EFFECTIVENESS OF DRONES FOR FRESHWATER TURTLE SURVEYS AIMED TOWARD DETECTING THE CRYPTIC WESTERN CHICKEN TURTLE. *(DEIROCHELYS RETICULARIA MIARIA).*

by

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THESIS

Presented to the Faculty of

The University of Houston-Clear Lake

In Partial fulfillment

Of the Requirements

For the Degree

MASTER OF SCIENCE

in Environmental Science – Biology

THE UNIVERSITY OF HOUSTON-CLEAR LAKE

MAY, 2023

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Acknowledgments

This project would not be possible without the help of many. Thanks to all the amazing staff at the Environmental Institute of Houston at the Univeristy of Houston - Clear Lake who aided in setting up the WCT project, traveling long distances, enduring grueling hot sampling days, and listing to me drone on. Overall project funding and support coming from the Texas Comptroller of Public Accounts. Being able to fly drones at properties with Mr. Welch, W. Newman, M. House, and B. Foshee with the Coastal (formerly Katy) Prairie Conservancy, Bill Kirby with Sabine River Authority of Texas, Kyle Brunson with Gus Engling WMA, private landowners, the Garcia's', Harris's, and Rushin's, Dr. Paul Crump and Ben Anderson with Texas Parks and Wildlife, and U.S. Fish and Wildlife Service.

Thank you to everyone who took the time for turtles. To all the contributors, landowners, mentors, coworkers, friends, and family who have supported me in this phenomenal experience combining nature and technology. Especially those who have helped the WCT team at EIH. From UHCL's amazing professors Dr. George Guillen, Dr. Marc Mokrech, Dr. Jenny Oakley and Dr. Cindy Howard to the core WCT team Mandi Gordon, Jimmy Welch and Danielle DeChellis and all the turtle technicians, true and honorary Aurora Alvarez, David Bontrager, Sarah Bullard, Ashley Collins, Danielle Davidson, Grey Dennis, Kelly Garcia, Nick Hughes, Trey Hughes, Jaimie Kittle, Story Lesher, Isabel Marzullo, Taylor McKenzie, Victoria Ontiveros, Adriana Puzon, Sarah Schrock, Cecilia Silva, Taylor Thomisee, Cecilia Thompson, Mathew VanBemmel, Tori Vourganas, Haley Welshoff, and Emily Yargeau. Thank you so much, y'all rule!!!

ABSTRACT

EFFECTIVENESS OF DRONES FOR FRESHWATER TURTLE SURVEYS AIMED TOWARD DETECTING THE CRYPTIC WESTERN CHICKEN TURTLE. *(DEIROCHELYS RETICULARIA MIARIA).*

Jason Nagro
University of Houston-Clear Lake, 2023

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The rise in the use of drones in wildlife research has shown promising results for conservation practices. Few studies have focused on drone surveys for aquatic freshwater turtles. This study evaluated the effectiveness of drones for detecting freshwater turtles with the primary target species being the Western Chicken Turtle (WCT; *Deirochelys reticularia miaria*). Two drones were employed to investigate their effectiveness for detecting freshwater turtles. 1) Videos and thermal imagery were utilized using a DJI Mavic 2 Enterprise (M2) and 2) static multispectral imagery using a DJI Phantom 4 (P4MS). Binocular aided visual surveys (BAVS) were conducted simultaneously with M2 surveys to compare and contrast methodologies. A total of 20.7 hours of video footage yielded 1916 freshwater turtle detections and 57090 photos with 1915 detections.

BAVS had a cumulative time of 58.1 hours with 1096 turtle detections. Six turtle groups were detected with the M2, five with the P4MS and four with BAVS. Groups that were identified by all methods were Slider Turtles (*Trachemys sp.*), North American Softshell Turtles (*Apalone sp.*), and Common Snapping Turtles (*Chelydra serpentina*). A majority of all detected freshwater turtles displayed no reaction to the drones presence. The M2 had a statistically significant ($p = 0.015$) higher monthly catch per unit effort (CPUE) for freshwater turtles when compared to BAVS. Six WCT were detected using drone surveys (M2 = 5 and P4MS =1) while BAVS failed to detect WCT. Drone surveys were successful at detecting and identifying freshwater turtles such as the WCT when compared to BAVS, but quality data collection relies upon many internal and external factors such as camera resolution and essential habitat features. Drones are powerful tools when surveying freshwater turtles and other wildlife collecting vast amounts of data. Their implementation in future research studies concerning wildlife conservation with freshwater turtles have evident benefits in overall site accessibility, field team safety, and non-invasive rapid data collection.

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INTRODUCTION

Freshwater environments come in many different forms all with their own unique characteristics such as hydrology, geomorphology, flora, and fauna. Freshwater wetlands, rivers, streams, lakes, and ponds support multiple over-arching natural and anthropogenic systems and processes worldwide (Baron et al., 2002; Mitsch & Gosselink, 2000; Mulholland et al., 1997). Anthropogenic activities such as agriculture and landscape modification can influence the physico-chemical attributes and ecology of these freshwater systems (Bhowmik, 2020; Mitsch & Gosselink, 2000). Due to the high value associated with freshwater, there has been a rapid expansion of urbanization, agriculture, and industrialization in these environments (Davidson, 2014; Gibbs, 2000; Mulholland et al., 1997; Zedler, 2004). Because of this rapid expansion, there has been destruction and fragmentation of natural freshwater habitats (Revenga et al., 2005; Zedler, 2004). As a result of this, native freshwater and riparian flora and fauna have experienced declines. Specifically, communities of herpetofauna, which includes reptiles and amphibians, that utilize these freshwater habitats, have suffered population declines, extirpation, or even extinction (Gibbons et al., 2000).

The order Testudines which includes turtles (freshwater and marine) and tortoises (terrestrial) has 357 recognized species (living and extinct). (Rhodin et al., 2021). A majority of these species, 345, are freshwater or terrestrial and 61% are threatened or already extinct due to habitat loss that stems from human expansion (K. Buhlmann et al., 2008; Rhodin et al., 2021). In the United States, 75% of all turtle species are found in the southeastern region (K. Buhlmann et al., 2008). Turtles are important components of their ecosystems. They store high biomass and influence energy flow, mineral cycling, bioaccumulation, seed dispersal, and bioturbation (Lovich et al., 2018). If turtle biodiversity and populations continue to decline due to habitat loss there is potential for detrimental impacts to these ecosystems, specifically freshwater environments.

Monitoring freshwater turtles by tracking changes in their populations and range can provide valuable data about their population viability and status. To accomplish this, many methods both traditional and novel have and are being employed to assess freshwater turtles. Traditional non-invasive methodology for observing freshwater turtles includes using binoculars, spotting scopes, or setting camera traps to identify and count (Escobar et al., 2018). Other more invasive methods involve capturing individuals using dip nets, hoop traps, fyke nets, canine surveys, basking traps, or conducting snorkel surveys (Dodd, 2016). It is important to select which method that best achieves the goals of the research project. Advances in new technology available in the form of drones may provide a powerful tool for freshwater turtle surveys.

Drones, also referred to as small unmanned aerial systems (sUAS) or unmanned aerial vehicles (UAV), have rapidly grown in their usage within environmental conservation and research by using aerial imagery to identify and enumerate wildlife and assess habitat, especially in areas of difficult terrain or for cryptic and hard to find species (Butcher et al., 2021; Chabot et al., 2022; Howell et al., 2021; La Vigne et al., 2022; Landeo-Yauri et al., 2020). These drones vary in size from nano (less than 5 kilograms with limited flight times and ranges) to large (≈ 200 kilograms with long flight times and ranges) each with their own strengths, limitations, and applications to the study of spatial ecology (Anderson & Gaston, 2013).

There are numerous benefits to using drones such as surveying a large surface area in a short amount of time, reduced stress to wildlife, and coverage of areas not accessible by foot (Brooke et al., 2015). Drones can provide valuable data from areas that are difficult to access by foot in both a timely and cost-effective manner compared to other ground based methods (Anderson & Gaston, 2013; Brooke et al., 2015; La Vigne et al., 2022; Schofield, Katselidis, et al., 2017). New observations of marine species behavior such as birthing, mating, and primary feeding have been documented using drones, expanding our knowledge of these previously undocumented behaviors (Brooke et al., 2015; Schofield, Katselidis, et al., 2017). Lastly, spatial ecology data collected by drones can be used to quantify habitat characteristics for endangered species (Butcher et al., 2021; Howell et al., 2021; La Vigne et al., 2022). Drones for wildlife surveys provide promising new tools for conservation ecologists as the technology and understanding of them grows (Christie et al., 2016).

Many turtles can be identified by their carapace shape, color, and head / skin markings, but the ultimate factor for identification using drones resides in the resolution quality of the camera sensors (Anderson & Gaston, 2013; Bevan et al., 2016; Biserkov & Lukanov, 2017; Christie et al., 2016; Schofield, Katselidis, et al., 2017). Having a high-resolution camera that is stable with an optimal angle can produce high quality imagery data for behavior studies. The effectiveness of drone surveys is not only determined by the resolution of the camera, but other environmental factors such as water clarity and the depth of the turtle (Bevan et al., 2016). Research studies involving drones have conducted successful surveys on both freshwater (Rio Grande Cooter [*Pseudemys gorzugi]*) and saltwater (Green Sea Turtles [*Chelonia mydas*]) turtles each with their own benefits (Bogolin et al., 2021; Davis et al., 2020; Schofield, Katselidis, et al., 2017; Schofield, Papafitsoros, et al., 2017). Drones can be used to document mass basking groups, allow for increased shore visibility, and identifying turtle tracks in the mud (Biserkov & Lukanov, 2017; Davis et al., 2020). Studies have shown using drones were more effective in open water surveys under both low and high densities of Green Sea Turtles (*Chelonia mydas*) compared to traditional boat surveys (Schofield, Katselidis, et al., 2017; Schofield, Papafitsoros, et al., 2017). Rapid assessments of freshwater turtles have been successfully conducted with drones as seen in Daniels 2018, as they were able to identify turtles but not down to a species level.

Other factors of consideration for successful drone surveys include elevation of the platform/unit, proximity to the animal, stressful disturbance to the organism, area accessibility, and allocated flight time (especially in relation to battery life) (Biserkov & Lukanov, 2017; Vallery, 2018; Wilson et al., 2017). There is evidence that there are minimal wildlife behavioral and physiological responses to drones especially when it comes to avian species (Christie et al., 2016; Vallery, 2018; Wilson et al., 2017). But more studies are needed to document the response of other wildlife species to drones, specifically freshwater turtles. Ideally photography and video should be taken at elevations that do not provoke a flight response from the organism, while still capturing the highest quality of image and video for counts and identification. Using a higher quality camera when turtles exhibit a basking behavior, are key to documenting data for freshwater turtles with drones (Bogolin et al., 2021).

Drones can be equipped with multiple sensors that serve a wide range of applications. For example, the use of multispectral imagery has aided in the detection, identification, and characterization of wildlife and vegetation (Sesnie et al., 2016). A multispectrum refers to a small number of bands collected on the electromagnetic scale, ranging somewhere between three to 10 bands depending upon the sensor (GISGeography, 2014). These bands can be used to create color composite images as well as a NDVI (Landsat Normalized Difference Vegetation Index) to investigate the density and health of vegetation. Multispectral imagery has been used in studies to map aquatic vegetation in wetlands with high success for classifying surface and emergent vegetation and mild success for submerged vegetation (Chabot et al., 2018). Because Chabot et al. 2018 demonstrates success in wetland habitats, there is potential that these multispectral sensors might prove useful for surveys of aquatic freshwater turtles that thrive in these habitats.

Another sensor on drones that might prove useful is the utilization of thermal imagery to detect the presence of wildlife, which has been demonstrated to be beneficial in other studies (Avery et al., 2014; Chrétien et al., 2015; Howell et al., 2021). Using both visible and thermal infrared imagery to aid wildlife management by having both a visible spectrum video taken along with thermal infrared imagery data can enable the detection of multiple species in one flight survey, however false positives from the environment must be monitored (Chrétien et al., 2015). A drone equipped with a near-infrared sensor that combines the electromagnetic scale and heat signatures can be quite valuable for surveying turtles. As seen with the impactful invasive Burmese Python (*Python bivittatus*) in Florida comparing the heat signature of surrounding habitat to the target species can help detect their presence (Avery et al., 2014; Driggers et al., 2019). By using VisNIR (visible near-infrared) and SWIR (short wave infrared) the reflectivity of the surrounding vegetation can be used to detect pythons. While live vegetation had a larger difference in reflectivity compared to the pythons which made them easily detectable, dead vegetation could also be used but with smaller differences (Driggers et al., 2019). There has been little research done with thermal use for freshwater turtles making this study unique in examining its application.

Drones have a vast number of possibilities for data collection for wildlife conservation. This study will test the application of drone methodology and their sensors for surveying freshwater turtles in Southeastern portion of the United States, specifically Texas.

The Southeastern portion of the United States represents a biodiversity hot spot containing 42 species in Testudinidae found in a wide variety of habitat (K. Buhlmann et al., 2008). One of these freshwater turtle species is *Deirochelys reticularia miaria* or the Western Chicken Turtle (WCT). *D. r. miaria* is one of three subspecies of *Deirochelys reticularia* and is found west of the Mississippi River (Ewert et al., 2006). The current range of the WCT within Texas covers the Eastern part of Texas extending all the way from the northeastern border with Oklahoma and Arkansas to the southeastern border with Louisiana and the Gulf coast (USFWS & ECOS, 2016) (Figure 1). Western Chicken

Turtles (*Deirochelys reticularia miaria*) face many threats to their habitat, primarily anthropogenic ones. Humans have converted natural wetland habitat that the WCT utilizes into metropolitan areas or for agricultural purposes (Ryberg et al., 2014). This species migrates between these intermittent wetlands making this habitat loss directly impactful to the WCT (Chyn et al., 2020; Ryberg et al., 2014). Due to the rapid expansion of urbanization within the state of Texas, these cryptic turtles have become increasingly difficult to locate due to the loss of their natural habitat.

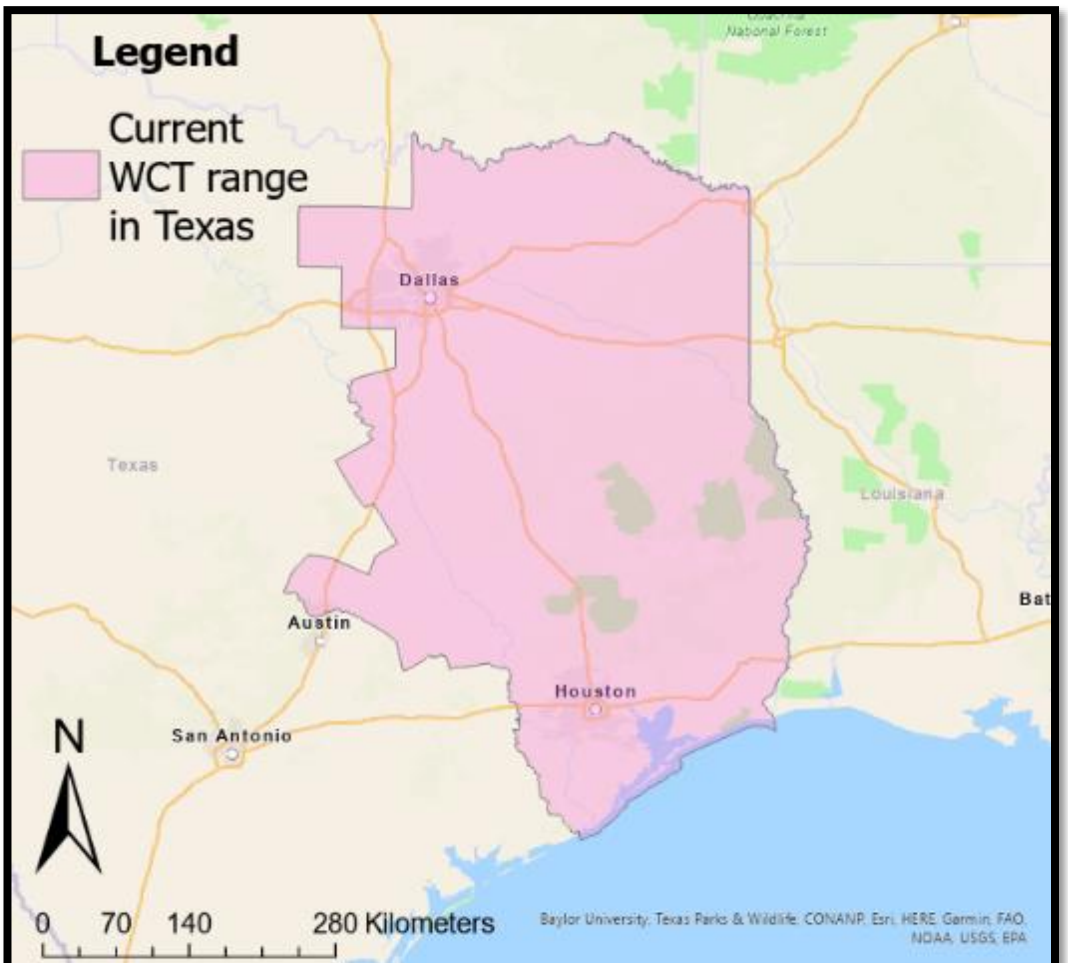

Figure 1: Current range map of the WCT in Texas, made in ArcGIS Pro. Shapefile data from United States Fish and Wildlife Service Environmental Conservation Online System. Last updated 02/24/2020 (USFWS & ECOS, 2016).

The WCT is a small to medium sized freshwater turtle with the distinct characteristic of having a very long yellow striped neck

Figure 2). The largest sizes in plastron length that WCT reach for females is 200 mm and males is 120 mm (K. A. Buhlmann et al., 2009). All Chicken Turtles (*Deirochelys*) have yellow stripes along their head and neck with a black background which is similar to other freshwater turtle species in East Texas. Western Chicken Turtles (*Deirochelys reticularia miaria*) can easily be mistaken for Slider Turtles (*Trachemys*) and Cooter Turtles (*Pseudemys*) if not examined carefully. Like other turtle species, the only sexual dimorphism that is observed is where the females are typically larger compared to the males (Ewert et al., 2006). Another distinguishing feature of the WCT is found at the posterior portion of the turtle on the legs. Western Chicken Turtles (*Deirochelys reticularia miaria)* have prominent vertical yellow strips that look like "pajama pants" (Figure 3).

The WCT carapace is a smooth pear oval shape and lacks serrated ridges on the posterior edges that are common on Red-eared Sliders, *Trachemys scripta elegans*. Another crucial characteristic is at the anterior part of the carapace the first vertebral scute overlaps the first and second marginal scutes (Figure 4). These morphology characteristics are key to identifying the WCT through various methodologies.


Figure 2: Western Chicken Turtle (*Deirochelys reticularia miaria*) long neck display. Photo by Mandi Gordon.

Figure 3: “Pajama Pant” stripped posterior legs of a Western Chicken Turtle (*Deirochelys reticularia miaria*). Photo by Jimmy Welch.

