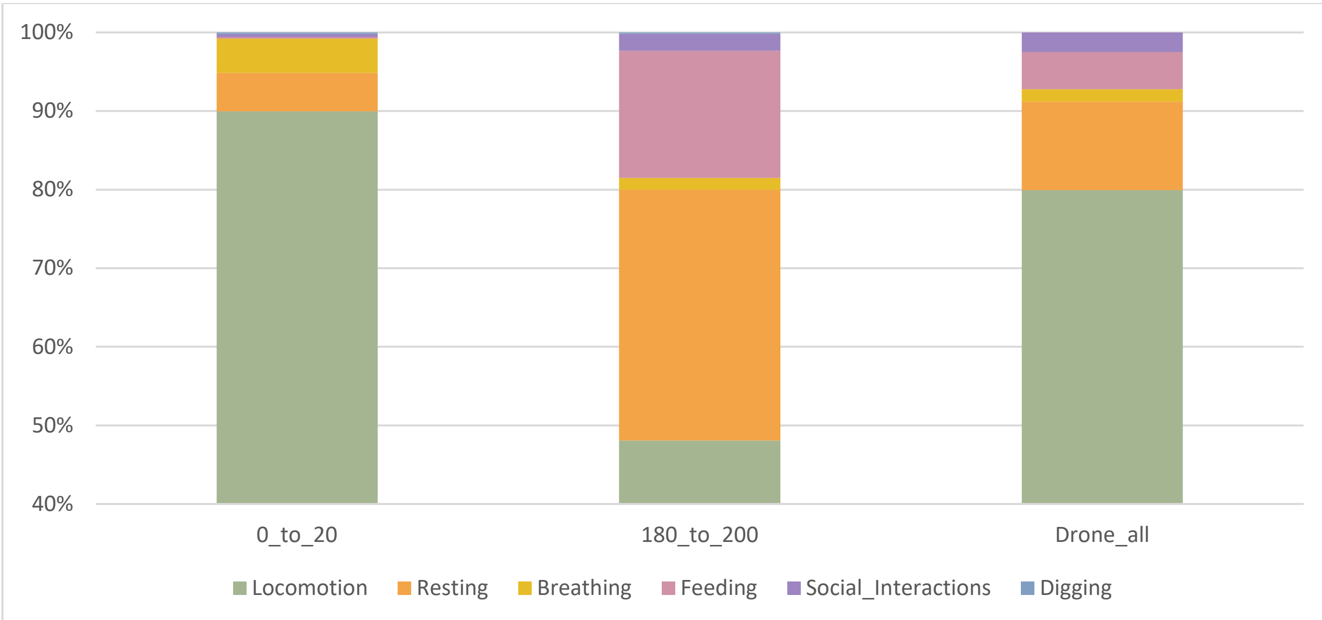

Figure 1-6 Independent time segments for attached camera (TurtleCam) and drone footage in each behavioral state from green turtles in the Bahamas, 2018 - 2020

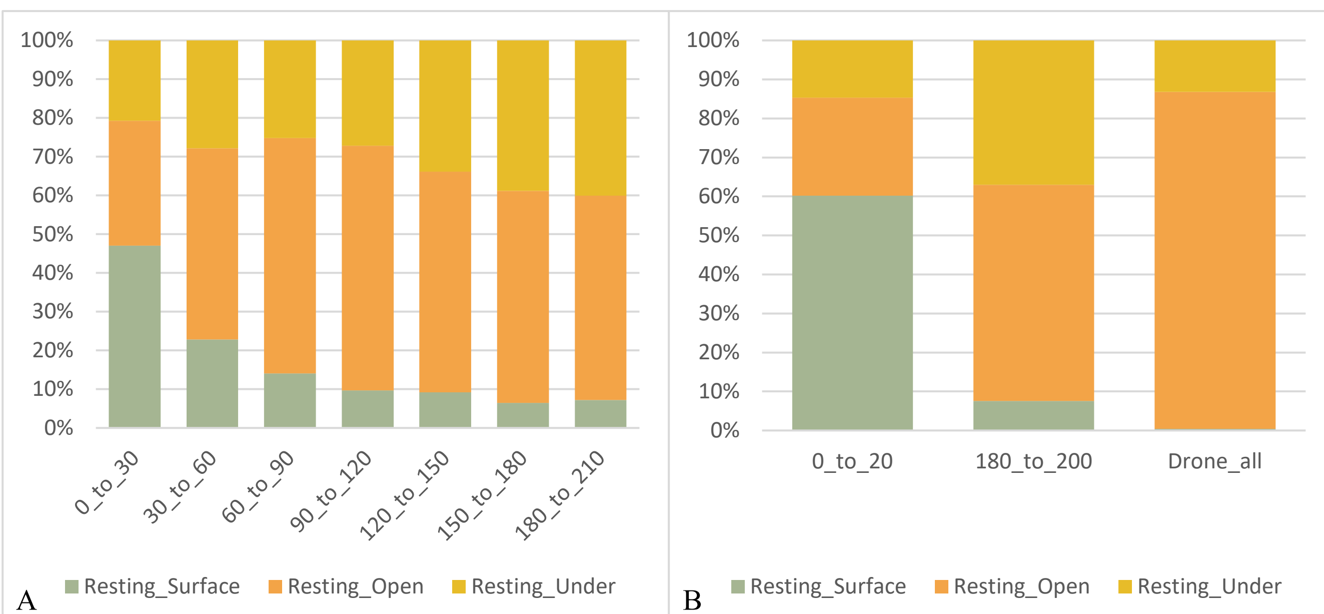

Figure 1-7 Types of resting by green turtles with attached cameras (TurtleCam) (A) and compared to sea turtles recorded by drone (B), in the Bahamas, 2018 - 2020

1.4.3 Behavior comparison by size class for TurtleCam turtles

Small turtles (SCL 35 – 45 cm) spent the most time in locomotion out of all of the size classes (67%). Medium sized turtles (SCL 45 – 55 cm) spent the most time feeding compared to the other size classes (7%). Large turtles (SCL 55 – 65 cm) spent the most time resting compared to the other size classes (31%) (Fig. 1-8). All size classes spent the same amount of locomotive time swimming (94%) and crawling (6%).

