ASSESSING DIFFERENT MONITORING TECHNIQUES FOR JUVENILE GREEN TURTLES (*CHELONIA MYDAS*) IN THE BAHAMAS

by

Laura St. Andrews

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THE PURDUE UNIVERSITY GRADUATE SCHOOL
STATEMENT OF COMMITTEE APPROVAL

Dr. Frank V. Paladino, Chair
Department of Biology

Dr. Nathan Robinson
Oceanografic De Valencia

Dr. Jordan Marshall
Department of Biology

Dr. Robert Gillespie
Department of Biology

Approved by:
Dr. Jordan M. Marshall

I dedicate this thesis to my effervescent teachers and family, of many places and species

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ABSTRACT

Sea turtles are integral components of many marine ecosystems. Green turtles (*Chelonia mydas*) are generally herbivorous, feed primarily on seagrasses, and are endangered in the Caribbean. The species utilizes extensive marine habitats for foraging and migratory routes, and because of its broad distribution, it is difficult to conduct population assessments. Here, I assessed commonly used techniques for monitoring green turtles in the wild. Specifically: (1) biopsy sampling for molecular assays and (2) unoccupied aerial vehicles (UAVs) deployment and boat-based surveys for population monitoring.

Skin biopsies are collected from sea turtles for a variety of molecular analyses; however, very little information exists on the natural healing rates at the site of the biopsy in the wild. In Chapter 2, I monitored the healing rates of 17 juvenile green turtles in Eleuthera, The Bahamas, for up to 488 d after taking a 6mm biopsy tissue sample. Complete tissue repair and maturation was observed after a year and a half, and there was no evidence of infection at any point during the healing process. While scarring persisted for several months, biopsy sampling had minimal long-term impact.

UAVs are increasingly being used to monitor marine megafauna. In Chapter 3, I evaluated the efficacy of using UAVs to detect sea turtles when compared to boat-based surveys. During UAV surveys, the UAV was flown along preprogrammed routes in four creek systems. A boat survey was conducted simultaneously on the same path. I used regression analyses for each survey type to assess the effects of environmental variables on turtle detection rates My results indicate that there were no statistically significant difference between the numbers of turtle detected via boat or UAV surveys; however, there were clear differences in the time and potential cost associated with either method.

CHAPTER 1. ASSESSMENT OF GREEN TURTLES IN THE BAHAMAS

1.1 Conservation status of green turtles in the Caribbean

It is estimated that green turtle (*Chelonia mydas)* populations have declined by 97% from their pre-Columbian numbers in the Caribbean (Nietschmann, 1979; Jackson, 1997; Eckert et al., 2020). Such declines are a result of many threats that date as far back as the mid-17th century, and early European colonization of the Caribbean. The main reasons for the initial decline were organized fishing, harvesting eggs, and harvesting nesting turtles on Caribbean beaches (Jackson et al., 2001; Bjorndal et al., 2005). Such demand for protein was in part due to limited agricultural yield for colonizing Europeans on islands (Jackson, 1997). Indeed, consumption of green turtles was the main source of meat until the 1730s on Caribbean islands until a dramatic crash in abundance was observed in 1800 (Long, 1774; Lewis, 1940; Car, 1956; King, 1982; Jackson, 1997).

Although consumption of turtle meat and eggs remains a threat to green turtles in some Caribbean islands, fisheries bycatch, habitat degradation from coastal development, pollution, and shipping traffic are growing threats (Witzell, 1994b; Bjorndal et al., 2000; Jackson et al., 2001; Seminoff, 2004). Habitat loss is now a major risk to sea turtles (Hamann et al., 2010) that also influences the health of marine and terrestrial habitats, such as nesting beaches and coastal seagrass beds (León and Bjorndal, 2002; Bjorndal and Jackson, 2003; Bjordnal, 2020). Foraging green turtles contribute to seagrass meadow productivity by altering succession, growth and decomposition processes and by influencing other grazer behaviors (Thayer et al., 1984). Studies in the Atlantic Ocean show that the presence of green turtles in near-shore habitats increases ecosystem resilience, especially to eutrophication (Thayer et al., 1984; Valentine and Heck, 1999; NAS, 2000; Duffy et al., 2003; Heck et al., 2006). Yet increased organic matter, often found in areas of high human presence, can lead to harmful algal blooms, which have resulted in lethal and sub lethal effects in turtles (Perrault et al., 2020). Increased threats are problematic as many turtles are already vulnerable to human induced fatalities, such as boat strikes, toxin accumulation, ingestion of, and entanglement by plastic products, coastal development and incidental bycatch (Hamann et al., 2010; Mentaschi et al., 2018). Additionally, the long lived green turtle is slow to reach sexual maturity (between 26- 40 yrs.), making its populations vulnerable to such high levels of anthropogenic mortality (Frazer and Ladner, 1986; Whitham, 1991; Limpus and Chaloupka, 1997; Musick, 1999; Gerrodette and Taylor, 1999; Seminoff, 2004; Bjorndal et al., 2005; Lazar, 2010).

To protect critical habitats and better understand populations, we need to identify turtle hotspots and regions of habitat biodiversity (Seminoff et al., 2008; Robinson et al., 2016). Current population assessments focus on nesting females because they come ashore to lay clutches of eggs. Nesting beach surveys are a logical first attempt to survey populations because female turtles exhibit site fidelity to nesting grounds. In a worldwide effort, surveys were compiled from 34 index sites from three generations and 19 subpopulations of green turtles (106-148 y) (Seminoff, 2002). Results from this study revealed a 48-67% decline in nesting females. Yet these assessments were only representative of nesting females and the hatchlings that emerge from these identified nesting beaches, and not indicative of male juvenile green turtle population numbers as they do not come ashore to nest (Seminoff, 2002). As such, robust efforts to assess post-hatchling juvenile life stages and male green turtle health are needed to understand the health of the entire endangered Caribbean population (Hamann et al., 2010).

1.2 Life cycle and habitat requirements

In the Atlantic, green turtle hatchlings swim from their natal beaches to the epipelagic, oceanic zone, and have been recorded foraging near or in *Sargassum* driftlines (Carr and Meylan, 1980; Lalli and Parsons, 1993, Bolten, 2003; Salmon et al., 2004). When these turtles reach their juvenile stage at roughly 30-40cm in straight carapace length, they shift habitats from pelagic waters to coastal environments. When in coastal waters, they forage primarily on seagrasses and algae (Limpus and Walter, 1980; Bjorndal and Bolten, 1988; Musick and Limpus, 1997; Bolten, 2003; Lazar, 2010).

In their sub-adult years, green turtles in the Caribbean graze on seagrass beds, primarily consisting of turtle grass (*Thalassia testudinum)*, manatee grass (*Syringodium filiforme)* and shoal grass (*Halodule wrightii)* (Thayer et al., 1984; Bjorndal et al., 2000; Seminoff, 2004; van Tussenbroek et al., 2014). *T. testudinum* is a native seagrass rich in phosphorus, nitrogen, and carbon, particularly at its base, where green turtles bite (Bjorndal, 1980; Thayer et al., 1984; Zieman et al., 1984; Bjorndal, 1997; Duffy et al., 2003; Heck et al., 2006; Christianen, 2012; Christianen, 2013; Hernandez and van Tussenbroek, 2014; Christianen, 2018).Green turtles also feed on microalgae and invertebrates, but seagrasses constitute an estimated 92% of green turtle diet in the Caribbean and Atlantic (Bjorndal, 1980; Bjorndal, 1997; Leon and Bjorndal, 2002; Duffy et al., 2003). *T. testudinum* constitutes 87% of a green turtle's total diet in the Caribbean while shoal and manatee grass combined are another 5%. Such seagrasses are an important source of protein for these turtles (Bjorndal, 1980; Thayer et al., 1984; Williams et al., 2014). Further, green turtles rely on a specific composition of carbon, nitrogen, phosphorus and soluble sugar found in seagrasses (Bjorndal, 1997; Christianen et al., 2018; Whitman, 2018) and they prefer native to invasive seagrass species in the Caribbean. Such preference for native seagrasses is likely because they possess higher nutritional value than invasive seagrasses, such as *Halophila stipulacea* (Fourqurean et al., 1995; Willette et al., 2014; Christianen, 2018; Whitman, 2018).

Green turtles exhibit foraging site fidelity as both sub adults and adults (Coyne, 1994; Broderick et al., 2007; Lazar, 2010; Whitman, 2018). This is likely due in part to lowered energetic costs associated with staying in one location (Bjorndal 1980; Broderick et al., 2007; Whitman, 2018). Once in their adult life stage, green turtles migrate between foraging, mating and nesting habitats.

1.3 Study area: Eleuthera, The Bahamas

In Chapters 2 and 3, I assessed monitoring techniques routinely used to study green turtles on Eleuthera Island, The Bahamas. Eleuthera is one of the 700 islands and cays that make up 300,000 km^2 Bahamian archipelago (Carew and Mylroie, 1997), bordered by the Atlantic Ocean to the East and the Caribbean Sea to the West. Habitats of the eastern and western coasts of Eleuthera differ (Patterson et al., 2006; Murchie and Cooke, 2015). The western side of Eleuthera sits on the Great Bahamas Bank, which is composed of four major lithofacies; coralgal, ooid, grapestone, and lime mud along the seafloor (Carew and Mylroie, 1997; Hearty, 1998). The eastern side of Eleuthera has jagged limestone cliffs as well as high wave energy from the Atlantic (Hearty, 1998).

Eleuthera contains an abundance of shallow, salt-water creeks and bays surrounded by abundant mangrove forests. Creek is the local term for shallow salt-water inlets and bays, but they are not associated with fresh-water river systems (Bjorndal et al., 2003). The island of Eleuthera is non-industrial and sparsely populated. The greatest threats to marine species in nearshore environments on Eleuthera are habitat degradation from increasing coastal development, shipping traffic and marine debris (Brumbaugh, 2014; Ambrose et al., 2019; Clegg et al., 2019). As of 2020, there was no existing protection for marine habitats on Eleuthera, even though the western and eastern banks of southern Eleuthera have suitable turtle habitat (Lagueux, 2001; Brumbaugh 2014; Knowles et al., 2017).

