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Using small drones to photo-identify Antillean manatees: a novel method for monitoring an endangered marine mammal in the Caribbean Sea

Sarah Sofía Landeo-Yauri[1,*], Eric Angel Ramos[2,3], Delma Nataly Castelblanco-Martínez[2,4,5], Carlos Alberto Niño-Torres[2,5], Linda Searle[6]

[1]Posgrado de Ciencias del Mar y Limnología, Universidad Nacional Autónoma de México, Ciudad de México 04510, Mexico
[2]Fundación Internacional para la Naturaleza y la Sustentabilidad, Chetumal 77014, Mexico
[3]The Graduate Center, City University of New York, New York, NY, 10016, USA
[4]Consejo Nacional de Ciencia y Tecnología, Ciudad de México 03940, Mexico
[5]Universidad de Quintana Roo, Chetumal 77039, Mexico
[6]ECOMAR, PO Box 1234 Belize City, Belize

ABSTRACT: Population assessments and species monitoring for many endangered marine megafauna are limited by the challenges of identifying and tracking individuals that live underwater in remote and sometimes inaccessible areas. Manatees can acquire scars from watercraft injury and other incidences that can be used to identify individuals. Here we describe a novel method for photo-identification of Antillean manatees *Trichechus manatus manatus* using aerial imagery captured during flights with a small multirotor drone. Between 2016 and 2017, we conducted 103 flights to detect and observe manatees in Belize, primarily at St. George's Caye (SGC) near the Belize Barrier Reef. Review of aerial videos from these flights resulted in 279 sightings of manatees (245 adults, 34 calves). High-resolution images of individual manatees were extracted and classified according to image quality and distinctiveness of individual manatees for photo-identification. High-quality images of manatees classified as sufficiently distinctive were used to create a catalog of 17 identifiable individuals. At SGC, 21 % of all sighted adult manatees (N = 214) were considered photo-identifiable over time. We suggest that the method can be used for investigating individual site fidelity, habitat use, and behavior of manatee populations. Our photo-identification protocol has the potential to improve long-term monitoring of Antillean manatees in Belize and can be applied throughout clear, shallow waters in the Caribbean and elsewhere.

KEY WORDS: Drones · Trichechidae · Photo-ID · Manatee · *Trichechus manatus manatus*

1. INTRODUCTION

The Antillean manatee *Trichechus manatus manatus* is a subspecies of the West Indian manatee *T. manatus* and is considered Endangered by the IUCN (Self-Sullivan & Mignucci-Giannoni 2008) due to threats such as entanglement, poaching, watercraft collision, and habitat loss (Castelblanco-Martínez et al. 2012). In the Regional Management Plan for the West Indian manatee (Quintana-Rizzo & Reynolds 2010), long-term and longitudinal studies of individually identifiable animals are recommended to determine the survival rates, reproduction rates, site fidelity, and movement patterns of manatee populations. However, the techniques typically used to identify individuals, such as capturing and tagging animals, are expensive, invasive, and require extensive effort. Devising affordable alternative methods

*Corresponding author: sslandeo@gmail.com

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for tracking manatees in regions with limited available research funds is essential to the species' longterm preservation (Quintana-Rizzo & Reynolds 2010).