Of the time spent resting, large turtles spent the most time in the open compared to the other size classes (53% of total resting time for large turtles) with small turtles spending the least (34% of total resting time for small turtles). Small turtles spent the most time resting under substrate (47%) and at the surface (19%) with large turtles spending the least (31% and 17% respectively).

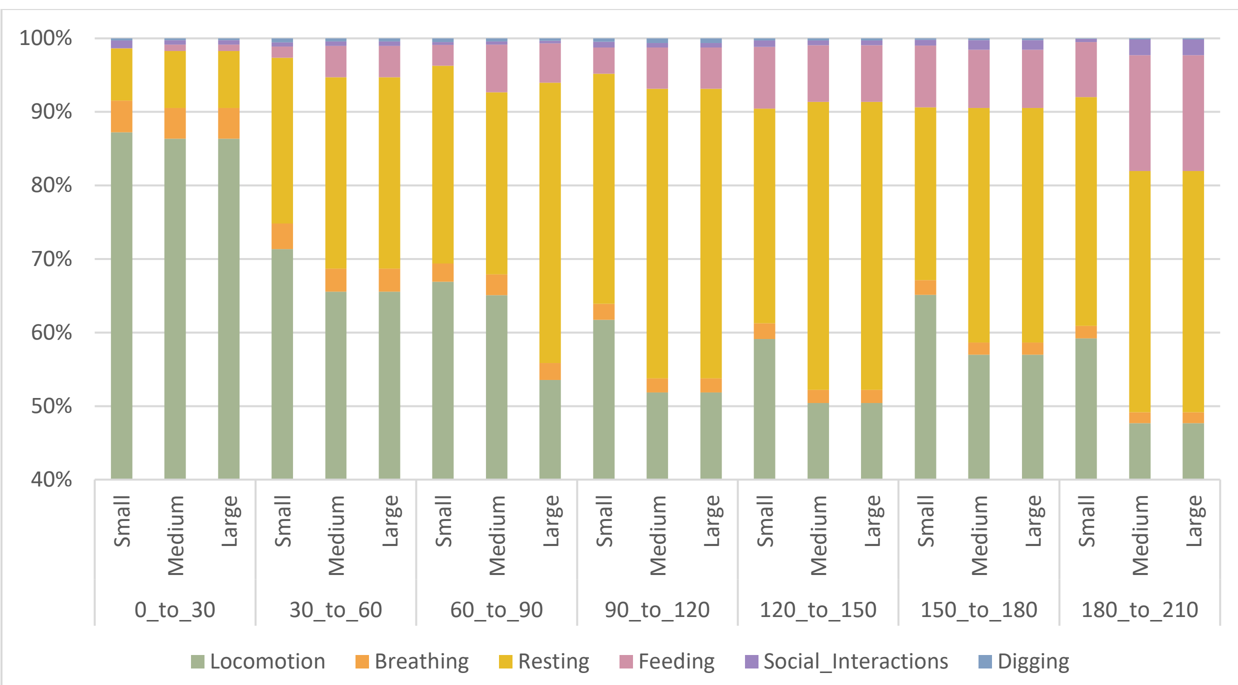

Figure 1-8 Percentage of time green turtles spent in each behavioral state for each size class in the Bahamas, 2018 – 2020. Small = 35 – 45 cm SCL, medium = 45 – 55 cm SCL, large = 55 – 65 cm SCL

1.4.4 Breaths

The interval between breaths (mins in apnea) increased from 0.5 mins (SD = .06) in the first 30 mins of the TurtleCam footage, to 1.5 mins (SD = .14) in the last 30 mins of TurtleCam footage (Fig. 1-9A). The drone footage recorded a breath interval of 1.6 mins (SD = .12) which was significantly different to 0 to 20 mins of TurtleCam footage (M = 0.48, SD = .03, P<.001) but not significantly different to 180 to 200 mins of TurtleCam footage (M = 1.46, SD = .14, P=.923) (Fig. 1-9B). Small turtles consistently took shorter intervals between breaths compared to medium turtles (small: 0 to 30 mins: 0.48 mins, 180 to 210: 1.35 mins; medium: 0 to 30 mins: 0.47 mins, 180 to 210: 1.57 mins) (Fig. 1-10). Large turtles took the longest intervals between breaths except in the 180 – 210 min time segment (large: 0 to 30 mins: 0.76 mins, 150 to 180 mins: 2.01 mins, 180 to 210: 1.44 mins) (Fig. 1-10).

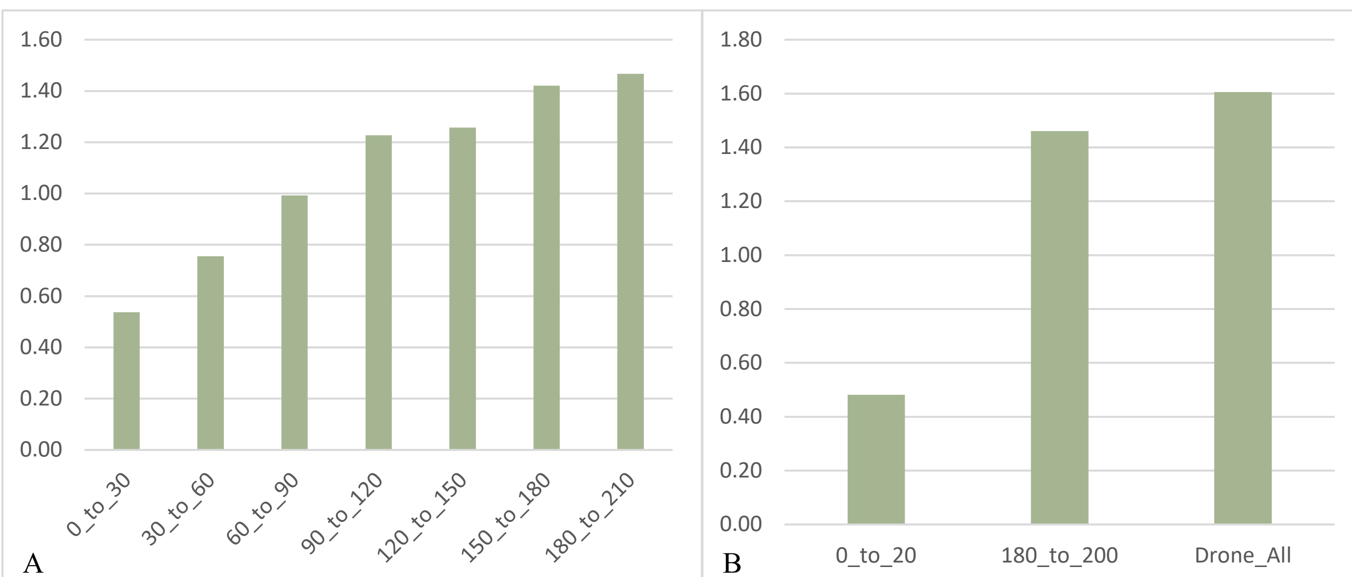

Figure 1-9 Length of breathing interval in mins for green turtles with attached cameras (TurtleCam) (A) and compared to sea turtles recorded by drone (B), in the Bahamas, 2018 - 2020