A population of northwest Atlantic green turtles (Wallace et al., 2010) utilizes the mangrove estuaries and shallow, oolithic sandy areas embedded with sea grasses around the southern portion of Eleuthera (Carew and Mylroie, 1997; Rankey and Reeder 2009).*Thalassia testudinum, Syringodium filiforme,* and *Halodule wrightii* are the three most common grasses in the Great Bahamas Bank, and thus, surrounding Eleuthera (Bjorndal, 1980; Thayer et al., 1984; Bjorndal, 1997; Duffy et al., 2003; Heck et al., 2006; Christianen, 2012; Christianen, 2013; Hernandez and van Tussenbroek, 2014; Christianen, 2018). The shallow creeks (2-5m depth) fluctuate up to 80 cm with diurnal tidal cycles and host a wide range of plant and animal life. Tidal cycles are necessary to follow as turtles move in with the rising tide. *Rhizophora mangle* and *Avicenna germinans* mangroves surround the four creeks. Benthic fauna including echinoderms, sharks, such as *Ginglymostoma cirratum* (nurse sharks) and *Negaprion brevirostris* (lemon sharks), and rays, *Hypanus americanus* (southern stingrays) (Murchie et al., 2010; Murchie et al., 2013; O'Shea et al., 2018; Shipley et al., 2018; Robinson et al. 2020).

1.4 Monitoring techniques for sea turtles

An active long-term monitoring program of green turtles began in 2011 in four creek systems of southern Eleuthera; Rollins Creek, Deep Creek, Starved Creek and Half Sound. Opportunistic recaptures of green turtles occur on Eleuthera as biologists conduct routine monitoring studies of green turtles in mangrove creeks and bays on the island. In the subsequent chapters, I investigated commonly used techniques for sea turtle monitoring globally. The following studies sought to answer questions about whether researching endangered species can be done more effectively, and with minimal impact to the animal. Monitoring green turtles in the Caribbean is of particular importance because of their endangered status, and because there is a large amount of suitable habitat for them in the region. Incorporating data from the long term monitoring project helped to better inform suitable sample sites to conduct the following studies.

In Chapter 2, I assessed the long-term healing of wild, juvenile green turtles after biopsy sample collection. At present, there is very little scientific literature, and no long term data, on healing rates in wild sea turtles. For this study, I monitored the healing rates of turtles after tissue collection, and until turtles were considered fully healed. Turtles were recaptured as part of a long-term monitoring project, and would have been recaptured regardless of this study. To better understand methods of sea turtle healing, I also reviewed studies of other reptile species.

In Chapter 3, I compared an emerging technology, unoccupied aerial vehicles (UAV), with a traditional technique for monitoring turtles, boat surveys. Because there is potential for aerial and boat based surveys to provide large-scale population data on juvenile green turtles, it would be useful for researchers to know if there is a more reliable, or accurate technique (Bjorndal et al. 2005). Boat based surveys are a commonly used practice to collect population, morphometric, and molecular data from turtles in water (Hamann et al., 2010; Seminoff et al., 2014). Although this technique can be time, cost, and labor intensive, it also makes it possible to cover large areas of green turtle habitats on Eleuthera, and to collect physical samples from turtles in the water. UAV surveys do not allow for the physical capture of turtles to assess body conditions, or to collect samples, but they can fly undetected over animals, and can allow for large scale population monitoring at potentially lower training and equipment costs (Bevan et al., 2018; Hodgson et al., 2018; Rees et al., 2018).

Both chapters in this study highlight the importance of effective research for endangered species. Inherently, working with endangered species requires diligence, effectiveness and minimal long-term impact. As new technologies emerge, researchers are faced with options for surveying target species. In the following chapters, I describe the long-term effects of biopsy sampling on sea turtles in the wild, and the accuracy of two different survey techniques to better inform future research and management decisions.

CHAPTER 2. HEALING OF SKIN BIOPSIES IN WILD JUVENILE GREEN TURTLES

2.1 Introduction

Skin biopsies are routinely collected from sea turtles for a variety of molecular assays, such as stable isotope, heavy metal, or genetic analyses (Dutton and Balasz, 1995; Robinson et al., 2016; van der Zee et al., 2019). While these tools can provide valuable ecological insights, dermal biopsy collection remains an inherently invasive process. There is also a possibility that the biopsy sites could become infected or leave permanent scarring (Swaim et al., 2001; Gutner et al,. 2015). In the following study, I expected that small biopsies will heal at varying rates in juvenile turtles in the creeks of Eleuthera because of differences in body composition and timing of opportunistic recaptures. Based on existing literature for healing rates in other reptiles, I also estimated that rapid healing (~two months) would be seen without significant long term impact.

Wound healing of cutaneous lesions is different in reptiles than in mammals. Healing in reptiles involves reconstruction of tissue by collagen synthesis, contraction of cellular matrix deposits, re-epithelization, and the disappearance of inflammatory markers (Smith et al., 1988; Keller et al., 2014; Negrini et al., 2016). Cutaneous healing in turtles is affected by ambient temperatures as well as differences in tissue formation as turtles do not have the cutaneous muscles or beds of granulation tissue, that mammals do, and turtle skin tissues are less mobile than mammals (Maderson and Roth, 1972; Smith et al., 1988; Keller et al., 2014; Negrini et al., 2016; Negrini et al., 2017). Cutaneous healing has been found to be slower in reptiles than in mammals, and studies have shown that fresh water turtle wounds take longer to heal than fishes, lizards and snakes (Negrini et al., 2016). Indeed, Negrini et al. monitored cutaneous biopsy healing in *Trachemys scripta elegans* and found that re-epithelization was completed 14 d post-biopsy, and new tissue differentiation was present by 28-42 d after sampling. Inflammation of the biopsy site was recorded up to 28 d after tissue collection. Complete healing, and remodeling however, was observed between 42-135 d. These data indicate that turtles take longer to heal than other reptiles in similar temperature ranges (*Thamnophis sirtalis* = 5-10 d, *Urosaurus ornatus* = 14 d), which may also make the species more susceptible to infection (Brattstrom, 1965; Monagas and Gatten, 1983; Smith et al., 1988; French et al., 2006; Keller et al., 2014; Negrini et al., 2017). Reptiles are frequently documented basking, as this is one way to optimize immune system function (Brattstrom, 1965; Crawford et al., 1993; Zimmerman et al., 2010). As a result of these findings, I estimated that juvenile green turtles in The Bahamas will take longer to heal than lizards, snakes and even freshwater turtles partly because they do not come out of the water to bask. Since temperature plays an important role in immune function, I also monitored monthly water temperatures throughout the study period (average 26-29 °C ([Weather Atlas]).

2.2 Methods

A team of trained biologists captured juvenile green turtles in southern Eleuthera, The Bahamas, between August and December of 2019. Turtles were captured using a modified "rodeo" technique as described in Ehrhart and Ogren (1999). Upon capture, turtles were tagged, weighed, measured, and visually assessed for injuries or abnormalities. I collected a skin sample from 68 juvenile green turtles (42.0-63.5 cm curved carapace length) using a 6 mm diameter biopsy punch (Integra™ Miltex®). I sterilized the collection site on the left or right shoulder using alcohol wipes before sampling. I then inserted the biopsy punch to a depth of 1–2 mm to remove just the dermal tissue (Figure 2.1). To avoid pulling the dermal tissue with force from the underlying base, I held the dermal skin from the biopsy with forceps and used the biopsy punch to cut horizontally through the tissue to extract the sample. Immediately following sample collection, I cleaned the area with Betadine wipes. If bleeding occurred, I applied pressure with sterile gauze until bleeding stopped. Upon each recapture, I photographed the biopsy site (Figure 2.1) and categorized the stage of healing, using six categorical phases, each time an individual was recaptured (Table 2.1).

To analyze the recapture data, I used a LOESS regression, or locally weighted scatterplot smoother. Smoothing parameters (α = 0.6) tested data difference from the means within neighborhoods of data when data was graphed. Neighborhoods of data were set at this α to include all data points. Most notably, this regression displayed and allowed for recognition of healing trends from turtles sampled over the study period (Cleveland and Devlin, 1988).

2.3 Results

Of the original 68 turtles, 17 were opportunistically recaptured over the next year and a half. Unfortunately, there was a nine month gap in sampling between March and December of 2020 because of field work restrictions due to COVID-19. Ten of the 17 recaptured turtles were recaptured once, three were captured twice, two were captured three times, one was captured four times, and one individual was captured five times. In total, there were 31 recapture events. No detectable signs of infection at the biopsy scar site were observed in recaptured individuals at any phase.

I encountered five individuals in Phase 1 (Open, Healing). Turtles in Phase 1 were encountered between 7-9 d post-biopsy sampling, and all exhibited rapid healing, already possessing formed granulation tissue (Figure 2.2). Two individuals were encountered in Phase 2 (Closed, Indented). Turtles in Phase 2 were recaptured between 19 and 28 d. I observed Phase 3 (Closed, Discolored) in five turtles between 29 and 77 d post-biopsy. Phase 4 (Healed, Visible) was observed for eight individuals between 67 and 176 d after tissue collection. Thus, even though superficial damage caused by biopsies is healed within a few weeks, external disfiguration can last for many months. After 176 d, no turtles had reached Phase 5. However, sampling was not possible for nine months after 176 d elapsed. When COVID-19 field restrictions lifted, Phase 5 (Healed, Indistinguishable) was observed in five individuals that were captured between 455-488 d post-biopsy. No discernable scars were observed after 455, 468 or 488 d post-biopsy in these individuals. As shown by the LOESS regression, healing occurred rapidly in Phases 1 and 2, and then gradually slowed. Some turtles were captured multiple times, which could have resulted in some bias in my LOESS regression, as turtles could have been considered in the same neighborhood representing multiple encounters (Figure 2.2). However, in order to include all data in this analysis, I still determined LOESS regression to be the best method for representing the trends in my data due to my limited sample size.

Table 2.1 Description of healing phases.