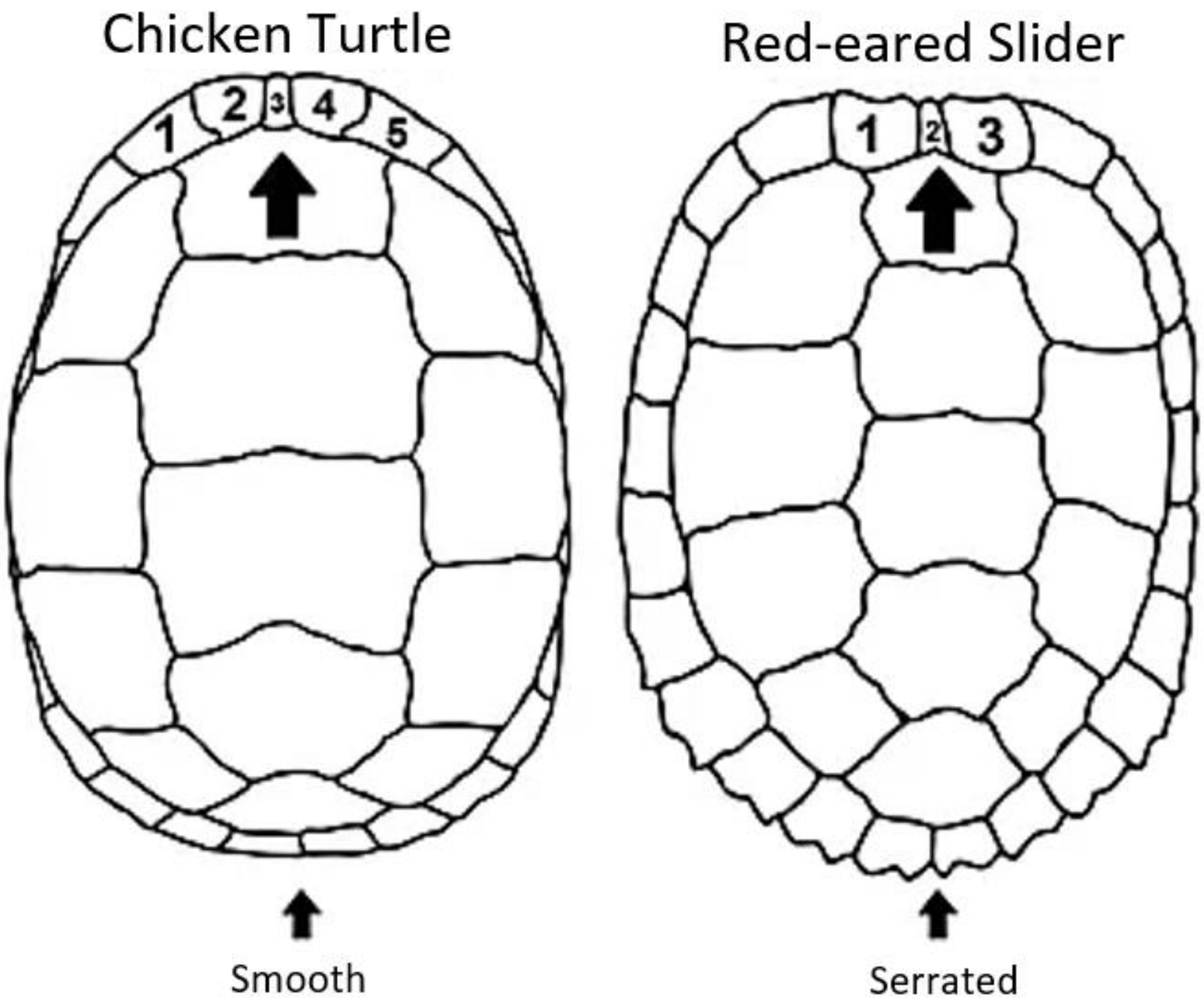

Figure 4: Carapace outline comparison of Western Chicken Turtle (*Deirochelys reticularia miaria*) and Red-eared Slider (*Trachemys scripta elegans*). Made by Jason Nagro.

Seasonal fluctuations in hydrology are extremely important for the WCT as it is found to migrate between ephemeral (short lived) wetlands that border prairies when they dry up or for mating and nesting purposes (Ryberg et al., 2014). This active movement period is limited to the months of March to July which plays a role in their elusiveness (Ryberg et al., 2014). Wetlands can be difficult to navigate by foot due to the fluctuating saturation levels and thick sticky mud. The safety of crew members and impacts to native vegetation need to be taken into consideration while planning an excursion into a wetland. Drones can provide a practical alternative data collection method in wetlands compared to ground surveys. *Deirochelys* prefer shallow intermittent wetlands, but not necessarily year round (K. A. Buhlmann et al., 2009). Western Chicken Turtles (*Deirochelys reticularia miaria*) also tend to avoid brackish waters, but certain subspecies have been found on islands surrounded by saline water on St. Vincent Island, Florida (Ewert et al., 2006). The growth rates of WCT are dependent upon the aquatic habitat quality and the hydroperiodicity of wetlands, with a seasonal change between resource rich wetlands and nearby terrestrial refuge environments (K. A. Buhlmann et al., 2009). Buhlmann et al. 2009 documented that WCTs had less time to occupy aquatic habitat and acquire food resources such as aquatic insects and crawfish when habitats were subjected to shorter hydroperiods and extended droughts. Individuals can visit anywhere from one to six different wetlands during migration and aestivation with a rather large home range, while some, researchers postulate that this species might exhibit partial or irruptive nomadism (Bowers et al., 2021).

Identifying the WCT visually can present complications as its size and carapace coloration is similar to other coexisting freshwater turtles along with the wide range of freshwater habitats it is found in. Another complication that arises often is the limited ground accessibility of freshwater habitats such as wetlands, rivers, and lakes. However, by collecting aerial imagery with drones there can be permanent data retention and the ability to allow multiple experts to identify turtle species. The goal of this study was to 1) assess and determine possible species and counts of freshwater turtles using drone technology at locations specifically targeted for the Western Chicken Turtle (*Deirochelys reticularia miaria*); 2) employ and test drone methodology for collecting imagery data and 3) to make recommendations for future investigators interested in the continued use of drones for freshwater turtle conservation and research.

Objectives

1. Evaluating the use of drones and acquired aerial imagery for freshwater turtle surveys, specifically aimed toward detecting the Western Chicken Turtle *(Deirochelys reticularia miaria).*
2. Comparing and contrasting catch per unit effort (CPUE) from drone surveys and their sensors (multispectral and thermal) to each other along with a traditional method, binocular aided visual surveys (BAVS) for aquatic freshwater turtles.
3. Make recommendations on drone methodology for future studies regarding aquatic turtles in freshwater environments.

MATERIALS AND METHODS

Study area and site selection

Surveys were conducted at 11 sites in East Texas representing two types of aquatic turtle habitat types including lentic habitats (LE = ponds and lakes) and emergent wetland habitats (WTL = wetlands) (Figure 5). The sites that were sampled represented a wide variety of freshwater habitat that the WCT could potentially occupy and within the known WCT range in the state of Texas. Overall habitat type was determined by ground truthing the waterbody, water depth and retention, hydrology, and vegetation. Before choosing sites, desk reconnaissance was performed using Google Earth and Google Maps. An ideal site was determined by investigating key environmental elements such as amount of open-air space with minimal canopy cover, availability of safe launch and landing zone where the pilot can maintain line of sight (LOS) of the drone, and amount of prime basking habitat for freshwater turtles (Biserkov & Lukanov, 2017; Jones IV et al., 2006; Junda et al., 2015; Shah Alam & Oluoch, 2021). Candidate sites were selected when these observed conditions were maximized. It is important to note that this study was a part of a much larger project involving WCT (Gordon et al., 2023). In that larger study, more sites were sampled using a variety of methods including BAVS, walking surveys, road surveys camera traps, eDNA, hoop/fyke net trapping, and canine surveys. Western Chicken Turtles (*Deirochelys reticularia miaria*) were detected with these traditional and novel techniques in that study (Gordon et al. 2023). This study focused on comparing drones and BAVS at sites where both methods were used simultaneously.

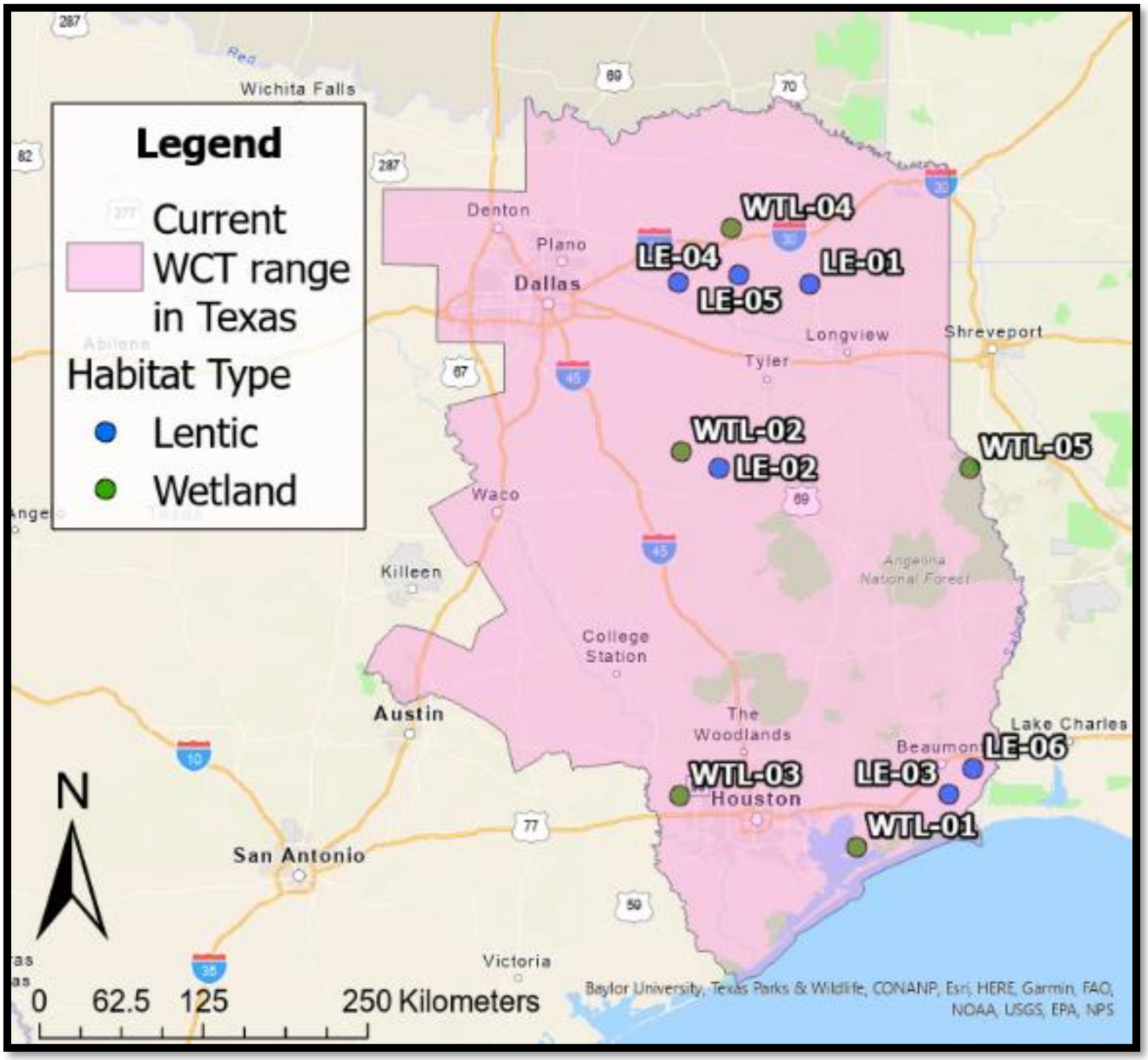

Figure 5: Sites surveyed for WCT with drones and BAVS in years 2021 and 2022. Pink layer is the current known WCT range in Texas from United States Fish and Wildlife Service Environmental Conservation Online System. Last updated 02/24/2020 (USFWS & ECOS, 2016). Blue dots are lentic habitat sites and green dots are wetland habitat sites. Map created in ArcGIS Pro.

Drone surveys

Pre-flight preparation

There are currently many legal steps required before flying drones for research purposes. A Federal Aviation Administration (FAA) Part 107 remote pilots license is required to fly drones for commercial purposes in the United States. To obtain this license, pilots are required to pass a knowledge test about airspace and the rules of aviation. Then, all drones need to be registered with the FAA (FAAdronezone.com) affiliated with the pilot's Part 107 license. In order to use drones for wildlife research, the state of Texas also requires an Aerial Wildlife Management (AWM) permit for all activities that are being conducted in the air (available online at https://tpwd.texas.gov/business/permits/land/wildlife_management/aerial_wl_management/). Each site needs to be validated and approved by the associated landowner regarding drone activities and boundaries. Quarterly reports were submitted to the Texas Park and Wildlife Department (TPWD) depicting when and where flights took place, which drone platforms were flown, the type of survey, and which types of wildlife were examined.

Once survey locations were identified and approved by the landowners(s), the area was evaluated for restrictions and prohibited areas such as a no-fly zone and Notice to Airmen (NOTAM). NOTAMs need to be checked and monitored every time during the pre-flight preparation procedure. Prior to field surveys weather conditions were monitored closely to determine if flight was possible. Acceptable conditions and/or variables that were considered included: temperatures were within the platforms operating range (0^{o}C - 40^{o}C), condensation, projected wind speeds were < 12 mph. High temperatures can contribute to overheating the drone's battery which can result in motor power loss and connection failure. Excessive condensation can cause damage to internal electrical systems, can result in moisture within the camera lens and eventually will lead to a full platform shut down (MacLeod, 2016). Lastly, excessive wind will affect flight stability, data collection and overall mission safety.

Flight plans were developed and carried out using Litchi or DJI software. Variables evaluated during flight planning were location of Pilot in Command (PIC), "home" point (location that drone will return to in case of emergency), "launch" zone (flat ground with open air space), altitude, aircraft orientation (direction drone is facing), camera orientation (gimbal), waypoints, speed, smoothing flight path, and actions (starting video recording, stopping video recording, and taking photos). These flight plans were constructed to cover a majority of the targeted freshwater habitat to maximize capturing imagery of freshwater turtles. Each drone platform and software have unique settings that can be modified to create these flight plans. Flight time was around 22 mins which is based on the power of the lithium-ion battery for the drone platform (assuming 15% critical limit for the battery and non-ideal conditions). A critical limit point of 15% was used to safely allow for the drone to return to home point regardless of position during flight mission. The drone communicates to the remote controller with a built-in Wi-Fi to the remote controller that is connected to the respective phone/iPad. Cell phone coverage availability was checked for the site, though flight plans and base maps were uploaded to the control device (android phone or iPad) in case no cell coverage was available on site. Next, the aircraft and support equipment were prepared. Additional pre-flight preparations included charging drone batteries, the Remote Controller (RC), phone/iPad, preparing blank micro-SD cards and backups, check propellers and backups, securing a launch pad (3 ft x 3 ft as reasonable wooden board) for areas with extreme soil saturation and/or tall vegetation. A retrieval net, dry towels, and silica desiccant for electronics was brought along in case the aircraft got wet or fell into the water.

Drone platforms

Two drone models were used over the course of the study, all manufactured by DJI (Shenzhen, China https://www.dji.com/?from=store-nav) (Figure 6). The DJI Mavic 2 Enterprise Dual (herein referred to as the "M2") (https://www.dji.com/mavic-2-enterprise/specs) recorded video data on the visible spectrum and thermal level simultaneously. It has a 12-megapixel sensor and recorded visible spectrum video at 30 fps (frames per second) and thermal at 8.7 fps. This was the first drone flown at every site and conducted simultaneously with binocular assisted visual surveys (BAVS) targeting freshwater turtles, due to having a higher quality camera resolution. The other platform used was the DJI P4 Multispectral (herein referred to as the "P4MS") (https://www.dji.com/p4-multispectral/specs) recorded image data on a visible and multispectral scale. It has a 2.07-megapixel sensor and captures images at the following bands. Blue (450 nm), green (560 nm), red (650 nm), red edge (730 nm), and near-infrared (860 nm) (Figure 7). These bands are the reflection of energy waves back into the capture lens moving from shorter wavelengths (blue) to longer ones (near-infrared). These bands are generally used to create color composites and a Normalized Difference Vegetation Index (NDVI) which can both be used to investigate the density and health of vegetation. This model was flown directly after completing the mission with the M2 but was not conducted alongside BAVS.

Figure 6**:** Drone or Small unmanned aerial system (sUAS) platforms used for aerial surveys: DJI Mavic 2 Enterprise Dual (left) and DJI P4 Multispectral (right).

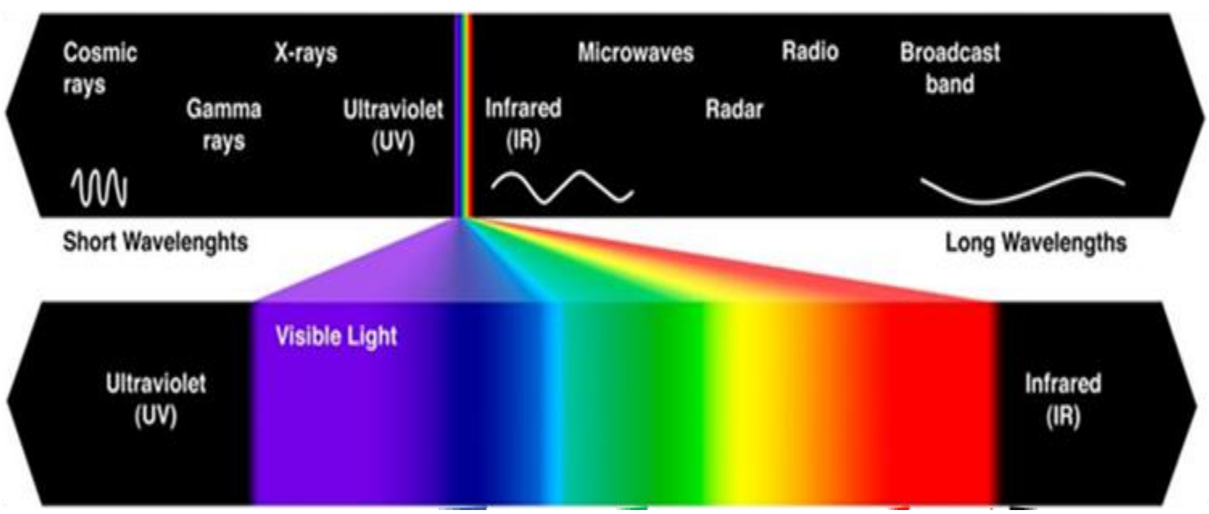

Figure 7**:** Display of electromagnetic scale highlighting the visible light spectrum where color bands can be collected for multispectral imagery.

Drone field surveys

At the site, the locations of visual observers were established to assist the pilot during the operation. It is important to have visual observers to aid the pilot in maintaining line of sight (LOS), monitor for any potential hazards such as other aircrafts or animals in the air and on the ground, and responding to emergency situations (loss of control, crash, or fly-away). These visual observers were also conducting BAVS for turtles while assisting the PIC. During the first visit at each site the launch zone, visual observer locations, and flight space were determined based upon the current conditions observed by the PIC. An acceptable launch zone location composed of open space, flat ground, which provided good overview vantage point and high visibility of the site. Visual observer locations were spread out to maximize viewing area while keeping LOS of the drone. Flight space needed to be open air space which minimized canopy cover that extended over the targeted freshwater habitat. The pilot would then use one of the associated drone applications either DJI Pilot (iOS v1.1.5) or Litchi (iOS v4.25.0-g) for the M2 (video/thermal) and DJI GS Pro (iOS v2.0.17) for the P4MS (images/multispectral) to create pre-planned transect flight paths (Figure 8 and Figure 9).