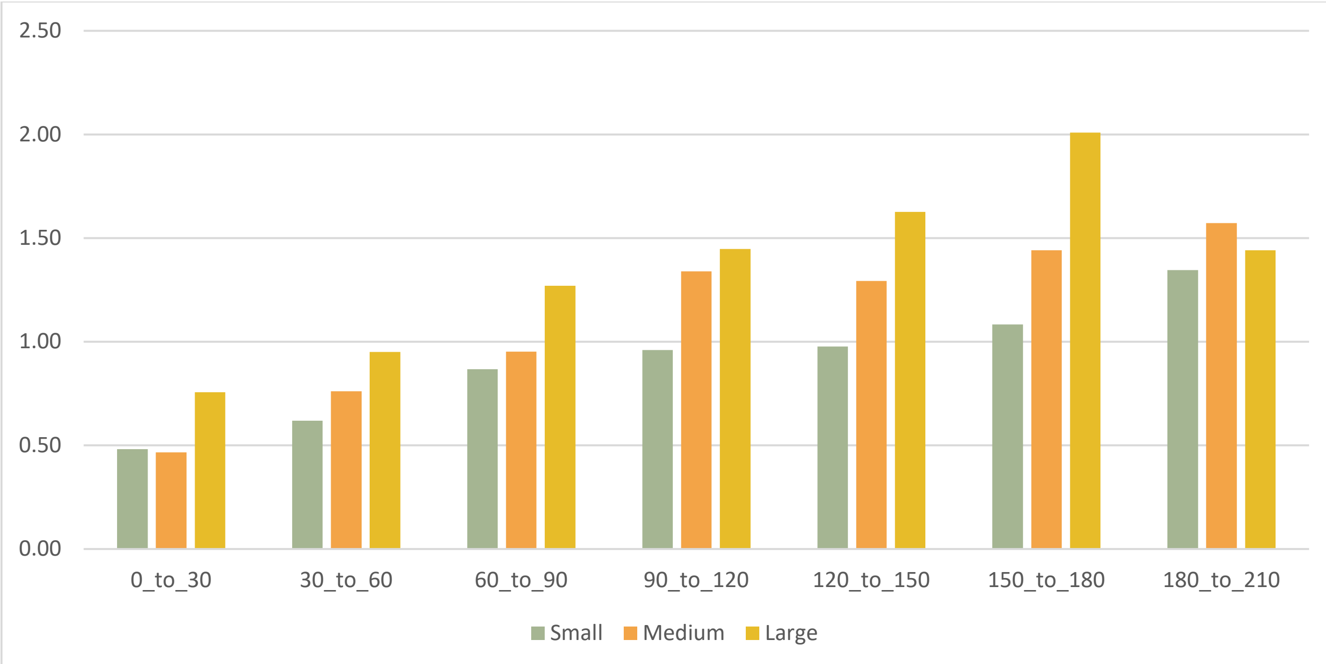

Figure 1-10 Breaths interval (in mins) for green turtles with attached cameras (TurtleCam) at 30-min intervals after camera attachment and release, grouped by size classes, in the Bahamas, 2018 – 2020**.** Small = 35 – 45 cm SCL, medium = 45 – 55 cm SCL, large = 55 – 65 cm SCL

1.5 Discussion

As sea turtle populations continue to be threatened, their study aims to learn how and if we can aid in their survival and the recovery of populations (Ceriani et al., 2019). To study marine animals, researchers sometimes need to handle wild subjects who may experience stress. We can consequently be conscious of our impact on these animals as researchers, and continue to develop ways to reduce any handling stress. Furthermore, to ensure the data we collect are representative of natural behaviors, we should understand how our manipulation can result in unnatural behaviors and how long such effects last. I utilized bio-logger (animal-borne camera, TurtleCam) and UAV (drone) technologies to determine how each tool differed in terms of data collected, and to determine the effect of handling on green turtle behavior. Behaviors seen at the beginning of the TurtleCam footage differed from that seen later in the footage, and differed from behaviors recorded by the drone. Behaviors recorded by the TurtleCams after 180+ min were similar to those seen on the drone footage and thus more natural, as drones have been reported to not disturb sea turtles at a height of 10 m and above (Bevan et al., 2018).

1.5.1 Behavior

The most commonly observed behavior was locomotion, which was more common in the first 30 mins accounting for 86% of the total time compared to 48% in the last 30 mins. This could signify movement as a response to capture, which aligns with findings by Thomson & Heithaus (2014). They recorded persistent swimming in the first 30 mins, which they attributed to movement away from the capture site or exploring the area once away from the site. The drone footage recorded locomotion 80% of the time, which is higher than the last 30 mins and conflicts with the findings by Thomson & Heithaus (2014). However, as sea turtles were generally spotted by drone due to their movements, the high frequency of locomotion could be due to starting the recording whilst in that state, and only recording for ~14 mins. It is likely that I missed turtles that were resting or performing other non-movement behaviors during the time of drone deployment.

Resting occurred more frequently in the last 30 mins of TurtleCam footage; accounting for 33% of all behaviors, but the drone footage was similar to the first 30 mins of TurtleCam footage with 11% of time spent resting. This again may be due to the inability to spot resting turtles as easily as those in movement behaviors. However, these results could indicate that turtles captured in the previous hours and have swum more persistently beforehand require more resting. Thomson & Heithaus (2014) found that when they delayed the start time of video recording by a day, swimming was vastly reduced and resting and foraging increased. My results support this claim, with feeding occurring minimally in the first 30 mins, accounting for less than 1% of the total time, and increasing to 16% of the total time in the last 30 mins.

