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Quantifying minimum survey effort to reliably detect Amazonian manatees using an unoccupied aerial vehicle (UAV) at an *ex situ* soft-release site

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Abstract

Detection of many threatened aquatic mammals, such as manatees (*Trichechus* spp.), using traditional visual observation methods is associated with high uncertainty due to their low surfacing times, cryptic behaviors, and the environmental heterogeneity of their habitats. Rapid advancements in technology provide an opportunity to address these challenges. In this study, we aimed to quantify survey effort of unoccupied aerial vehicles (UAVs) for detecting the Vulnerable Amazonian manatee (*T. inunguis*). Using a closed population of manatees that is being rehabilitated within a lake at the Rainforest Awareness, Rescue, and Education Center in Iquitos, Peru, we calculated the number of repeat surveys needed to detect at least one individual with 95% (n = 3.10) and 99% (n = 4.76) confidence. We used both generalized linear mixed-effect models and Bayesian single-species and single-season detection models to determine the effects of the environment (water depth, water transparency, cloud cover, wind speed), time of day, and behavior (breathing, foraging, milling) on the time-to-detection and detection probability, respectively. Both models indicated a significant interaction between water depth and water transparency, causing an increase in the time-to-detection (β = 0.032; 95% CI = 0.028, 0.037) and a decrease in the probability of detecting manatees (α = -0.65; 95% CI = -1.3, -0.007), which was calculated to be 0.62 (95% CI = 0.23, 0.94). Due to the similarities between the lake and *in situ* habitats, the results of this study could be used to design *in situ* UAV survey protocols for Amazonian manatees or other difficult-to-detect freshwater aquatic mammals and to monitor *ex situ* animals pre- and post-release, which should ultimately contribute to a better understanding of their spatial ecology and facilitate data-driven conservation efforts.

Keywords:
Conservation, detection probability, drone, monitoring efforts, research methodology, *Trichechus inunguis*

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Introduction

The Amazonian manatee (*Trichechus inunguis*) is one of four extant species belonging to the Order Sirenia and is categorized as Vulnerable by the IUCN Red List, primarily due to anthropogenic threats, such as illegal hunting (Domning, 1982; Marmontel et al., 2016). The Amazonian manatee is the only Sirenian species that exclusively inhabits freshwater ecosystems, and the species is endemic to the Amazon basin in four South American countries: Colombia, Ecuador, Peru, and Brazil (Best, 1982; Rosas, 1994; Reynolds et al., 2018). Similar to its counterpart, the African manatee (*T. senegalensis*), Amazonian manatees are severely understudied, with key information about the species' distribution and ecology lacking due to their low population densities and elusive behaviors in response to anthropogenic threats, including low frequency of surface behaviors while retaining a low profile once at the surface and modification of activity patterns (Rathbun et al., 1983; Castelblanco-Martínez, 2004; Gonzalez-Socoloske et al., 2011; Marmontel et al., 2016; Reynolds et al., 2018). The low water clarity of the ecosystems the species inhabits and their preference for areas with floating vegetation also increases the difficulty of visually detecting animals (Montgomery et al., 1981; Arraut et al., 2010; Landeo-Yauri et al., 2017; Ruano et al., 2021). For example, the visibility of the Amazon River and its tributaries is generally restricted to less than 2 m, with the exception of clearwater and blackwater rivers that have a transparency of about 1.1 – 4.3 m and 1.3 – 2.9 m, respectively (Montgomery et al., 1981; Rosas, 1994). The species' dark color can further prevent detections at the subsurface (Rosas, 1994).

In addition to water clarity and vegetation cover, several other environmental factors can influence the probability of detecting manatees. For the Florida manatee (*T. manatus latirostris*), environmental factors such as air and water temperatures, wind speed, and water depth have been found to affect the resting behavior of animals, and therefore surfacing intervals (Edwards et al., 2021). Depth has also been found to influence detection of Amazonian manatees in the wild (Rosas, 1994; Arraut et al., 2010; Marmontel et al., 2012). Movements and habitat use of the species correspond to the high and low-water seasons, showing a general preference for accessibility to deeper areas during both seasons (Best, 1984; Rosas, 1994). During the high-water season, Amazonian manatees will move into flooded forest areas (Best, 1984). During the low-water season, Amazonian manatees will relocate to perennial lakes or canals of deep water, which are typically blackwater (Best, 1984).

To address the challenges of directly detecting Amazonian manatees, researchers primarily rely on alternative methods to visual observations to advance scientific knowledge about the species' biology and conservation, such as community surveys (Hoffmann et al., 2021), indirect signs of presence (*i.e.,* feeding signals or feces; de Souza et al., 2021), active acoustics such as side-scan sonar (Gonzalez-Socoloske et al., 2009; Ruano et al., 2021; Gonzalez-Socoloske & Olivera-Gómez, 2023), and radio-tracking (Montgomery et al., 1981; Arraut et al., 2010; Landeo-Yauri et al., 2017; Guzmán Téllez, 2020). Indirect signs of Amazonian manatee presence, such as feeding signals, are easier to detect in comparison to directly detecting animals (Timm et al., 1986; de Souza et al., 2021). Side-scan sonar has become an effective tool for detecting manatees in murky waters (Gonzalez-Socoloske & Olivera-Gómez, 2023). However, there are several limitations of this method, including difficulties with interpreting resulting images to confirm manatee presence, avoidance and displacement behavior of manatees upon initially detecting the survey boat, and inaccessibility of habitats by boat (Machuca Coronado, 2015; Puc-Carrasco et al., 2016; Gonzalez-Socoloske & Olivera-Gómez, 2023). Much of the recent knowledge obtained about the species has stemmed from, or is supported by, research conducted through radio-tracking animals, and is spearheaded by local rescue, rehabilitation, and release centers. Radio-tracking and the other indirect observation methods described can offer practical, and somewhat inexpensive for the indirect methods, solutions to obtaining more information about the ecology of the Amazonian manatee. However, a lack of direct or physical sightings of animals using these methods can reduce the amount of information obtained that is critical to both scientists and rehabilitators to properly manage the species. For example, direct observations are useful to assess the body condition and behavior of animals (Guzmán Téllez, 2020). Additionally, many radio-tracking studies are limited by sample size, which reduces the amount of inferences that can be confidently made (Landeo-Yauri et al., 2017).

For aquatic species that spend a significant amount of time beneath the water's surface, the overhead perspective from observers onboard an aerial vehicle (hereafter 'occupied aerial surveys') increases the probability of directly detecting animals within the survey site (Hodgson, 2004). Occupied aerial surveys are used to obtain minimum population estimates and to acquire knowledge of the ecological requirements of the Florida manatee (Craig & Reynolds, 2004). However, occupied aerial surveys are not without their limitations, including high cost, safety concerns, and the inability to survey inaccessible habitats, which reduce their applicability in more remote areas, such as within the Amazon Basin (Sasse, 2003; Koski et al., 2009; Hodgson et al., 2013; Linchant et al., 2015). Furthermore, occupied aircraft are constrained to certain altitudes, can cause noise disturbance that may affect the surfacing behavior of animals, and are associated with high uncertainty in observer bias (Würsig et al., 1998; Patenaude et al., 2002).

Advancements in technology have been used to curtail many of the aforementioned challenges associated with traditional occupied aerial survey methods for detecting aquatic mammals. While traditionally used in military operations, drones or unoccupied aerial vehicles (UAVs) have become available within the last decade for commercial, recreational, and scientific purposes (Han et al., 2015). UAV systems have been adapted as tools by wildlife scientists and are increasingly used to research the movement, ecology, behavior, health, and habitat use for an array of aquatic organisms (Linchant et al., 2015; Raoult et al., 2020). Additionally, UAVs are more cost-effective and safer compared to occupied aerial surveys (Koski et al., 2009; Hodgson et al., 2017). Equipped with a variety of sensor types, UAVs can also collect high-resolution data that can be stored and analyzed for missed detections of animals during real-time surveys (*i.e.*, observer bias) (Marsh & Sinclair, 1989).

To date, researchers have used UAVs to study two species of sirenians, the dugong (*Dugong dugon*) and the West Indian manatee (*T. manatus*), including both subspecies of the latter, the Florida and Antillean manatees (*T. m. manatus*). Applications of UAVs in these studies have included monitoring the occurrence and behaviors of individuals (Hodgson et al., 2013; Ramos et al., 2018; Infantes et al., 2020; Landeo-Yauri et al., 2021), identifying individuals (Landeo-Yauri et al., 2020), determining body size and condition of individuals (Castelblanco-Martínez et al., 2021; Ramos et al., 2022), and estimating abundance (Edwards et al., 2021). However, these studies have primarily been conducted in clear, shallow waters. To the best of our knowledge, researchers have not yet assessed the use of a UAV to visually detect Amazonian manatees, but its potential use has been considered by researchers (de Souza et al., 2021). While their profile at the surface is greater than what is observed for sirenians, Oliveira-da-Costa et al. (2020) successfully used UAVs to detect two Amazonian dolphin species, the tucuxi (*Sotalia fluviatilis*) and the Amazon river dolphin (*Inia geoffrensis*), in the Brazilian Amazon despite low water transparency.