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

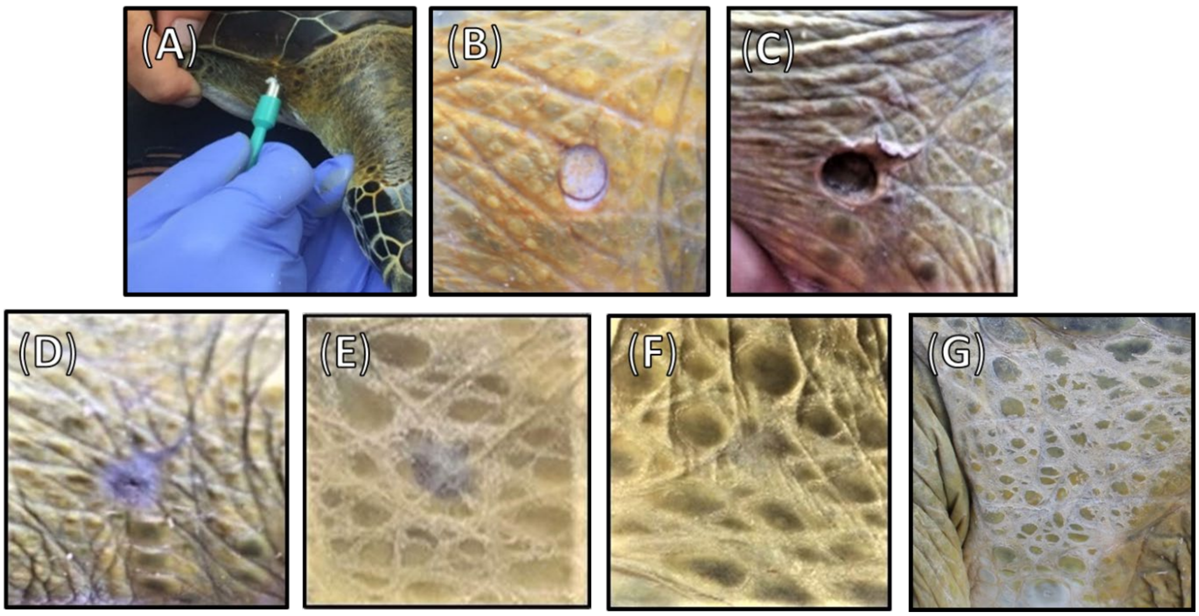

Figure 2.1 (A) Tissue collection sites on shoulders of juvenile green turtles. (B) Biopsy site immediately after tissue collection. (C-F) Representative photos of days since biopsy sampling and the phase of healing. (C) Phase 1; 9 d (D) Phase 2; 28 d (E) Phase 3; 50 d (F) Phase 4; 91 d (G) Phase 5; 488 d. Photos taken by Laura St. Andrews (A-F) and Liberty Boyd (G).

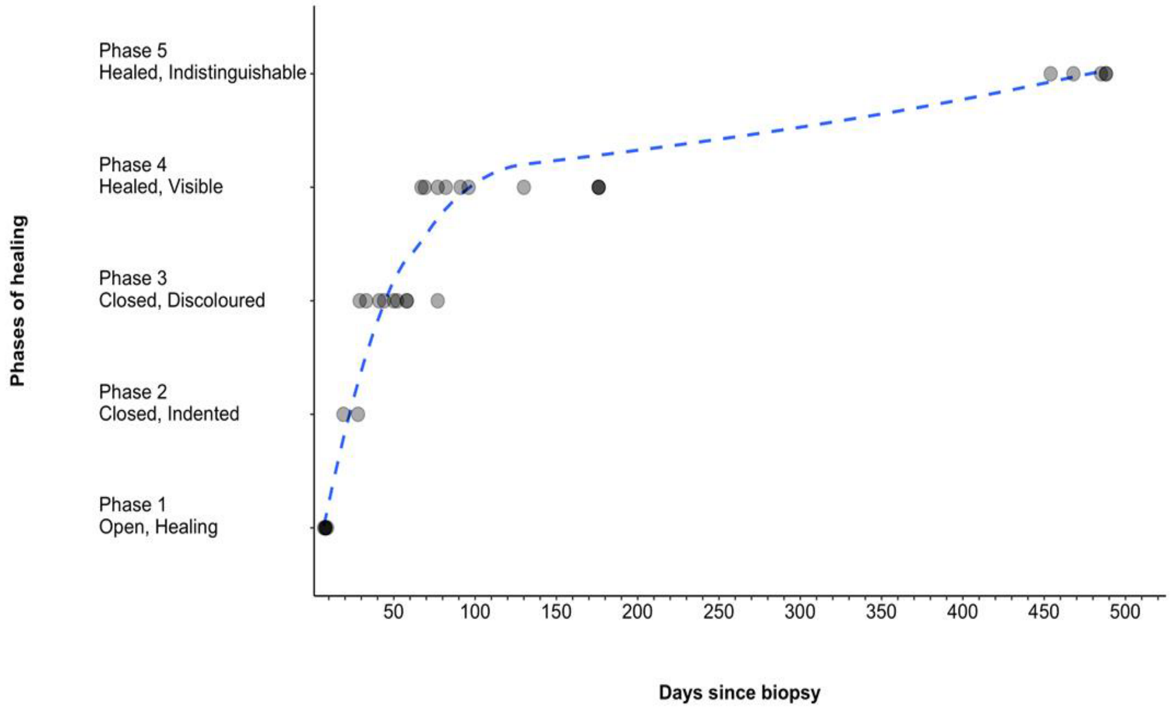

Figure 2.2 Phases of healing post biopsy sampling. The grey dots represent all recaptured turtles combined. The dotted line shows a LOESS regression for the number of days to reach each healing phase.

2.4 Discussion

In my findings, initial healing was rapid and there were no noticeable detrimental effects (long term wounding or infection) observed in any turtles. The impact of biopsy sampling from this study supports the results of previous studies of reptile healing (Brattstrom, 1965; Monagas and Gatten, 1983; Smith et al., 1988; Negrini et al., 2016) and provides preliminary data to suggest that this is a relatively safe technique for collecting molecular assays from sea turtles. As this is the first long-term study of healing in wild juvenile green turtles, it is relevant to compare the healing rates to other reptiles in particular. Studies of cutaneous biopsy healing in *Trachemys scripta elegans* found that re-epithelization was completed 14 d post-biopsy, but that wound healing was considered complete from 42-135 d post biopsy (Negrini et al., 2016). This is in line with findings from my study, as initial healing of a regenerated epidermis was clearly observed within 28 d in juvenile green turtles, but total healing was not observed as rapidly. However, it is also possible that the results from my study would have shown different trends in healing in Phases 4 and 5 if I had been able to sample turtles healing during the 176-455 d window that was halted due to COVID-19 restrictions. Indeed, complete (Phase 5) healing might have been observed closer to 176 d if I had been able to continue with data collection. Additionally, water temperatures could have impacted healing rates, as ambient temperature has been documented to increase healing in other species (Negrini et al., 2016). I would anticipate healing to be more rapid in turtles that inhabit tropical waters than sub-tropical habitats, thus the warm temperatures in the Caribbean may have sped up healing in turtles that I sampled.

In this survey, my sample size was limited and the days in between sampling do not conclude when each Phase occurs with precision. The time required to reach each phase was objectively determined as healing is a continual process. Individuals may have reached new phases before I was able to recapture them, and there are likely subtle transitions between the phases. As evidenced by the LOESS regression, healing occurred rapidly in Phases 1 and 2, and then gradually slowed. Yet, as some turtles were captured multiple times, this could have biased my LOESS regression as the same turtles were observed more than once in the same Phase. This suggests that the healing Phases described here should be interpreted with some caution, as the exact time between each Phase will likely vary in ways the regression did not show. Variation in healing phase is expected, however, as turtles were different sizes and ages in the study. Such differences in healing could also be explained by individual differences in body composition, nutrient availability, microbial colonies, and depth of biopsy as has been observed in other reptilian studies (Bennett, 1996; Bjorndal 1997; Caillouet et al.„ 1997). Despite individual variation between Phases, results from this study suggest biopsy sampling of sea turtles is a relatively safe method for researchers to continue to use (when combined with aseptic techniques) as no detrimental long-term effects were observed. The data also show that sea turtle biopsy healing is similar to freshwater turtles, such as *Trachemys scripta elegans* (Negrini et al., 2016*)*. Future studies on wound healing of freshwater turtles, or sea turtles may be able to be used interchangeably to understand testudines' healing processes in more detail.

CHAPTER 3. COMPARING BOAT AND UAV SURVEYS FOR MONITORING JUVENILE GREEN TURTLES IN ELEUTHERA, THE BAHAMAS

3.1 Introduction

Limited data exist for population trends and high-use habitats for green turtles (*Chelonia mydas*) due to a lack of access to large study areas and inaccessibility to remote regions. To address the dearth of remote and difficult population data, researchers have conducted surveys to count sea turtles by boat or low-flying aircraft (Marsh and Saalfield, 1989; Witt et al., 2009; Seminoff et al., 2014; Rees et al., 2018). However, both techniques are expensive, time consuming, and require staff training (Crouse, 1984; Shoop, 1985; Garmestani et al., 2001; Koh and Wich, 2012; Rees et al., 2018). These limitations can also inhibit the size of the survey area (Witt et al., 2009). Boat surveys also require technical expertise of crew members and can be limited by difficult to reach coastal areas, weather, fuel costs, or by lengthy travel time to research areas. Additionally, the sound of boats could deter, or disrupt sea turtles in boat surveys, biasing surveys (Mansfield, 2006; Work et al., 2010). However, rapid advances in technology, such as unoccupied aerial vehicles (UAVs) present the opportunity to reduce survey costs and limitations and to expand the distance of surveys to monitor this population of turtles (Rees et al., 2018). One area that this could be especially beneficial would be in the The Bahamas as it is known to host extensive seagrass beds and large sea turtle populations.