I saw a reduction in stress responses (swimming) as well as an increase in recovery responses (resting) within the full timeframe of the TurtleCam (~3.5 h) before sea turtles reportedly return to their pre-capture stress levels. Gregory et al. (1996) found that levels of a stress indicator hormone, corticosterone, reduced after the first hour following capture, which may align to the reduced swimming and increase of other behaviors seen in the TurtleCam footage. They found that levels do not return to near baseline until after 6 h after capture, which may mean that the behavior I recorded for the last 30 mins of TurtleCam footage (~3.5 hours after capture) may still be stress or recovery responses, and those behaviors seen in the drone footage aligned to base level of stress. Hunt et al. (2019) also found that 6 h in a saltwater pool enabled sea turtles to recover from transportation at least in terms of some of the stress indicators (glucose, potassium, and corticosterone), which supports my findings.

Small turtles spent the most time in locomotive states, with medium-sized turtles feeding more than other sized turtles, and large turtles resting for the greatest amount of time out of the size classes. Larger turtles generally took longer intervals between breaths, with an exception in the last time segment (180 to 210 mins). This exception could be due to there being less available footage for this time segment, with available seconds reducing by 74 – 85% compared to the first 30 mins. This could lead to a few turtles skewing the results.

1.5.2 Breathing

Breath interval (the length of time in apnea between breaths) increased over time on the TurtleCam footage to become similar to the drone recording. A breathing interval of 1.5 to 1.6 minutes could be a natural rate for juvenile and sub adult green turtles in the Bahamas. Furthermore, turtles spent less time at the surface taking breaths as time from capture increased. Minimal time resting at the surface was recorded on the drone, which could show that the natural behavior of juvenile and sub adult greens is to not rest at the surface taking breaths.

1.5.3 Conclusions

This study shows that sea turtles may exhibit stress and recovery responses for over 3.5 h, but such responses reduce over time to become close to that seen in drone footage, which does not have a capture event. I also show that some behaviors, such as resting at the surface whilst taking multiple breaths, may be a response to capture. Further research could include reviewing natural behaviors of turtles seen by drones that have longer battery lives and can provide detail on longer-term behaviors.

CHAPTER 2. COMPARING THE ACCURACY OF USING FLIPPER OR HEAD SCALE PATTERNS FOR SEA TURTLE PHOTO-IDENTIFICATION

2.1 Abstract

Photo identification (photo-ID) is a non-invasive and increasingly reliable method for mark-recapture in sea turtles. Most photo-ID studies in sea turtles distinguish individuals using scale patterns of the head, even though the scale patterns on the flippers are more complex than the head and thus could provide a more robust area for identification. Here, I compared the accuracy of using the head or fore flipper photos for distinguishing between juvenile green turtles (*Chelonia mydas*) using the Automatic Photo-Identification Suite (APHIS) software. Using various sections of the flipper, I achieved between 53 and 88 % successful matches with the most accurate encompassing the digits. Using the dorsal view of the head as a reference area resulted in 61% correct identifications compared to 55% using the lateral view of the head. Within the reference area, the number of scales used in the identification affected the accuracy of the matches, with 10 scales of the head providing the most accurate identifications, and 14 scales on the flipper. After optimizing the head and the flipper by using the optimum number of scales and only photos that followed the ascribed quality protocol, I then achieved 80% correct identification using the head and 100% correct identifications using the flipper. I conclude that flippers can provide accurate areas to use for photo-ID in sea turtles rather than head photos, especially when captured.

2.2 Introduction

Mark-recapture data provides valuable information about movement patterns, growth rates, population size, or demographic rates of animal populations (Powell et al. 2000, Casale et al. 2009, Pradel 1996, Fonseca et al. 2008). In most cases, individuals of the species under study are marked on first capture so subsequent recaptures and associated data can be recorded (Lettink & Armstrong, 2003). Photo identification (photo-ID) is often employed by wildlife biologists as an alternative to conventional tagging techniques such as external plastic or metal tags (Balazs, 1999) or implanted Passive Integrated Transponder (PIT) tags (Gibbons & Andrews, 2004). The accuracy of photo-ID often tends to be lower than physical tagging efforts, yet a clear benefit is that it is non-invasive and does not inherently require direct handing of wild animals (Schofield et al., 2008, Dunbar et al., 2021). The use of photo-ID most typically relies on pattern matching using specific features, such as scale or spot patterns (Brooks et al., 2010). In sea turtles, it is common to use the scale patterns on the head (Calmanovici et al., 2018, Carpentier et al., 2016) with some studies reporting accuracies of 80 – 97% (Calmanovici et al., 2018, Dunbar et al., 2021). However, a recent study also validated the use of flipper scales for photo-ID in sea turtles, achieving reporting accuracies of 82 – 93% (Gatto et al., 2018). No studies to date have directly compared identification using head or flipper scale patterns using the same technique. As accuracy is a key concern in photo-ID studies, there is therefore a need to test the accuracy of photo-ID techniques in sea turtles for both head and flipper scales.

A clear advantage of using head scales for photo-ID in sea turtles is that it provides a rigid structure that does not contort with movement. However, photos taken of the head, particularly at night on nesting beaches, can require shining bright lights into the eyes of the turtle. The use of torches or flashing lights can cause sea turtles to display a disturbance response, including aborting the nesting attempt (Waayers et al., 2006). Arguably flipper photos could provide more accuracy than head photos as scale patterns on the flippers of sea turtles are more complex and thus provide greater diversity for identification. However, turtle flippers are flexible, semi-rigid appendages (Wyneken et al, 1997) and this may reduce accuracy when assessing scale patterns, especially from a 2D perspective. Furthermore, the angle of the image can affect the ability for tools to identify individuals, with increasing the horizontal angle of the photo reducing match likelihood (Speed et al., 2007).