Photo-identification (photo-ID) is typically the most affordable and non-invasive method to identify individual animals using unique features on the surface of an animal's body (Würsig & Würsig 1977, Gope et al. 2005). Photo-ID methods are often used during the monitoring of most whale and dolphin populations to distinguish individuals through comparisons of the scarring patterns and unique markings on the dorsal fins, flukes, or body surfaces to catalogs of previously identified individuals (Urian et al. 2015). In sirenians, studies employing photo-ID have been conducted on dugongs *Dugong dugon* (Shawky et al. 2017) and West Indian manatees (Beck & Reid 1995) using surface scarring patterns to distinguish animals, and with Amazonian manatees *T. inunguis* using the light-colored ventral patch of most individuals of this species (Ely et al. 2017). Images of animal scarring and identifying marks are typically gathered with high-resolution cameras from boats (Reid et al. 1991, Anderson 1995, Langtimm et al. 2004), shore (Reid et al. 1991, Langtimm et al. 2004, Goldsworthy 2018), underwater photographs (Langtimm et al. 2004, Shawky et al. 2017) and underwater videos (Arce 2012), or during captures for health assessments (Stamper & Bonde 2012). Sirenians often bear scars, mutilations, and congenital deformities on their bodies that are stable enough to reidentify individuals over time (Beck & Clark 2012). For example, cuts from boat propellers are a common cause of scars in *T. manatus* (Beck et al. 1982, Self-Sullivan 2007), whereas *D. dugon* scarring can also be attributed to wounds inflicted by tusks of adult males (Anderson 1995). Photo-ID data have enabled the investigation of important demographic factors of Florida manatees *T. m. latirostris*, such as reproductive rates (Kendall et al. 2004), annual adult survival rates (Langtimm et al. 1998, 2004), reproduction traits (Rathbun et al. 1995), and movement patterns (Reid et al. 1991). Criteria that must be considered for successful photo-ID of sirenians include: (1) imaged individuals must have at least 1 permanent feature (e.g. scar, mutilation); (2) good quality images depicting the feature should be obtained; and (3) images of the entire body must be available (Beck & Reid 1995). These criteria help ensure that all permanent body marks are detected and that the individual can be identified over time and in different areas.

In the Caribbean Sea, capturing images of wild manatees is challenging due to the small size and low density of their populations (Quintana-Rizzo & Reynolds 2010). Manatees are often found alone or in groups of fewer than 10 individuals (Hartman 1979). In contrast, large aggregations of manatees (>100) like those documented during the winter season in Florida, USA, provide more opportunities for obtaining high-quality photographs of individuals with distinct features (Beck & Reid 1995). Additionally, their shy and inconspicuous behavior, minimal time at the surface (Hartman 1979), strong responses to oncoming vessels (Rycyk et al. 2018), and sometimes to swimmers (D.N.C.-M. pers. obs.), can make it difficult to find manatees and to approach them to detect body scars and identifiable features at the surface.

Manned aerial surveys are commonly used to remotely detect and study sirenians (e.g. Ackerman 1995, Marsh 1995, Morales-Vela et al. 2003, Alves et al. 2016) and many other species of marine mammal (e.g. Bengtson et al. 2005, Keller et al. 2006, Panigada et al. 2011) because aerial surveys provide overhead views which facilitate the detection and counting of individuals. In recent years, unmanned aerial systems or drones have demonstrated their use in supplementing, or in some contexts, replacing, manned aerial surveys (Hodgson et al. 2013). Drones possess several major advantages over manned aerial surveys: they can record detailed flight logs and permanent visual records, are safer for the operator, and can be less costly than manned aerial surveys (Hodgson et al. 2013). Manned aerial surveys can cover larger areas and fly longer compared to small drones, but lack the maneuverability of multirotor drones and the potential of drones for extensive sampling in small areas and at low altitudes (Colefax et al. 2018). Drones have been successfully used in a broad range of applications including the detection and counting of marine megafauna (Hodgson et al. 2017); photogrammetry to assess cetacean body condition (Durban et al. 2015, 2016, Christiansen et al. 2016); collection of exhaled breath condensate from large baleen whales (Domínguez-Sánchez et al. 2018), and behavioral studies (Ramos et al. 2018, Torres et al. 2018). Drone-based photo-ID studies of bowhead whales *Balaena mysticetus* (Koski et al. 2015), killer whales *Orcinus orca* (Durban et al. 2015), and seals (Pomeroy et al. 2015) have confirmed the efficacy of this method for identifying numerous marine mammals, but its application to sirenians has not been thoroughly examined.

Photo-ID of sirenians with drone-based imagery is a promising avenue of research. To our knowledge, Ramos et al. (2018) conducted the only study to date that tested the use of small drones for photo-ID of manatees, but a description and validation of the method has not been made until now. Here we used part of the same dataset as Ramos et al. (2018) to describe and examine the efficacy of a novel method using small drones to photo-identify Antillean manatees. Images of all manatee sightings were extracted from aerial videos, selected according to image quality as well as distinctiveness of manatee features, and used to identify and catalog distinctive individuals. Our proposed photo-ID method has promise as a low-cost aerial platform to improve the capacity for monitoring and researching wild manatee populations in the Caribbean.