UAVs are useful tools to conduct broad-scale population, behavior and habitat assessments. UAVs are cost and time effective for sea turtle surveys when compared to plane and large-scale boat-based surveys, particularly if repeat surveys are necessary. Further, by programming UAV software, post-processing video footage can be automated. This has the potential to reduce human error from field based observations (Hodgson et al., 2018; Robinson et al., 2020). UAVs can fly lower than planes and record high-quality images of species, habitat and depth in difficult to reach coastal habitats (Jones et al., 2006; Koh and Wich, 2012; Allan et al., 2015; Rees et al., 2018; Schofield et al., 2019; Yaney-Keller et al., 2019). Coastal development, fisheries bycatch and recreational and commercial boat traffic can severely disrupt and harm sea turtles, yet all of these threats can be monitored by UAVs (Mansfield, 2006; Work et al., 2010). Because of the high use potential of UAVs to detect and monitor wildlife, as well as monitor threats to endangered species, it is pertinent to evaluate UAV effectiveness.

Caribbean-wide recovery plans for green turtles seek to restore populations to their once abundant numbers by mitigating anthropogenic threats (Bacon et al., 1984; Ogren, 1989; NMFS and USFWS, 1991; Eckert et al., 1999; Eckert and Abreu Grobois, 2001; Fleming, 2001; Jackson et al., 2001; IUCN, 2010). Yet the enforcement of recovery plans is difficult, as habitat surveillance and population monitoring require intensive effort in marine ecosystems (Magnuson et al., 1990; Bräutigam and Eckert, 2006; Jones, 2006). Because green sea turtles face endangerment in the Caribbean, further research into where these animals forage and migrate could lead to effective coastal management strategies in known foraging grounds (Schoeman et al., 2020). Here, I hypothesize that 1) UAV surveys will be more accurate than boat surveys to detect juvenile green turtles because of their vantage point over the water (flown at 30 m) 2) increased wind speed, increased cloud cover and an incoming tide will decrease the frequency of turtle detections using both UAV and boat surveys because the view will be less clear in these conditions and 3) the detection of turtles will vary using boat or UAV surveys between creeks and within creeks as the creek systems have different depths and levels of seagrass abundance.

3.2 Methods

We conducted boat and UAV surveys in 2018-2019 in southern Eleuthera at four creeks: Rollins Creek, Deep Creek, Starved Creek and Half Sound (Figure 3.1). The benthic substrate at each site was mainly sand and rock. I chose these sites for their abundance of seagrasses, *T. testudinum* and *S. filiforme*.

To test whether the turtle numbers varied between boat-based and UAV surveys across all creeks, I adapted methods from Robinson et al. (2020). I flew the DJI Phantom 4 Pro (DJI Inc., Shenzhen, China), a small, consumer-scale UAV, parallel to the flow of the creek. I used Litchi software to automate the flight paths, and to record all video imagery.

I chose survey routes and lengths based on creek accessibility and flight capabilities of the UAV. The prerecorded survey length of Deep Creek was 1.75 km, Rollins Creek was 1.41 km, Half Sound was 2.0 km and Starved Creek was 2.23 km. For all creek systems, I set the UAV to fly at a fixed, 30 m altitude. I chose this altitude as it was high enough to not create ripples at the water's surface and preliminary studies suggest that UAVs remain undetected by turtles at this altitude. I programmed the UAV to travel at ~7.5km h^{-1}, a rate that enabled the full survey route to be covered within the UAV's battery life (30 min).

Immediately following UAV deployment, a team of trained individuals followed the set UAV route in a small boat (5.5 x 2 m). A designated recorder documented all turtles sighted by the observers. Between 2018 and 2019, thirteen surveys were conducted in Rollins Creek, nine in Deep Creek, twelve in Starved Creek and five in Half Sound. Before each boat survey began, I recorded tidal state, wind speed, percent cloud cover, and the start and end time of the survey.

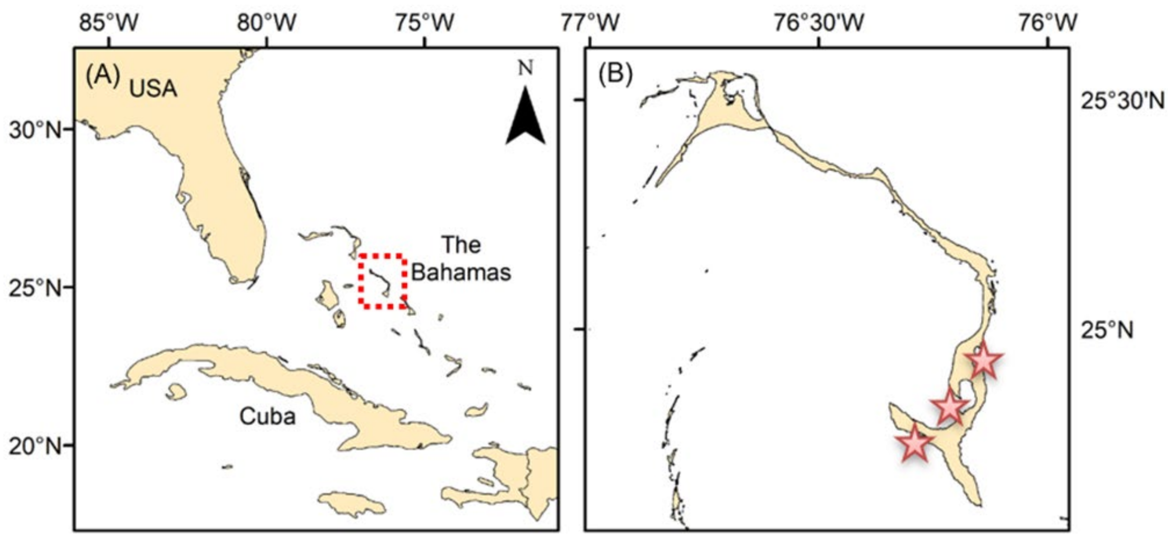

Figure 3.1 (A) Map of The Bahamas. (B) A map of Eleuthera to specify the sampling locations for juvenile green turtle sampling at mangrove creeks. Half Sound is the northernmost creek, Starved creek is the westernmost creek and Deep and Rollins creek are the two southernmost creeks and are indicated by one combined star because of their close proximity. Figure adapted from Robinson et al. 2020.

3.3 Field of View: Boat and UAV calculations

I attained the relative accuracy of spatial resolution of the UAV by calculating the ground sampling distance (GSD), or the photogrammetric resolution of the distance between the center points of each pixel taken from still frame images of UAV footage (Lee and Sull, 2019). Imagery resolution is an important consideration for marine research as many benthic markings and objects can obscure results if images are not clear. Further, for this study, a low resolution, or low GSD could skew survey results.

The GSD is the combination of the dimensions of the UAV's sensors, its aspect ratio, or the ratio of a UAV image's length and width, the focal length of the camera and the distance of the UAV from the object it is filming of photographing. I flew the UAV at a height of 30m at a 90 degree angle for all aerial surveys in this study. The sensors of the DJI Phantom Pro 4 are 13.2 x 8.8 mm, which results in a 3:2 aspect ratio that corresponds to a 20 megapixel sensor (5472 by 3648 pixels= 19,961,856 pixels). The focal, or lens, length, which is the lens' angle of view and magnification, of the DJI Phantom Pro 4 is 8.8 mm. I calculated GSD for each using the previously outlined metrics (GSD= cm/px). GSD for height was .88 cm/pix and GSD for width was 0.82 cm/pixel GSD for height and width were calculated as:

$$GSD_h = \frac{Flight\ height * Sensor\ height}{Focal\ lenght * Image\ height}$$

$$GSD_w = \frac{Fligh\ height * Sensor\ width}{Focal\ length * Image\ width}$$

The field of view is the range that the camera captures; this metric allows researchers to record the angular size of objects within a frame. The field of view, or angular view, of the UAV model is 84 degrees. The image footprint or distance covered in a single frame of UAV footage is a calculation of the actual size of the area that reviewers see. This metric is also an important consideration when comparing this to the distance observers on a boat can see. The image footprint of the field of view at 30 m elevation was 1347.31 m^2.

During boat surveys, I was unable to calculate the exact distance between the boat and the turtles. When surveys were conducted with little cloud cover, wind and glare, I estimated the detection distance to be a 25 m radius surrounding the vessel. Surveys that were conducted with high glare, wind or cloud cover could have limited detection. However, I did not conduct boat or UAV surveys in inclement weather.

3.4 Analyses

I first assessed which technique (boat vs. UAV) detected more turtles during various weather conditions (cloud, wind and tide), time of day and within and between different creeks. To do so, I tested the distribution of turtle count data for normality using Shapiro-Wilks tests (Royston, 1982; Royston, 1995). I then used a paired t-test to determine whether there was a difference in the total number of turtles detected in all creeks using boat and UAV surveys.

To compare random and fixed effects against turtle detection, I conducted generalized linear mixed effects regressions (lmer) for each survey technique separately using the “lme4” package (Bates et al., 2015). The dependent variable for both models was the number of turtles detected. The fixed variables in the initial boat and UAV models were cloud cover, wind speed, and incoming or outgoing tide. Tide was considered a binary variable (indicated as a 1 for incoming tide or 0 for outgoing tide) in these models. Random effects in the boat model included time of survey start and the number and training level of observers on the boat. The only random effect in the UAV survey model was time of the survey start. I used reverse stepwise selection to fit the model of the linear regression and linear mixed effect regression. This allowed for determination of which fixed and random variables explained the number of turtles detected; i.e. I removed random and fixed variables that showed zero variance. When no random effects contributed to the detection of turtles, I removed the variables and ran a simple linear regression.

To test boat and UAV models, I included the interaction of fixed variables wind, tide and cloud cover on the number of turtles detected. I used ANOVA tests to compare whether the random or fixed variables, or their interactions, significantly contributed to the detection of turtles. To include observer training in the boat model, I categorized groups into more trained than untrained, less trained than untrained or equal trained and untrained observers on the boat. This analysis was conducted to evaluate whether the training level of the observers contributed to differences in the number of turtles spotted on boat surveys. The slope for the final, most parsimonious boat survey was tested using “lm.beta.lmer” (King, 2021). For the UAV model, the interactions of all of fixed variables were tested against turtle detection in the four creek systems using the “lmer4” and “car” libraries. Results revealed a singular fit to the UAV model. The slope of the UAV model was tested using the “lm.beta” package (Behrendt, 2014). The beta slope for both models was the standard deviation of the independent variables multiplied by the standard deviation of the dependent variable.