Here, I compared the accuracy of using the head or the fore flippers to identify juvenile green turtles (*Chelonia mydas*) within the Automatic Photo-Identification Suite (APHIS) (Moya et al. 2015). APHIS is a free software that has been successfully used in the identification of reptiles from scale patterns including lizards (Rotger et al., 2016), snakes (Rotger et al., 2019), and sea turtles (Gatto et al., 2018). To compare the accuracy of head and fore-flipper photos, I have four key objectives. (1) I determined which area of the head and which area of the fore flipper provide the most accurate identification. (2) I assessed whether photo quality influences identification accuracy. (3) I assessed whether increasing the number of scales used within the defined area influences the accuracy of the identifications. (4) I compared the accuracy between using the optimized head or flipper areas.

2.3 Methods

From August 2018 to March 2020, I routinely hand-captured sea turtles from four tidal creeks in Eleuthera, The Bahamas (Fig. 1-1), via hand-capture (rodeo) or seine netting (for details see Ogren & Ehrhart, 1999). I checked turtles for metal flipper tags, and tagged them if no previous tags were present. In addition, I weighed and measured each turtle for both straight and curved carapace length and width (for details see Bolten, 1999). Finally, I collected 3 photos for photo-ID (details below). I then released turtles within 50 m of their original capture location.

2.3.1 Photo quality protocol

From each individual, I collected both dorsal and lateral photos of the head and dorsal photos of the flipper. The photo quality protocol I created described that I fully extended the head to show the proximal or parietal scales for the lateral and dorsal views and took the photo perpendicular to the head (Fig. 2-1 A, B). To minimize any distortion from the flexing of the flippers, the photo quality protocol described that I laid each flipper laid flat using the palm of my hand or a clipboard before the photo was taken (Fig. 2-1 C, D). I took photos perpendicular to the flipper and I ensured that I framed the entire flipper in the photo.

Finally, I positioned the turtle in either in full sunlight or in full shadow if sunlight was not possible. I took photos using a camera phone (Galaxy S8, Samsung, n.d.) as this was readily available in the field, and use of DSLR in other studies produced a similar accuracy (Hoefer et al., 2021).

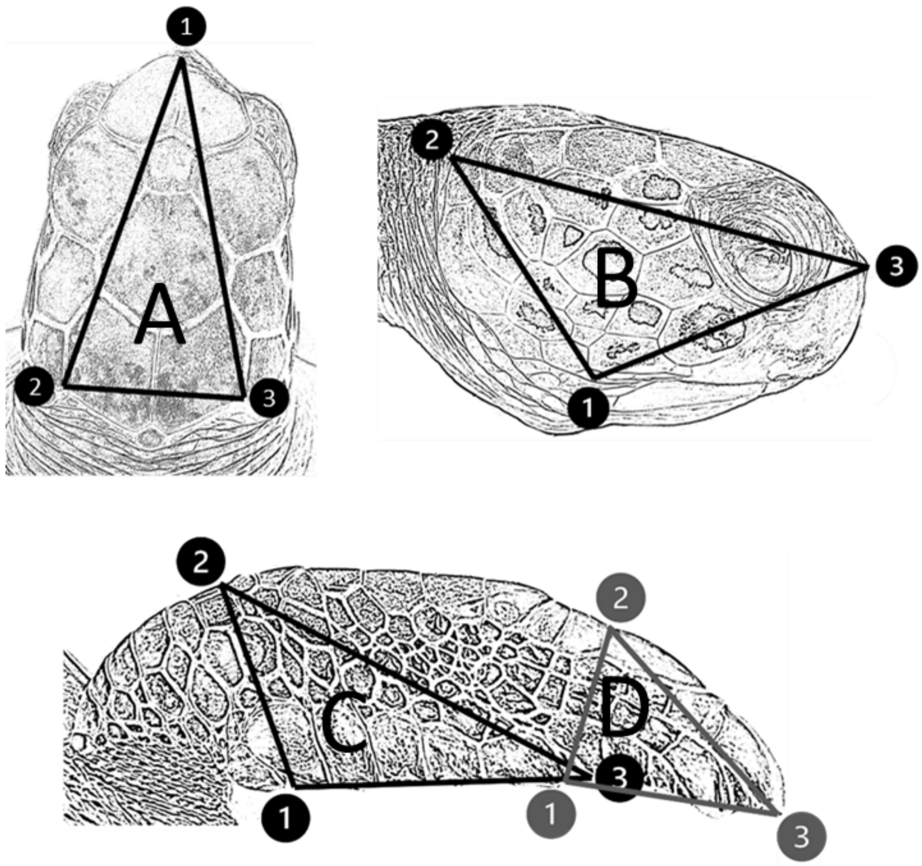

Figure 2-1 Example of photos taken of the dorsal view of the head, lateral view of the head the right flipper of green turtles, along with the placement of reference points (1) bottom reference, (2) left reference, (3) right reference, in the Bahamas, 2018 - 2020

2.3.2 Processing images

I identified four different study areas: the lateral view of the head, dorsal view of the head, digits of the fore flipper, and wrist of the fore flipper. I selected two photos for each individual: the first capture photo and one recapture photo from the same individual. This was to ensure all individuals had an equal number

(2) of photos within the database. Otherwise, there could be a bias towards individuals with the highest number of photos. I did not include any individuals with missing or severed flippers.

APHIS uses the Spots Pattern Matching (SPM) routine, which compares user-defined spots patterns (see details in Moya et al., 2015). The SPM routine requires the placement of 3 reference points that will outline the area to be used for pattern recognition, hereafter referred to as the "reference area". Within the reference area, I defined a scale pattern by placing marks (spots) at the intersections, or corners, between the scales. APHIS spatially corrects the scale pattern to align the reference points exactly, before comparing all scale patterns in the database and assigning a rank to matched photos. APHIS calculates the rank by summing the metric distances between pairs of every mark from the comparison and database photos, then dividing by the square of the total number of paired marks, with the lowest scores denoting a more likely match. The rank denotes how likely it is a correct match with the inputted photo, from the best match (1st rank) to worst match (in this case, 51st rank). To determine the accuracy of the matches, I graded them as follows: A correct match when the correct individual is the top ranked match. A partial match when the correct individual is within ranks 2-10. No match if the correct individual is outside of the top 10 ranked matches.