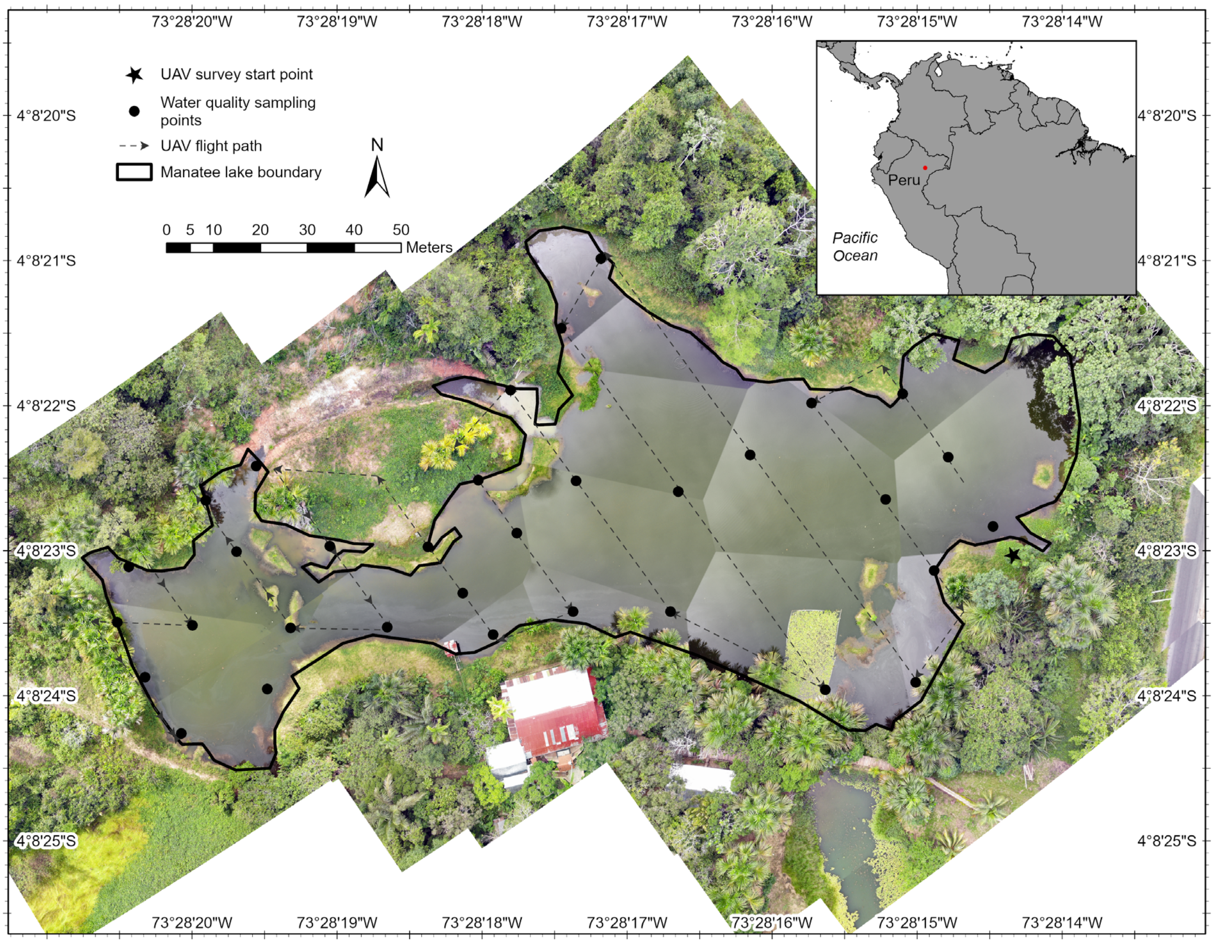

Figure 1. Unoccupied aerial vehicle (UAV) flight path and location of environmental sampling points at the study site, a closed man-made lake at the Rainforest Awareness, Rescue, and Education Center in Iquitos, Peru. The orthomosaic map created from UAV images is used as the basemap.

The goal of the present study was to evaluate the use of a UAV to detect Amazonian manatees by exploring the most influential environmental and survey covariates that are hypothesized to affect the time-to-detection and detection probability under controlled conditions at an *ex situ* soft release site. Studies that investigate detectability associated with new survey methods usually occur in the wild, where true occupancy and abundance are unknown at the survey time, but the area is known to have a high density of animals that can be reliably detected (*e.g.,* Hodgson et al., 2017). In this study, we evaluated the use of a UAV at an *ex situ* enclosed man-made lake with a known number of Amazonian manatees that was held constant throughout the research, given the rarity of detecting manatees in the wild. Similar to the natural habitats the species resides in, the manatees within the lake are only detectable near or at the surface due to the poor water clarity of the lake. To accomplish the goal of this study, we aimed to answer the following research questions: 1) How do environmental covariates (water depth, water transparency, wind speed, and cloud cover), time of day, and the surfacing behavior of manatees affect the time until a manatee is detected and the detection probability using a UAV?, 2) Under our survey conditions, what is the probability of detecting manatees using a UAV?, and 3) How many repeat UAV surveys are necessary to be 95% or 99% confident that true absence was obtained at the study site?

Material and Methods

Study site

This study was conducted over an enclosed man-made lake (4°08'22" S, 73°28'14" W) located at the Rainforest Awareness, Rescue, and Education Center (RAREC) in Iquitos, Peru, nestled within the Amazon Rainforest (Fig. 1). The lake mimics the natural environment of a blackwater oxbow lake, as visibility is greatly restricted. At RAREC, injured, orphaned, or illegally captured manatees are rehabilitated and then moved to the man-made lake, which serves as a soft-release site. After health and behavioral assessments indicate successful adaptation, these animals are released back into the wild. The lake is surrounded by various species of native vegetation that can be foraged upon by the manatees, including grasses. Manatees are also indirectly provided with a supplemental food source, water lettuce (*Pistia stratiotes*), to assist with their rehabilitation, confined to a feeding station within the lake. The true abundance was known at the study site, with five manatees of various ages, including three males (ages 8, 9-10, and 15) and two females (ages 5 and 15), residing in the lake during the study period. The study took place from 29 April - 16 May 2022, which corresponded to the end of the wet or high-water season at the study site, and included a period of high winds from the southwest referred to by the locals as the cold winds from San Juan.

UAV surveys

We used a DJI Mavic 2 Enterprise Dual quadcopter for all surveys, connected to an iPad tablet (Apple Inc.) to stream the live video feed during flights. We used noise-reducing propellers to reduce potential disturbance to manatees during flights. Additionally, we recorded and saved continuous videos of all flights to a memory card using the onboard visual sensor mounted with a three-axis gimbal for stabilization. The sensor has a video resolution of 4K (3840 x 2160 pixels) and a frame rate of 24 frames per second.

The drone pilot first delineated the study area by manually flying the UAV and marking the boundaries of the lake using the DJI Ground Station Pro (DJI GS Pro) v.2.0.15 (DJI, 2021) flight planning application. Transects within the boundary were then created using the DJI GS Pro application by specifying an 80% front and side overlap, a flight height of 60 meters above the lake, a shooting angle parallel to the main path, and a course angle of 126°. These parameters resulted in 11 line transects that covered an area of 9,800 m^2, encompassing the entirety of the lake (Fig. 1). The sum of the length of the transects was 853 m. We selected a flight altitude of 60 meters to avoid disturbing the manatees while allowing us to also observe behaviors as the UAV sensor cannot zoom (Landeo-Yauri et al., 2021). Specifying an 80% front and side overlap resulted in approximately 16 m between transect lines and was used to compensate for sun glint that could obstruct the detection of animals and to maximize the chances of detecting animals with low surfacing rates (Finkbeiner et al., 2001; Hodgson et al., 2013). Additionally, for all surveys, the drone pilot manually maintained a speed of approximately 2.0 m/s. These flight parameters are conservative to increase the probability of detecting the manatees.

All surveys began at the same starting location on the east side of the lake, near where manatee caretakers provide the supplemental food source (Fig. 1). It is important to note that we did not conduct surveys when the caretakers were replenishing the supplemental food source. Additionally, we excluded two surveys due to the presence of caretakers in or on the water. We designed two survey types using the same flight path described above to address the research questions. Surveys of type 'short' consisted of manually flying one pass over the lake following the predesigned flight path, averaging six minutes and 51 seconds of flight time. Surveys of type 'long' consisted of manually flying the predesigned flight path repeatedly and continuously for 60 minutes for surveys conducted on 3 – 7 May and 40 minutes for all other survey dates or until the UAV pilot opportunistically detected a manatee on the live video feed to maximize the number of flights conducted. Detection effort based on detection via the live video feed versus during the review of the video footage post-flights is not considered in this study, as efforts to detect animals via the live feed were opportunistic and varied significantly among surveys due to having a single observer.

Each drone battery lasts approximately 20 minutes under our flight conditions, and the batteries were quickly changed if the duration of the flight lasted longer than the life of a single battery after landing the UAV. A total of three batteries were used for long surveys conducted on 3 – 7 May, and a maximum of two batteries were used for all other survey dates. The drone pilot promptly continued the flight from the stopping point during long surveys that exceeded the use of a single battery. Both short and long surveys were conducted to increase the robustness of the response variables (time-to-detection and detection/non-detection) for data analyses. For example, long surveys are unlikely to result in missed detections due to the selected survey time and terminating the survey after making an opportunistic sighting via the live video-feed, as well as increasing the chances of detecting more than one animal. By conducting short surveys, we were also able to increase the sample size with minimal effort. We included both short and long surveys in the study design since, for many researchers, it might not always be feasible to use multiple batteries due to financial reasons or safety concerns regarding launching and landing the UAV. Most cost-conscious commercially available UAVs have a battery life less than the aerobic dive limit of manatees, and the inclusion of short surveys accounts for this.

To maximize the total number of surveys performed, we conducted surveys as often as possible during the daytime (from 06:30 h to 18:00 h), except when raining, and the survey type (short vs. long) was performed opportunistically in no particular order. To consider each repeat survey conducted per day as independent from one another, we waited at least 25 minutes between surveys. We selected the wait period between surveys per day based on the estimated aerobic dive limit, which is between 19 to 22 minutes for Amazonian manatees (Gallivan et al., 1986). We conducted a total of 106 surveys, including 47 short surveys and 59 long surveys. We aimed to conduct the same total number of short and long surveys, as well as the number of surveys across different times of day (morning, afternoon, and evening). However, towards the end of the study, we aimed to maximize the total number of surveys performed by conducting long surveys when no rain was forecasted, which resulted in variability in the number of each type of survey per time of day. The total number of surveys conducted also depended on the day, with a mean number of surveys conducted per day of 4.70 (SD = 2.57, min = 1, max = 8) and 4.92 (SD = 2.36, min = 1, max = 9) for short and long surveys, respectively. Of the short surveys, 23 were conducted during morning hours (06:30–12:00), 17 during the afternoon (12:00–17:00), and seven in the evening (17:00–18:00). For long surveys, 21 were conducted in the morning, 34 in the afternoon, and four in the evening.