I tested the difference in detection of turtles between boat and UAV surveys within each creek system using paired t-tests and Wilcoxon signed rank tests. Subsequently, I ran repeated measures tests to analyze variation between creeks for all surveys. Next, I conducted a post-hoc Tukey test to compare the means of the surveys (Bakeman, 2005). UAV and boat surveys were tested separately. Both datasets were unbalanced as the number of surveys was not consistent across the four creeks.

I assessed the total time spent conducting boat and UAV surveys as well as the total time post-processing data from both techniques. To do so, I assessed the amount of time it took to download, send and review all of the UAV footage to the three reviewers, and all of the data input. Data input from boat surveys was brief as turtle count results were immediate.

All statistical tests were conducted with $\alpha = 0.05$ using R (R Core Team 2021).

3.5 Results

3.5.1 Turtle detection between survey types and creeks

We conducted a total of 39 combined UAV and boat surveys. The mean rate at which turtles were recorded from each survey was highest in Half Sound (Boat= 0.009 m^{-1} ±19.058 SD, UAV=0.012 per m^{-1} ±20.693 SD), was lower in Rollins Creek (Boat= 0.005 per m^{-1} ±3.945 SD, UAV=0.005 per m^{-1} ±3.952 SD), lowest in Starved Creek (Boat= 0.003 per m^{-1} ±5.066 SD, UAV=0.001 per m^{-1} ±2.304 SD) and Deep Creek (Boat= 0.001 per m^{-1} ±0.916 SD, UAV=0.002 per m^{-1} ±2.657 SD). Such high standard deviation in the mean number of turtles in the different creeks suggested a more thorough explanation of the creeks was necessary, such as determining if environmental conditions could explain the variation. Between creeks there was variation of average number of turtles between survey techniques, although the number of surveys and time of year that surveys were conducted was not consistent across the four creeks (Table 3.1, Figure 3.2). Average UAV surveys were 10 min in Rollins Creek, 13 min in both Deep Creek and Half Sound, and 15 min in Starved Creek. The average duration of boat surveys was 15 min Rollins Creek, 18 min in Deep Creek and Half Sound, and 23 min in Starved Creek. The total time spent conducting UAV surveys was 8 hr 58 min and the total time conducting boat surveys was 11 hr 40 min.

The Shapiro-Wilks test confirmed that data were non-normally distributed (UAV: W = 0.543, p-value = <0.001, Boat: W = 0.615, p-value = <0.001), but log- transformation of the data successfully met the assumption of normality (UAV: W = 0.958, p-value = 0.148, Boat: W = 0.976, p-value = 0.577) (Conover, 1999). Chi squared tests revealed that no random effects of categorical and continuous variables were collinear. As collinearity was not observed, the interaction between cloud cover, wind speed and tide were included in this testing to understand whether the combination of different environmental conditions led to differences in detection of turtles using the two survey techniques. Results from paired t-tests of log transformed data sets of UAV and boat surveys revealed no significant difference between the combined total number of turtles detected in all creeks for the two survey techniques (p-value = 0.896).

Table 3.1 Average turtle count per creek (2018 and 2019 combined).

	Deep Creek	**Half Sound**	**Rollins Creek**	**Starved Creek**
Survey length (m)	1750	2000	1410	2230
Average detection of turtles- Boat	1.2	18	7.2	6
Average detection of turtles-UAV	4.2	24.4	6.6	2.8
Average turtles/m- Boat	0.001	0.009	0.005	0.003
Average turtles/m- UAV	0.002	0.012	0.005	0.001
Highest turtle detection				
Boat	3	53	13	20
UAV	7	56	14	7
Lowest turtle detection				
Boat	0	1	2	1
UAV	0	4	1	0

Distribution of data was variable across all four creeks, when normally distributed, or normally distributed after log transformation, I conducted a paired t-test and when data was not normally distributed, and a nonparametric Wilcoxon signed rank test to test the significance of survey types and detections in each creek. Results of the Wilcoxon signed rank tests for Deep Creek revealed a significant difference between the two survey techniques and turtle detection

(V = 35, p-value = 0.021). Paired t-test results from Starved Creek also revealed significance between the two survey techniques and the detection of turtles (t value = -3.41, df = 11, p-value = 0.006). Paired t-test results for the other two creeks revealed no significant difference between detection of turtles and survey techniques for Half Sound (t value = 1.8743, df = 4, p-value = 0.134) or Rollins Creek (t value = -1.4254, df = 12, p-value = 0.18) (Figure 3.2).

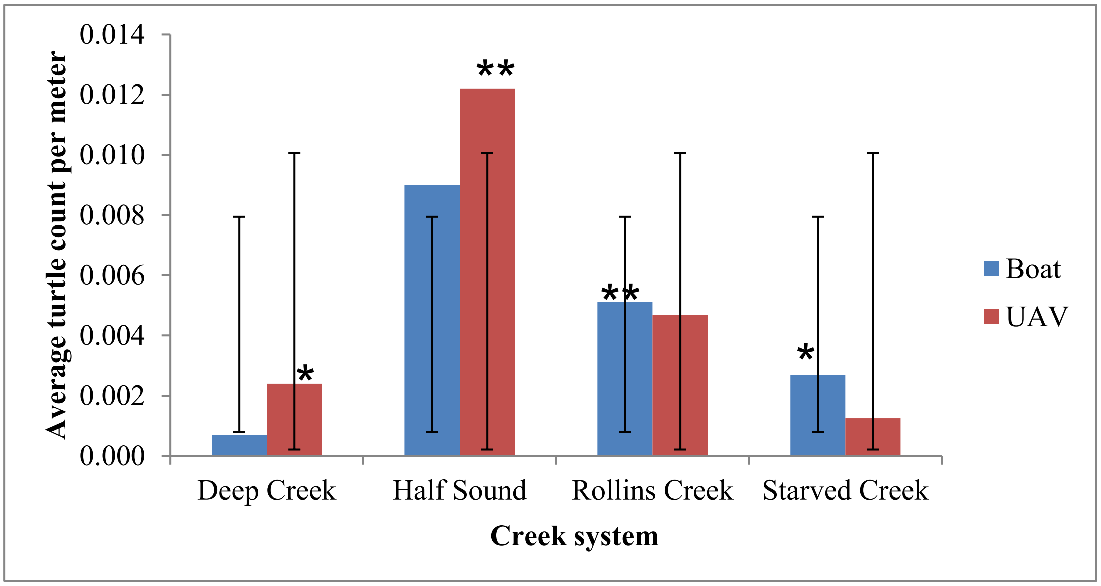

Figure 3.2 Average turtle detections per meter in each creek system. Standard deviation represented by error bars. *UAV (unoccupied aerial vehicle) and Boat surveys significantly different within: Deep Creek (p = 0.02*), Starved Creek (p = 0.01*). **UAV and Boat surveys significantly different between: Half Sound and all other creeks (UAV), Half Sound and Deep Creek (p = 0.01*), Half Sound and Starved Creek (p = 0.04*).

The repeated measures ANOVA indicated that there was a significant difference in the number of turtles seen on UAV surveys between creeks (df (3), F =8.46, p <0.001). More turtles were counted in Half Sound than all other creeks. Tukey multiple comparisons confirmed all differences between Half Sound and other creeks as significant. However the detection of turtles between Deep-Rollins, Deep-Starved and Starved-Rollins creeks were not statistically different using UAV surveys (Figure 3.2, Table 3.2).

The repeated measured ANOVA test for boat surveys also revealed that the detection between creeks was statistically significant (df (3), F = 4.585, p-value = 0.008). Tukey multiple comparisons revealed that the detection of turtles, using boat surveys, in Half Sound had significantly higher detection of turtles than Deep and Starved creeks. However, the detection of turtles using boat surveys was not statistically different between Deep- Rollins, Deep-Starved, Starved- Rollins or Half Sound - Rollins creek (Figure 3.2, Table 3.2).

Table 3.2 Significance of Tukey comparison results for boat and UAV surveys between creek systems

	Boat		**UAV**	
Creek system	**t-value**	**p-value**	**t-value**	**p-value**
Deep Creek: Half Sound	3.684	0.004*	4.305	0.001*
Deep Creek: Rollins Creek	1.697	0.337	0.657	0.911
Deep Creek: Starved Creek	1.327	0.549	-0.375	0.981
Half Sound: Rollins Creek	-2.506	0.075	-4.022	0.002*
Half Sound: Starved Creek	-2.761	0.042*	-4.822	0.001*
Rollins Creek: Starved Creek	-0.377	0.981	-1.124	0.674

Most notably, the reason for the significant differences for both surveys is due to surveys in Half Sound. Surveys in Half Sound were only conducted between April-June; two in Half Sound in 2018 and three in 2019. Two of these three 2019 surveys had much high detection rates than any survey in any of the four sample creeks. Starved, Rollins and Deep creeks were surveyed on more occasions (n=12, n=13, n=9 respectively), and in a wider range of months.

3.5.2 **Observer variability and duration of surveys**

There was at least one trained observer, with an average of three trained observers and two untrained observers per boat survey. In 17 surveys there were more untrained than trained observers, in 19 surveys there were more trained than untrained, and in three surveys the training levels were equal. No significant variance was found for the number of turtles detected with the number of trained or untrained observers on boat surveys (Table 3.5).

In this study, the UAV surveys resulted in less time spent surveying the same route, and less fuel needed to conduct the survey. However, the post processing of data must also be factored into the cost and time. Boat surveys required quick data input into Excel files (~1 h), whereas video files from UAVs need to be downloaded, saved and often transferred through cloud storage for review. Three independent reviewers, and myself, viewed the UAV footage for this study on separate occasions. If each reviewer watched the videos without pausing or recording data, I estimate that it took ~35 hrs to review all footage by 4 reviewers. However, it is necessary to stop the videos to record data. Further, the transfer and download of the large data files (3.8 GB for 5 min of video) requires substantial time and planning. Therefore, I estimate that the total time spent transferring videos and recording turtles per survey was 1.3 hrs and the total time for all surveys was 50 hrs. With this estimate, the actual time of field time and data processing time for UAV surveys is much greater than boat surveys.