2.3.3 Optimizing the reference areas

When comparing photos from the dorsal view of the head, the three reference points were placed in (1) between the prefrontal scales, closest to the nares (bottom reference), (2) back left corner of the left parietal scale, closest to the neck (left reference), (3) back right corner of the right parietal scale, closest to the neck (right reference) (Fig. 2A). When comparing photos of the lateral view of the head, the three reference points were placed in: (1) the corner of the mouth (bottom reference), (2) top proximal scale determined by the scale which extends furthest to the left than any other towards the top of the head (left reference) (3) tip of the nose (right reference) (Fig. 2B).

I compared flipper photos using two different reference areas: one at the wrist of the fore flipper as used by Gatto et al. (2018) and the other using the digits of the fore flipper. Reference points for the wrist of the fore flipper are placed as described by Gatto et al (2018; Fig. 2C). The area using the digits of the fore-flipper has reference points: (1) the left corner of the first non-thickened scales, distal from the axilla (bottom reference), (2) the left top corner of the longest scale along the anterior edge of the flipper (left reference), (3) the tip of the furthest right scale (right reference) (Fig. 2D).

Within the reference areas for the wrist and digits of the fore flipper, I placed 60 marks in the corners of scales within the reference area. For the lateral and dorsal view of the head, I marked all corners of scales within the reference area, totaling up to 60 marks (limited by how many scales were available). From this comparison, I determined the most accurate area to use from this point onwards. Due to small sample numbers, I selected the Fishers Exact test to determine significance between the reference areas (Kim, 2017).

2.3.4 Photo quality comparison

Once I sorted photos into whether they followed the protocol described above correctly or not (Fig. 2-2), I compared head and flipper photos to see whether the quality protocol influenced the accuracy of the identifications. Photos taken at an angle that distorted scales, were blurry or out of focus, or did not encompass the entire reference area did not follow the protocol. I initially started with a database of 51 individual green turtles, which reduced to 32 head photos and 35 flipper photos after removing non-compliant photos. Significance between the photos that followed the photo quality protocol and those that did not was tested using Fishers Exact test (Kim, 2017). From this point on, I only used those photos determined to follow the photo quality protocol.

Figure 2-2 Example photos of the head of a green turtle following the quality protocol (left) and not following the protocol (right), in the Bahamas, 2018 - 2020

2.3.5 Number of scales

To test whether the number of scales used within the reference area influences the accuracy of photo matching, I analyzed the database using increments of scales. I initially started by using 6 scales within the reference area, increasing to 10 scales and finally 14 (Fig. 2-3). Number of scales was chosen to ensure the minimum number of marks (15 marks minimum = 6 scales), and then increased by 4 scales each time. I then used the treatment with the highest number of correctly identified individuals to determine the optimal number of scales. A one-way ANOVA tested the significance of using different number of scales, by analyzing the means between three groups (Ross & Wilson, 2017).

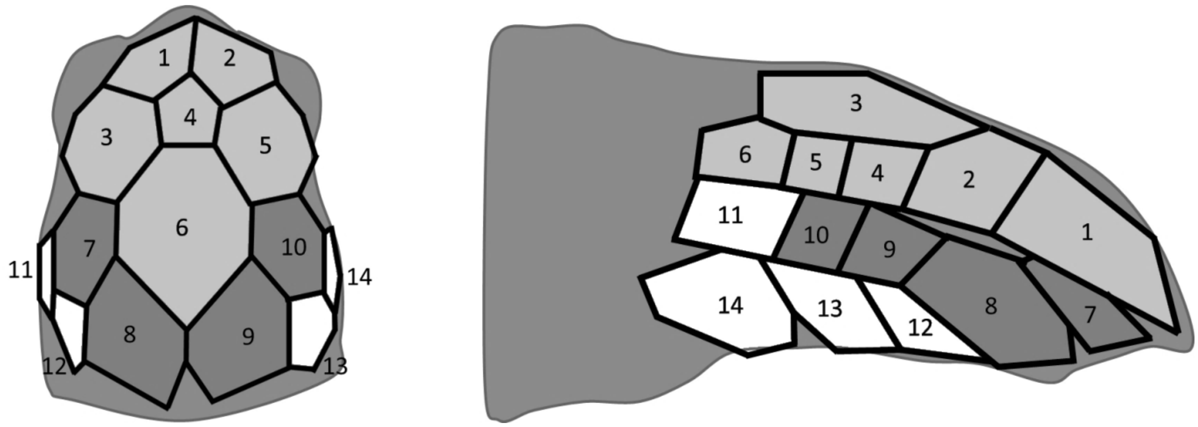

Figure 2-3 Numbering of scales used to create the scale pattern for the dorsal view of the head (left) and digits of the flipper (right) of green turtles captured in the Bahamas, 2018 – 2020

2.4 Results

My photo-ID database included 51 individuals with at least two captures. All turtles were juveniles or sub-adults and had straight carapace lengths, from notch to notch, between 233 mm to 605 mm (M = 405 mm, SD = 11.88).

2.4.1 Optimizing the reference areas

Of the two head scale areas, the dorsal view resulted in 31 (61%) correct matches (the correct individual as the top ranked match), whereas the lateral view resulted in 28 (55%) correct matches (Fig. 2-4). Of the two fore-flipper scale areas, the digits resulted in 45 (88%) correct matches, whereas the wrist resulted in 27 (53%) correct matches (Fig. 2-4), which was significantly different (P = <.001).

2.4.2 Photo quality protocol

Of the individuals photographed using the dorsal view of the head, 32 followed the photo quality protocol, and of these 69% resulted in a correct match. Those that did not follow the photo quality protocol were correctly matched 47% of the time (n = 19) (Fig. 2-5), which was significantly different to those that followed the protocol (P = <.001). Of the photos using the digits of the fore flipper, 35 followed the photo quality protocol, of which 94% correctly matched. Those that did not follow the photo quality protocol correctly matched 75% of the time (n = 16) (Fig. 2-5), which was significantly different to those that followed the protocol (P = .029).