Environmental sampling

We used an unmotorized canoe to navigate around the lake to measure water depth (m) and water transparency (m) at three points, the two water edges and the midpoint, along each of the 11 line transects that made up the UAV flight path (n = 33) (Fig. 1). Water depth was measured at each of the 33 sampling points using a handheld depth finder, DepthTrax 1H, which was inserted approximately 0.04 m into the water column, perpendicular to the surface of the water. We measured water transparency using a Secchi disk and averaged the depths at which: 1) the user lost sight of the disk as it was inserted into the water column on the shaded side of the canoe to minimize the effect of the sun on measurements, and 2) the depth when the user was able to see the disk reemerge at each of the sampling points (Tyler, 1968). We took these measurements once per survey day, but repeat surveys were conducted within a day following rain and prior to subsequent UAV surveys. As the lake is man-made and enclosed, rain is the only factor significantly impacting water depth and water transparency.

We uploaded all flight logs to the online application, Airdata™ UAV (www.airdata.com), to retrieve weather conditions for each survey, including percent cloud cover and wind speed (mps). Historical weather data used by Airdata™ comes from Dark Sky, providing hyperlocal weather forecasts down to 0.001° of latitude and longitude (Apple Inc.). This application is often used by UAV pilots to determine if weather conditions are suitable for flying. These variables are recorded as a single value for each battery used in a survey. Therefore, if multiple batteries were used in a long survey, we recorded the average percent cloud cover and wind speed.

UAV survey video processing

An experienced UAV observer reviewed all resulting video footage in the raw MP4 format from the UAV surveys to reduce observer bias, irrespective of whether a manatee was opportunistically detected during a survey. For each survey, we noted the start and stop times so that only the times when the UAV was flying the transects were included in analyses to calculate survey effort and the time-to-detection. Due to poor water clarity, flight altitude, and the proportion of the body a manatee displayed when surfacing, we were unable to confidently identify unique individuals. Therefore, we defined independent detections as those when an animal was detected and then observed submerging prior to subsequent detections. For example, a manatee milling at the surface, and thus detectable multiple times throughout the survey due to the large overlap between transects, was only included in the analyses as the time it took to first detect the animal, whereas an animal that briefly surfaced to breathe followed by immediately submerging could be redetected within a given survey.

When reviewing the video footage from surveys, we also broadly categorized surfacing behaviors as either breathing, milling, or foraging and noted any behaviors that could indicate that the manatees were disturbed by the UAV. Breathing was categorized as an animal briefly surfacing followed by immediately submerging. Milling was categorized as an animal swimming either at the surface or just beneath it, so that the outline of the animal was still visible. Foraging was categorized as either an animal observed directly consuming vegetation or an animal that was stationary and facing vegetation, but direct consumption of the vegetation was not observed. The observer manually and repeatedly reviewed the video footage until no new detections were made. The time it took to review a single video was estimated to be twice as long as the survey duration with exceptions due to weather conditions affecting visibility. Although other species were present within the lake, including various species of fish and turtles, none can be easily mistaken for a manatee, which improves our confidence of each

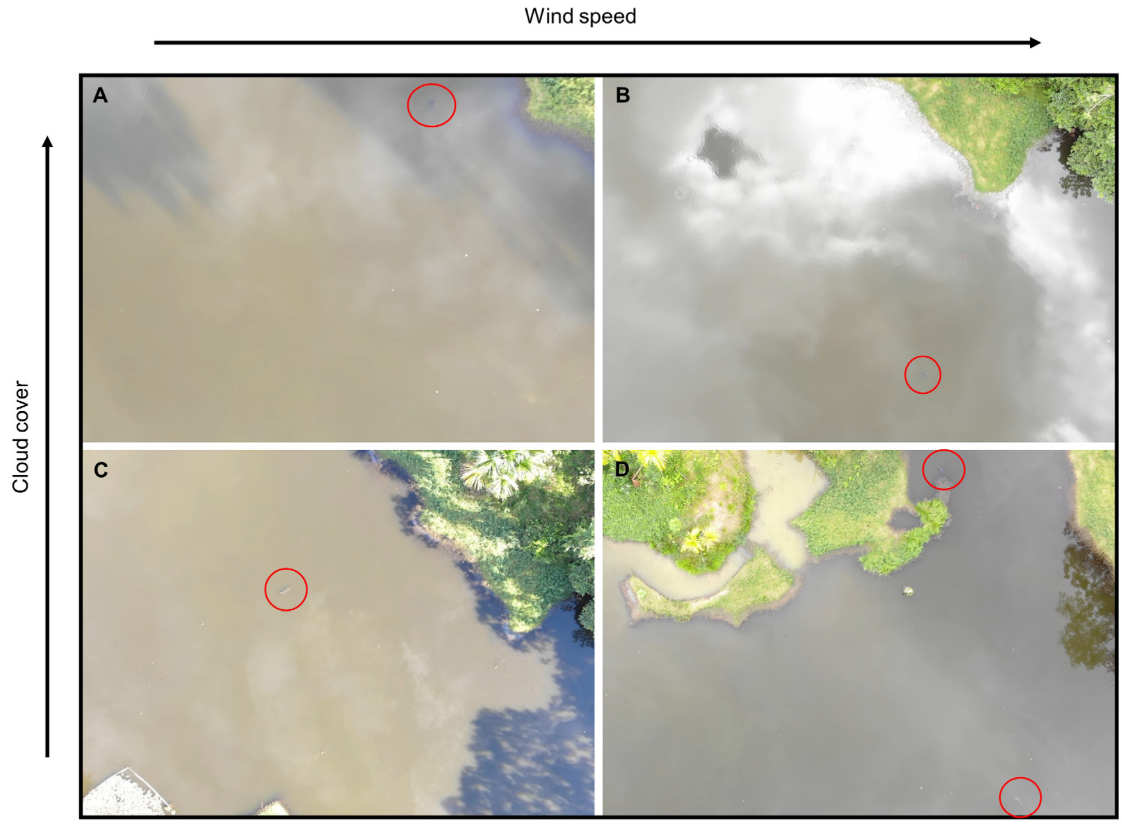

Figure 2. Example detections of Amazonian manatees (*Trichechus inunguis*) under various cloud covers and wind speeds. Manatee detections are circled in red, where A) survey ID: 57, wind speed: 0.54 mps, cloud cover: 98%, B) survey ID: 61, wind speed: 1.7 mps, cloud cover: 90%, C) survey ID: 76, wind speed: 0.67 mps, cloud cover: 62%, D) survey ID: 28, wind speed: 1.12 mps, cloud cover: 77%.

Table 1. Parameter estimates of the optimal (global) generalized linear mixed-effect model results assessing the time-to-detect *ex situ* Amazonian manatees (*Trichechus inunguis*) using an unoccupied aerial vehicle. All continuous covariates are scaled.

Model parameters		Estimate	Standard error	*p* value	95% CI
Intercept		5.93	0.14	< 0.05	5.65, 6.21
Cloud cover		0.023	0.0037	< 0.05	0.016, 0.030
Observed behavior					
(Reference: Breathing)	Foraging	0.33	0.0097	< 0.05	0.32, 0.35
	Milling	-0.12	0.0079	< 0.05	-0.13, -0.10
Survey effort		0.59	0.0043	< 0.05	0.58, 0.60
Time of day					
(Reference: Afternoon)	Morning	0.23	0.0048	< 0.05	0.22, 0.24
	Evening	0.061	0.0078	< 0.05	0.046, 0.076
Water depth		0.0019	0.0029	0.53	-0.0039, 0.0076
Water transparency		-0.019	0.0051	< 0.05	-0.029, -0.0086
Water depth*water transparency		0.032	0.0024	< 0.05	0.028, 0.037
Wind speed		-0.065	0.0036	< 0.05	-0.073, -0.058

detection. Arapaima (*Arapaima gigas*) are the most similar to manatees in terms of size and appearance. However, this fish species exhibits significantly different surfacing behaviors (full body visible and increased movement at the surface due to side-to-side tail movement), and individuals at the study site had a red coloration indicative of the reproductive period. These characteristics helped to avoid misidentification with manatees.

All detections were plotted onto a high-resolution orthomosaic map (Fig. 1) to assign them to the nearest transect. The orthomosaic map was created by stitching together overlapping photos of the delineated transects using ArcGIS Pro v.2.7.0 (ESRI, 2020). Detections were then plotted by scaling the orthomosaic map to match the video and calculating the angle and distance from fixed features on the map. Each detection was subsequently assigned to the nearest transect using the Near tool in the Analysis toolbox.

Data analyses

We omitted six long surveys and one short survey from data analyses. The short survey was omitted due to the corruption of the video file that inhibited post-survey review. Six long surveys were omitted due to the presence of caretakers in or on the water (n = 2), significant differences in flight altitudes (n = 2), and flights conducted prior to measuring water depth and water transparency that were followed by rain (n = 2). All surveys used in analyses are provided in Supplementary Material Table S1.

To assess the effects of our covariates, including water depth, water transparency, wind speed, cloud cover, time of day, and surfacing behavior on the time-to-detection (seconds) for each manatee that met our criteria of detection within a survey in both short and long surveys, we constructed generalized linear mixed-effect models (GLMMs) (Bolker et al., 2009) in the Poisson family using the glmer function in the lme4 package (Bates et al., 2015) in R, v.4.2.2 (R Core Team, 2022). We first fitted a global model with fixed effects for the abovementioned covariates, and all continuous covariates were scaled for standardization. We included survey effort (total survey duration in seconds) as a fixed effect, which allowed us to pool data from both short and long surveys without biasing the results due to the longest survey time for short surveys being restricted to approximately seven minutes. We also specified an interaction between water depth and water transparency. To account for temporal autocorrelation due to repeat surveys per day, we specified the survey date as a random effect. To account for spatial autocorrelation within each survey, the nearest transect to each detection location was included as a nested random effect within date. We investigated the potential for collinearity among fixed effects by calculating their variance inflation factor (VIF) within the global model (Zuur et al., 2010). All fixed effects had a VIF < 2 and were therefore retained in the models. We tested the global model for overdispersion, which was not evident (*p* value = 0.74). Model selection was then performed based on the second order Akaike's Information Criterion (AIC) using a backwards-stepwise approach with the drop1 function in the lme4 package in R (Burnham & Anderson, 2002). Only the models that had an AIC difference (Δi) < 10 are reported. Models with Δi > 10 have essentially no support and are not considered further (Burnharm & Anderson, 2002).