The number of turtles detected per review for UAV footage was considered. This step in post-processing data contributed to a large portion of additional time when factored into total survey time, and is important for further consideration of which survey type makes most sense to employ. I calculated the percentage of difference between the number of turtles detected by reviewers 1 and 2 (R1,2), 1 and 3 (R1,3) and reviewers 2 and 3 (R2,3), as well as the average percentage difference in detection between the three sets of reviewers. Between R1,2, in 29 of the 39 UAV surveys there was a difference of 0 or 1 turtles, in 5 of the surveys there was a difference of 2 turtles and in the other four surveys there were differences of 4, 5, 6 and 7 turtles. Between R1,3, there was a difference of 0 or 1 turtles detected in 30 surveys, a difference of 2 turtles in 4 surveys, a difference of 3 turtles in 2 surveys, and differences of 4, 5 and 7 turtles in the remaining 3 surveys. Between R2,3, there was a difference in turtles detected by the reviewers of 0 or 1 in 32 surveys, a difference of 2 turtles in 5 surveys, and of 3 and 8 in two other surveys. The average difference in the number of turtles detected between R1,2 was 15%, between R1,3 3 was 10.8 % turtles and between R2,3 was 15.3% turtles. The average percent difference between the three sets of reviewers was 13.7%. The total number, and average, of turtles detected for each reviewer was compared against my final review of all footage.

3.5.3 **Boat survey modeling**

First, I tested environmental variables (cloud cover, tide and wind speed) during boat-based surveys against detection of turtles using a generalized linear mixed model. Random variables in this model were time of survey start and observer training level. ANOVA results revealed a significant interaction between incoming tide and cloud cover, but all other variables and interactions had no significant effect on the number of turtles detected (Table 3.3).

Table 3.3 Significance of variables after ANOVA testing of boat surveys.

Variable	**Chisq**	**Df**	**p-value**
Tide	1.0	1	0.311
Wind	0.1	1	0.710
Cloud	2.7	1	0.104
Tide:Wind	0.2	1	0.644
Tide:Cloud	8.8	1	0.003 *
Wind:Cloud	1.3	1	0.262
Tide:Wind:Cloud	3.8	1	0.052

Next, I used reverse variable selection to remove wind from the model as it contributed least to the detection of turtles, in the ANOVA test (p-value= 0.710). When wind was removed and a new generalized linear mixed effect regression was run, results showed a singular model. The singular model was fit in an ANOVA test and the results revealed that the random variable time of survey start had no effect on variation among number of turtles seen during boat surveys (Table 3.4).

Table 3.4 Results of fixed (ANOVA) and random effects from singular model of boat surveys

Random variables			
	Variance	Std. dev.	
Time of survey start	0.00	0.00	
Observer training	0.03	0.19	
Fixed variables			
	Chisq	Df	p-value
Tide	0.3	1	0.578
Cloud	3.4	1	0.065
Tide: Cloud	7.5	1	0.006 *

I removed the random variable, time of survey start, and ran a final regression with tide and cloud as fixed variables and the observer training number as the only random variable. Results from the two-way regression in the ANOVA test showed that the interaction of tide and cloud cover significantly impacted the number of turtles detected (p-value = 0.006), but when analyzed separately, tide and cloud cover do not significantly impact the number of turtles detected (Table 3.5). The training level of observers did not significantly affect the number of turtles detected on boat surveys, but was retained throughout the modeling to absorb error.

Results of the final, most parsimonious model for all creeks revealed a negative slope for tide and cloud cover and a positive slope (more turtle detection) for the interaction between cloud and tide (Table 3.5). Final model**:** Turtle detection= 2.82- 1.06*incoming- 0.031*cloud+ 0.03*(incoming: cloud).

Table 3.5 Results of final boat model and ANOVA.

Fixed effects			**Correlation of Fixed Effects**			
Variable	Std. error	t-value	Variable	Intercept	Tide	Cloud
(Intercept)	0.42	6.76	Tide	-0.76		
Tide	0.51	-2.08	Cloud	-0.85	0.7	
Cloud	0.01	-3.29	Tide:Cloud	0.73	-0.86	-0.86
Tide:Cloud	0.01	2.73				
Random effects			**ANOVA results of fixed effects**			
Variable	Variance	Std.Dev.	Variable	Chisq	Df	p-value
Observers	0.03	0.19	Tide	0.3	1	0.577
Residual	0.61	0.78	Cloud	3.4	1	0.065
			Tide:Cloud	7.5	1	0.006*

The results from surveys in Half Sound showed significantly higher detection than surveys in other creeks. However, Half Sound surveys were limited (n=5) and thus despite the model above, these surveys could have biased the final model results. Thus, it was prudent to run a generalized linear mixed effect model without Half Sound to interpret results of turtle detection against environmental conditions in the other creek systems. When detection of turtles using boat survey method in Deep Creek, Starved Creek and Rollins Creek was tested, the random variables, time of survey start and observers training had zero variance on the model and were excluded. Because of this, the final model, excluding Half Sound results was a simple linear regression testing turtle detection against tidal variation, wind speed and cloud cover. In this final regression, there were no significant differences in the number of turtles detected when tested against environmental variables (p-values >0.05) (Table 3.6). These results suggest that Half Sound data contributed to the significance in the number of turtles detected in varying weather conditions for boat surveys shown above (Table 3.5). Thus, in Half Sound environmental conditions may significantly impact turtle detection for boat surveys, but the significant differences were not observed for the other three creek systems.

Table 3.6 Significance of environmental variables against turtle detection using boat survey technique for Starved Creek, Rollins Creek and Deep Creek (excluding Half Sound). No statistical significance was observed.

Variable	Std. Error	t-value	p-value
Tide	1.36	-0.42	0.682
Wind	0.08	-0.45	0.646
Cloud	0.03	-1.36	0.185
Tide:Wind	0.12	-0.18	0.858
Tide:Cloud	0.03	0.99	0.332
Wind:Cloud	0.002	0.81	0.424
Tide:Wind:Cloud	0.002	-0.57	0.575

3.5.4 **UAV survey modeling**

Next, I tested environmental variables as fixed effects (cloud cover, tide and wind speed) against detection of turtles using generalized linear mixed effect regression for UAV surveys. The only random variable in this model was the time of survey start. I used an ANOVA test to evaluate the effects of each fixed variable, and random variable, on turtle detection. Results revealed a significant interaction between tide and cloud cover (p-value = 0.001) only (Table 3.7).

Table 3.7 Significance of variables after ANOVA testing of UAV surveys. ":" indicates interaction of variables.

Variable	Chisq	Df	p-value
Tide	1.1	1	0.299
Wind	0.8	1	0.358
Cloud	2.7	1	0.099
Tide:Wind	1.1	1	0.3
Tide:Cloud	10.8	1	0.001*
Wind:Cloud	0.7	1	0.414
Tide:Wind:Cloud	0.3	1	0.608

Because the standard deviation of the time of survey start revealed zero effect on error in the model, I removed this variable from subsequent models. As time of survey start was the only random variable, once it was removed, I used a simple linear model to test the effects of cloud cover, wind and tide on the number of turtles detected using UAV surveys. Results from this revealed that wind was the least significant variable for detecting turtles using UAV surveys (p-value = 0.352), and was subsequently removed from the model (Table 3.8).

Table 3.8 Results from linear regression of environmental conditions during UAV surveys.

Variable	Std. Error	t-value	p-value
(Intercept)	0.73	4.68	<0.001*
Tide	1.08	-0.99	0.328
Wind	0.07	-0.94	0.352
Cloud	0.02	-2.46	0.019*
Tide:Wind	0.11	-0.17	0.868
Tide:Cloud	0.02	1.97	0.058
Wind:Cloud	0.001	0.91	0.369
Tide:Wind:Cloud	0.002	-0.51	0.612

Next, I ran a simple, two-way linear regression with tide and cloud cover. The results from this model revealed tide, cloud cover, and the interaction of tide and cloud cover were all statistically significant. No random effects were observed for the UAV model, thus, a simple linear regression was fitted, as the mixed effects regression requires inclusion of random variables. Results of this final, most parsimonious, model revealed a negative slope for tide and cloud cover uniquely, but a positive slope for the interaction between cloud and tide (Table 3.9). Final model: Turtle detection= 2.85- 1.16*incoming -0.03*cloud +0.04 *(incoming: cloud).

Table 3.9 Results of final linear regression of UAV surveys and environmental variables.

Variable	Std. Error	t-value	p-value
(Intercept)	0.39	7.36	<0.001*
Tide	0.49	-2.34	0.025*
Cloud	0.009	-3.75	<0.001*
Tide:Cloud	0.01	3.43	0.002*

However, for UAV surveys, results from Half Sound could have also biased overall results. Therefore, I ran another generalized linear mixed effect model without Half Sound to interpret results of turtle detection against environmental conditions in the other creek systems using UAV surveys. When detection of turtles using UAV survey method in Deep Creek, Starved Creek and Rollins Creek was tested against environmental variables, the random variable, time of survey start had zero variance on the model and was excluded. The final model, excluding Half Sound results was a simple linear regression testing turtle detection against tidal variation, wind speed and cloud cover. As with boat surveys, in this final regression for UAV surveys, there were no significant differences in the number of turtles detected when tested against environmental variables (p-values >0.05) (Table 3.10). These results also suggest that Half Sound data contributed to the significance in the number of turtles detected in varying weather conditions for UAV surveys (Table 3.5). Thus, in Half Sound environmental conditions may significantly impact turtle detection for UAV surveys, but this significant difference was not observed for the other three creek systems.

Table 3.10 Significance of environmental variables against turtle detection using UAV survey technique for Starved Creek, Deep Creek and Rollins Creeks (excluding Half Sound). No statistical significance was observed.

Variable	Std. Error	t-value	p-value
Tide	1.1	0.916	0.368
Wind	0.07	1.155	0.259
Cloud	0.02	0.207	0.838
Tide:Wind	0.11	-1.35	0.188
Tide:Cloud	0.03	-0.11	0.916
Wind:Cloud	0.002	-1.05	0.306
Tide:Wind:Cloud	0.002	0.82	0.419

3.5.5 Comparing boat and UAV environmental conditions models

I standardized slopes into the same scale before comparing UAV and boat models. Results of the standardized coefficients for UAV surveys revealed that incoming tide had the least influence on the model (-0.664), cloud cover has the next least (-1.031) and the interaction of tide and cloud cover had the greatest influence on the model (1.334) and contributed to the most turtles detected. Similarly, for the final boat model, standardized coefficients revealed that incoming tide had the least influence on the model (-0.619), cloud cover has the next least (-0.952) and the interaction of tide and cloud cover had the greatest influence on the model (1.13) and contributed to the most turtles detected.