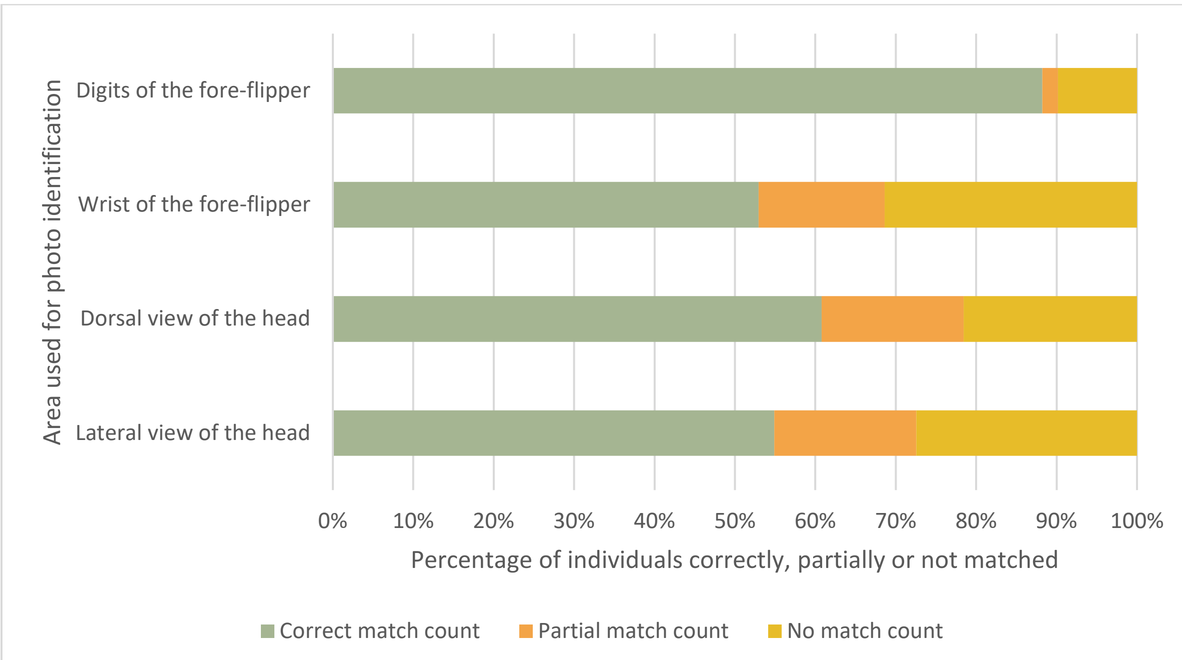

Figure 2-4 Percentage of individual green turtles from the Bahamas (2018 – 2020) correctly, partially or not matched / identified using different areas of the head (dorsal and lateral) and flipper (wrist and digits)

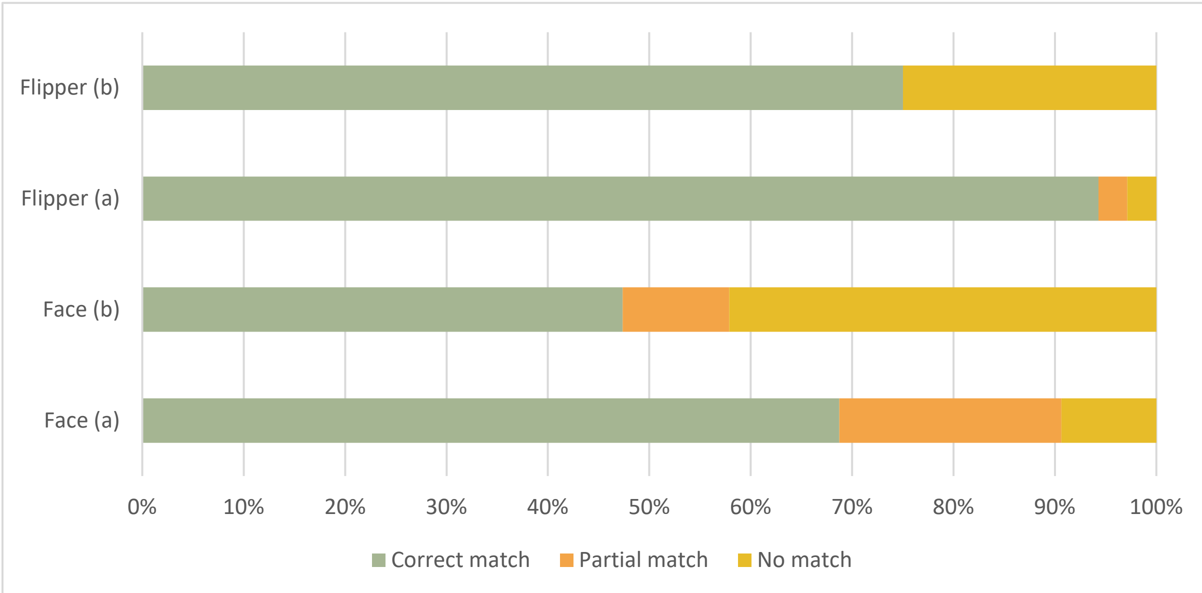

Figure 2-5 Percentage of individuals correctly, partially or not matched when following the photo quality protocol and when not following, (a) = protocol followed, (b) = protocol not followed

2.4.3 Number of scales

From this point onwards, only those photos following the photo quality protocol were used (face = 32, flipper = 35). When using 6 scales on the dorsal view of the head, individuals were correctly matched 63% of the time, which increased to 91% when using 10 scales and decreased to 81% when using 14 scales (Fig. 2-6). When using 6 scales on the digits of the fore-flipper, individuals were correctly matched 86% of the time, which increased to 89% when using 10 scales, and increased to 100% when using 14 scales (Fig. 2-6).

A final comparison between the optimized head (dorsal view, quality photos, 10 scales) and the optimized fore flipper (digits, quality photos, 14 scales) resulted in the flipper providing 100% identifications whereas the head provided 91% correct identification (P=.009).