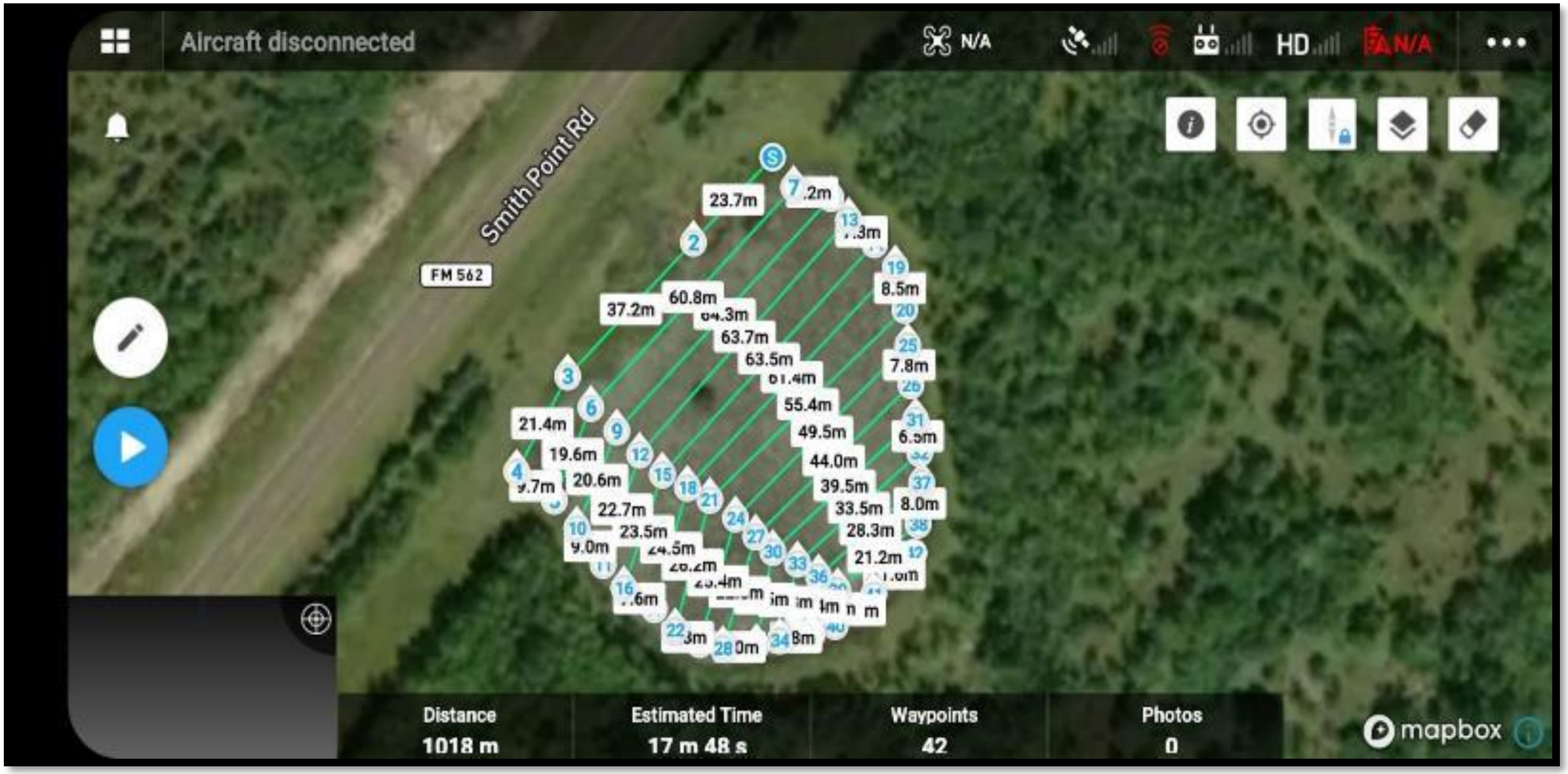

Figure 8: User interface for DJI Pilot demonstrating a pre-planned transect for the M2. The top section shows connection information for the drone, satellites, remote controller, and battery level. Bottom information displayed consists of flight distance, time, waypoints, and number of photos. The M2 flies along the pathway lines with generated waypoints to complete the mission while recording video for the entire duration.

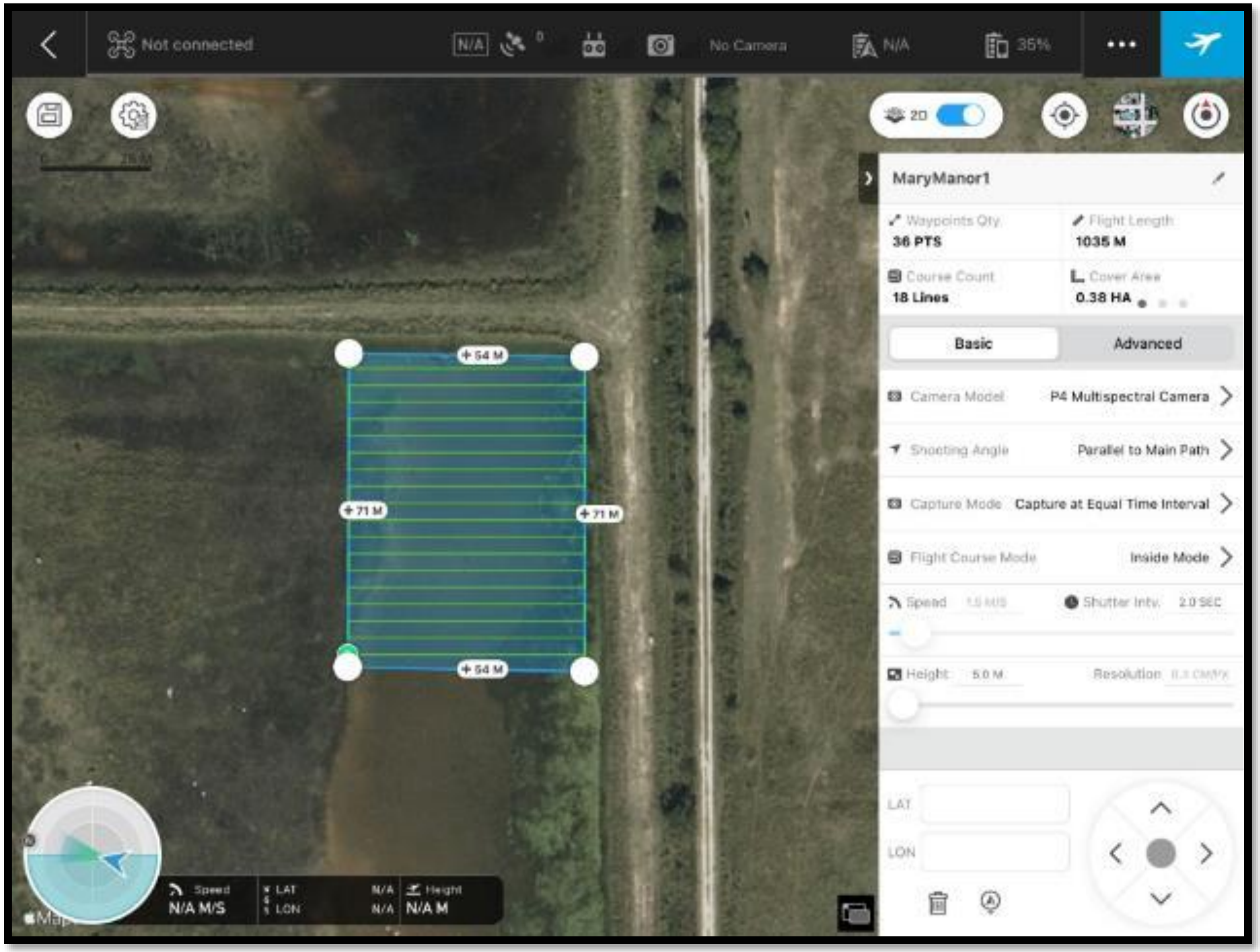

Figure 9: User interface for DJI GS Pro demonstrating a pre-planned transect for the P4MS. Top information includes connectivity for drone, satellites, and remote controller along with battery levels. Information that is displayed on the right includes waypoints, length, area covered, and drone information. Left side shows imagery basemap with flight mission pathway

At all sites the M2 drone was flown first due to the drone's greater image resolution and stronger megapixel camera which was used for turtle species identification. Then, directly after the M2 drone survey, the P4MS drone was flown. Static imagery for the P4MS was collected with a 10% overlay (e.g., 10% of the frame overlapped between images) at an equal time interval of 2.26 seconds in DJI GS Pro, which generated a full image of the survey area during data processing. Manual flights (controlled by the PIC at all times with the remote controller (RC) using the associated software apps) were conducted at sites where a plot transect was impractical due to complex terrain and canopy cover. Once in flight, the visual observers facilitated and helped the PIC, identifying any flying object and hazards, maintaining LOS on the drone, estimating the location of the drone in case of a crash (establishing direction using a two-point procedure and estimating distance), while recording the date and exact time when the pilot started the mission. The PIC executed the mission monitoring the drones flight path and using manual controls when necessary to avoid collisions with mobile or static objects. Flights were conducted at a target altitude of 5 meters which produced high quality imagery for identification without causing stress to the surrounding wildlife. Five meters was selected as the target altitude based on literature and local testing during training which involved capturing imagery of freshwater turtles at varying altitude with both drone platforms at an approved facility that allowed for safe flying within the FAA rules and regulations (Biserkov & Lukanov, 2017; Daniels, 2018; Davis et al., 2020).

In favorable conditions, the gimbal (angle of camera) was tilted at -90^{o} (straight down) and the heading (direction drone is facing) remained constant to reduce screen tearing which is when the display becomes distorted from quick sporadic movements (Figure 10). Slight gimbal tilt (approx. -45^{o}) was used in situations involving dense canopy cover over banks (Figure 11), sun glare, water reflections, and skittish turtles that would retreat before the drone could fly directly overhead (Figure 12). If needed the gimbal was adjusted mid-flight. The target speed of the drone was no more than 1 m/s allowing for smooth video playback. Additionally, areas with high turtle activity or target habitat (e.g., multiple basking locations or shallow water) were targeted by the PIC for detections. Flight paths consisted of a full coverage of the site when possible, including water and banks. Sites that were excessively large such as lakes, had limited survey coverage due to limited battery life, insufficient spare batteries, and extensive time requirements. In these instances, the flight mission included the surrounding area near the launch zone along with areas that were identified as turtle hotspots by visual observers. Binocular aided visual surveys (BAVS) occurred simultaneously along video M2 drone surveys. This was done to help confirm turtle species with aerial imagery and to compare the effort between the two methods. The M2 was chosen for comparison and flown first due to it having a more powerful, higher quality camera. After sampling, data and imagery was brought back on the SD cards, uploaded, filed, and compiled for analyses using the proper associated imagery software.

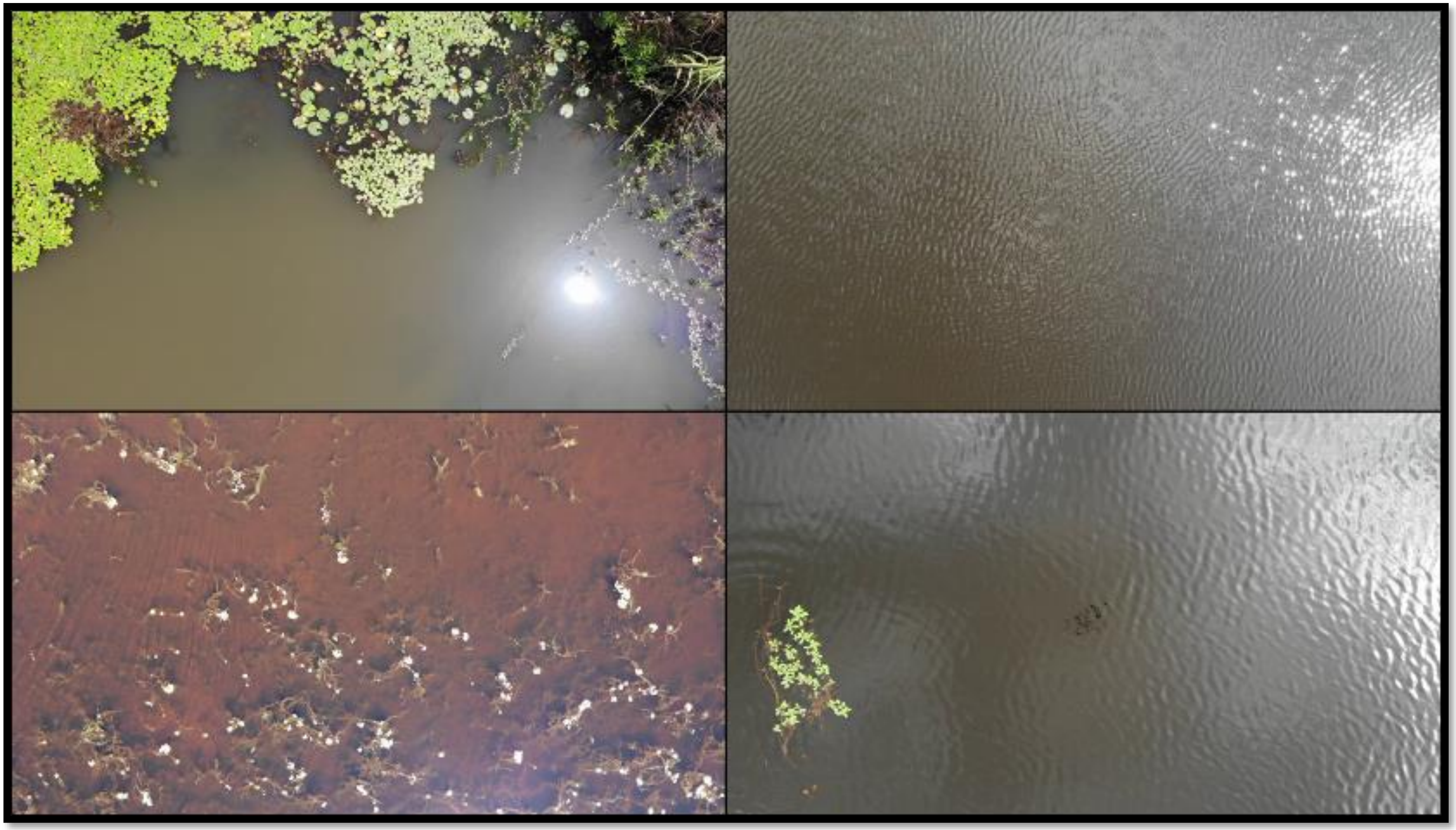

Figure 10: Images showing how different environmental conditions can affect the quality of collected imagery data. Top left - heavy glare and sun reflection, top right - wind action causing ripples at the water's surface, bottom left - ideal conditions, and bottom right - cloud reflections off the water's surface.

Figure 11: Section of pond at OW-03 with low hanging canopy cover that extends over the water's surface. Overhanging canopy cover can influence data collection from drone surveys.

Figure 12: Unknown turtle detected at OW-01 by using a slight gimbal angle tilt (45°) method to view areas underneath low overhanging canopy cover.

Binocular aided visual surveys (BAVS)

Binocular aided visual surveys (BAVS) were conducted at all sites simultaneously alongside the initial M2 drone survey to determine turtle species composition and compare methodologies. Scanning from a distance using binoculars or a spotting scope is a non-invasive method that allows the observer to view individuals to avoid provoking a flight response (Figure 13). Multiple observers scanned the water surface for swimming and surface breaching turtles attempting to breathe. Areas that provide room for basking in direct sunlight were prioritized. Standing still while observing a large area of view was preferred over walking along the bank. This allowed sufficient time for turtles to surface and become acclimated to the observer's presence. Certain sites required walking along the water's edge due to the irregular water body shape and viewing access availability.

Figure 13: Example of binocular aided visual surveys (BAVS) being conducted for freshwater turtles in the field. Visual observers standing at the waterbody's edge and scanning the surrounding environment for turtles.

Observers used any accessible locations along the bank of the wetland that maximized the viewing area covered. When approaching the vantage point, adjacent areas were scanned first to detect possible turtles that might be disturbed when approaching. Binocular aided visual surveys were conducted for a minimum of 20 minutes. Observers utilized a variety of binoculars including brand, size, and zoom. Total time allocated to surveying at each site was dependent upon size of the waterbody, obstructions to viewing, and general time limitations. In areas where aerial coverage is not blocked by obstructions and visibility is good, observers remained in one location for the full 20+ minutes of surveying. In areas where observable aerial coverage was minimal due to obstructions or vegetation, scanning was conducted for a minimum of 10 minutes before relocating (Armstrong, 2016). For small waterbodies with high visibility, a single observer was able to cover the full aerial extent of the waterbody. Conversely, in large waterbodies with low visibility, three observers were in some cases restricted to only a small portion of the viewable area. Additional notes were made about viewing restrictions at each survey location. Each site was broken down into locations with latitude / longitude coordinates, search time at the location (minutes), and number of turtles of all species observed at each location.

Data that was recorded when a turtle or group of turtles was spotted includes detection time (24-hr; HH:MM), distance (m) and bearing relative to the observers location (°) to observed turtle(s), species observed (if possible; or recorded to lowest taxonomic group), confidence in turtle identification (0 [unknown turtle] – 3 [100%]), number of individuals observed, and behavior or activity of individuals. An example datasheet is illustrated in the appendix (Appendix 1). Each observer used a compass and laser range finder to record the bearing and distance. Lastly, observers recorded any reactions that turtles displayed when the drone was in flight. This included if there was a reaction (yes/no), an estimated lateral horizontal distance of the turtle from the drone (m), and how the individual reacted based on categorical responses.

Data Analysis

Visible and thermal spectrum videos were viewed and analyzed using VLC Media Player, a cross-platform multimedia player developed by the VideoLAN non-profit organization (https://www.videolan.org/). This free to download, open-source software allows the data analyst to zoom in, slow downplay back speed, and extract snippets or clips of video imagery (Figure 14).

Data recorded for each detection from the M2 were similar to that for BAVS and included: Time detected (24-hr; HH:MM), video time stamp (MM:SS), location in image, species (recorded to lowest taxonomic level), number of individuals detected, and behavior (basking, swimming, etc.). If a turtle reacted to the drone, the level of reaction was scored on a scale between 0-4 with 0 being no reaction and 4 being most reactive (Table 1). If a reaction was indeterminable from video analysis, a score of “Unk” (unknown) was recorded. Unknowns were not used in analyses.

Figure 14: User interface of VLC Media Player demonstrating the zoom feature on a North American Softshell Turtle (*Apalone sp.*). Top left corner is the full screen of the recorded video with zoomed in section in white box. Zoom control is triangle directly below full video box. Control of playback speed and ability to record sections of video at bottom left.

Table 1: Reaction score range from 0 (no reaction) to 4 (most extreme reaction) and including unknowns (Unk) in first column, type description in second column, and examples of reaction types observed in the third column during M2 drone video analysis.

Reaction Score	Reaction Type	Examples
0	No reaction	No reaction
1	Reaction but did not submerge	Followed with head, slight movement
2	Submerged but did not retreat	Submerged but stayed at surface or resurfaced before/after drone platform passed or during platform elevation change
3	Submergence and retreat	Submerged and swam away to cover or out of view of the drone imagery
4	Quickly retreated	Submerged rapidly creating a splash or obvious water disturbance
Unk	Unknown	Unknown reaction, submerged before entering frame, ripples in edges of frame with no observable behavior in imagery

Visible static images and the associated multispectral bands were analyzed using the default image viewer such as Microsoft Photos with the associated computer as long as it has the zoom capability. The analyst scanned each image and its associate bands for turtles. Data that was recorded for the P4MS included file name, species (to the lowest taxonomic level), location on screen, bands in which the turtle is visible, behavior or activity, number of individuals and identification confidence (0 [unknown turtle] – 3 [100%]).

For each M2 drone survey the time of solar noon was found using the National Oceanic and Atmospheric Administration (NOAA) Solar Calculator (https://gml.noaa.gov/grad/solcalc/) by inputting the appropriate date (day, month, year) and coordinates (latitude, longitude). Solar noon is the point in time at which the sun is at the highest point directly overhead at a specific location. Then the difference in minutes

(+/-) was found from solar noon to determine when each survey was conducted, and turtle detection was made. In this study, solar noon is represented by zero.

Data were archived and manipulated using Microsoft Excel Version 221. Statistical analyses were conducted using the software SigmaPlot Version 15 (15.0.0.123). Statistical analysis tests that were run involved; paired t-test with a Shapiro-Wilk normality test, if normality failed then a Wilcoxon Signed rank test was then run; Kruskal-Wallis one-way analysis of variance (ANOVA) on ranks test with a Dunn's Method, *post hoc* pairwise multiple comparison; repeated measures of ANOVA on ranks with a Shapiro-Wilk normality test with a Tukey pairwise comparison in *post hoc* analysis. A *p*-value of 0.050 was used for determining statistically significant results. Box plot figures were also generated by using the SigmaPlot. Other figures and tables were generated with Microsoft Excel Version 221.

RESULTS

Total effort and freshwater turtle detections

A total of 11 sites were examined with drones during the years of 2021 and 2022 for the months spanning March through July. Sites located in East Texas were split into two freshwater habitat categories by ground truthing: Emergent wetlands (WTL, *n* = 5), lentic systems (lakes and ponds: LE, *n* = 6) (Figure 5).

Figure 15: Site overview drone imagery of four sampled sites. The top two images detected WCT with drone surveys. Top left: LE-03, one WCT detected with P4MS. Top right: WTL-01, 5 WCT detected with M2. Bottom left: LE-06. Bottom Right: WTL-04.

At all 11 sites, BAVS were conducted simultaneously alongside drone surveys with the M2 (video and thermal) and directly after these surveys, the P4MS (photo and multispectral) was flown over the same target location at the site. A total of 20.7 hours of video footage were recorded for the M2 and a total of 58.1 hours of BAVS (sum of all observers) were conducted. The P4MS collected a total of 9515 visible images each with six multispectral bands for a grand total of 57090 photos collected for all sites. Despite extensive planning, some flights were cancelled due to safety concerns (high winds, high temperatures, rain, and poor visibility), technology failures (connectivity issues with drone and remote controller and poor satellite and GPS signals) or time restraints. Both drone surveys detected similar a similar number of freshwater turtles. The M2 imagery yielded a total of 1916 freshwater turtles and the P4MS detected 1915 turtles (Figure 16).