To account for imperfect detections, we also estimated the detection probability for all surveys (short and long; n = 85) where a single pass over the lake was made using Bayesian single-species and single-season detection models (Doser et al., 2022). We omitted 13 surveys of type long and one of type short from the analysis, as these surveys were terminated prior to completing the flight path over the lake due to quickly detecting a manatee. We truncated the remaining videos of long surveys to equate to a singular pass over the lake (averaging six minutes and 51 seconds). We transfigured our data into binomial data by assigning a value of '1' to a particular survey if at least one manatee was detected and '0' if no manatees were detected after completing one pass over all 11 transects.

To generate the detection history, we specified one site and 85 sampling occasions or, in terms of the matrix, one row and 85 columns. We used the mean of the values recorded for water depth and water transparency per survey day, which was separated by pre- and post-rain measurements if applicable, and the recorded values of wind speed and cloud cover per

Table 2. Comparison of Bayesian models for estimating the probability of detecting *ex situ* Amazonian manatees (*Trichechus inunguis*) using an unoccupied aerial vehicle. Model names are written based on lme4 syntax (Bates et al., 2015). All models had a random effect of date (1|Date), a fixed effect of survey effort, and an interaction term between water depth and water transparency (water depth*water transparency). Models (m.1 - m.14; global model (m.g); and null model (m.null)) were ranked using the Widely Applicable Information Criterion (WAIC) with a small value indicating a better fit (Gelman et al., 2014), ΔWAIC indicates the difference of WAIC between the best model and the model under consideration, weight indicates the Akaike weight or the probability that the model under consideration is the best model, and evidence ratios (*wi/wj*). We also provide the Bayesian *p* value indicating model fit and the average estimated detection probability and their associated 95% confidence intervals.

Model ID	Detection probability model	WAIC	ΔWAIC	Weight	Evidence ratios	Bayesian *p* value	Detection probability	95% CI
m.1	(1|Date) + water depth*water transparency + survey effort	115.29	0.00	0.14	1.00	0.48	0.62	0.23, 0.94
m.null	~1	115.48	0.18	0.13	1.10	0.48	0.62	0.52, 0.72
m.2	(1|Date) + cloud cover + water depth*water transparency + time of day + survey effort	116.39	1.10	0.08	1.73	0.47	0.62	0.19, 0.97
m.3	(1|Date) + cloud cover + water depth*water transparency + survey effort	116.45	1.15	0.08	1.78	0.47	0.62	0.21, 0.96
m.4	(1|Date) + wind speed + survey effort	116.76	1.46	0.07	2.08	0.47	0.62	0.31, 0.91
m.5	(1|Date) + time of day + water depth*water transparency + survey effort	116.79	1.49	0.07	2.11	0.47	0.62	0.21, 0.96
m.6	(1|Date) + wind speed + cloud cover + water depth*water transparency + survey effort	116.80	1.51	0.07	2.13	0.46	0.62	0.19, 0.96
m.7	(1|Date) + wind speed + water depth*water transparency + survey effort	117.07	1.78	0.06	2.43	0.47	0.62	0.21, 0.95
m.g	(1|Date) + wind speed + cloud cover + water depth*water transparency + time of day + survey effort	117.17	1.88	0.06	2.56	0.46	0.62	0.17, 0.98
m.8	(1|Date) + time of day + survey effort	117.35	2.05	0.05	2.79	0.47	0.62	0.30, 0.93
m.9	(1|Date) + cloud cover + survey effort	117.49	2.19	0.05	3.00	0.47	0.62	0.31, 0.91
m.10	(1|Date) + wind speed + water depth*water transparency + time of day + survey effort	117.49	2.20	0.05	3.00	0.47	0.62	0.19, 0.97
m.11	(1|Date) + wind speed + time of day + survey effort	117.60	2.31	0.04	3.17	0.49	0.62	0.27, 0.94
m.12	(1|Date) + wind speed + cloud cover + survey effort	118.69	3.40	0.03	5.46	0.47	0.62	0.28, 0.92
m.13	(1|Date) + cloud cover + time of day + survey effort	118.80	3.51	0.02	5.78	0.47	0.62	0.28, 0.94
m.14	(1|Date) + wind speed + cloud cover + time of day + survey effort	120.50	5.21	0.01	13.53	0.47	0.62	0.26, 0.95

survey as detection covariates. We assessed collinearity among fixed effects using the Spearman correlation coefficient, and r ≥ 0.6 indicated collinearity (Dormann et al., 2013). If variables were found to be correlated, we excluded the biologically less relevant variable. All continuous covariates used in candidate detection probability models were scaled for standardization.

For all models, occupancy (ψ) was null (intercept-only), and we included a random effect of survey date to account for temporal autocorrelation. Similar to GLMMs, we specified an interaction between water depth and water transparency when these covariates were included in a candidate model. Additionally, all candidate models included effort, which is defined as the time it took to complete a single pass over the lake following the transects, as another detection covariate. Bayesian single-species and single-season detection models were constructed using the function PGOcc in the spOccupancy package (Doser et al., 2022) in R, v.4.2.2 (R Core Team, 2022). This package uses Pólya-Gamma data augmentation (Polson et al., 2013) and Markov chain Monte Carlo (MCMC) algorithms for increased computational efficiency and allows for the inclusion of random effects and interaction terms following the lme4 syntax (Bates et al., 2015; Doser et al., 2022). We ran three Markov chains with 15,000 iterations with a burn-in of 3,000 and a thin rate of two, giving 3,000 posterior samples. We assessed convergence of the models using the Gelman-Rubin statistic ($\hat{R}$), with $\hat{R} < 1.10$ indicating adequate mixing (Gelman & Rubin, 1992) and by examining the trace plots. All models showed adequate mixing (Supplementary Material Fig. S1).

Model fits were assessed using posterior predictive checks with the function ppcOcc in the spOccupancy package (Doser et al., 2022). Bayesian *p* values > 0.05 indicated adequate fit. Since we only had one site, model selection was performed using the Widely Applicable Information Criterion (WAIC) to identify the best-performing model (Watanabe, 2010). We also calculated the Akaike weight and evidence ratios to compare the models and determine the probability that a particular model is the best (Burnham & Anderson, 2002). The average detection probability estimated by the optimal model was then used to calculate the number of passes over our study area needed to be 95% and 99% confident in the presence/absence of an Amazonian manatee that is undetectable until surfacing using the following equation (Reed, 1996; Kéry, 2002):

$$N_{min} = \log(1-F)/\log(1-p)$$

where N_{min} is the minimum number of flights, using the methods described above, over the area of interest, F is the percent confident (95% or 99%), and p is the average detection probability.

Results

Environmental sampling

Water depth values measured at 33 points across the lake for all survey occasions ranged from 0.25 m to 2.30 m (M = 1.01 m, SD = 0.39 m). The average depth of the entire lake per survey

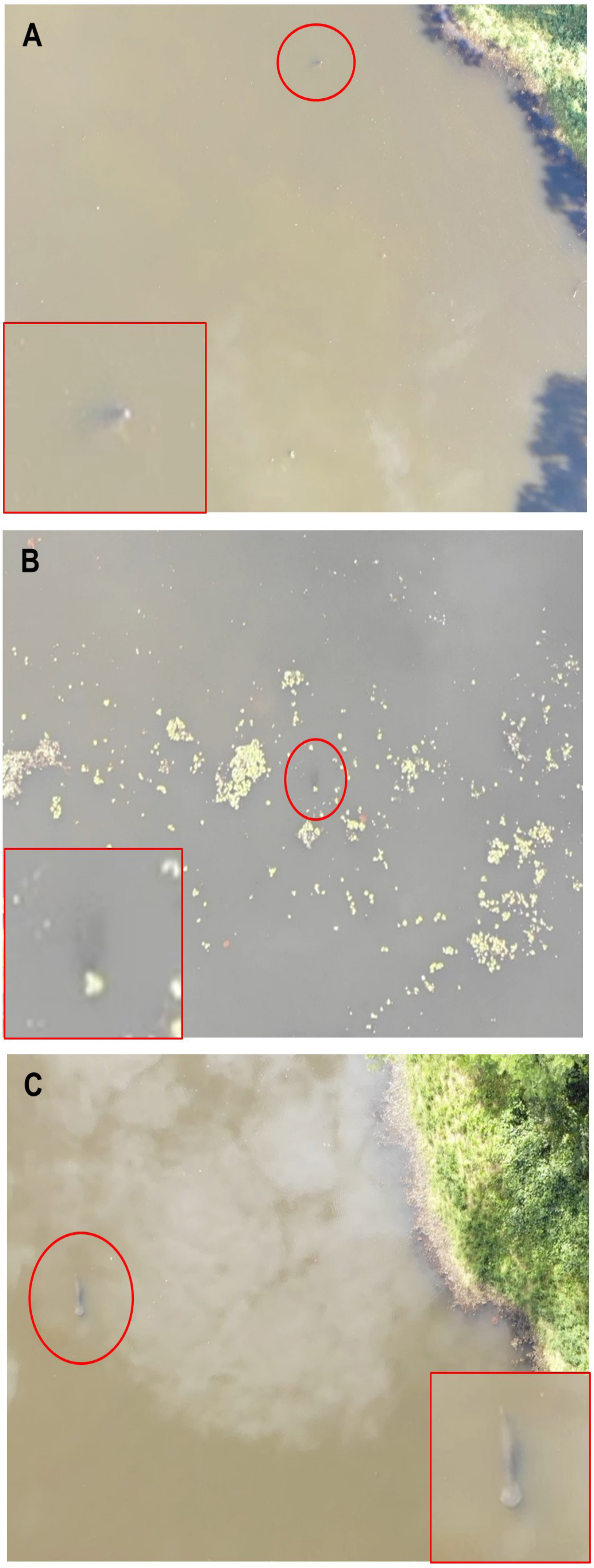

Figure 3. Example detections of Amazonian manatees (*Trichechus inunguis*) based on the categorized behaviors. Manatee detections are circled in red, where A) behavior: breathing, survey ID: 76, wind speed: 0.67 mps, cloud cover: 62%, B) behavior: foraging, survey ID: 80, wind speed: 0.54 mps, cloud cover: 76%, and C) behavior: milling, survey ID: 9, wind speed: 1.15 mps, cloud cover: 95.33%. For each detected behavior (3A-C), an inset zoomed-in image is provided in the red rectangles.

occasion was 1.01 m (SD = 0.05 m), with a minimum average depth of 0.94 m and a maximum average depth of 1.12 m. Water transparency ranged from 0.25 m to 0.95 m across the lake (M = 0.60 m, SD = 0.11 m). The average water transparency of the entire lake per survey occasion was 0.60 m (SD = 0.05 m), with a minimum average water transparency of 0.46 m and a maximum average depth of 0.66 m.