2. MATERIALS AND METHODS

2.1. Study area

The coast of Belize is comprised of a diverse array of marine ecosystems and habitats, including sandy and mangrove cayes, coastal lagoons, estuaries, and a system of fringing reefs of the Mesoamerican Barrier Reef System and 3 offshore atolls (Cho 2005). Important areas for manatees include the cayes adjacent to Belize City, the Belize River, the Southern Lagoon, Placencia Lagoon, Corozal Bay Wildlife Sanctuary, and the Port Honduras area (Auil 1998).

Drone flights were conducted from June to July 2016 and July 2017 at St. George's Caye (SGC), a small crescent-shaped island located 10 km east of Belize City and 1.5 km west of the Belize Barrier Reef (Fig. 1). Extensive seagrass flats and calm waters prevail on the leeward side of the island, and mixed seagrass/sand patches and a sandbar on the windward side. Opportunistic surveys were also performed at the mouth of the Belize River, the Drowned Cayes, Man O' War Caye, and in Placencia Lagoon (Fig. 1).

2.2. Drone flights

Our protocol for drone flights corresponds to that of Ramos et al. (2018), who aimed to record manatee behavior and test if manatees respond to the presence of a small drone. From these flights and associated data, we used a subset to examine their use for manatee photo-ID.

Two commercial drones, the DJI Phantom 3 Professional (P3) and Phantom 4 (P4) quadcopters, were used for flights. Each drone was equipped with a high-definition camera (1/2.3′ CMOS, 12.4 MP sensor; FOV 94° 20 mm f/2.8 lens) recording 4K video in MP4 format, mounted to its 3-axis gimbal transmitting a live-feed to the tablet-mounted remote control. The