Figure 16: Still frame from a M2 video survey at LE-05 highlighting heavy basking activity for freshwater turtles. Both Slider Turtles (*Trachemys sp.*) and North American Softshell Turtles (*Apalone sp.*) are present in frame.

Any turtle that was seen within the video or static images was recorded as a detection, so the possibility of recounts is possible. BAVS that were conducted simultaneously alongside M2 surveys detected a smaller number of freshwater turtles with 1096 detections. Freshwater turtles that were detected by each method were grouped into eight groups by genus with the exception of Mud/Musk Turtles (subfamily – Kinosternidae) and unknown turtles (suborder – Cryptodira).

Two graph figures were created to highlight the number of freshwater turtle detections for each method, BAVS, M2 and P4MS. 1) Grand total of all freshwater turtles detections split into three groups, Slider Turtle (*Trachemys sp.*), unknown turtles (Cryptodira) and other turtle groups (Figure 17) and 2) the total number of detections of the other freshwater turtle groups which includes, North American Softshell Turtles (*Apalone sp.*), Snapping Turtles (*Chelydra sp.*), Mud/Musk Turtles (Kinosternidae), Chicken Turtles (*Deirochelys sp.*), Map Turtles (*Graptemys sp.*), and Cooter Turtles (*Pseudemys sp.*) (Figure 18). Excluding unknown turtles, the M2 identified the greatest number of turtle groups (n = 6) followed by the P4 (n = 5) then BAVS (n = 4). Many turtles that were detected were able to be identified down to the species level along with other herpetofauna. The results and analyses in this study will focus on these eight freshwater turtle groups. A full list of all herpetofauna observed and their relative abundance by all three methods down to the lowest taxonomic level that was identified is illustrated in the appendix (Appendix 2).

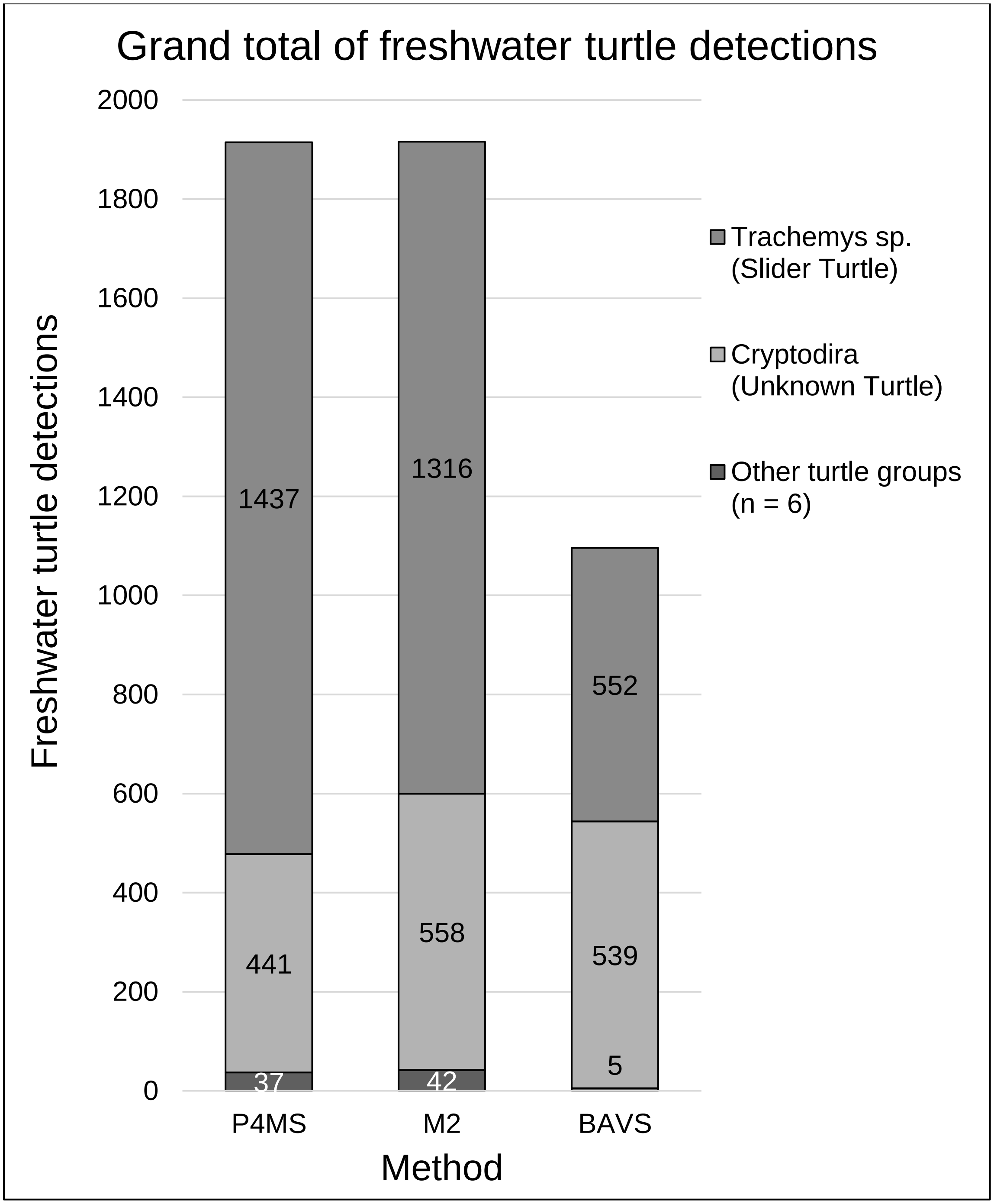

Figure 17: Grand total of freshwater turtle detection for each method, P4MS, M2 and BAVS. Split into three groups by greatest number of detections from top to bottom starting with Slider Turtles (*Trachemys sp.*), unknown turtles (Cryptodira) and other turtles groups (*n* = 6) each with the total amount of detections for each freshwater turtle group.

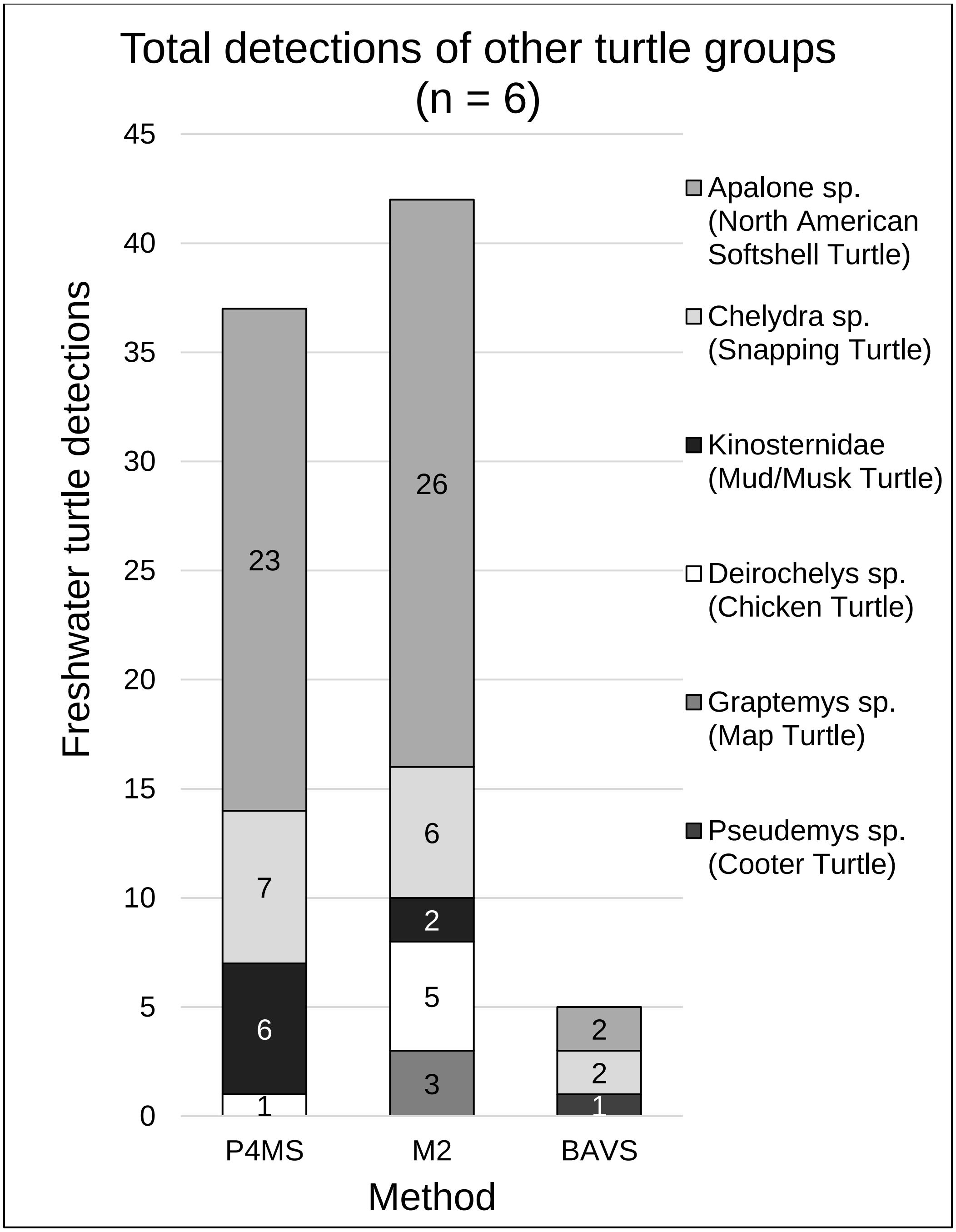

Figure 18: Total number of freshwater turtle detections for the six other turtle groups that were listed in Figure 17 for each method P4MS, M2, and BAVS. Turtle groups are organized in order of the greatest number of detections per group from top to bottom. Total number of detections for each turtle group is listed inside the corresponding column.

For all three methods combined and individually had the same top three taxonomic groups of freshwater turtles by number of detections and relative abundance (Table 2 and Table 3). They are as follows in order with associated abundance percentages; 1. Slider Turtles, *Trachemys sp.* (All = 67.08%, P4MS = 75.00%, M2 = 68.72%, BAVS = 50.36%), 2. Unknown turtles, Cryptodira (All = 31.22%, P4MS = 23.02%, M2 = 29.14%, BAVS = 49.18%), and 3. North American Softshell Turtles, *Apalone sp.* (All = 1.04%, P4MS = 1.20%, M2 = 1.36%, BAVS = 0.18%). When using BAVS, there was a higher relative abundance for unknown turtle detections (49%) when compared to both drone surveys (M2 = 23% and P4MS = 29%).

Table 2: Table representing the freshwater turtle detections by each method for the eight taxonomic groups. Total detections are listed for each individual method used: P4MS, M2 and BAVS along with the grand total. Scientific names were retrieved December 7th, 2022 from the Integrated Taxonomic Information System (ITIS), www.itis.gov, CC0 https://doi.org/10.5066/F7KH0KBK.

Freshwater turtle groups and their total detection count by method						
Freshwater Turtle			**P4MS**	**M2**	**BAVS**	**Total**
Taxonomic Level	**Scientific name**	**Common name**	**Count by method**			
Genus	*Trachemys sp.*	Slider Turtle	1437	1316	552	3305
Genus	*Apalone sp.*	North American Softshell Turtle	23	26	2	51
Genus	*Chelydra sp.*	Snapping Turtle	7	6	2	15
Genus	*Deirochelys sp.*	Chicken Turtle	1	5	-	6
Genus	*Graptemys sp.*	Map Turtle	-	3	-	3
Genus	*Pseudemys sp.*	Cooter Turtle	-	-	1	1
Subfamily	*Kinosternidae*	Mud/Musk Turtle	6	2	-	8
Suborder	*Cryptodira*	Unknown Turtle	441	558	539	1538
Total			1915	1916	1096	4927

Table 3: Table representing the relative abundance (%) of freshwater turtle detections by each method for the eight taxonomic groups. Relative abundance is listed for each individual method used: P4MS, M2 and BAVS along with the grand total. Scientific names were retrieved December 7th, 2022 from the Integrated Taxonomic Information System (ITIS), www.itis.gov, CC0 https://doi.org/10.5066/F7KH0KBK.

Freshwater turtle groups and their relative abundance (%) by method						
Freshwater Turtle			**P4MS**	**M2**	**BAVS**	**Total (%)**
Taxonomic Level	**Scientific name**	**Common name**	**Relative Abundance (%)**			
Genus	*Trachemys sp.*	Slider Turtle	75.04	68.68	50.36	67.08
Genus	*Apalone sp.*	North American Softshell Turtle	1.20	1.36	0.18	1.04
Genus	*Chelydra sp.*	Snapping Turtle	0.37	0.31	0.18	0.30
Genus	*Deirochelys sp.*	Chicken Turtle	0.05	0.26	-	0.12
Genus	*Graptemys sp.*	Map Turtle	-	0.16	-	0.06
Genus	*Pseudemys sp.*	Cooter Turtle	-	-	0.09	0.02
Subfamily	*Kinosternidae*	Mud/Musk Turtle	0.31	0.10	-	0.16
Suborder	*Cryptodira*	Unknown Turtle	23.03	29.12	49.18	31.22

A repeated measures ANOVA on ranks was ran for total detections by turtle group at each site and determined there was a statistically significant difference ($p < 0.001$) in the median values among groups. Then a Tukey pairwise multiple comparison test was run to compare individual groups. There were statistically significant differences ($p < 0.050$) in detections for Slider Turtles (*Trachemys sp.*) and unknown turtles (Cryptodira) when compared to the other turtle groups for all three methods (BAVS: Chi-square = 38.094, df = 4; M2: Chi-square = 53.528, df = 6; P4MS: Chi-square = 39.970, df = 5). Except for Slider Turtles (*Trachemys sp.*) and Unknown turtles (Cryptodira) compared to North American Softshell Turtles (*Apalone sp.*) with the M2 and unknown turtles (Cryptodira) compared to North American Softshell Turtles (*Apalone sp.*) with the P4MS. However, these *p*-values for comparing the detections of North American Softshell Turtles (*Apalone sp.*) were close to 0.050 (M2: Slider Turtles (*Trachemys sp.*), $p = 0.064$ and unknown turtles (Cryptodira), $p = 0.083$; P4MS: unknown turtles

(Cryptodira) $p = 0.069$). There was no statistical difference between the other detected turtle groups within the three methods. These results show that for all three methods, Slider turtles (*Trachemys sp.*) and unknown turtles (Cryptodira) have a higher likelihood of detection when compared to the other turtle groups. with the exception of North American Softshell Turtles (*Apalone sp.*) in drone surveys (M2 and P4MS).

<u>Western Chicken Turtles (*Deirochelys reticularia miaria*)</u>

Five Western Chicken Turtles (*Deirochelys reticularia miaria*) were identified with video (M2), all at site WTL-01 with two occurring in the year 2021 (04/27/2021 ($n = 1$), 06/30/2021 ($n = 1$)) and three in the year 2022 (03/16/2022 ($n = 3$)) (Figure 19). Two of the observed WCT on 03/16/2022 are likely to be the same individual that moved by swimming to a new location where it was observed again during the drone survey. One WCT was observed alongside a Red-eared Slider (RES; *Trachemys scripta elegans*) allowing for a comparison of each species unique morphology such as the broad-yellow band and snake like head in the WCT and red-ear with a rounded head in the RES. One WCT was identified with multispectral imagery (P4MS) at site LE-03 on 04/26/2022 ($n = 1$) with the red, red-edge and near-infrared bands best highlighting unique characteristics such as the broad-yellow band on the front legs and snake like head (Figure 20). BAVS failed to detect any WCT at the sites that were surveyed with both the M2 and P4MS drones.

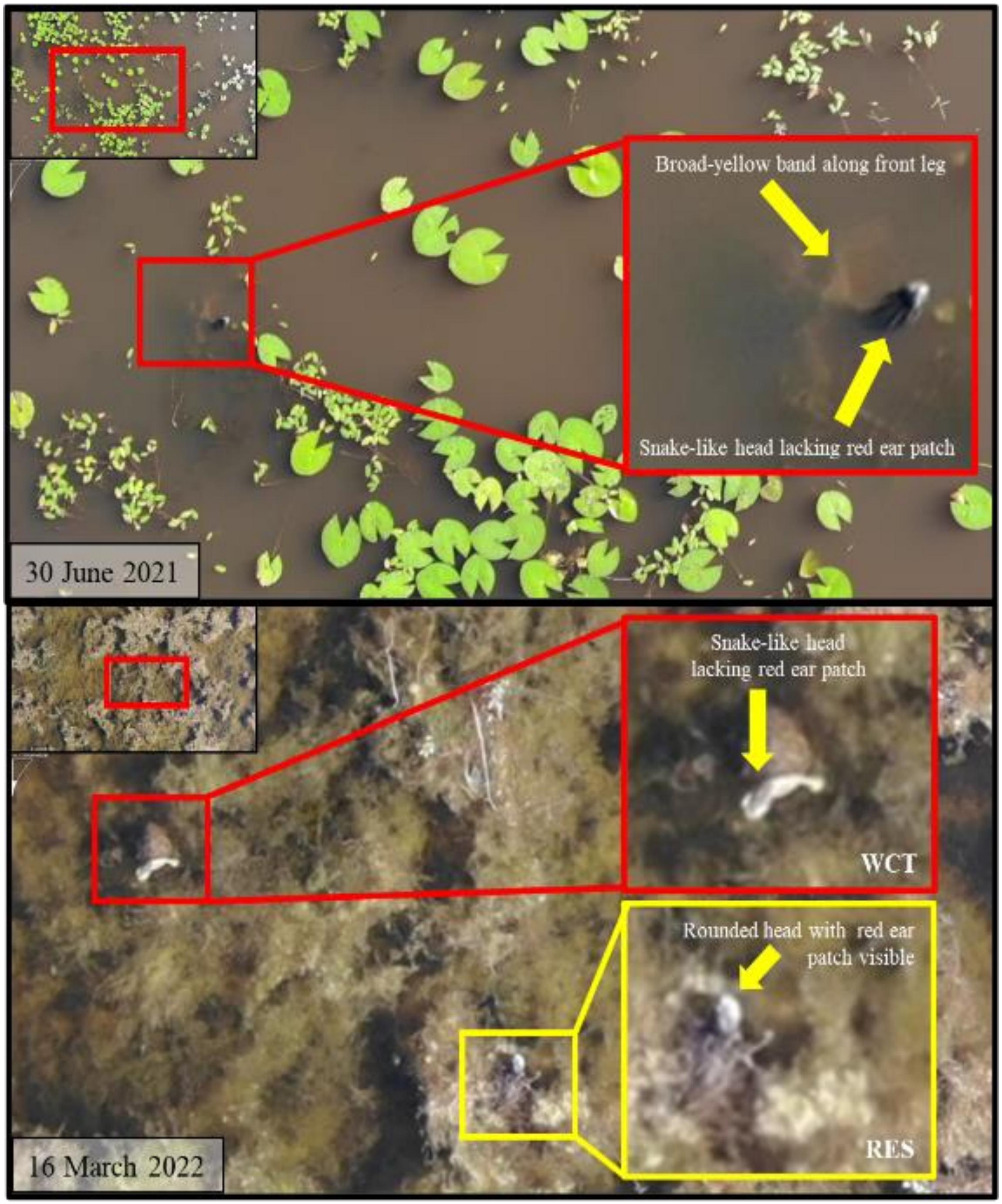

Figure 19: Images of Western Chicken Turtles (*Deirochelys reticularia miaria*) observed with the M2 drone, both at the site WTL-01. Top left corner is the full view in VLC Media Player and larger image is zoomed in. Both images highlight features of the WCT. Bottom image compares WCT features in the red box to a Red-eared Slider (RES; *Trachemys scripta elegans*) in the yellow box.