Factors influencing proportion of time until a unique individual was detected

For easier and more meaningful interpretation of the results, we describe time-to-detection in minutes rather than reporting as it was measured (in seconds). Across all surveys, we made a total of 374 detections. For short surveys, the average time-to-detection was 1.6 minute (SD = 1.17), constituting 22% of the average total survey duration for all short surveys. For long surveys, the average time-to-detection was 20 minutes (SD = 15.91), constituting 53% of the average total survey duration for all long surveys. Examples of manatee detections under various wind speeds and cloud covers are presented in Fig. 2. In all surveys, both short and long, breathing was the most frequently observed surfacing behavior (79%), followed by milling (14%), and foraging (7%). Examples of these behaviors are presented in Fig. 3. There was a single occurrence of an obvious behavioral indicator of disturbance, where one individual was observed rapidly submerging while the UAV was approaching following the transects.

The optimal model was the global model, with an AIC of 160830, while the next best model had a Δi of 37 when dropping cloud cover from the global model (Supplementary Material Table S2). All covariates were significant in the global model except for the fixed effect of water depth, which had an insignificant direct effect on the time-to-detection (Table 1). Water depth was also the only parameter in the global model with 95% confidence intervals overlapping zero, indicating that no reliable conclusions can be made (Table 1). The time-to-detection significantly increased as cloud cover increased (Table 1). Conversely, there was a significant inverse relationship between the time-to-detection and wind speed as well as water transparency (Table 1). It took significantly less time to detect manatees milling at the surface compared to detecting manatees that were foraging (Table 1; Fig. 4). In comparison to surveys conducted in the afternoon, more time was required to detect manatees during morning or evening hours (Table 1; Fig. 4).

Detection probability

All covariate pairs had a Spearman correlation coefficient < 0.6 and were therefore retained in the models. Posterior predictive checks indicated good model fits for all candidate models (Table 3). The probability of detecting at least one individual when making one pass over the lake across all survey dates using the null model was 0.62 (95% CI = 0.52, 0.72) (Table 2). Based on the WAIC, the most parsimonious model above the null model showed an indirect effect of water depth and direct effects of water transparency and effort on the probability of detecting a manatee using a UAV (Table 3). When investigating the two-way interaction between water depth and water transparency on the probability of detecting a manatee using a UAV in this

Table 3. Detection coefficient estimates for all candidate models (m.1 - m.14; global model (m.g); and null model (m.null)) in Table 2. Occupancy was set to null in all models. Model parameters are written in lme4 syntax (Bates et al., 2015), where a random effect of date is specified by (1|Date) and an interaction term between water depth and water transparency is specified by (water depth*water transparency). We provide the mean, standard deviation (SD), and 95% confidence intervals (CI) for the model parameters as well as the Gelman-Rubin diagnostic ($\hat{R}$) and the effective sample size of the posterior samples to assess convergence. All continuous covariates were scaled.

Model ID	Model parameters		Mean	SD	95% CI	$\hat{R}$	Effective sample size
m.1							
	Intercept		0.37	0.33	-0.26, 1.0	1.0166	1502
	Water depth		-0.29	0.33	-0.97, 0.36	1.0024	2046
	Water transparency		0.76	0.47	-0.14, 1.7	1.0033	2584
	Survey effort		0.11	0.31	-0.52, 0.75	1.0003	2703
	Water depth*water transparency		-0.65	0.35	-1.3, -0.007	1.0013	2412
	(1|Date)		0.39	0.54	0.043, 1.7	1.0441	467
m.null							
	Intercept		0.50	0.22	0.071, 0.93	1.0038	3000
m.2							
	Intercept		0.33	0.47	-0.59, 1.3	1.0034	1449
	Time of day						
	(Reference: Morning)	Afternoon	-0.09	0.51	-1.1, 0.90	1.0	2982
		Evening	1.11	0.88	-0.57, 2.9	1.0045	2619
	Cloud cover		0.30	0.34	-0.34, 1.0	1.0027	1276
	Water depth		-0.33	0.39	-1.1, 0.40	1.0067	1295
	Water transparency		0.93	0.53	-0.062, 2.0	1.001	1845
	Survey effort		0.14	0.33	-0.52, 0.80	1.0034	2562
	Water depth*water transparency		-0.69	0.38	-1.5, 0.019	1.0027	1900
	(1|Date)		0.67	1.09	0.045, 3.2	1.0089	395
m.3							
	Intercept		0.41	0.36	-0.26, 1.2	1.0055	1209
	Water transparency		0.81	0.51	-0.18, 1.8	1.0033	1837
	Water depth		-0.38	0.38	-1.2, 0.33	1.0003	1154
	Cloud cover		0.26	0.34	-0.37, 0.97	1.0046	1472
	Survey effort		0.08	0.32	-0.55, 0.70	1.0011	2650
	Water depth*water transparency		-0.68	0.37	-1.5, 0.0057	1.0011	1924
	(1|Date)		0.63	0.85	0.046, 2.9	1.0226	401
m.4							
	Intercept		0.61	0.31	0.035, 1.3	0.9999	1285
	Wind speed		-0.14	0.30	-0.79, 0.38	1.0038	1168
	Survey effort		0.28	0.29	-0.28, 0.86	1.0001	2392
	(1|Date)		0.53	0.72	0.041, 2.5	1.0169	473
m.5							
	Intercept		0.34	0.44	-0.53, 1.2	1.0005	1935
	Water transparency		0.87	0.51	-0.13, 1.9	1.0143	2206
	Water depth		-0.24	0.35	-0.94, 0.44	1.0021	1859
	Time of day						
	(Reference: Morning)	Afternoon	-0.10	0.50	-1.1, 0.88	1.001	2644
		Evening	0.97	0.82	-0.59, 2.6	1.0065	3000
	Survey effort		0.16	0.33	-0.48, 0.82	1.005	2538
	Water depth*water transparency		-0.66	0.37	-1.4, 0.023	1.009	2304
	(1|Date)		0.48	0.57	0.046, 2.1	1.0309	528
m.6							
	Intercept		0.40	0.38	-0.29, 1.2	1.0015	1412
	Wind speed		-0.33	0.34	-1.0, 0.29	1.0159	1209
	Cloud cover		0.28	0.36	-0.40, 1.0	1.0134	1215
	Water depth		-0.39	0.41	-1.2, 0.40	1.0159	1377
	Water transparency		0.95	0.54	-0.075, 2.1	1.0018	2040
	Survey effort		0.12	0.33	-0.54, 0.75	1.0137	2664
	Water depth*water transparency		-0.70	0.38	-1.5, 0.018	1.0099	2035
	(1|Date)		0.83	1.13	0.043, 4.2	1.0401	382

Model ID	Model parameters		Mean	SD	95% CI	R̂	Effective sample size
m.7							
	Intercept		0.39	0.35	-0.25, 1.1	1.0056	1322
	Wind speed		-0.28	0.33	-0.99, 0.32	1.0063	1520
	Water transparency		0.89	0.51	-0.11, 1.9	1.0142	2268
	Water depth		-0.31	0.38	-1.1, 0.39	1.0047	1310
	Survey effort		0.15	0.33	-0.50, 0.82	1.0014	2789
	Water depth*water transparency		-0.67	0.36	-1.4, 0.0043	1.0143	2284
	(1|Date)		0.61	0.91	0.042, 3.0	1.0142	451
m.g							
	Intercept		0.34	0.49	-0.58, 1.3	1.0104	1533
	Wind speed		-0.28	0.35	-1.0, 0.39	1.005	1215
	Cloud cover		0.35	0.36	-0.32, 1.1	1.0162	1487
	Water depth		-0.37	0.41	-1.2, 0.43	1.0043	1126
	Water transparency		1.07	0.57	-0.030, 2.2	1.0089	1976
	Time of day						
	(Reference: Morning)	Afternoon	-0.04	0.52	-1.1, 1.0	1.0007	3000
		Evening	1.06	0.87	-0.60, 2.8	0.9996	2687
	Survey effort		0.17	0.35	-0.52, 0.84	1.0035	2524
	Water depth*water transparency		-0.72	0.40	-1.5, 0.016	1.0052	1856
	(1|Date)		0.88	1.21	0.050, 4.29	1.0422	402
m.8							
	Intercept		0.62	0.40	-0.13, 1.4	1.0034	1924
	Time of day						
	(Reference: Morning)	Afternoon	-0.19	0.47	-1.2, 0.72	1.0041	3000
		Evening	0.83	0.80	-0.71, 2.5	1.0006	2830
	Survey effort		0.30	0.29	-0.25, 0.88	1.0026	2535
	(1|Date)		0.38	0.44	0.041, 1.6	0.9996	617
m.9							
	Intercept		0.61	0.30	0.031, 1.2	1.0047	1052
	Cloud cover		0.06	0.29	-0.50, 0.64	1.0001	1707
	Survey effort		0.27	0.28	-0.27, 0.82	1.0028	2338
	(1|Date)		0.48	0.59	0.047, 2.2	1.006	506
m.10							
	Intercept		0.33	0.46	-0.53, 1.3	1.0039	1950
	Wind speed		-0.26	0.34	-0.96, 0.35	1.004	1432
	Time of day						
	(Reference: Morning)	Afternoon	-0.03	0.52	-1.0, 1.0	1.0001	3000
		Evening	0.95	0.85	-0.66, 2.7	1.0071	2846
	Water depth		-0.26	0.38	-1.1, 0.45	1.0131	1130
	Water transparency		0.98	0.53	0.0036, 2.1	1.008	2303
	Survey effort		0.20	0.34	-0.47, 0.88	1.001	3156
	Water depth*water transparency		-0.68	0.38	-1.5, 0.025	1.0003	2339
	(1|Date)		0.67	0.85	0.049, 3.2	1.0269	495
m.11							
	Intercept		0.65	0.41	-0.12, 1.5	1.0059	1814
	Wind speed		-0.08	0.31	-0.74, 0.49	1.0015	1203
	Time of day						
	(Reference: Morning)	Afternoon	-0.19	0.48	-1.1, 0.73	1.0037	3000
		Evening	0.79	0.81	-0.72, 2.5	1.0004	2801
	Survey effort		0.30	0.29	-0.25, 0.90	1.002	1978
	(1|Date)		0.57	0.81	0.044, 2.8	1.0334	446
m.12							
	Intercept		0.63	0.35	-0.015, 1.4	1.0084	1123
	Wind speed		-0.16	0.31	-0.81, 0.40	1.0023	1243
	Cloud cover		0.07	0.31	-0.52, 0.70	1.0019	1626
	Survey effort		0.26	0.29	-0.31, 0.83	1.0017	2204
	(1|Date)		0.72	1.10	0.048, 3.4	1.0298	412
m.13							
	Intercept		0.62	0.41	-0.18, 1.4	1.007	1633
	Cloud cover		0.10	0.29	-0.46, 0.68	1.0017	2142
	Time of day						