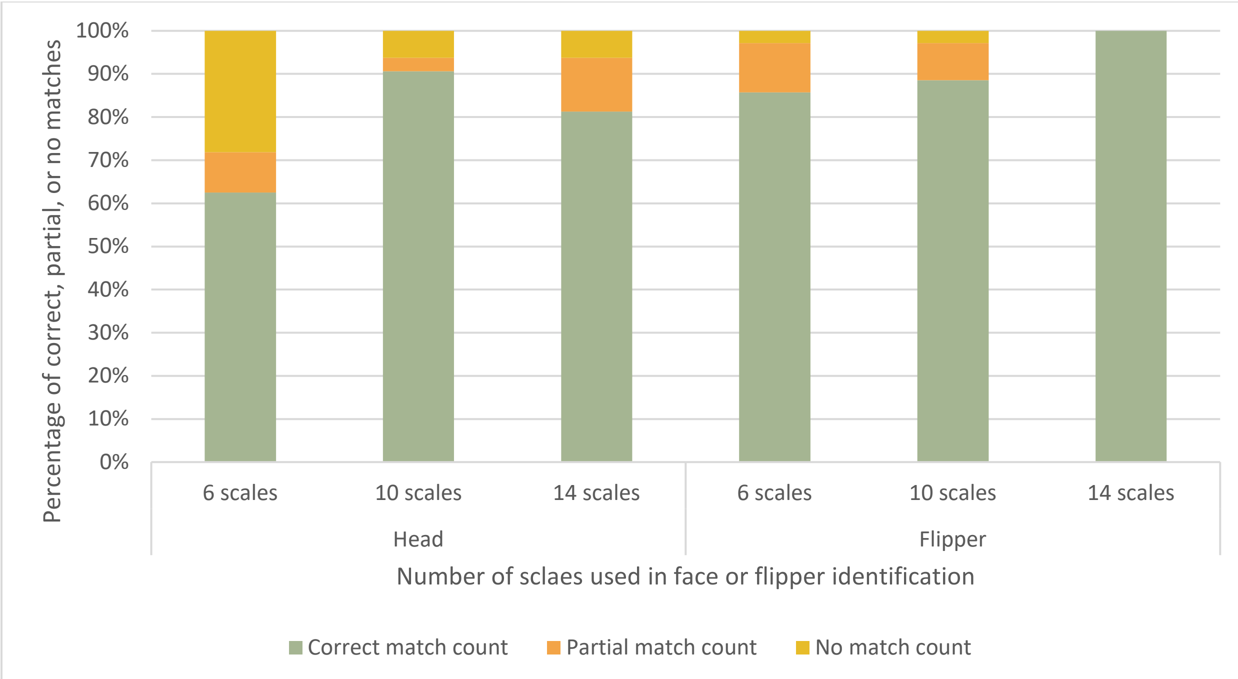

Figure 2-6 Percentage of individual green turtles from the Bahamas (2018 – 2020) correctly, partially or not matched / identified when 6, 10, and 14 scales were used in the photo-ID

2.5 Discussion

Photo-ID studies have increased in the past three decades facilitated by improved technologies (Markowitz et al., 2003); however, there remains a need to continually refine these methods to improve accuracy and efficacy. Furthermore, no previous studies have compared the accuracy of photo-ID for sea turtles using either head or flipper photos. Here, I compared the accuracy of using head or fore flipper photos for identification. This included which section of the head or fore flipper should be used, where the reference points should be placed, and how many scales to use. I then determined whether fore flipper photos could provide comparable or more accurate identifications than head photos. Our findings can be used as best practice guidelines for future photo-ID studies.

Different areas of the head have been used to identify sea turtles, including the lateral view (Fig. 2-1A) (Calmanovici et al., 2018, Jean et al., 2010, Schofield et al., 2020) and the dorsal view (Fig. 2-1B) (de

Urioste et al., 2016). For this reason, I tested different areas of both the head and the flipper to determine the most accurate location of the reference area. When I used the dorsal view of the head, I achieved more correct identifications than when I used the lateral view (61 % and 55%, respectively) which suggests this is a more accurate reference area. Using images of the wrist of the right flipper (Fig. 2-1C), Gatto et al. (2018) correctly matched individuals 89% of the time for adult green turtles and 93% for hatchlings. Our results followed the same method as Gatto et al. (2018) by removing any photos that did not follow the quality protocol. Our findings showed that this area of interest was not the optimal position as the digits of the fore flipper (Fig 2-1D) generated more correct matches (94%) than the wrist (54%). Gatto et al. (2008) utilized a smaller adult database (n = 14) which may have affected their results. They also conducted their research with turtles at different life stages, which may produce differing results to our study, which used only juveniles and sub-adults.

Previous studies using APHIS as a tool to identify individuals found that the resolution of the image did not affect the success of identifying individuals (Hoefer et al., 2021). However, studies using other tools and species have found that the angle photos are taken has an impact on the rate of identification (Speed et al., 2007). I separated our database into photos that followed the quality protocol, including the described angle, and those that did not. When the photos did not follow the protocol, the likelihood of correct identification reduced significantly, indicating that this is an important factor to consider when using photo-ID.

Then I compared the accuracy of using photos either of the head or fore flipper after optimization of the reference area, photo quality, and number of scales. I found that head photos provide significantly fewer correct identifications compared to the fore-flipper photos (head = 91%, flipper = 100%, P=.009). While photo-ID using the fore flipper is beneficial, it also has limitations such as when flippers are missing, which led to the exclusion of one individual in our database. However, in most instances, the percentage of wild sea turtle populations with missing flippers is likely minimal, with only 3% of our total database of 294 turtles having missing or damaged flippers. Photos of the head can also be taken in-water whilst a turtle is swimming, removing the need for any physical interaction with the subject as shown by Dunbar et al., (2021) who achieved correct identifications of free swimming turtles 84% of the time. However, as the fore flipper is moving and changing shape from flat to curved whilst in-water, it is unlikely to provide an option for identifying an individual without any physical contact. The fore flipper could however be a viable option for those studies conducted on nesting beaches. A high number of sea turtle monitoring projects take place on nesting beaches, whereby researchers collect measurements from nesting females (Mazaris et al., 2017, Philips et al., 2021). This provides an opportunity for photos of the flipper or other parts of the body, which may decrease the disturbance turtles experience taking photos of the face (Tabuki et al., 2021, Waayers et al., 2006).

I conclude that fore flipper photos can identify individuals more accurately than head photos when using APHIS. Within the flipper, the area to place reference points is at the digits of the fore flipper, following a defined photo quality protocol, and using 14 scales to create the scale pattern. Using these techniques simultaneously produces correct matches for 100% of individuals, which suggests this is a promising setup for future photo-identification studies. However, I suggest further research avenues to determine if flipper patterns remain suitable for photo-ID over an animals' entire life cycle and whether this technique is suitable for other sea turtle species.

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