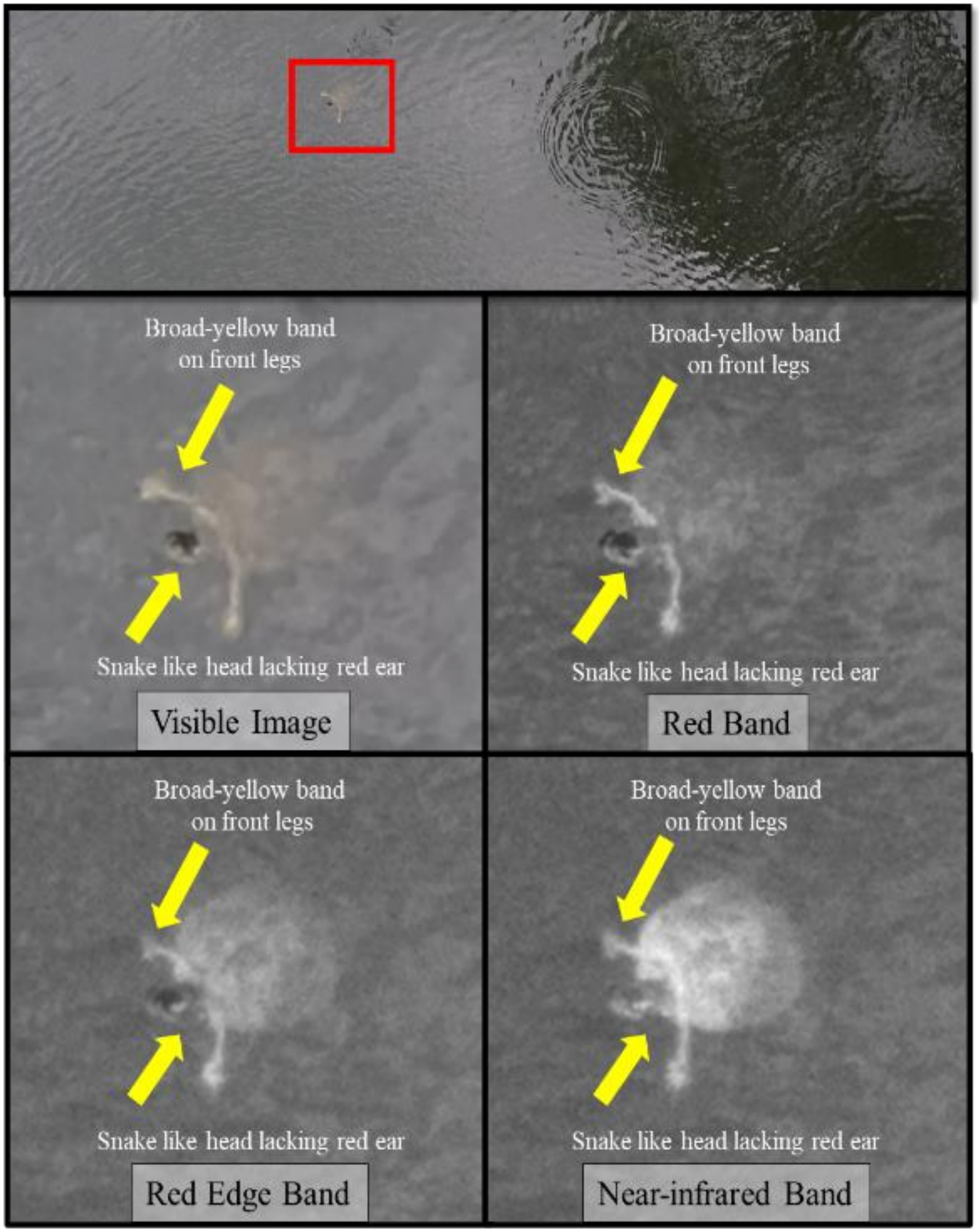

Figure 20: A WCT detected with the P4MS at LE-03 on 04/26/2022. The top image is an overview image with the WCT inside the red box. Below images highlight the WCT features such as the broad-yellow band and snake like head observed in the visible image, red, red edge, and near-infrared bands.

Method catch per unit effort (CPUE) comparison

To compare the three methodologies to each other, a catch per unit effort (CPUE) was calculated using catch as number of freshwater turtles detected and effort in total minutes of time searched within SigmaPlot. Each method took the total amount of freshwater turtle detections and divided by total survey time in minutes for each sampled month and grand total. Every turtle that appeared on screen during the video and each photo was recorded as a detection so multiple counts of the same turtle are possible. The P4MS camera setting within DJI GS Pro was set to take images at an equal time interval 2.26 seconds so the total amount of photos was mutiplied by this variable then divided by 60 to get a total effort of 359.59 minutes for the entire study. Effort was broken down by each month for the two years of sampling to generate a box plot of the three methods (Figure 21). A second box-blot was generated to highlight the comparison of BAVS and the M2 due to their simlutaneous survey conductance (Figure 22). The monthly mean CPUE for each method was BAVS = 0.31, M2 = 1.54, and P4MS = 5.33 and the monthly median CPUE for each method was BAVS = 0.28 M2 = 1.06, and P4MS = 1.96. A Kruskal-Wallis one-way analysis of variance (ANOVA) on ranks test on was run to compare the monthly CPUE median values between methods. There was a statistically significant difference between monthly CPUE median values for all three methods (H = 20.396, df = 2, p = < 0.001). After this an all pairwaise multiple comparison procedure (Dunn's Method) was run reduce the inflation of error rates. Both the M2 and P4MS highlighted a statistically significant difference when compareed to BAVS (M2: Q = 2.810, p = 0.015 and P4MS: Q = 4.456, p = <0.001) but there was no statistically significant difference between the M2 and P4MS (Q = 1.721, p = 0.256). This shows that BAVS resulted in a lower monthly CPUE than the M2 and P4MS for freshwater turtles.

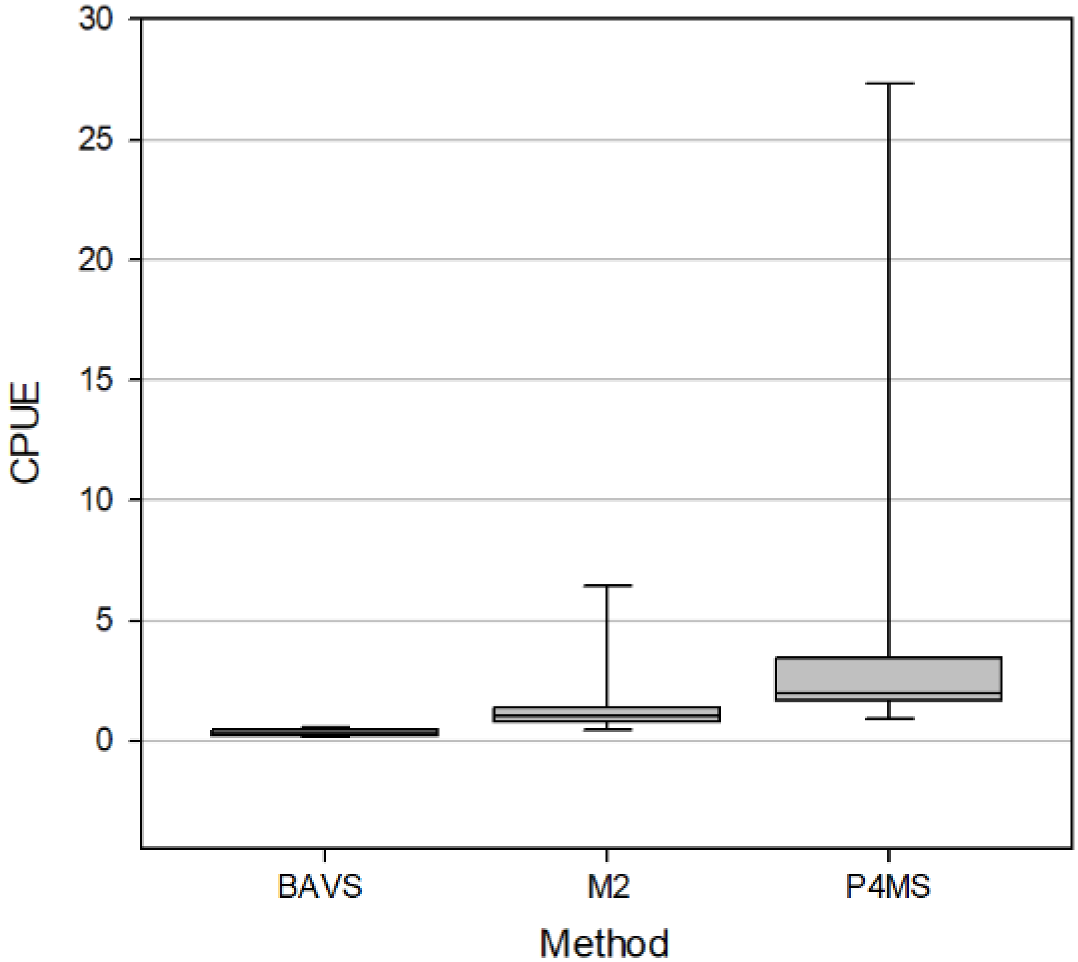

Figure 21: Box plot of monthly CPUE for freshwater turtles for all three methods. Black line inside boxes represents median value. Inner quartile range is represented by the grey box with the upper and lower quartiles being the outside edges. Whiskers and lines outside box represent the 95% and 5% percentiles.

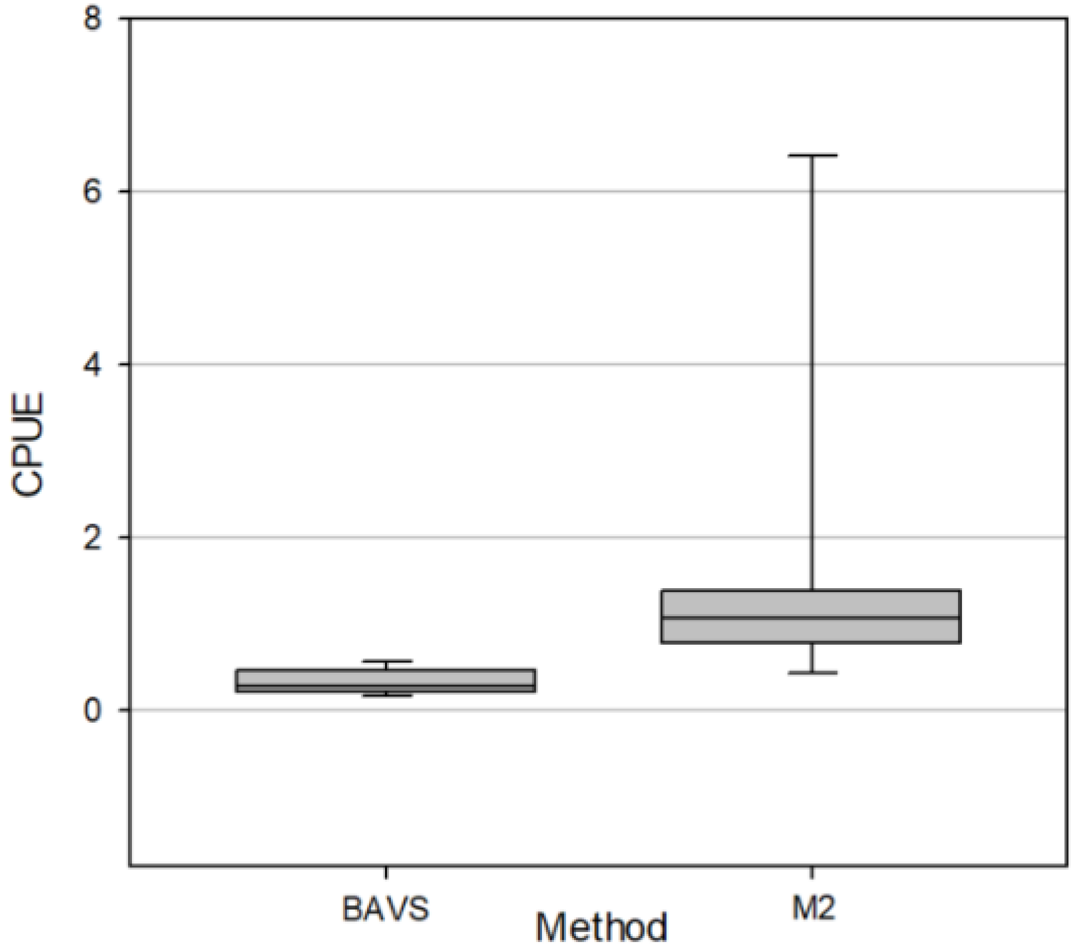

Figure 22: Box plot of monthly CPUE for freshwater turtles for BAVS and the M2 drone. Black line inside boxes represents median value. Inner quartile range is represented by the grey box with the upper and lower quartiles being the outside edges. Whiskers and lines outside box represent the 95% and 5% percentiles.

Sensors comparison

Multispectral

When using multispectral imagery for detecting freshwater turtles, we can see that they do offer a unique visual and can aid in identifying certain attributes. Almost as if an x-ray is being used, areas underwater that are hidden in visible images become revealed using multispectral bands (Figure 23 and Figure 24). From the total collected multispectral images, the total number of freshwater turtles that were identified by band are as follows, visible (*n* = 1876), blue (*n* = 1680), green (*n* = 1705), red (*n* = 1728), red edge (*n* = 1691) and infra-red (*n* = 1692) (Figure 25). The red band was second for the number of freshwater turtle detections when compared to the visible image. In terms of freshwater turtle abundance estimated using the P4MS turtles were grouped into six groups and are as follows in order: Slider Turtles, *Trachemys sp.* (75.04%), Unknown turtles, Cryptodira (23.03%), North American Softshell Turtles, *Apalone sp.* (1.20%), Snapping Turtles, *Chelydra sp.* (0.37%), Mud/Musk Turtle, Kinosternidae (0.31%), and single Western Chicken Turtle, *Deirochelys reticularia miaria* (0.05%) (Table 3). All freshwater turtle group detections and their associated proportions detected by each multispectral band are depicted in (Figure 25).

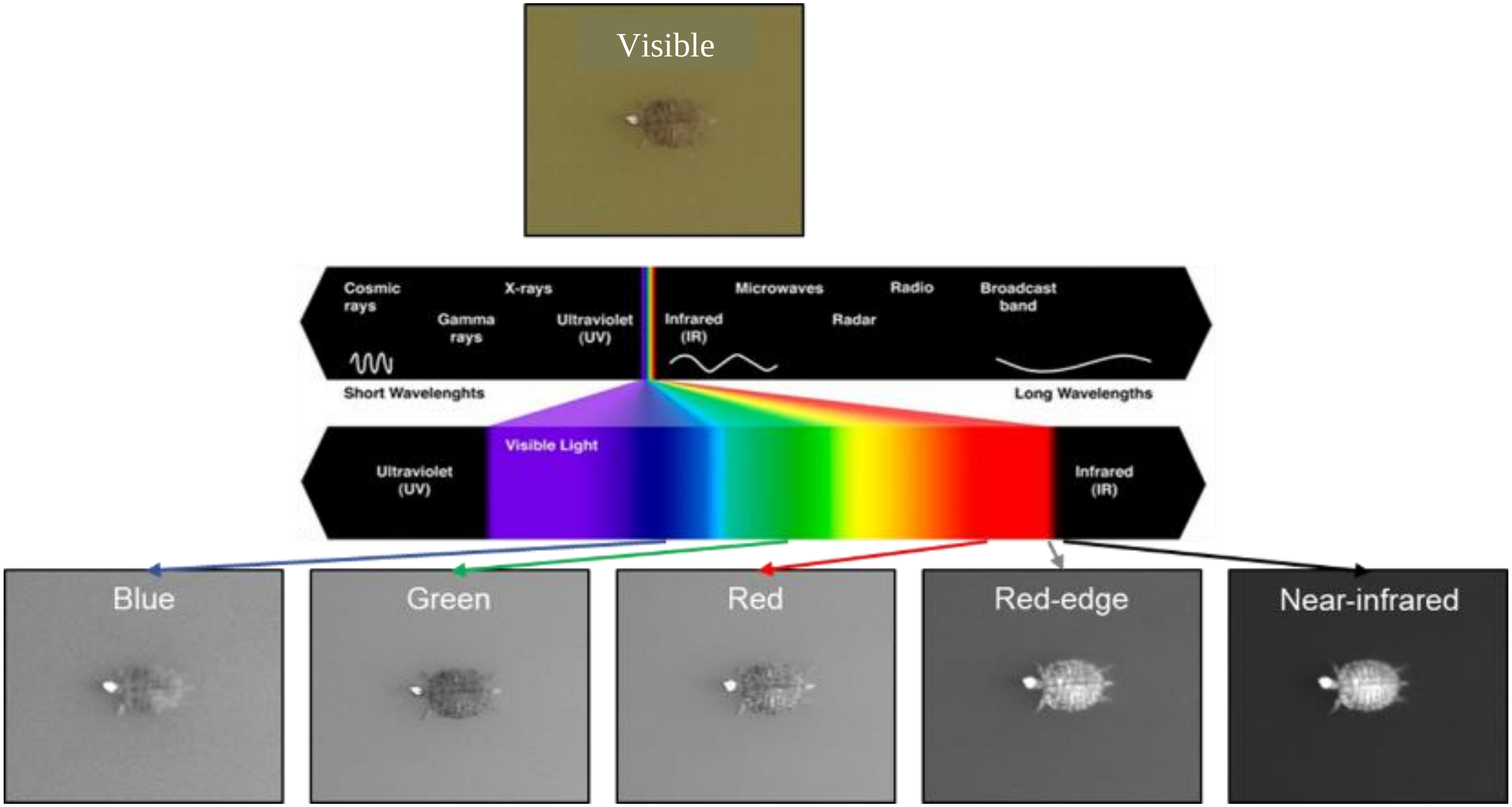

Figure 23: Multispectral bands and their associated wavelength along the visible light spectrum within the electromagnetic scale highlighting a top-down view of a Red-eared Slider, *Trachemys scripta elegans*.

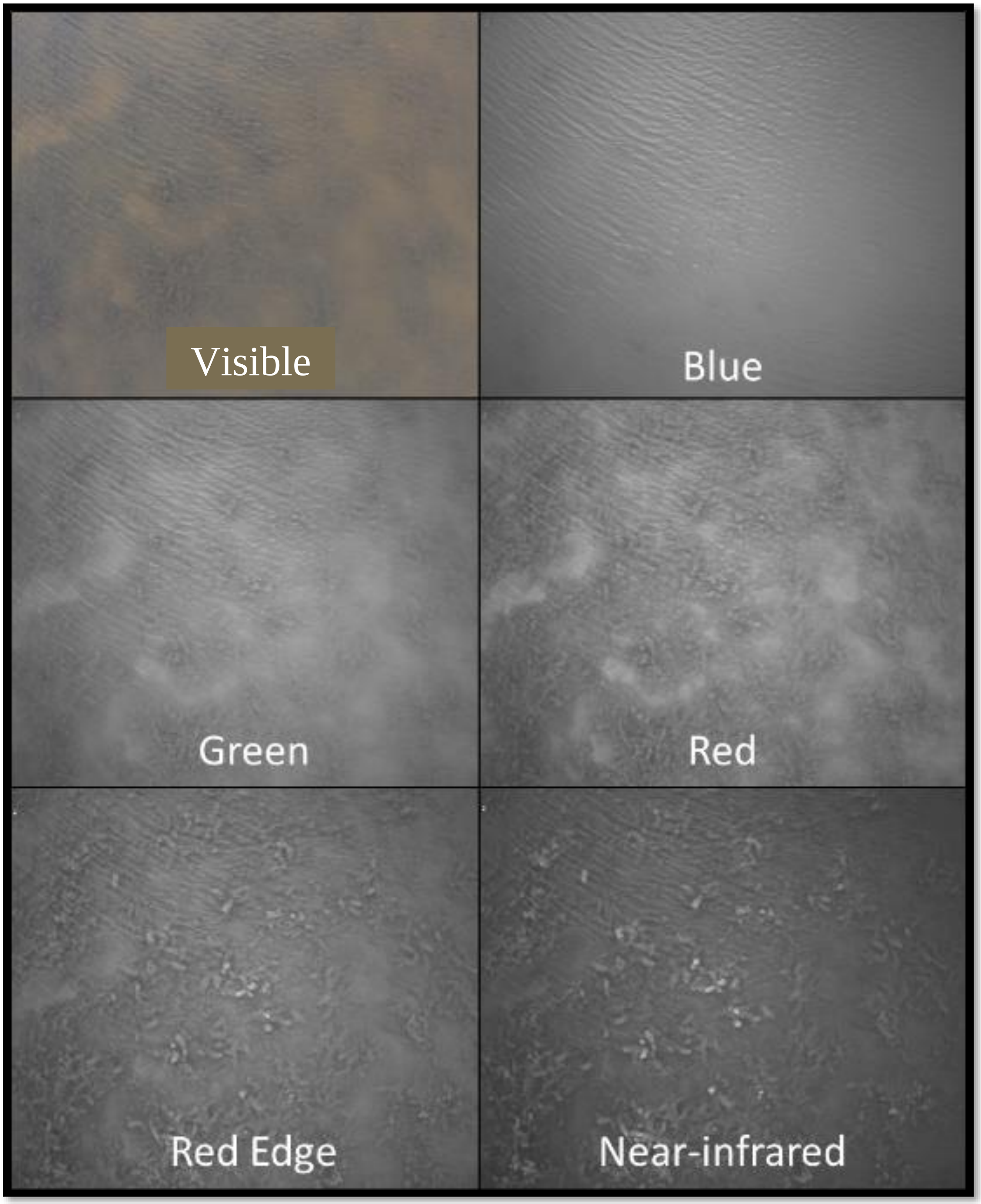

Figure 24: Images showing submerged aquatic vegetation underneath the water's surface in the different multispectral bands at WTL-02 on 03/22/2021. This image highlights how visibility becomes clearer in the red, red edge, and near-infrared bands. No turtles are present in frame.