Model ID	Model parameters		Mean	SD	95% CI	R̂	Effective sample size
	(Reference: Morning)	Afternoon	-0.19	0.49	-1.2, 0.76	1.0013	3000
		Evening	0.86	0.80	-0.67, 2.5	1.0045	2611
	Survey effort		0.29	0.28	-0.27, 0.87	1.0059	2588
	(1|Date)		0.43	0.54	0.040, 1.7	1.0448	582
m.14							
	Intercept		0.63	0.43	-0.20, 1.5	1.0007	1653
	Wind speed		-0.09	0.32	-0.79, 0.49	1.0195	1233
	Cloud cover		0.12	0.31	-0.46, 0.75	1.0015	1827
	Time of day						
	(Reference: Morning)	Afternoon	-0.18	0.49	-1.1, 0.76	1.0067	3000
		Evening	0.81	0.83	-0.76, 2.6	1.0011	2438
	Survey effort		0.29	0.29	-0.26, 0.86	1.0023	2375
	(1|Date)		0.63	0.86	0.045, 2.9	1.029	445

model, a significant indirect effect was observed (Table 3). This model resulted in an average detection probability of 0.62 (95% CI = 0.23, 0.94) (Table 2). Based on the evidence ratios, all models had some support (Table 2). All candidate models provided similar parameter estimates for the interaction between water depth and water transparency when present in the model (Table 3). All models showed a decrease in the probability of detecting a manatee when wind speeds were high (Table 3). Conversely, there was a greater probability of detecting a manatee under greater cloud cover and in the evening compared to UAV surveys conducted in the morning or afternoon (Table 3). However, all parameter estimates within all models have 95% confidence intervals that overlap zero, except for the interaction between water depth and water transparency, indicating that no reliable conclusions can be drawn from these parameters within the models (Table 3). For the significant interaction between water depth and water transparency, the models showed that when water depth is greater, there is a lower probability of detecting manatees where there is also low water transparency (Fig. 5). As water transparency increases, the effect of depth decreases, and there is a higher probability of detecting manatees, illustrated by a decrease in the separation between the minimum, mean, and maximum depths (Fig. 5).

The average detection probability across all candidate models was 0.62, which is equivalent to that of the null model (Table 2). Using this average detection probability, we estimated the number of repeat surveys required to have a 95% chance of establishing true absence at our survey site was n = 3.10, and n = 4.76 to have a 99% chance.

Discussion

Overall, our results identified the factors that affected the detectability of Amazonian manatees using a UAV in difficult-to-detect environments, such as where visibility is restricted to at or near the water's surface. We provided the first estimate of detection probability (p = 0.62; 95% CI = 0.52, 0.72) associated with using a UAV to detect at least one of the five Amazonian manatees at our study site and under our flight conditions. Additionally, we calculated that approximately three and five repeat surveys are necessary to have a 95% and 99% chance, respectively, of establishing true absence at our study site. The results of this study are the precursors to determining the effort needed to reliably detect Amazonian manatees using a UAV in complex *in situ* habitats where they go undetected due to availability bias. For *in situ* applications, the results of this study can be interpreted as the minimum effort required to detect Amazonian manatees in areas where the animals are confined to an area. In the wild, confinement could refer to foraging sites or relatively enclosed deep areas within a waterbody, including lakes and sections of rivers, during the low-water season or periods of droughts when the aquatic area becomes reduced due to the loss of connectivity with rivers, greatly restricting the movements of Amazonian manatees (Arraut et al., 2010). In this context, UAVs could be used to survey deep-water sites used by Amazonian manatees that are no longer accessible by boats during the low-water season. For reference, the density at our study site was 0.00051 manatee/m^2. Furthermore, based on our results, we also suggest that UAVs could be useful for surveying small areas of interest, pre-release monitoring, or when paired with other methods, such as telemetry or side-scan sonar.

Changes in environmental conditions at a study site can affect the detection of manatees due to variations in surfacing intervals or the time animals spend on the surface or bottom (Edwards et al., 2021). The results of our GLMMs and detection models agreed that the interaction between water depth and water transparency significantly impacts the ability of a UAV to reliably detect Amazonian manatees at our study site. We expected water depth and water transparency to influence time-to-detection and detection probability as they affect the proportion of time an animal is visible, which has been shown when conducting occupied aerial surveys for other sirenians (Pollock et al., 2006). The interaction between water depth and water transparency when estimating their effect on the time-to-detection revealed a threshold at which water depth begins to significantly impact the time-to-detection, irrespective of water transparency, as indicated by the point where the minimum, average, and maximum measured water depths overlap in Fig. 4. Prior to this threshold, detections made in areas of the lake with lower water transparency showed a significant increase in the time-to-detection, even in shallow waters (Fig. 4). However, although more survey effort is required to detect manatees in these scenarios, it does not necessarily mean they will go undetected by a UAV. Detection probability models showed that when the lake had lower water transparency on average, the probability of detecting a manatee was higher when the lake was also shallower on average. Again, a similar threshold is observed:

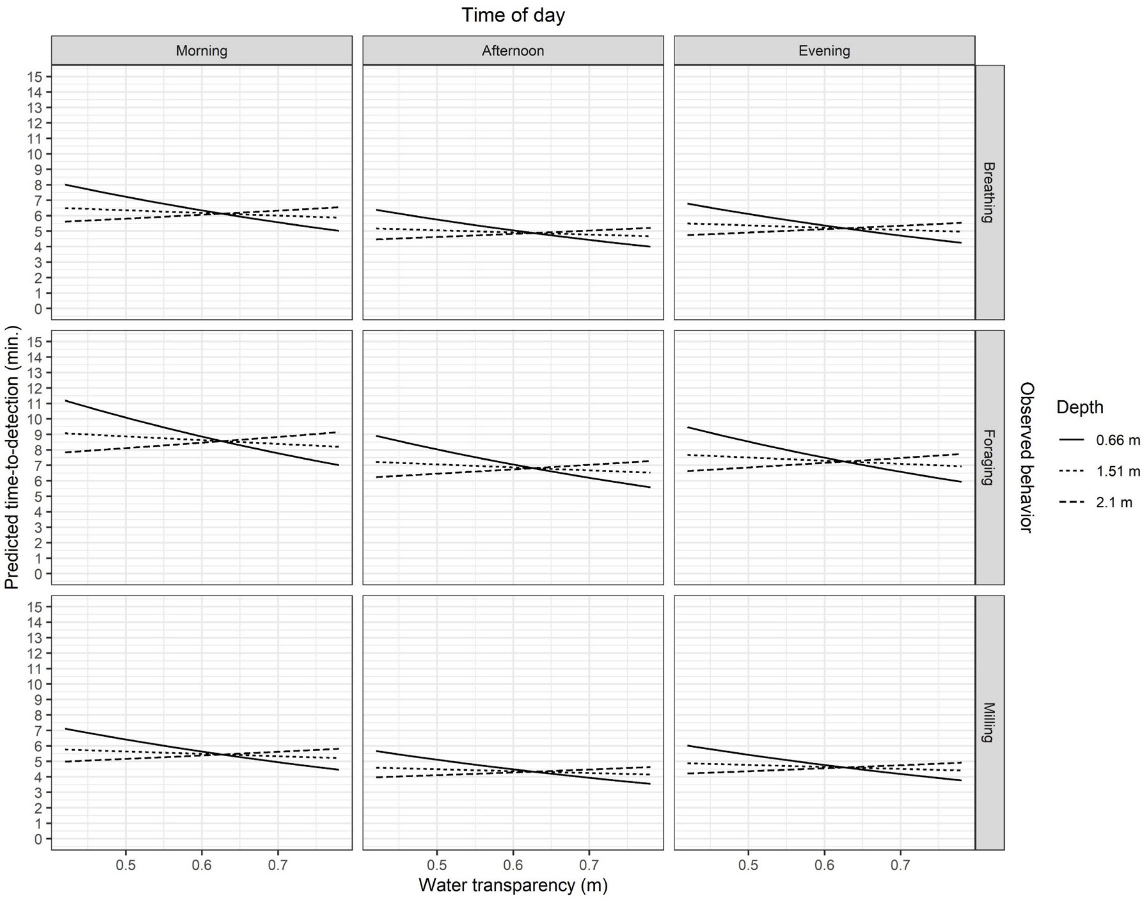

Figure 4. Generalized linear mixed-effects model results showing the interaction of water transparency (ranging from the minimum to the maximum measured values) and depth according to the time of day and observed behaviors of Amazonian manatees (*Trichechus inunguis*) and their effects on the time-to-detection. Depth is represented by the minimum (0.66 m), mean (1.51 m), and maximum (2.10 m) measurements where animals were detected. For the random effect of survey date in the model, we specified the date that corresponded to the median of the random intercepts (2 May 2021). Similarly, the median of the nested random effect of transect was specified (transect = 11). We used the average survey time (32.40 minutes), wind speed (1.01 mps), and cloud cover (90.39%).

as the water transparency of the lake improves, the probability of detecting a manatee is much higher and there is less of a difference between the probability of detecting a manatee in the observed minimum, average, and maximum average water depths (Fig. 5). Therefore, when considering the use of UAVs to detect *in situ* animals, researchers should adjust survey effort based on water depth and water transparency, given the dynamic nature of Amazonian aquatic habitats.