Next, I conducted an ANOVA to compare the slope of both models from the lm and lmer beta functions. Results from this ANOVA revealed that the two models did not differ significantly (p- value = 1, df = 1) between the number of turtles detected between the two survey techniques (Table 3.11). Environmental conditions that impacted turtle detection for both survey techniques independently were cloud cover and tide (incoming or outgoing). However, these environmental conditions did not contribute significantly to the difference in the number of turtles seen using either technique. For combined UAV surveys across all creeks, interaction plots revealed outgoing tide had the strongest positive effect on turtle detection at 10%, and the strongest negative effect on turtle detection was observed at 10% cloud cover with an incoming tide. Results from the interaction plots of boat surveys across all creeks also showed outgoing tide had the strongest positive effect on turtle detection at 10% and the strongest negative effect on turtle detection was observed at 10% cloud cover with an incoming tide. Results are summarized to show the interaction plots and values (Table 3.11). Interaction terms were included throughout modeling as the individual variables were not significant, and contributed to understanding the ways in which variables are linked.

Table 3.11 Comparison of slopes for both survey techniques for all creeks. Statistically significant p-values (<0.05) are indicated by *. T value is reported for fixed effects only. Fixed effect variables separated by a ":" represents the interactions of the variables.

	Boat			**UAV**		
Fixed variable	**T-value**	**p-value**	**Slope**	**T-value**	**p-value**	**Slope**
Cloud cover	-3.291	0.065	-0.953	-3.750	0.001*	-1.032
Tide	-2.081	0.578	-0.619	-2.343	0.025*	-0.665
Cloud cover: Tide	2.733	0.006*	1.128	3.427	0.002*	1.339

These results indicate no significant difference between the survey methods, and that they could be used interchangeably with varying cloud cover, wind speed and tidal states. However, the exclusion of Half Sound because of its significantly higher detection and limited survey size was also important to consider here. The slope of boat surveys for Starved, Deep and Rollins Creeks indicated that the greatest influence (highest number of turtles detected) was for the interaction of tide and cloud cover (1.229), while the least influential variable for turtle detection (lowest number of turtles detected) was cloud cover (-1.207) in the boat model. For the UAV model, the greatest influence on turtle detection was wind (0.55), while the least influential was the interaction of tide and wind (-0.954). However, ANOVA results comparing the slopes of both techniques revealed no significant difference between the survey techniques for detecting turtles when tested against environmental variables (Table 3.12). Thus, these results further show that the survey techniques can be used interchangeably with varying cloud cover, wind speed and tidal states for all creeks.

Table 3.12 Comparison of slopes for both survey techniques for Starved Creek, Rollins Creek and Deep Creek (excluding Half Sound). No statistical significance was observed. Fixed effect variables separated by a ":" represents the interactions of the variables.

	Boat	**UAV**	**ANOVA: Boat and UAV**	
Fixed variable	**Slope**	**Slope**	**F-value**	**p-value**
Tide	-0.378	0.771	2.595	0.119
Wind	-0.239	0.55	0.105	0.748
Cloud	-1.207	0.169	0.385	0.54
Tide:Wind	0.138	-0.954	1.222	0.279
Tide:Cloud	1.229	-0.122	1.871	0.183
Wind:Cloud	0.724	-0.859	0.328	0.572
Tide:Wind:Cloud	-0.566	0.756	0.529	0.474

Overall, statistical testing that excluded Half Sound from linear regression testing did indeed show no significant interactions between cloud cover and tidal variation. In fact, no environmental variables contributed significantly to turtle detection between these three creek systems. This further suggests that Half Sound is a significant anomaly when compared to the other creeks.

Time of survey start

In the above boat and UAV models, I tested whether the time that each survey started had an effect on the number of turtles detected. Here, I was interested in whether the time that the survey started contributed to the difference between the numbers of turtles detected using either method. Field observations suggested that sun position based on time of day may increase the level of glare, or reflection, that the UAV camera detects (high glare periods considered to be mid-day), and could negatively affect the ability to detect turtles using UAV surveys. Based on observations and sun angle, I would also expect the reflection on boat surveys to be a factor in turtle detection during specific time frames (before sunset when boat is level with water). However, I did not physically record glare during surveys during field surveys. The true level of glare or reflection is dependent on other variables, such as cloud cover, wind speed, light intensity, angle of sun and wave action.

I estimated the effects of the sun's reflection on turtle detection. First, I subtracted the number of turtles observed during boat surveys from the number of turtles detected on UAV surveys. This resulted in the average difference of turtles detected between the two surveys (Figure 3.3). I then compared this average difference to the time of day and correlated the cloud cover of each survey to a respective color (i.e. yellow dots represent 0-20 % cloud cover, etc.). The results show that cloud cover was lowest during all morning surveys, and highest during the later afternoon surveys. If cloud cover does factor into reflection, it could have prevented reflection during the afternoon sun, and could have explained why time of day did not factor significantly into turtle detection between the models. Yet, cloud cover in field observations was noted to reduce turtle detection, particularly for boat surveys, as high cloud cover obscured observer view. Additionally, during all surveys the speed of the UAV and boat surveys was consistent, and for UAV surveys, even if there was noticeable glare on the video recordings, the flight speed recorded transient glare across the screen. Thus, if glare was detected on the screen, it moved across the screen quickly enough for us to be able to spot turtles covered by temporary bouts of reflection.

3.6 Discussion

I quantified whether UAVs or boats are better at measuring the abundance of juvenile green turtles in four shallow mangrove creek systems in The Bahamas. In this study, I found 1) overall, UAV surveys were not more effective than boat surveys to detect juvenile green turtles 2) increased wind speed, increased cloud cover and incoming tide did not result in reduced turtle detection for both UAV and boat techniques 3) there were differences in turtle detection between creeks and within the four sample creek systems.

Results highlight the usefulness and accuracy of both survey techniques as effective methods for detecting juvenile green turtles as no significant difference was observed between the total number of turtles detected between the two methods (Duffy et al., 2018; Schofield et al., 2019; Robinson et al., 2020). The duration of boat and UAV surveys also differed on average; boat surveys took longer in the field than UAV surveys. Thus, UAV surveys resulted in less time spent surveying the same route, and less fuel needed to conduct the survey itself, although the amount of fuel to get to each survey site was the same for both techniques.

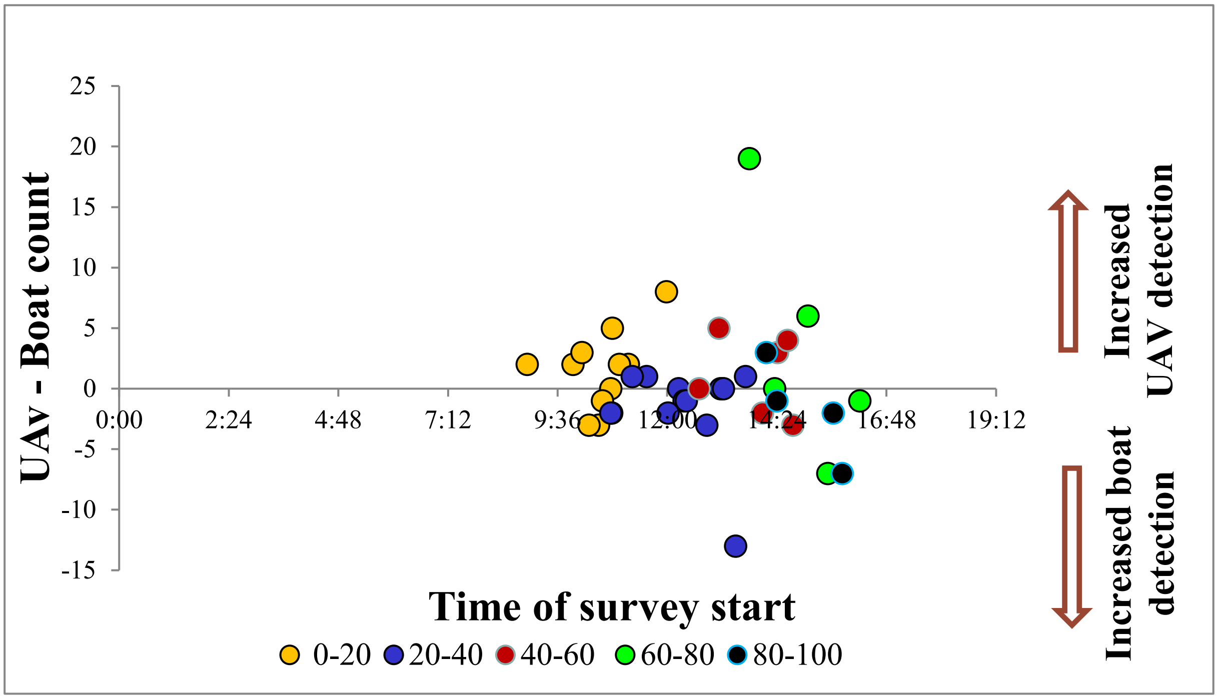

Figure 3.3 Effect of time of survey start and cloud cover on turtle detection. Values represent the number of turtles detected by boat surveys (-) the number of turtles detected by UAV surveys. Arrows indicate the number of turtles detected by UAV surveys increased as y-values increased, and decreased as y- values decreased. Colored points represent variations in percentage cloud cover.