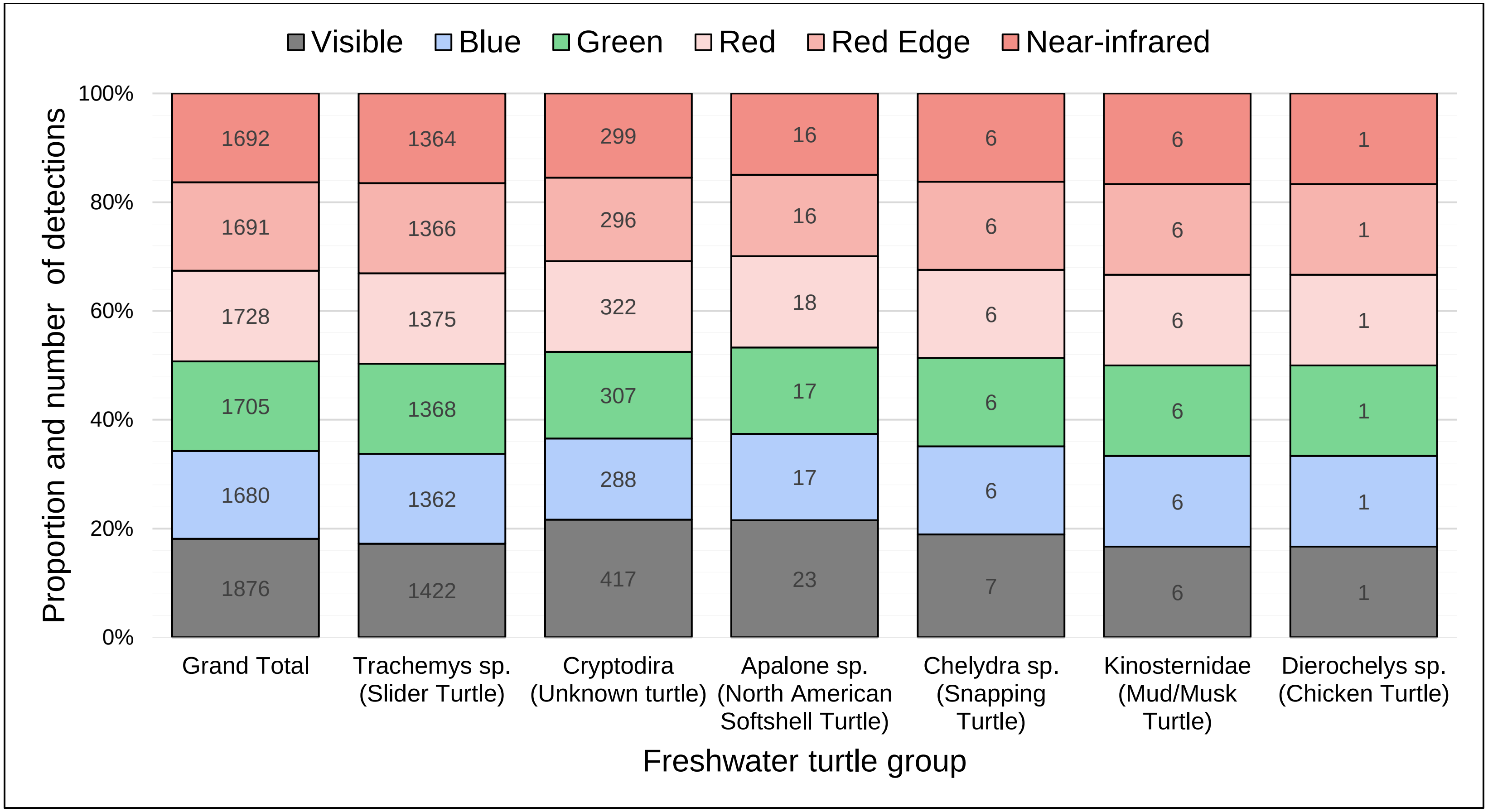

Figure 25:Proportion and total number of freshwater turtle detections by the P4MS for the visible image and multispectral bands, blue, green, red, red edge, and near infrared. Columns contain the grand total, and each detected freshwater turtle group listed in order of greatest number of detections.

First, a repeated measures ANOVA on ranks test was run on all freshwater turtle detections made by each image type, the visible image, and associated bands (blue, green, red, red edge, and near-infrared). A repeated measures ANOVA was used due to the likelihood of that the same detected turtle could be seen in multiple bands. This repeated measures ANOVA failed a Shapiro-Wilk normality test ($p < 0.050$) but was statistically significant for differences in the median values between groups (Chi-square = 28.985, df = 5, p = < 0.001). Then an all pairwise multiple comparison (Tukey test) was run to compare individual image groups, visible image, and bands. The visible band was statistically significant for differences between the median when compared to the blue ($p = 0.002$), red edge ($p = 0.036$) and near-infrared ($p = 0.026$) bands but not the green ($p = 0.080$) and red ($p = 0.839$) bands. All other comparisons between the multispectral bands found no statistically significant differences in the medians ($p > 0.050$). The results of this test show that the visible image detected significantly more freshwater turtles compared to the multispectral bands with the exception of the green and red band.

Even though a majority of turtles were found using visible imagery and had statistically significant differences, specific bands did allow for the detection of “hidden” turtles (Figure 26). Specifically, the red band (650 nm) displayed clear underwater images of submerged aquatic vegetation and the most freshwater turtles outside of the visible image ($n = 31$) (Figure 27) A total of 39 freshwater turtle detections consisting of Slider Turtles, *Trachemys sp.* ($n = 15$) and unknown turtles, Cryptodira ($n = 24$) were detected outside of the visible images with a majority detected using the Red band ($n = 31$), Red Edge band ($n = 27$), and Near-infrared band ($n = 30$) (Figure 26). To see if there was a significant difference in turtle detections that were made outside of the visible image a repeated measures ANOVA on ranks test, with a Shaprio-Wilk normality test, was run for the turtle detections outside of the visible band. A repeated measure ANOVA was used due to the likelihood that the same detected turtle could be seen in multiple bands. This test failed a Brown-Forsythe equal variance test ($p < 0.050$) and failed to detect a statically significant difference ($\text{Chi-square}_4 = 7.282$, $p = 0.122$) in the medians of detections between the bands observed outside of the visible image. Visible images were excluded from this test to compare the effectiveness of multispectral bands. The results of this test show that there is no difference in turtle detections by bands outside of the visible image.

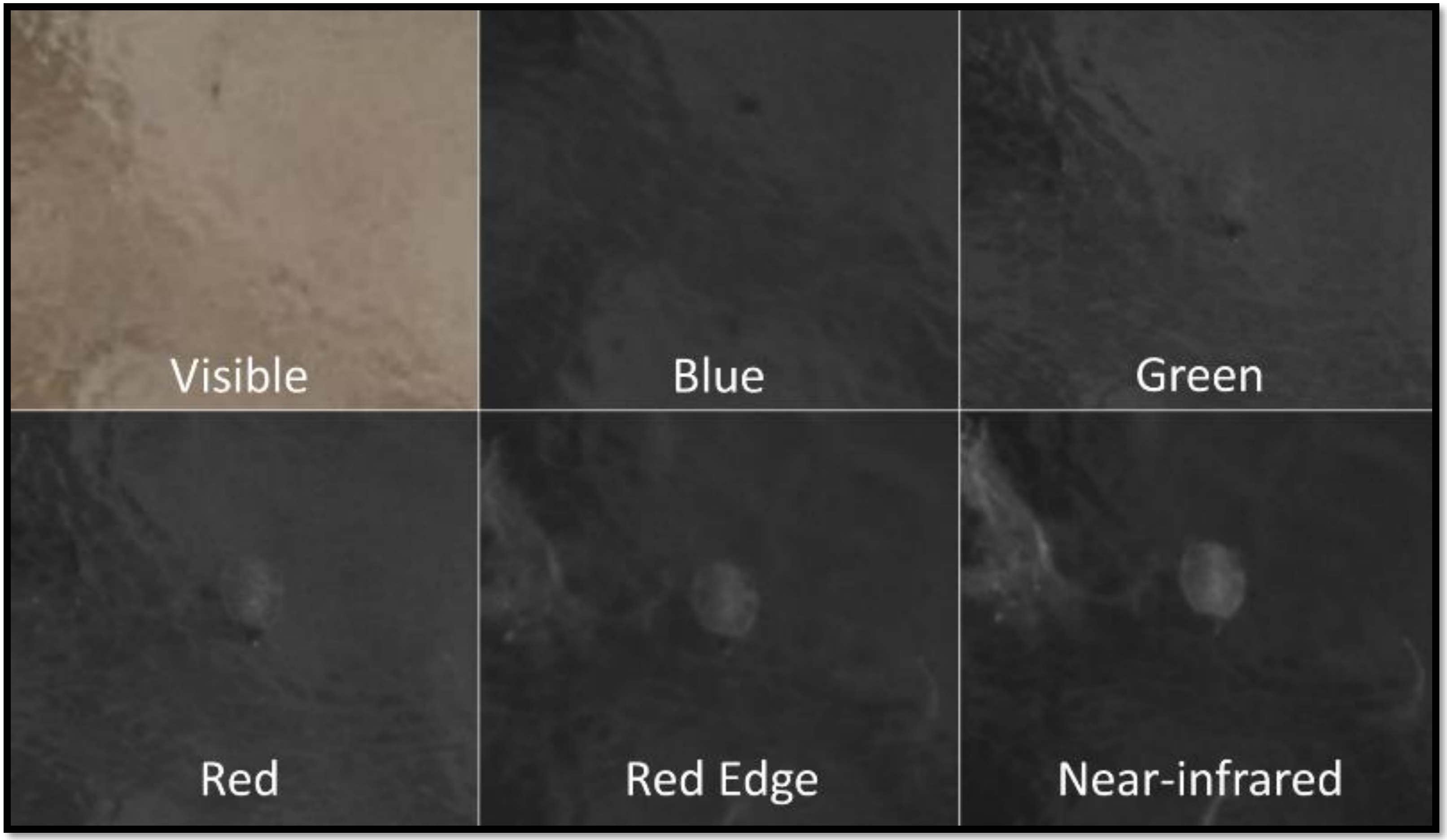

Figure 26: An unknown turtle (Cryptodira) at LE-02 on 06/01/2022. Cannot be seen in the visible image but the turtle becomes more visible as multispectral imagery progressed towards a longer wavelength as seen in the red, red edge and near-infrared band.

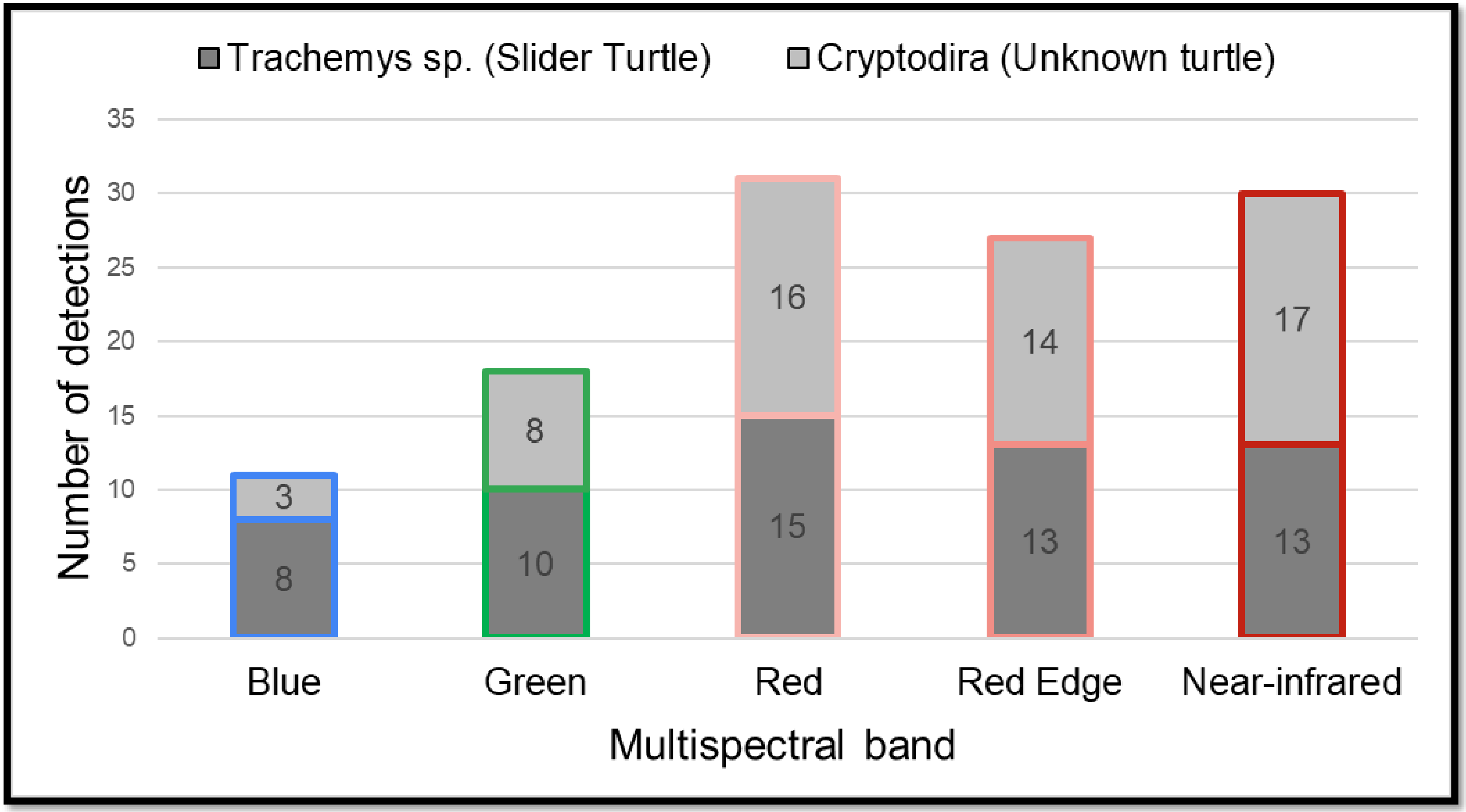

Figure 27: Total number of freshwater turtle detections made with the P4MS by band (blue, green, red, red edge, and near-infrared) when turtles were not detected within the visible image. Only two turtle groups were detected in these multispectral bands, Slider Turtles, *Trachemys sp.* (bottom) and unknown turtles, Cryptodira (top).

Thermal

Due to the large amount of data generated, the thermal imagery was utilized infrequently which generated minimal amounts of data. Thermal imagery was recorded at a lower resolution and fps, 640 x 360 @ 8.7 fps when compared to the visible imagery in the M2. The thermal heat sensor was unable to identify species of herpetofauna to a taxonomic level but could detect turtle silhouettes when they were basking out of the water along with potential basking hotspots (Figure 28). Even though identification was not possible, it can be clearly seen from the thermal imagery where hot spots that had a high density of basking turtles were present. This was confirmed by the simultaneous capture of thermal imagery alongside visible video footage in the M2. From this minimal data it was determined that the heat signatures of freshwater turtles were unable to be identified conclusively when found in water with the thermal sensor. Turtles that were on out of the water when basking or moving did display heat signatures, but without the use of the visible imagery that is collected simultaneously with the M2, it would be impossible to determine the heat signatures as turtles.

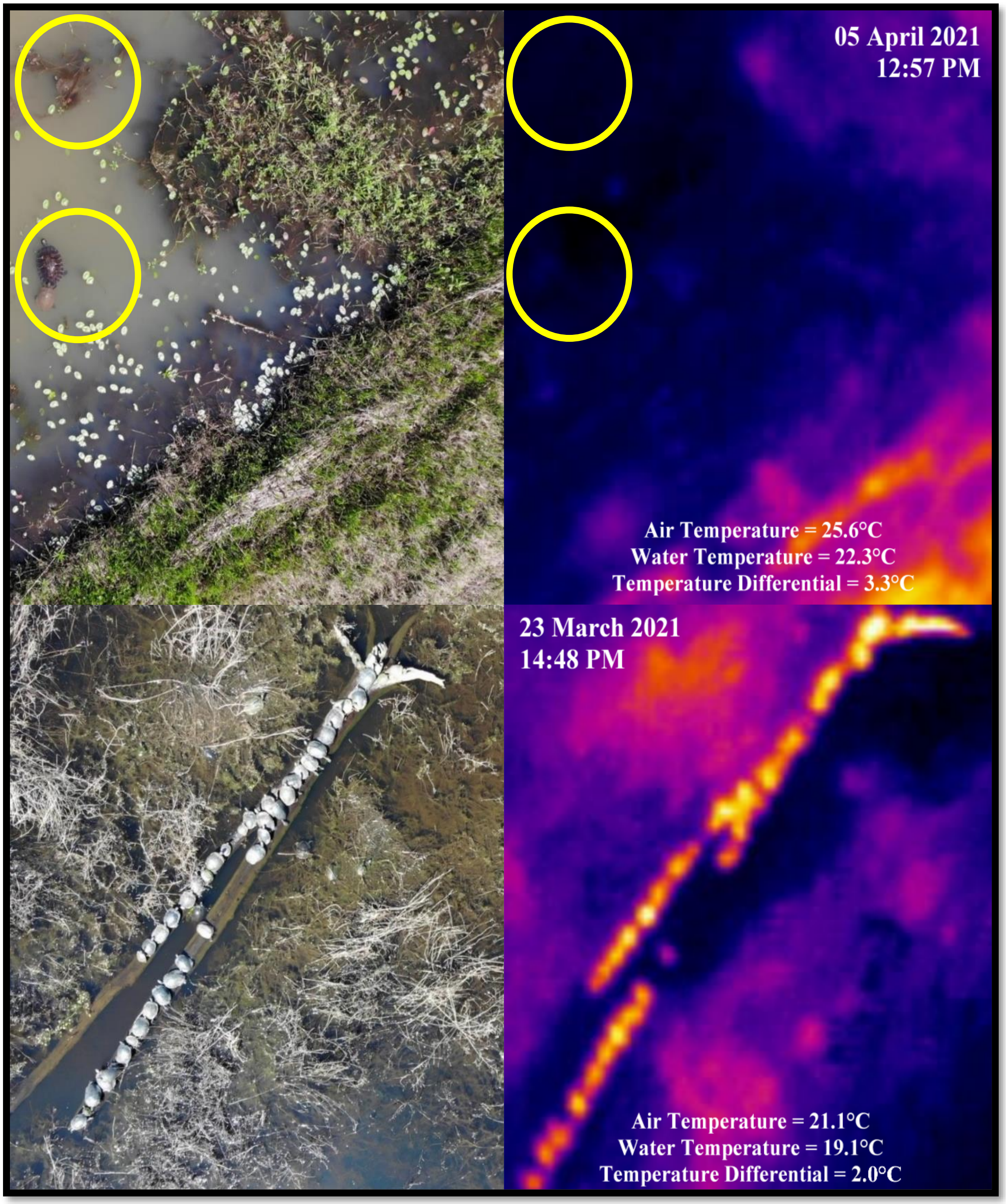

Figure 28: Images displaying the thermal sensor observing freshwater turtles. Top highlights turtles swimming that do not display heat signatures with corresponding water and air temperatures while the bottom image shows heat outlines of turtles basking out of water on a log with water and air temperatures.