The effect of cloud cover and wind speed also significantly affected the time-to-detection, albeit not as expected. Previous studies have shown that weather conditions, such as cloud cover and sea state, can prevent observers from detecting West Indian manatees during occupied aerial surveys, even when animals are at or near the water's surface (Eberhardt et al., 1982; Packard et al., 1985; Ackerman, 1995; Lefebvre et al., 1995; Wright et al., 2002; Edwards et al., 2007). However, Fonnesbeck et al. (2009) found that cloud cover significantly increased the probability of detecting manatees at a warm-water aggregation site during occupied aerial surveys. Sunlight is generally thought to improve visibility into the water column, but cloudy conditions can reduce glare, thereby increasing visibility (Fonnesbeck et al., 2009). Similarly, we found that greater cloud cover significantly increased the time-to-detection and increased the probability of detecting a manatee. We suggest that the latter is due to the ability to store a permanent video record of the UAV survey, which has also been suggested by Hodgson et al. (2013) when conducting UAV surveys to detect dugongs. This potentially overcomes the limitation of availability bias due to manatee surfacing behaviors being masked by clouds, as the observer reviewing the video footage has more time and can repeatedly scan for animals. Additionally, visibility into the water column was already greatly restricted at our study site due to low water transparency. As a result, sunlight likely does not improve visibility enough when surveying turbid waterbodies like the ones inhabited by Amazonian manatees. Although not measured in this study, anecdotal evidence from our surveys show that glare can completely mask an area, especially in the afternoon under direct sun. Comparatively, surfacing behavior did not appear to be masked by clouds. It should be noted that the time to review the UAV video footage took much longer to detect manatees with confidence when there was greater cloud cover. Additionally, when conducting synoptic surveys to detect Florida manatees in an occupied aircraft, researchers were more likely to detect animals below the water surface in smooth, clear water under low cloud cover and in bright sun (Ackerman, 1995). This may explain why the time-to-detection was greater for detections made when there was high cloud cover, as manatees were unable to be detected just beneath the water's surface.

Contrastingly, we found that greater wind speeds both decreased the time-to-detection and the probability of detecting manatees with a UAV. This may be due to a bias in our data, as cloud cover ranged 57–100%, whereas wind speed only ranged 0.36–1.7 mps. When reviewing the video footage, the observer did not notice a significant change in the lake's surface water state due to wind speeds. Another potential bias in our data is that wind speed and cloud cover were obtained remotely from historical weather data provided by Dark Sky. Although highly accurate at a fine spatial scale, there are likely discrepancies between the actual observed wind speed and cloud cover on the ground versus what was recorded remotely, and this discrepancy was observed in some videos. Therefore, an increased sample size across a wider range of wind speeds and on-the-ground measurements of wind speed and cloud cover might be needed to more adequately quantify the effects of these covariates on detecting an Amazonian manatee with a UAV.

Behavior also greatly affects the detectability of aquatic species with low above-water profiles, including sirenians (Wright et al., 2002). When resting, wild Florida manatees can be observed either hanging suspended at the surface or lying on the bottom (Edwards et al., 2007). Additionally, both subspecies of West Indian manatees have been found to rest at the bottom of deep-water holes (O'Shea et al., 1988; Smethurst & Nietschmann, 1999; Jiménez, 2002; Bacchus et al., 2009). When resting at the bottom, manatees tend to surface less frequently (Castelblanco-Martínez et al., 2015). Resting behaviors of Amazonian manatees have not yet been observed in the wild, but in managed care Amazonian manatees have been reported to only bottom rest (Mukhaetov et al., 1992). This could further explain why increased survey efforts are necessary to detect Amazonian manatees in deeper areas of the lake, as individuals might be resting at the bottom. For Antillean manatees, Bacchus et al. (2009) found that resting holes are used more frequently during the day rather than at night, potentially to avoid human activity, such as boats. Although information regarding *in situ* behavior of Amazonian manatees is sparse, they may have adapted to behave similarly in response to historic and present illegal hunting. This evasive behavior has been observed in African manatees, as they tend to rest during the day in the middle of large waterbodies, potentially in response to hunting pressure (Powell, 1996). We found that for all categorized behaviors (breathing, foraging, milling), there was a lower time-to-detection of these behaviors outside of morning hours. Additionally, we found that the probability of detecting an Amazonian manatee at our study site increased during evening hours. These results could be indicative of nocturnal activities of Amazonian manatees at our study site. However, given that we conducted more surveys in the morning and afternoon, this could also be due to a bias in our data sampling. For Antillean manatees, Castelblanco-Martínez et al. (2015) also found that manatees exhibited a greater frequency of surfacing activities during the night or early morning. However, we were unable to quantify the activity patterns of the Amazonian manatees at our study site. For better *in situ* application, future research is needed to determine how activity patterns or resting behaviors of Amazonian manatees affect detection with a UAV while specifying an interaction between environmental factors.

Our estimated detection probability is comparable to those using other methods to detect *in situ* Amazonian manatees. Using both direct and indirect survey methods, including visual observations from a canoe, feeding signals on aquatic vegetation, fecal samples, and acoustics, de Souza et al. (2021) estimated a detection probability of 0.50. Additionally, the probabilities of detecting Amazonian manatees using side-scan sonar for two waterbodies in Ecuador were estimated to be 0.51 and 0.48 (Ruano et al., 2021). While side-scan sonar has shown to be an effective tool for detecting the species in murky waters (Gonzalez-Socoloske & Olivera-Gómez, 2023), UAVs could assist with addressing some of the limitations of this survey method, given our high detection probability obtained in this study. For example, a key limitation of using side-scan sonar is the avoidance and displacement of manatees when detecting an oncoming motorboat (Machuca Coronado, 2015; Puc-Carrasco et al., 2016; Castelblanco-Martínez et al., 2018). In addition to missed detections, this can also result in a lack of visual confirmation of images captured by the sonar (Castelblanco-Martínez et al., 2018). The habitat being surveyed can compound the ability of researchers to visually confirm detections made with side-scan sonar, as researchers are not able to make observations when manatees are further from the boat, especially in meandering rivers (Castelblanco-Martínez et al., 2018). Therefore, UAVs could be used to hover at higher altitudes around the survey boat using side-scan sonar to attempt visual confirmation of detections. Our study showed that even at low altitudes (60 m), no obvious visual indicators of disturbance were observed, except in the case of one individual that rapidly submerged. Manatees were observed slowly submerging as the UAV approached, but we were unable to determine whether this was a natural behavior or due to the UAV. A road runs parallel to the lake on the east side, which might have produced enough noise to mask that of the UAV, but we did not quantify ambient noise during the surveys. Continuous improvements in the available technology compatible with UAVs, such as the development of a photoacoustic airborne sonar

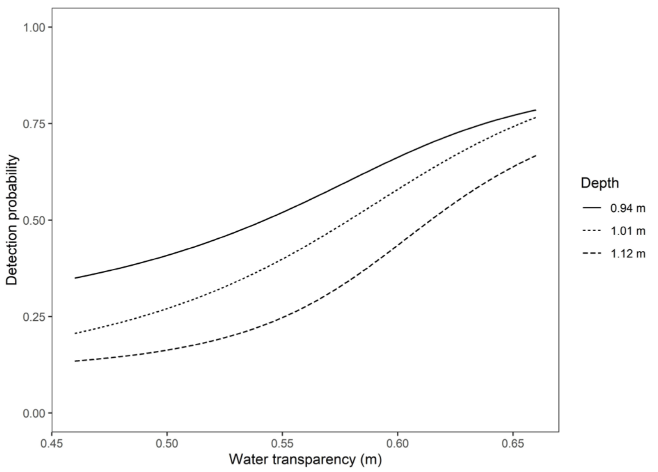

Figure 5. Bayesian detection model (m.1 from Table 3) results showing the significant interaction of water transparency (ranging from the minimum to the maximum measured values) and depth on the probability of detection. Depth is represented by the minimum (0.94 m), mean (1.01 m), and maximum (1.12 m) measurements where animals were detected. The random effect of date and survey effort were held at the mean. For reference, the mean detection probability in this model was p = 0.62.

system (Fitzpatrick et al., 2020), could improve the detection of Amazonian manatees.

The overhead perspective of a UAV also minimizes the likelihood that researchers would misidentify a manatee, which was also reported by Fürstenau Oliveira et al. (2017) when conducting aerial surveys utilizing a blimp with a mounted camera to detect the Araguaian river dolphin (*Inia araguaiaensis*) in Brazil and by Castelblanco-Martínez et al. (2018) when detecting Antillean manatees using side-scan sonar in French Guiana. Similar to our observations, Fürstenau Oliveira et al. (2017) also noted that reviewing videos of aerial surveys resulted in a greater ability, compared to boat surveys, to distinguish between species with similar surfacing patterns, such as between arapaimas and the Araguaian river dolphin. Additionally, observer bias can lead to errors in side-scan sonar image interpretation, and manatees can be mistaken for objects or other megafauna without visual confirmation (Castelblanco-Martínez et al., 2018). Due to the permanent video record associated with UAV surveys, UAVs can potentially eliminate this observer bias (Aniceto et al., 2018). Fürstenau Oliveira et al. (2017) also noted that videos from aerial surveys can be accurately reviewed even by inexperienced reviewers, whereas the reliability of visual counts made onboard a boat is highly dependent on observer experience.

Although our detection probability estimate was relatively high, this is likely due to our study site being a rather small and enclosed lake. We expect a lower detection probability when using a UAV to detect Amazonian manatees in the wild. However, careful selection of study sites may overcome this limitation. Our study site represented approximately 2% of the estimated home range size of rehabilitated and released Amazonian manatee subadults, which researchers estimated to be 0.53 km^2 (Guzmán Téllez, 2020). Guzmán Téllez (2020) also found that, within a single day, only 8.33% of movements were larger than 0.5 km, which is approximately one-fifth of the area of our study site. However, the home range sizes of wild Amazonian manatees in Brazil were found to vary between 2.34 to 34.74 km^2 (Arraut et al., 2010). Due to the variability in home range size estimates, future studies should investigate how survey area affects detection when using a UAV as a survey tool. It is possible that unoccupied aerial surveys could result in greater detectability of manatees when surveying large areas in comparison to boat surveys, as this was found by Fürstenau Oliveira et al. (2017) when comparing detectability of the Araguaian river dolphin using a blimp to conduct aerial surveys versus boat-based surveys. Similar to Amazonian manatees, the Araguaian river dolphin also exhibits elusive behavior by surfacing quickly and quietly. Rather than environmental variables having a significant effect on survey counts – although the effects of these variables were minimized by selecting optimal survey times (early morning) – survey area had the greatest effect on counts, as the blimp detected more dolphins than the boat when the survey area was greater (Fürstenau Oliveira et al., 2017). Therefore, when investigating how survey area affects detection using a UAV, researchers should also compare detectability using more traditional methods, such as boat-based surveys, to account for the low population densities of wild Amazonian manatees and avoid false negatives. However, smaller foraging sites might make ideal *in situ* survey areas to further test the efficacy of using a UAV to detect Amazonian manatees.