Time spent post processing data varied greatly; each boat survey took roughly 15 min, while UAVs took up to 1.3 hrs per survey. When I considered the difference in detection between the reviewers (1, 2 and 3), the average percentage difference between the reviewers was considerable at 13.7%. This suggests that there is human error in the review of video files that could impact the survey type that is chosen for research. Such variation could have important ecological significance as UAVs and boats can be used to conduct presence or absence surveys (Pollock et al., 2006; Rowat et al. 2009). Additionally, in other surveys there was a difference of 7 turtles, which is a considerable number on a short survey, as in-water sea turtle surveys are used to estimate population abundance and distribution (Williams et al., 2017; Schofield et al., 2019). Indeed, UAV surveys can be used for behavioral studies, as well as studies of elusive individuals, such as male turtles (Crawford et al., 2014; Bevan et al., 2018). Thus, the accuracy of detecting individuals is important for effective population monitoring.

Reduction of post-processing efforts could make the difference in time negligible between each technique. Advanced software, or coding are quick ways to automate this process, to save time and to reduce error in detection (Hone, 2008; Hogson et al., 2018). Field surveys for UAVs were quicker, and do result in slightly less fuel as it is not necessary to move the boat while flying the units. If researchers could automate the post-processing time, and could cut travel time by utilizing UAVs instead of boats, UAV surveys would be favorable if time is a limiting factor. As UAV technology continues to advance, limitations from human error and post-processing time will likely be able to be eliminated for more precision, cost, and time saving benefits (Colefax et al., 2018; Schoefield et al., 2019).

Further, UAV surveys can be conducted by shore and can eliminate the need to have a boat, fuel for the boat and additional team members. UAVs also make possible assessments of shallow and difficult to reach areas of creeks (Rees et al., 2018). The benefits of the boat surveys are that they eliminate the need to post-process data, send video data files to additional reviewers and they are not limited by battery life (Bevan et al., 2015). There are also different technological difficulties with boat and UAV surveys. Boats are typically a reliable method of surveying turtles, but require prior training to operate. UAV surveys also require trained team members that can safely pilot a unit in often windy and high glare conditions. Teams of biologists can conduct as many surveys as possible in creeks using boat surveys, provided they can carry enough fuel, whereas UAV surveys are limited to the number of batteries, and thus, flight time (Jones et al., 2006). The utilization of either technique based on cost and time are necessary considerations for determining which survey type to employ.

Although there was no significant difference in the total number of turtles tested for each technique, between and within creek variability in turtle detection was significant for some creek systems. Within creeks, I observed a significant difference between the two survey techniques and turtle detection for Deep Creek and Starved Creek. Starved Creek was the only system that had significantly higher detection of turtles using boat surveys. The survey route in this creek was shallow, and benthic conditions are very sandy. Perhaps, observers on the boat were more able to spot swimming turtles as seagrass beds are not as abundant on this route, and rocks and crevices would not obscure observer view. Deep Creek was the only system wherein significantly higher numbers of turtles were detected using UAVs. This difference may be due to the survey route as well, as some areas along the route had deep pools. Turtles in these pools could have been undetectable by boat observers if the animals were resting, or swimming deeply. Half Sound and Rollins Creek revealed no significant difference between detection of turtles and survey. Half Sound is the only creek located on the eastern side of Eleuthera, it has richly abundant seagrass beds and is the largest of the creek systems. Rollins Creek has swift currents and deep pools throughout the narrow survey route. Although each of the creek systems is similarly lined with mangroves, relatively sandy bottom and seagrasses, within creek variability is certainly present. As there was a significant difference in the number of turtles detected within some creeks using the different methods, future studies may benefit from more detailed mapping within the creeks themselves. For example, mapping abundance of seagrass beds, or depths or pools may help to explain the differences in turtle detection and may help researchers decide which method of surveying to use dependent on more specific benthic conditions.

Similarly, significant differences were observed in the number of turtles detected between Half Sound and all other creek systems. Between the other creek systems (Deep, Rollins and Starved creeks), there were no significant differences for UAV surveys. Yet, Half Sound turtle detection using UAV surveys was significantly different than Deep, Rollins and Starved creeks. For boat surveys, Half Sound was not statistically different than Rollins Creek, only. This could be due to similarities between the benthic conditions in Rollins Creek and Half Sound; deep pools, rockier bottoms, or more seagrasses that could have made detection similar for both creeks. Half Sound may be an important resting point for juvenile turtles along the eastern coast. The eastern coastline has high wave energy (Hearty, 1998), and this particular inlet is sheltered as there is a small entrance to the system, but a large area within the inlet. The significance in turtle detection here may make it an ecologically significant area for green turtles, and continued studies to further categorize conditions should be considered.

I also attempted to assess the effects of the sun's reflection on turtle detection based on the time of day that surveys were conducted. In generalized linear mixed models, time of day was included as a random variable for both boat and UAV surveys and had no effect on the variance of turtle detection for either technique. I anticipated that the number of turtles detected during UAV surveys would be impacted by the time of day because at high noon, the glare on the UAV recording device could make detection more difficult because I flew the UAV at 30 m. Thus, when the UAV and sun were directly overhead, more glare could be detected on the video recording during post processing that would negatively affect the frequency of turtle detection

(Fuentes et al., 2015; Schofield et al., 2019). Yet, the distribution of data revealed that the time of day did not contribute to the difference in the number of turtles detected between survey types. The difference in the detection of turtles was sporadically shown across the time period that I conducted surveys (surveys all took place between 8 am and 5 pm). I also included cloud cover in this figure, to express that although the sun may be directly overhead; it is not necessarily the most intense at the noon hour if percentage of cloud cover is high. Interestingly, all of the surveys conducted with high cloud cover were during the time periods that I would have expected UAV glare to be highest.

However, there are likely more effective physical ways to measure reflection while surveying, for both UAV and boat surveys that may help researchers decide on survey method based on time of day. Such methods for quantifying glare, could involve measuring the sun's angle, the light intensity, the wave action and water turbidity (Fuentes et al., 2015; Schoield et al., 2019). Additionally, conducting surveys throughout the day with varying cloud cover may yield different results. It may be relevant to note here that the speed (~7.5km h^{-1}) and the height (30 m) the unit was flown may contribute to the lack of difference between survey techniques at different times of the day. For example, the UAV was flown at a speed that was slow enough to observe turtles (even if there was some glare on the screen), as enough time elapsed to detect the turtles at some point during the video length. The boat survey turtle detection was not different, possibly because I flew the UAV at a similar speed. However, boat speed automation was not possible; tide and wind speed likely factored into actual speed. The height of the UAV could also be impacted by glare. Researchers deciding if there is an "optimal" time of day for choosing either techniques, should consider the impact that glare could have at lower or higher UAV heights and varying speed; to start this testing, I recommend flying units at a height of 30 m and fixed speed of ~7.5km h^{-1}. The height (30 m) to fly the unit during these surveys was chosen because when flown at this height there was no observation of a turtle reacting to the UAV (Bevan et al., 2018),

Testing the effects of environmental conditions other than glare (wind, cloud cover, tidal variation) against turtle detection for both survey techniques indicated that the number of turtles detected during the interaction of outgoing tide and 10% cloud cover was the only significant effect on turtle detection for boat surveys. This interaction in both models resulted in higher numbers of turtles detected. Additionally, the rate of detection decreased for boat and UAV models when the tide was incoming, and was lowest when the tide was incoming and cloud cover was 10%. Because the two way interactions significantly impacted the number of turtles detected, the main effects, or fixed variables, are dependent at some level. Here, I observe that variation between increased cloud cover is dependent on incoming tide, so the interpretation of the model should not consider the variables in isolation (Aiken and West, 1991, Zar, 2010). Results from the UAV model ANOVA also showed that tide, and cloud cover were significant factors for the number of turtles detected independently, and that there was a significant interaction between the two variables. Yet, because of this significant interaction term, the two variables cannot be analyzed independently.

The comparative slopes for both UAV and boat models show that the interaction is strongest, and best for detecting turtles, between decreased cloud cover and outgoing tide. The results from both models indicate that this was the only variable interaction that contributed significantly to the detection of turtles. Essentially, though the interactions of some environmental conditions are a factor, these interactions factored into both surveys, and do not make one method better at detecting turtles based on varying environmental conditions. Statistical testing revealed that the two survey techniques can be used interchangeably with varying wind, cloud cover and tide. The results of regression testing revealed that there were significant interactions between cloud cover and tide when all creek systems were compared to each other. However, the significance of these interactions was observed for both UAV and boat models, and therefore there was no significant difference between the impacts of environmental variables on turtle detection between the two models during ANOVA testing. Further, when Half Sound data was excluded, there was no significant difference in any environmental variable or interaction between the variables, suggesting that the significance that was shown was a result of limited Half Sound surveys.

Given all considerations, total boat and UAV turtle detection in this study did not result in significantly different results. Although 10% cloud cover and outgoing tide did increase the number of turtles detected, these environmental variables were a factor in both survey techniques, and were observed during Half Sound surveys only. Thus, this significant interaction should be interpreted conservatively, and these environmental conditions did not contribute significantly to differences between the survey techniques. Within and between creek variability observed between the two survey types was also noted for Half Sound and all other creeks except between Half Sound and Rollins Creek during boat surveys. In all of the surveys, Half Sound had considerably higher detection of turtles, and it may be ecologically significant area for further studies to map seagrass abundance, wave action, and overall size. Indeed, results from UAV studies conducted for other survey goals, could map seagrass beds with the correct software (Hays et al., 2018).

Overall, I found that population monitoring of the endangered Caribbean green turtle can be conducted effectively using boats or UAVs for surveys. However, when planning which survey type to employ, researchers may benefit from doing an initial analysis of benthic conditions, such as rocks, seagrass beds and reefs. A preliminary survey could account for the between and within creek variability that was observed here for the two different techniques. The cost of survey duration; potential for automation of post-processing time and travel distance, are considerable factors in deciding whether to use boat and UAV surveys as both techniques proved reliable here. Future technological advances for UAVs such as extended battery life, reflection reduction, built in detection and automation of wildlife may make this survey techniques more efficient for researchers to use in difficult to access, or extensive survey regions for many species of wildlife (Jones et al., 2006; Koh and Wich, 2012; Rees et al., 2018). Both survey techniques assessed here further support the significance of in-water surveys in sea turtles as necessary tools to consider both male and females in populations (Chaloupka and Musick, 1997; Seminoff et al., 2003; Fuentes et al., 2015). At present, this study shows that the two techniques can be used interchangeably for detection of turtles along the shores of Eleuthera.

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