Solar noon relative to detections

A total of 77 M2 drone surveys were conducted at the 11 sites. A majority of these surveys occurred before solar noon (solar noon = 0) with the most surveys occurring at the time interval of three to four hours before solar noon (n = 18). Surveys and number of detections were sorted into hour intervals in minutes at their difference from solar noon (Figure 29). The greatest number of freshwater turtle detections occurred during the first hour (1:60) after solar noon (n = 900). A Kruskal-Wallis one-way ANOVA on ranks was run to compare the median number of turtles from the M2 survey detections at each listed time interval. After failing the Shapiro-Wilk normality test ($p <$ 0.050) the ANOVA on ranks determined there was a significant difference in the median values between time intervals from solar noon (H = 108.315, df = 6, p = < 0.001). Then an all pairwise multiple comparison procedure (Dunn's Method) was run to investigate the time intervals to one another. There was a statistically significant difference for only the time interval of 1:60, being the first hour directly after solar noon when compared to the other groups ($p < 0.004$). This test highlights shows that turtles were more likely to be detected in the first hour interval (1:60) just after solar noon occurred with the M2.

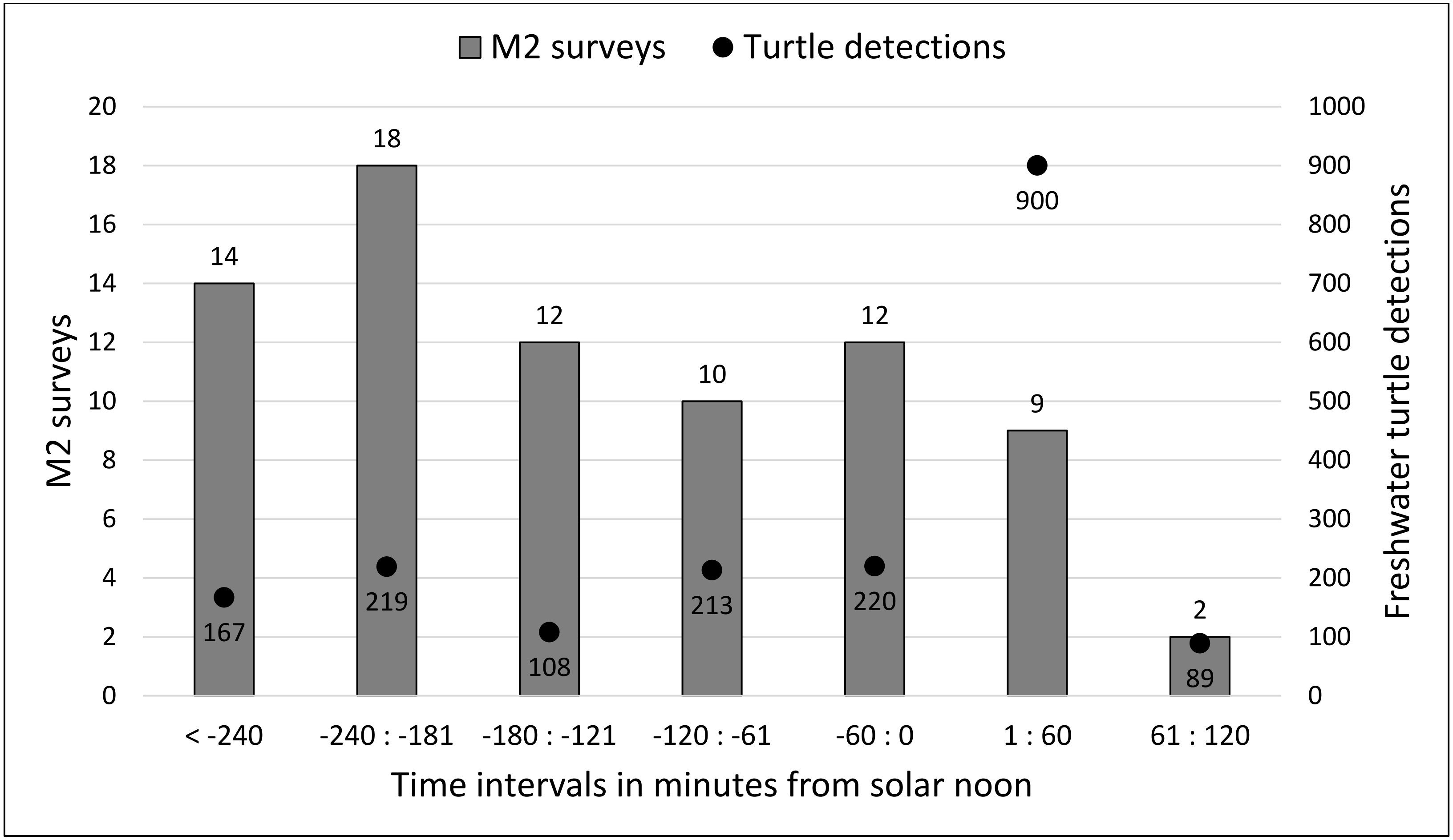

Figure 29: Number of freshwater turtle detections relative to solar noon (solar noon = 0). Left x-axis is the number of M2 drone surveys conducted represented by the grey bars with total number displayed at the top. Right x-axis is the total number of turtle detections represented by the black dot with the number displayed below. Y-axis represents the one-hour time intervals relative to solar noon.

Habitat relative to detections

Sites were categorized into two freshwater habitats: lentic (LE; *n* = 6) and wetlands (WTL; *n* = 5). Across all three methods (M2, P4MS and BAVS) more freshwater turtles were detected in lentic habitats compared to wetland habitats. The M2 (1429:487) and BAVS (828:268) had roughly three times and the P4MS (1615:300) had roughly five times as many freshwater turtle detections in lentic habitats compared to wetlands. The number of freshwater turtles observed by each method across the two habitat types, lentic and wetlands, can be seen in Figure 30.

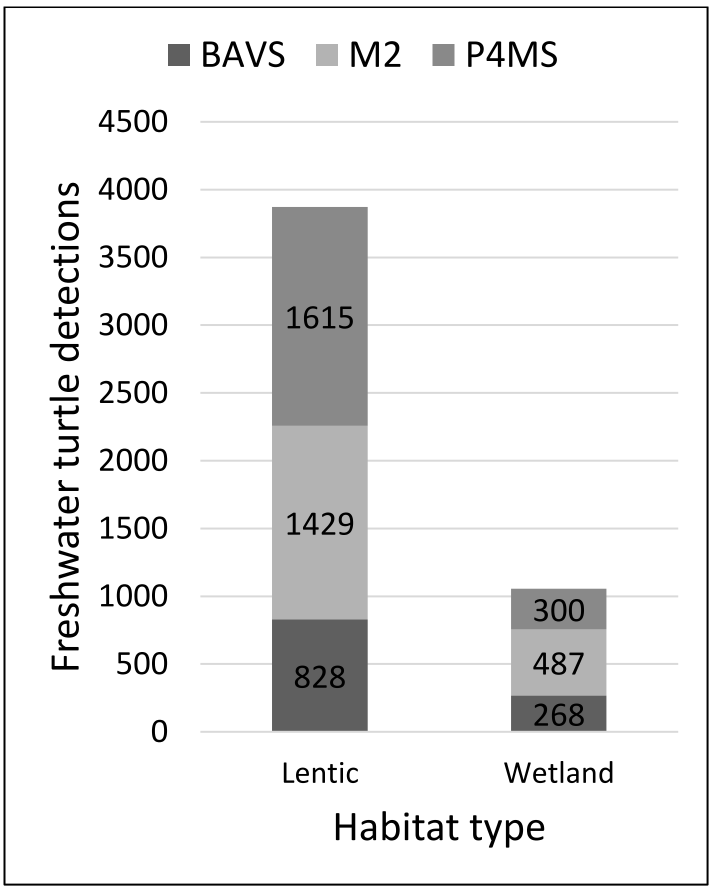

Figure 30: Number of freshwater turtle detections (x-axis) for lentic and wetland habitat types (y-axis) by each method, BAVS, M2, and P4MS (grey-scale boxes).

First, a Welch's t-test (not assuming equal variances) was run to compare the total number of freshwater turtle detections from all three methods for each habitat type, lentic and wetland. After passing a Shapiro-Wilk normality test, a statistically significant difference (t = 3.800, df = 2.329, $p = 0.049$) was detected for means between lentic and wetland habitats. Results from this test show that in lentic habitats, more freshwater turtle detections occurred compared to wetlands when using all methods for detection. Then two one-way ANOVA tests were run to test the mean groups for the total number of freshwater turtle detections made by each method (BAVS, M2, and P4MS) for these two habitat types. This test failed to detect a significant difference for lentic ($p = 0.783$) or wetland ($p = 0.533$) habitats across all three methods. These results show there was no significant difference in turtle detections made by any individual method BAVS, the M2, and the P4MS in lentic or wetland habitats. Lastly, three Welch's t-tests were run to investigate turtle detections by each method, for each habitat type. Both the M2 and P4MS failed the Shapiro-Wilk normality test, so a Mann-Whitney Rank sum test was run in place. Both tests failed to result in statistically significant differences for the median values (M2: $p = 0.537$; P4MS: $p = 0.537$). BAVS passed the Shapiro-Wilk normality test but failed to be significant ($p = 0.103$). The results of these three tests show that there were no statistically significant differences between habitat types when using any individual method. However, the *p*-values for both drone surveys, M2 and P4MS, were higher compared to BAVS.

Turtle behaviors and reactions

The majority of turtles displayed no reaction to the M2 drone's presence (77%, *n* = 1473) (Table 4 and Figure 31). Five initial conditions or behaviors of freshwater turtles were documented, in order of the greatest number of detections: swimming, basking, foraging (eating observed on land or in water), dead, and mating. A total of 432 individuals (22%), which were either swimming (*n* = 353) or basking (*n* = 79), reacted to the drone (Figure 32), with the most frequent reaction for both behaviors being a 2 (submerged but did not retreat) (swimming *n* = 125, basking *n* = 33) (Figure 33). A Welch's t-test (equal variance not assumed) was run comparing the number of turtles reactions from all reaction scores for swimming and basking behaviors observed with the M2. After passing a Shapiro-Wilk normality test, a statistically significant difference (t = -5.021, df = 3.747, *p* = 0.009) was observed between the means of swimming and basking turtles that reacted to the M2 drone. The results of this test showed that of the turtles that were in frame and reacted to the M2 drone, more were observed to be displaying a swimming behavior.

Table 4: Table showing the number of reactions and associated percentage of total for the reaction score of freshwater turtles on the scale of 0-4 and including unknowns.

Reaction Score	Reaction Type	# of Reactions	Percent of Total (%)
0	No reaction	1473	76.9
1	Reaction but did not submerge	88	4.6
2	Submerged but did not retreat	158	8.2
3	Submergence and retreat	99	5.2
4	Quickly retreated	87	4.5
Unk	Unknown	11	0.6
	Total	1916	100.0

Figure 31: A Red-eared slider (*Trachemys scripta elegans*) with algae on its carapace observed basking on a fallen log displaying no-reaction to the M2 drone at LE-05. Approximately one-meter altitude.

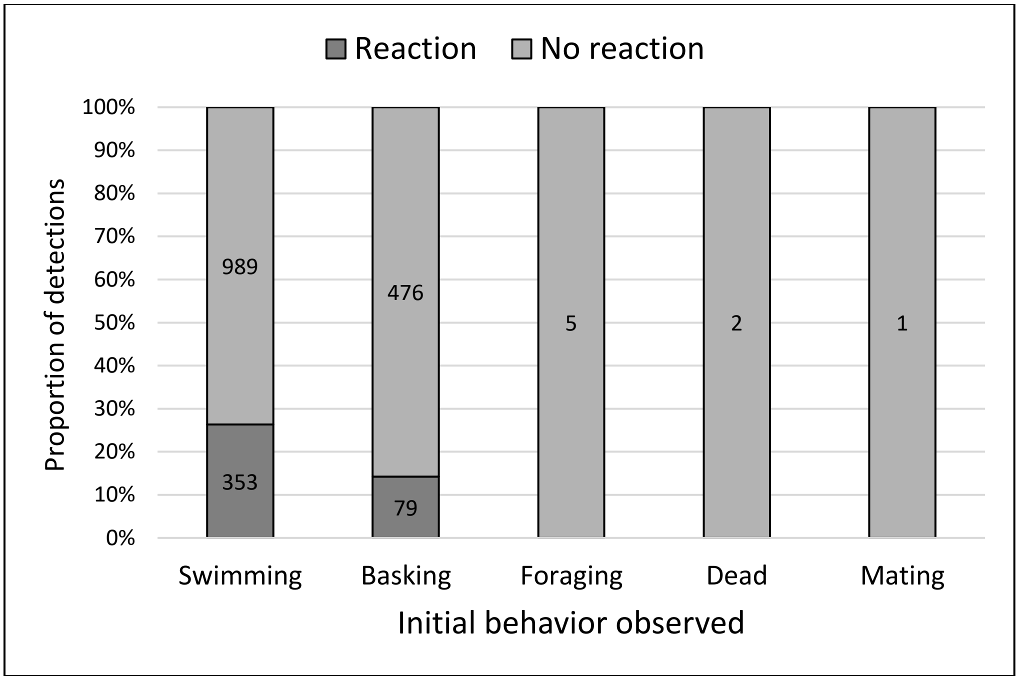

Figure 32: Proportion (x-axis) and total number of freshwater turtle detections (data labels) for each initial behavior observed (y-axis) with M2 drone surveys for turtles that reacted (dark grey) and did not react (light grey) to the drone.

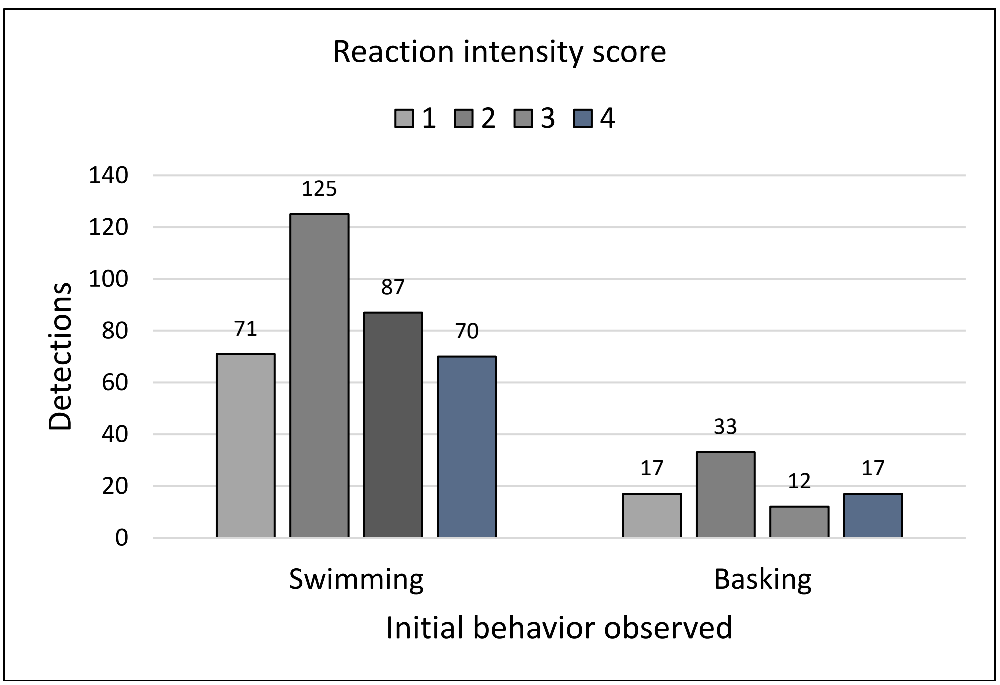

Figure 33: Number of detected freshwater turtles (x-axis) that reacted to the M2 drone for the behaviors of swimming and basking for their corresponding reaction score of 1 (minimal reaction) least to 4 (extreme reaction).

DISCUSSION

Overview

Drones such as the M2 and P4MS can provide multiple benefits for surveys of aquatic freshwater turtles with the ability to detect and identify cryptic species such as the Western Chicken Turtle, *Deirochelys reticularia miaria*. There are however many important factors to consider before adopting this technology for surveys including, start-up costs, quality of the sensors on the drone, skill of pilot-in-command, environmental conditions, and technological issues. If these factors are addressed and missions are executed using well planned protocol, drones can provide valuable data on wildlife populations and habitats that would otherwise be unattainable. Drones provide unique benefits when compared to more traditional methods of surveying for aquatic turtles like BAVS. They facilitate the collection of large amounts of habitat data from a unique aerial perspective in a safe non-invasive way that does not disturb the organism under study. This technology eases the collection of a vast amount of imagery data that can be permanently archived for later analysis.

If a researcher is considering using drone surveys, the initial investment of training and purchasing the correct drone models with up-to-date sensors can be time consuming and expensive (Vishwath N.C et al., 2022). A PIC must undergo proper training regarding the FAA regulations and acquiring the legal documents to fly, such as the Part 107 License, drone registration, and any additional permits required at the state and federal level in the United States. Even though the advancement in drones has allowed for smaller and cheaper models to become more accessible for commercial and recreational uses, obtaining a model with a high-quality camera resolution can cost thousands or even tens of thousands of dollars. In addition, careful consideration must be given to the selection of the type of sensors depending upon the study. With a wide range of applications regarding the use of multispectral and thermal imagery along with their growing capabilities, these additional sensors should be considered. Due to the ease of being able to capture visible imagery data simultaneously these other sensors (multispectral and thermal), should be utilized whenever available if the drone platform supports additional sensors. This extra data may prove useful in later analyses. These sensors are rapidly increasing in camera resolution, functions, and accessibility which will facilitate the ability to answer new questions about and more easily monitor wildlife with drones (Fust & Loos, 2020). When deciding on what model of drone system to employ, an in-depth investigation of costs, capabilities, and objectives should be conducted.

Freshwater turtles

The M2 and P4MS can be effectively used for freshwater turtle detection and identification when certain criteria and conditions are met. The morphology traits of the target species must be examined closely as size and external markings play an important role in identifying specimens using drone imagery (Bevan et al., 2016; Bogolin et al., 2021; Schofield, Katselidis, et al., 2017; Vallery, 2018). Turtles that are smaller in size, such as juvenile turtles and Mud/Musk Turtles (Kinosternidae), can be difficult to confirm identity due to a lack of resolution quality which might explain the lack of detections in the gathered data. Larger species such as North American Softshell Turtles and Common Snapping Turtles and ones with unique characteristics were much easier to identify during video playback. Slider Turtles (*Trachemys sp.)* especially the abundant Red-eared Sliders (*Trachemys scripta elegans*) were also easily identifiable due to their unique carapace markings, serrated anterior carapace, and colorful red ear. Many other studies involving drones and wildlife rely heavily upon external characteristics for identification purposes (Bogolin et al., 2021; Chabot et al., 2018; Rowe et al., 2022). It is recommended to consider what the target species external characteristics look like, especially from a top-down or aerial side point of view before implanting drone surveys for detection-based studies.

The M2 exhibited a statistically significantly ($p = 0.015$) higher CPUE when compared to BAVS based counts for freshwater turtles. This higher CPUE demonstrates that the M2 excelled at detecting more freshwater turtles compared to BAVS. Drones excel at capturing imagery data in a quick and non-invasive manner (Daniels, 2018; Valle & Scarton, 2021), and this was seen with our methodology comparison of the M2 to BAVS. The M2 captured more turtle detections and identified more turtle groups when compared to BAVS which demonstrates drone usefulness for data about populations and diversity for freshwater turtle species and other wildlife (Biserkov & Lukanov, 2017; E. M. Corcoran, 2021). The comparison of CPUE generated by the P4MS to other methods cannot be made due to our P4MS flight missions occurring after the two other methods were conducted. This confounds the ability to separate out the effects of the P4MS, but there were still significantly more turtles detected with the P4MS ($p < 0.001$). There is concern that there was alteration in turtle detections and behavior acquired with the

P4MS due to it being the second flight mission. But the P4MS flights were conducted primarily to test the sensor's capability with multispectral images (bands) for detecting freshwater turtles. Being able to fly directly over the wetland or lentic habitat with a unique top-down perspective, facilitated the detection of shallow submerged freshwater turtles. These turtle detections have a possibility to be missed by BAVS based on our observations. The use of drones for top-down identification methods can prove useful for many areas regarding wildlife conservation regarding both animals and plants, especially when unique characteristics are present in the target species (Chabot et al., 2018; Sesnie et al., 2016). Particularly in environments that lack ground accessibility with tall vegetation or structures that obscure lateral vision from surveying on the ground (Dronova et al., 2021).