Amazonian manatees spend long periods of time, six to eight hours per day, foraging on aquatic vegetation (Best, 1981). Additionally, Amazonian manatees consume approximately 8% of their body weight per day (Rosas, 1994). When feeding to simulate natural foraging conditions, captive Amazonian manatees exhibited longer surfacing intervals compared to when they were resting at the bottom, walking along the bottom, or surfacing outside of feeding times (Kikuchi et al., 2010). Although we found that the time-to-detection was greater when observing Amazonian manatees exhibiting foraging behavior, this is likely due to the location of preferred foraging sites within the lake relative to the survey starting location rather than implying a decrease in surfacing time. Most foraging behaviors were primarily observed at the northern side of the lake along the 4th transect from the starting location where there is an enclosed area surrounded by grasses (Fig. 1). Additionally, we even observed a manatee foraging with a good proportion of its body outside of the water in this area. Furthermore, Kikuchi et al. (2010) found that there was no significant difference in surfacing intervals when caretakers provided *ex situ* manatees with a food source versus when manatees were actively eating outside of feeding times on fallen leaves. Therefore, we suggest that UAVs would be useful tools to efficiently detect Amazonian manatees when conducting surveys at potential feeding sites, which are considerably smaller in area compared to surveying entire home ranges. de Souza et al. (2021) found that the probability of detecting Amazonian manatees in the wild increased in areas with high macrophyte coverage. Given the species' preference to consume floating or emergent plants (Domning, 1980; Best, 1981; Rosas, 1994; Colares & Colares, 2002; Arraut et al., 2010; Guterres-Pazin et al., 2014; Guzmán Téllez, 2020), detecting feeding behaviors at the surface in the wild should be possible using a UAV. This would also allow researchers to monitor feeding behaviors post-release to ensure rehabilitated animals are successfully adapting. Although a comparison of UAV survey designs (*i.e.,* transects vs. hovering) is outside of the scope of this study, small areas, such as foraging sites, may be more effectively surveyed by hovering the UAV for the duration of the estimated aerobic dive limit of Amazonian manatees (19 to 22 minutes; Gallivan et al., 1996). This could improve the detection of aquatic species that can only be detected when surfacing to breathe and could overcome a major limitation of current UAV technology that is commercially available.

A limitation of small, multirotor UAV technology is the decreased flight times with current battery technology (Raoult et al., 2020). Most small, multi-rotor UAVs, like the one we used in this study, have a battery life shorter than 30 minutes (Oleksyn et al., 2021). While researchers have suggested using multiple batteries to overcome this limitation, which we followed for our long survey design, we also want to highlight the solution of selecting optimal survey locations. In this study, and under the described environmental conditions specific to the surveys, covering an area of 9,800 m^2 took approximately seven minutes, and we only needed to conduct about three repeat surveys to ensure a 95% chance of accurately detecting an animal. Therefore, researchers could conduct multiple repeat surveys at foraging sites, where surfacing intervals are greater, using a single battery, and still maintain a good probability of detection. This method could replace or supplement the use of traditional, and costly, survey techniques with the use of a small, multirotor UAV. The use of UAVs is also a much safer method for field biologists compared to conducting occupied aerial surveys used for other sirenians, including the West Indian manatee and dugong (Sasse, 2003; Koski et al., 2009; Hodgson et al., 2013; Linchant et al., 2015; Edwards et al., 2021).

While researchers have used occupied aerial surveys to monitor many other aquatic mammals due to the overhead advantage, this is not a feasible survey method for Amazonian manatees. Previous studies have relied on radio-tracking as a primary method to monitor Amazonian manatees, especially post-release of rehabilitated animals (Landeo-Yauri et al., 2017; Guzmán Téllez, 2020). Prior to release, researchers fit a belt that contains a VHF radio-tag around the manatee's peduncle. Researchers then track the tagged manatees by navigating around the release site onboard a small motorized or unmotorized boat using an antenna and receiver that detects a manatee's preassigned radio-tag frequency (Ryan, 2011; Marmontel et al., 2012). However, radio-tracking tagged manatees can be logistically challenging, as researchers need to track released manatees daily and some habitats are not reachable by boat due to emergent or floating vegetation (Landeo-Yauri et al., 2017). Additionally, researchers are not usually able to make behavioral observations or assess body conditions due to the difficulties of observing Amazonian manatees in the wild. This was noted by Guzmán Téllez (2020) who, when radio-tracking released manatees, was never able to see the animals directly. Given the high detection probability obtained in our study (0.62), UAVs could be used by researchers in tandem with radio-tracking to make these behavioral observations that are critical to evaluating the animal's ability to adapt to their new, wild environment. We also encourage the exploration of equipping an antenna and receiver to a UAV to detect the radio frequencies of tagged animals to overcome the aforementioned challenges (Saunders et al., 2022). We strongly recommend the use of UAVs as a survey method that is used to support existing methodologies, such as radio-tracking, traditional boat-based surveys, and side-scan sonar. While the use of UAVs has yet to be incorporated, previous studies have found that a multimethod approach is necessary to effectively detect elusive species (Mattfeldt & Grant, 2007), including manatees in turbid waters (Castelblanco-Martínez et al., 2018).

To date, only two previous studies have been conducted using UAVs to detect a sirenian species, the Antillean manatee, in captivity (Landeo-Yauri et al., 2021; Ramos et al., 2022). The goals of these studies were to assess the effect of UAV flights on the behavior of captive manatees (Landeo-Yauri et al., 2021) and to evaluate manatee body size and condition for indicators of overall health (Ramos et al., 2022). Using the results of this study, and adapting the survey effort accordingly, researchers could explore the use of UAVs to passively monitor the health and behaviors of animals being rehabilitated to be released back into the wild. Our study showed a high probability of detection using UAVs to fly parallel line transects in a captive environment that mimics the environmental heterogeneity and conditions of the wild environment. This would greatly reduce the need for additional contact between caretakers and the animals being rehabilitated prior to release. The greatest threat to the Amazonian manatee is illegal hunting by people, and direct human contact between caretakers and species that consider humans as predators has been found to cause welfare issues (Sherwen & Hemsworth, 2019). Although excluded from the study, the only detections made on the far west side of the lake occurred when caretakers were in or on the water. This could indicate that human presence does impact Amazonian manatee behavior even in an *ex situ* setting. Therefore, UAVs also have the potential to be cost-effective tools for passively monitoring the behavior and body condition of captive sirenian species.

In conclusion, our results provided a minimum survey effort when using a UAV as a tool to detect aquatic species, particularly those with long surfacing intervals and in waters with poor visibility. In areas where there is a high density of animals, researchers should conduct a minimum of three repeat surveys while also specifying a high overlap between transects to reliably detect any animals present. More survey effort (flight time and repeat surveys) should be conducted even for areas with a high density of animals where there is also deep water and low water transparency. Fig. 4 can serve as a guide for adjusting the survey effort accordingly. However, these predictions represent only the values for water depth and water transparency within our observed range. Future studies should compare detection probability across a broader range of values. We also strongly suggest that researchers consider foraging sites as optimal study sites to conduct UAV surveys. Focusing UAV surveys on smaller foraging sites overcomes a limitation of UAV surveys in general, which is their limited battery life. Given the success at an *ex situ* site that mimics the natural environment, we encourage future studies to further investigate the effects of the significant environmental covariates on UAV detection at *in situ* study sites. We have also carefully considered and presented future applications of UAVs to detect Amazonian manatees in tandem with existing methods, which require further exploration. The results of this UAV survey protocol and its continued development will be useful for monitoring cryptic aquatic mammals around the world in poor visibility habitats, such as Amazonian manatees, both *in situ* and *ex situ*, contributing to a better understanding of their ecology and conservation.

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This study assessed a passive survey methodology, and therefore no animals were directly handled or manipulated. The methods used in this study were reviewed by the George Mason University Institutional Animal Care and Use Committee (IACUC) and were deemed exempt. We are grateful to all volunteers and staff members at RAREC for their support of this project and the work they do to conserve wildlife in Peru. This study constituted part of S. Farinelli's PhD dissertation. Therefore, we would like to thank committee members Dr. Elizabeth Freeman and Dr. Kathleen Hunt for their thoughtful edits and comments on this chapter of the dissertation. We would also like to thank Dr. Travis Gallo for his input on our study design and Dr. Lynne Baker, Dr. Jeffery Doser, and Dr. Brian Griffiths for their statistical advice. Finally, we are grateful to the anonymous reviewers of this manuscript, Dr. Daniel Gonzalez-Socoloske, and Dr. Miriam Marmontel for their constructive edits and comments that greatly improved the paper.

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Supplementary Material

Table S1. All surveys conducted to evaluate detecting *ex situ* Amazonian manatees using an unoccupied aerial vehicle that were used in generalized linear mixed-effect (GLMM) and detection models. Survey effort is given in minutes. Total survey effort was used as the survey effort covariate in GLMMs, whereas survey effort (one pass) was used as the survey effort covariate in detection models.

Table S2. Results of the backwards stepwise approach to model selection to determine factors that influence the time-to-detection when using an unoccupied aerial vehicle to detect Amazonian manatees at an enclosed soft-release site.

Figure S1. Trace plots of detection covariates in the global model (m.g in Table 3) indicating adequate mixing. Detection covariates include wind speed, cloud cover, water depth, water transparency (SDD), time of day (TOD2 = afternoon, TOD3 = evening, morning is used as the reference), survey effort (Effort), and the interaction between water depth and water transparency (Depth:SDD). All continuous covariates in the model were scaled for standardization.