When specifically targeting WCTs, the M2 drone was able to detect five WCT, all at the same site on three separate dates (WTL-01) and the P4MS detected only one WCT (LE-03). Binocular aided visual surveys failed to detect any WCT at sites where both M2 and P4MS drone surveys were conducted, though BAVS is a viable method for WCT detection (Gordon et al., 2023). This data supports the conclusion that these two drones should be effective for surveying WCT at these site locations. But, due to the cryptic nature of the species and evidence that WCT can be successfully detected through a variety of methods (Bowers et al., 2021; K. A. Buhlmann et al., 2009; Gordon et al., 2023; Ryberg et al., 2014), drones might not always be the best suited depending upon the research question at hand. Further research is needed to confirm the ability of drones to detect other species of freshwater turtles at other locations with varying habitat types.

Multispectral sensors

Multispectral imagery can aid in detecting freshwater turtles and visibility into freshwater habitats, by utilizing the different wavelengths given off in the electromagnetic scale from the turtles and their habitat. We observed some turtle characteristics could be identified in certain bands such as the red, red edge and near-infrared which facilitated the observer's ability to see into water under ideal circumstances. As wavelengths increased in the multispectral bands, such as the red band, red edge band, and near-infrared band visibility increased. Characteristics such as the individual scutes on turtle carapaces and skin markings were highlighted in these bands, aiding in identification of the WCT and other freshwater turtles. The one WCT that was identified using multispectral imagery highlighted the prominent yellow lateral lines that run along the front legs as it was facing upwards in the water towards the drone. This supports the use of multispectral imagery for observing unique external characteristics on freshwater turtles such as the WCT. Regarding other species outside of freshwater turtles, multispectral imagery has demonstrated its usefulness for wildlife conservation (Houegnigan et al., 2022; Sesnie et al., 2016). Even when turtles were not present in multispectral imagery, there are benefits for using the P4MS in aquatic habitats. With the red, red edge, and near-infrared clarifying the underwater environment, there is potential to investigate these submerged habitats. Data can be collected on underwater elements such as vegetation, fish, invertebrates, and other aquatic organisms (Houegnigan et al., 2022; Taddia et al., 2020).

Many additional factors can affect the quality of the images created in different bands produced by multispectral imagery. Current weather conditions such as the wind, cloud cover, and position of the sun can affect image quality similar to the M2. Habitat conditions like water clarity, water depth, vegetation, and substrate also influence multispectral results (Chabot et al., 2018; Houegnigan et al., 2022; Taddia et al., 2020). In some worst-case scenarios, all bands after other than the visible image were displayed completely black and were unusable. The influence of these variables requires more detailed testing to better understand how to collect the best possible imagery using multispectral bands in aquatic environments. It is recommended that to properly assess the functionality of multispectral for the desired project variables such as the target and environmental conditions before deployment. Otherwise, if these variables are not considered and adjusted for, more work will be needed during the data processing step.

Thermal

The thermal sensor was unable to identify freshwater turtles to a specific taxonomic level but could detect locations where turtles were residing or present. Additional research is needed to further explore its function and potential applications. Due to the massive amount of data collected in this study and low power of turtle identification from the thermal sensor, thermal imagery was only utilized infrequently. The brief use of the thermal sensor in this study is important to mention due to the implications that temperature can have in the study of wildlife conservation (Lembrechts et al., 2022; Ratnayake et al., 2019; Schofield, Papafitsoros, et al., 2017). Investigating and comparing the air, water, and substrate temperatures at where turtles are choosing to bask could provide valuable information about key habitat indicators (Boyer, 1965). As thermal sensor technology expands for use in drones, this might become an application worth investigating in the future. These basking hotspots could be targeted for conducting in depth habitat analysis to aid in conservation and restoration projects regarding ectotherms. The use of thermal imagery coupled with the simultaneous use of visible imagery would be useful in characterizing the thermal ecology of turtles and other ectotherms. Especially in environments that experience small microhabitat temperature shifts due to wind, shade, and water level fluctuations (Mulholland et al., 1997; Smith & Ballinger, 2001). Thermal sensors and imagery can provide unique data for a vast variety of habitats and organisms due to the importance of temperature (Chrétien et al., 2015; Howell et al., 2021). Their application in future wildlife conservation studies has merit if temperature is an important measurable for the project. Similar to the multispectral sensor, proper preliminary assessment of environmental conditions before deployment of thermal imagery is highly recommended.

Environmental conditions

One of the most influential factors that affected the ability of using drones for conducting surveys was surrounding environmental conditions. Current weather at the site can produce excessive rain, heat, and condensation, which can have detrimental effects on drones and should be avoided at all costs. Milder conditions such as wind, cloud cover, position of sun, and background noise can influence data collection. Careful pre-planning and monitoring of local conditions is necessary for successful surveys to be conducted. Being flexible with flight dates is advised. Wind can blow over surface water, causing ripples or waves, making it almost impossible to detect submerged turtles. The opposite is also true, if the water surface is calm and glassy, reflections of the clouds and specific angles of the sun can drastically affect the viewer's ability to spot turtles in water. It is up to the pilot-in-command (PIC) to determine the best position, gimbal angle (tilt of camera), and drone flight path to obtain the best quality data for the research project.

When focusing on ectothermic species such as freshwater turtles, the sun plays a vital role in their activity. With a significant amount ($p < 0.004$) of the turtle detections occurring in the hour just after solar noon (1:60) with the M2. Conducting surveys closer to solar noon is advised for ectotherms like freshwater turtles to provide more detections. But sampling at this time period runs the risk of compromising clear imagery data collected by the drone due to the potential water glare. However, this glare can be combated by using lens filters that are designed to remove sun glare such as polarized and neutral density filters. When targeting other species with drones, determining the active and dormant times are advised dependent upon the study.

Freshwater habitats

By targeting diverse freshwater habitats spread over eastern Texas, these two drones, M2 and P4MS were able to safely fly and collect large amounts of data at all 11 sites after proper training and careful practice. Being able to establish a safe take-off and landing location with open airspace can be difficult in extremely vegetated rural areas. Bringing along a "launch pad" 3 x 3 ft piece of plywood or other material that provides a stable flat surface to set on ground is critical to ensure smooth take-offs and landings.

This is particularly important in areas containing highly saturated soil or containing tall vegetation. Canopy cover, however, needs to be carefully assessed when planning and flying missions and should be adjusted for a visit-by-visit basis. Previous research with birds and mammals has shown that canopy cover can negatively impact drone imagery and that surveys evaluating non-arboreal species should focus on large areas with less heterogenous and decreased canopy cover (E. Corcoran et al., 2019; E. M. Corcoran, 2021). Due to a higher collision risk, it can be unsafe to fly drones in dense canopy cover which in result will limit data collection. For example, in this study lentic habitats that had dense canopy cover along the banks might have had turtles that were missed during drone flights. The risk of collision increases when a pilots line of sight (LOS) is compromised. These drones are equipped with anti-collision sensors, but they are not a failsafe against collisions occurring. It is advised to use extreme caution even for skilled pilots. In some instances, using varying gimbal tilt angles can provide visual access to these locations when unable to fly directly over the target spot. The gimbal can be adjusted during flight missions if needed with the remote controller. This relies heavily upon the skill of the PIC to be able to safely maneuver in challenging locations while maintaining quality data collection.

It is highly recommended to undergo many hours practicing manual flights with your drone prior to actual data collection. Gaining the right “touch” of the controls and understanding the flight mechanics behind your drone model can make the difference between smooth and clear or coarse and fragmented imagery data. Similar to conducting BAVS, avoiding sudden jerky movements can also reduce the chances of disturbing wildlife before the drone is able to capture data. The size of the targeted waterbodies also influences mission success (Fettermann et al., 2022). As the site area increases, more flight time is needed, meaning more batteries must be used. Once again, careful pre-planning for missions and allocating resources based on site characteristics is extremely important for utilizing drones properly.

We detected a significant difference ($p = 0.049$) in turtle detections between habitat types, with more detections occurring in lentic habitats over wetlands. Even though there was no statistically significant difference for the methods between habitat sight, BAVS were much lower compared to the drone surveys. The difference in lentic open water and wetland habitat turtle detections is likely due to the presence of emergent and floating aquatic vegetation that can limit LOS. In Lentic open water environments, it was much easier to spot turtle heads from a lateral point-of-view with BAVS compared to wetlands with emergent vegetation. This was not an issue for the drones as they can fly over vegetation and provide multiple points-of-view to document detections. Other freshwater habitat types with varying vegetation cover need further investigation with drone surveys, however there was success in riparian habitats (Davis et al., 2020). Drones have demonstrated their benefits for a wide variety of habitats globally, especially in difficult areas of access (La Vigne et al., 2022). More environments will become accessible for surveying with the skill of pilots increasing along with anti-collision software.

Disturbance

The potential disturbance drones can cause needs to be considered when surveying wildlife. As drones are unnatural objects that occupy airspace and generate noise there might be potential impacts upon fauna including avoidance behavior. A majority of turtles did not react to the drone and even those that did react displayed only a milder reaction score. In certain instances, the M2 drone was able to hover over for an extended amount of time and get extremely close to the turtle without the turtle exhibiting a reaction. This suggests that drones might have only minimal impacts on freshwater turtles. However, there are other wildlife present that drones might disturb especially avian species. Birds occupy airspace and/or nest in trees at similar flight altitudes which present them at risk to drone presence but recent studies have shown that birds also display minimal reactions to drone presence (Vallery, 2018; Wilson et al., 2017). Other organisms warrant investigation of disturbance that could be heavily impacted by drone presence such as flying invertebrates that play important roles in ecology like bees, dragonflies, and wasps (Batzer & Boix, 2016; Bilton et al., 2001).

These drones not only collected imagery of freshwater turtles with low disturbance, but information on the diverse herpetofauna found at each location. Even species from other animal groups like mammals, birds, and fish were detected in these freshwater habitats with minimal disturbance. In some special circumstances, it was noticed that sound pollution might play a role in the turtles' reaction to the drone. At sites located in nosier areas, such as ones adjacent to roads with heavy traffic, turtles appeared less sensitive to the noise the drone produced. The hearing of turtles is poorly understood compared to other reptiles, but what is known is that turtles have higher hearing thresholds (around 500 Hz) (Willis, 2016). Turtle hearing is much lower when compared to humans (20 Hz – 20000 Hz) and birds (1000 – 2000 Hz) (Beason, 2004; Le Prell et al., 2013). This may contribute to delayed or swift reactions from turtles to the drones presence. Future investigation of this hypothesized relationship between background and drone sound levels and wildlife disturbance is warranted. Ideally, as newer drones get quieter this will become less of an issue. Even with this possible drawback, drones are one of the most non-invasive methods for studying wildlife safely.

Figure 34: M2 drone images of other herpetofauna detected during freshwater turtle surveys. Right: American Alligator (*Alligator mississippiensis*) swimming. Top right: Diamond-backed Watersnake (*Nerodia rhombifer*) basking. Bottom right: Two American Bullfrogs (*Lithobates catesbeianus*) floating at surface.

Figure 35: M2 drone images showing other animal species detected. Top left: Roseate Spoonbills (*Platalea ajaja*) foraging. Bottom left: Nutria (*Myocastor coypus*) swimming. Right: Multiple gar (Lepisosteidae) swimming.

Recommendations

Future studies conducting surveys with drones need to consider the current status of the available technology as well as the rules and regulations. In the United States regulations that need to be considered are found at both the state and federal level including the FAA. Staying up to date with the technology and rules will allow researchers to efficiently plan and make decisions for projects based upon the most current understanding of drone capabilities and regulations. As drone technology continues to evolve so will the applicability of drones for research studies. From this study regarding the methods of the M2, P4MS, and BAVS for detecting freshwater turtles, it is recommended to use the M2 as it was efficient in terms of both identifying and detecting when compared to the other two methods. The M2 requires less time and captures large datasets that can be replayed.

The most important aspect for imagery data comes from the quality of camera resolution available. Resolution becomes even more relevant when the target subject is smaller, such as freshwater turtles like the WCT. Better resolution can aid in identifying morphology defining attributes. It is likely probable that a large portion of the turtles that were identified as unknown in this study, could have been identified to a lower taxonomic group with a higher camera resolution. Another approach that could potentially solve issues with the quality of the imagery data is the use of lens filters. Polarized or neutral density filters that affect the amount of light penetrating the lens could aid in reducing heavy glare and reflections allowing for cleaner data collection. This can be particularly useful when they survey location involves water. The size of the drone needs to be considered as well. Size is not an issue when studies occur in consistently open-air space but can become an issue for accessibility and maneuverability when operating below the canopy. Lastly, it is important to remember that the weight of the drone can influence its stability in stronger winds.

For collecting drone imagery data for freshwater turtles there are certain flight mission settings that should be practiced. Maintaining a low slow flying altitude of around 5-10 meters at 1 m/s will allows for best quality data (Biserkov & Lukanov, 2017; Bogolin et al., 2021; Daniels, 2018; Davis et al., 2020). The drone should face the same direction the entire flight (heading) and keep the gimbal angle consistent (where the camera is facing). These settings are a guideline for freshwater turtles. and need to be tweaked to each specific flight scenario. Pilots and researchers have to consider many things, involving different drone models, camera resolutions, site locations, current weather conditions, and target species for their associated project.

One aspect of drone imagery that is progressing is the use of automated trained AI models to detect the desired target (E. M. Corcoran, 2021; Lee et al., 2018). By training an algorithm to detect unique attributes within the imagery data, it can accurately detect targets and cut-down the amount of effort needed to analyze data (E. Corcoran et al., 2019; Rivas et al., 2018). With drones collecting such large datasets, manually processing, and recording detections requires a large time investment. As these automatic AI models become more accessible, easier to implement, and consistent in their accuracy, they should be considered for analyses.

It is recommended that sensors such as multispectral and thermal, be utilized for aquatic turtle surveys if available. Researchers will need to allocate additional time for data analysis when working with additional sensors since this will generate a higher quantity of data. Additional derived data can be generated using these sensors including normalized difference vegetation indexes (NDVI) using the multispectral bands and hotspots with thermal sensors. A NDVI quantifies vegetation by measuring the difference between near-infrared and red light which provides a value on the scale of -1 (unhealthy) to 1 (healthy) vegetation (GISGeography, 2017). Hotspots could provide valuable information about optimal thermal habitat that basking turtles prefer to occupy. When using the P4MS and investigating the multispectral bands, it is recommended to utilize the red, red edge, and near-infrared bands as they can clarify the visibility into water. Thermal imagery collected by the M2 does require further investigation and is recommended if temperature plays a critical role in the research question at hand. Further exploration of the use of multispectral bands and thermals sensors is warranted.

Conclusion

Conclusions that can be made from this study are that the drones, M2 and P4MS detected significantly more freshwater turtles when compared to BAVS. The multispectral bands allowed for clearer images to be produced for submerged habitats and aided in detecting "hidden" turtles. Thermal imagery produced by the M2 was of low quality but can have implications for future research about temperature differences for freshwater turtles and their habitats. Location and time of day will influence the quality of imagery data collected along with the number of detections. Open air space, like lentic habitats resulted in significantly more freshwater turtle detections. Flying at the hour interval after solar noon also resulted in significantly more turtle detections. Lastly, a majority of the freshwater turtles detected displayed no reaction to drone presence and those that did react, a significant number of turtles were initially displaying a behavior of swimming.

The use of drone technology is rapidly growing in the field of wildlife conservation and provides a powerful tool for researchers. This applies to common and cryptic species of aquatic turtles in freshwater habitats. By observing and detecting a variety of turtle species such as the WCT, drones can conduct successful surveys for a vast variety of data using multiple sensors. This imagery data can be permanently stored and analyzed using multiple approaches. Operators and researchers must be aware of the current rules and regulations for this ever-expanding field. There should continue to be multiple opportunities for future researchers interested in using drones for wildlife research.

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APPENDIX A: BAVS DATASHEET

2020-23 WCT Visual Survey Datasheet (front)

Site ID ________ **Date** ____________ **Start Time** ______ **End Time** ______ **Initials** ______

Search Location #	Latitude	Longitude	Search Time at location (min)	Total # Observations

									For use in UAV survey only		
Location #	Time Obs.	Distance (m)	Bearing (°)	Species (Common Name)	Behav.[a]	Est. Count	Conf.[b]	BAVS Observation Notes *If WCT, assign number & record habitat info (back)	UAV Reaction	Est. Linear Dist. (m)	UAV Reaction Notes

[a] B = basking, S = swimming, M = movement, F = foraging/eating, C = copulation, D = dead, O = other
[b] 3 = High, 2 = Moderate, 1 = Low, 0 = "It's a turtle"

Notes: ____

____

DE Initials & Date ________ QC Initials & Date ________ Page ____ of ____

Appendix 1: Image of a blank BAVS datasheet used for surveys conducted alongside the M2 drone surveys.

APPENDIX B: HERPETOFAUNA OBSERVED

Appendix 2: Table of all herpetofauna observed during the study by each method with their respective relative abundance down to lowest taxonomic level. Scientific names were retrieved December 7th, 2022 from the Integrated Taxonomic Information System (ITIS), www.itis.gov, CC0, https://doi.org/10.5066/F7KH0KBK.

Table of Herptofauna Observed				P4MS (Image)	M2 (Video)	BAVS		
Major Group	**Taxonomic Level**	**Scientific Name**	**Common Name**	**Count**			**TOTALS**	**Relative abundance (%)**
Crocadillians	Species	*Alligator mississippiensis*	American Alligator	8	13	6	27	0.005
Frogs	Genus	*Acris sp.*	Cricket Frog	-	-	2	2	< 0.001
Frogs	Order	*Anura*	Unknown frog/toad	8	12	2	22	0.004
Frogs	Species	*Lithobates catesbeianus*	American Bullfrog	13	11	19	43	0.009
Frogs	Species	*Dryophytes cinereus*	Green Tree Frog	-	-	1	1	< 0.001
Snakes	Genus	*Nerodia sp.*	North American Watersnake	-	-	1	1	< 0.001
Snakes	Species	*Regina grahamii*	Graham's Crayfish Snake	-	-	1	1	< 0.001
Snakes	Species	*Nerodia erythrogaster*	Plain-bellied Watersnake	-	-	1	1	< 0.001
Snakes	Species	*Nerodia fasciata confluens*	Broad-banded Watersnake	-	2	1	3	0.001
Snakes	Species	*Nerodia rhombifer*	Diamond-backed Watersnake	-	1	2	3	0.001
Snakes	Suborder	*Serpentes*	Unknown snake	3	3	2	8	0.002
Turtles	Genus	*Graptemys sp.*	Map Turtle	-	3	-	3	0.001
Turtles	Genus	*Sternotherus sp.*	Musk Turtle	1	1	-	2	< 0.001
Turtles	Genus	*Trachemys sp.*	Slider Turtle	1318	1054	282	2654	0.527
Turtles	Genus	*Apalone sp.*	North American Softshell Turtle	13	22	2	37	0.007
Turtles	Species	*Pseudemys concinna*	River Cooter	-	-	1	1	< 0.001
Turtles	Species	*Chelydra serpentina*	Common Snapping Turtle	7	6	2	15	0.003
Turtles	Species	*Kinosternon subrubrum*	Eastern Mud turtle	-	1	-	1	< 0.001
Turtles	Species	*Apalone mutica*	Smooth Softshell Turtle	-	1	-	1	< 0.001
Turtles	Species	*Apalone spinifera*	Spiny Softshell Turtle	10	3	-	13	0.003
Turtles	Subfamily	*Kinosternidae*	Mud/Musk Turtle	5	-	-	5	0.001
Turtles	Suborder	*Cryptodira*	Unknown turtle	441	558	539	1538	0.305
Turtles	Subspecies	*Trachemys scripta elegans*	Red-eared Slider	119	262	270	651	0.129
Turtles	Subspecies	*Deirochelys reticularia miaria*	Western Chicken Turtle	1	5	-	6	0.001
Unknown	Unknown	Unknown	Unknown	1	-	-	1	< 0.001
			TOTALS	1948	1958	1134	**5040**	
			Total Taxonomic Levels	14	17	17	**25**	