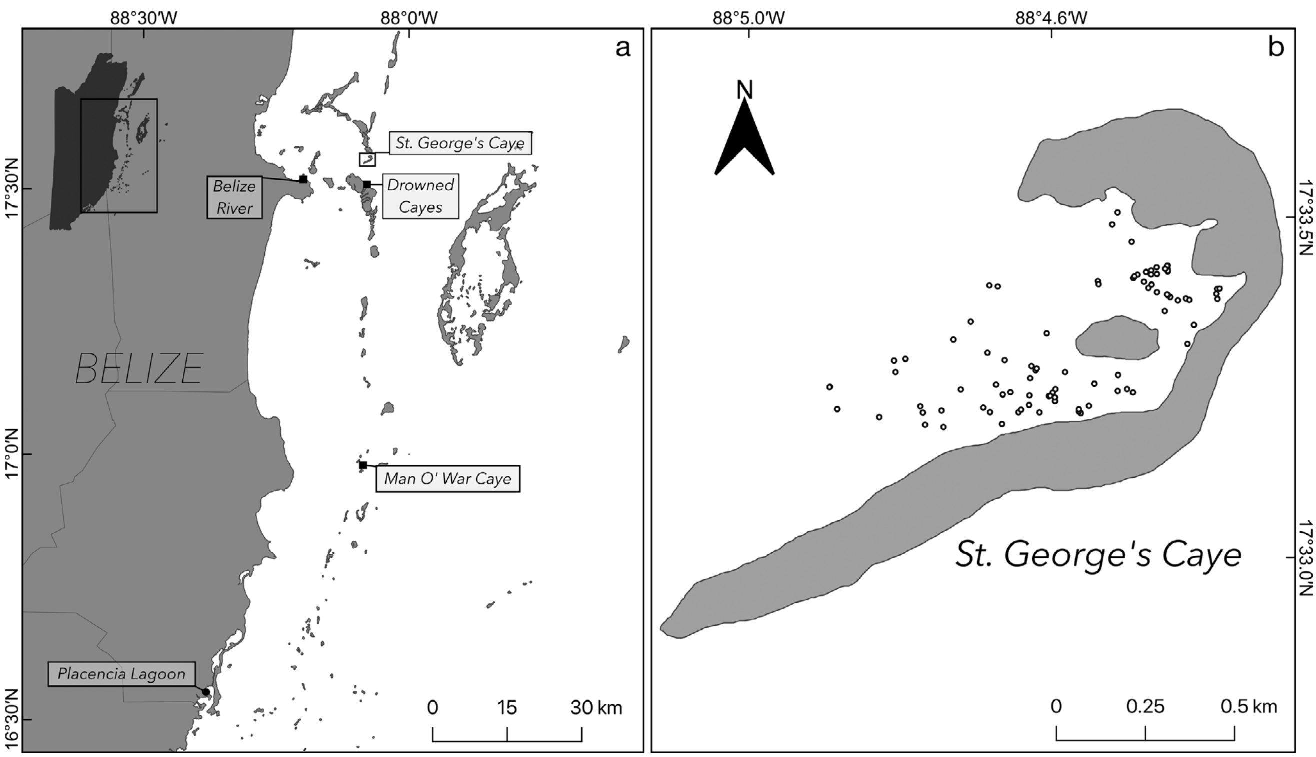

Fig. 1. (a) Study area in Belize (inset map shows the study area in relation to the whole country). Light gray lines demarcate districts. (b) Open circles represent sightings of Antillean manatees during small drone flights at St. George's Caye

drone was remotely piloted from shore for most flights and from a small (7–13 m long) motorboat (Drowned Cayes) or a 13 m long catamaran (Man O' War Caye, Placencia Lagoon). The pilot (E.A.R.) launched the drone to an altitude of 100 m at the beginning of flights to search for manatees on designated transects or to perform behavioral follows of previously detected manatees. Transects were saw toothed and followed mainly the entire leeward side of the caye, covering approximately 1.6 km^2.

The protocol entailed at least 1 flight h^{-1} (6–20 flights d^{-1}) following a predetermined saw-toothed route covering the study area to determine manatee locations. In the same or following flight, prior detections of manatees were used to fly to the same spot to approach an individual manatee or group of manatees. The animals were approached in one of several ways: vertical approaches to test animal responses (ranging from 5 to 100 m in altitude; Ramos et al. 2018), horizontal follows, or stable hovering over animals for focal behavioral follows.

Additional flights were conducted to monitor manatee occurrence in large nearby resting holes, i.e. depressions in the substrate where manatees frequently rest and seek shelter in Belize (Bacchus et al. 2009), and to observe their behavior. During flights, binoculars were used to maintain a visual line of sight with the drone.

2.3. Data analysis

Each drone video was reviewed post-survey at least twice by an experienced observer. Extracted images of manatees were acquired at different altitudes depending on whether they were recorded during horizontal or vertical approaches and stable hovering. The time at which images were extracted from each video was matched to drone flight altitude in the GPS-logged track. We selected the images in which most of each individual's body was visible and clear. One to 7 high-resolution images (3840 × 2160 pixels) of each manatee were extracted from videos in VideoPad v. 5.05 (NCH Software).

To standardize and facilitate the assessment of features on the manatees, images were coded, cropped, and rotated to align them lengthwise to the body of each manatee. Each image was then scored for 'quality' and 'distinctiveness.'

A preliminary sighting database was assembled including flight information: location, date, and time of video, sighting coordinates, number of manatees detected, and proportion of calves and adults. This database was used to compile information of all sightings, irrespective of whether the animal photographed was cataloged. The geographic position (latitude and longitude) of individual manatees at the time at which they were first detected was identified by overlaying the GPS track of each flight onto an orthomosaic map of the region (see Ramos et al. 2018) in ArcGIS Pro (ESRI). The manatee's location in the video was matched to stable habitat features in the map (e.g. boat scars in the substrate, edges of seagrass beds).

Table 1. Image quality assessment criteria for photo-ID of Antillean manatees. See Fig. 2 for representative images

Factor	Description	Scoring
Resolution/ clarity	Sharpness of image	2 = excellent 4 = average (grainy) 9 = poor (blurred)
Contrast	Range of tones in the image	1 = ideal 2 = excessive or minimal
Distortion	Portion of manatee body distortedby water surface movement	1 = none to little 2 = partial 8 = complete
Partial	Portion of manatee body visible. Body parts can be covered by turbidity and/or light reflection	1 = all body visible 2 = body partially visible
Angle	Angle of dorsal view to camera	1 = perpendicular 2 = moderately angled

A method of scoring images to evaluate the 'quality' of manatee photos was adapted from previous studies on humpback whales *Megaptera novaeangliae* (Friday et al. 2000) and bottlenose dolphins *Tursiops truncatus* (Urian et al. 1999, Rosel et al. 2011). Image quality was measured according to the following characteristics: resolution/clarity, contrast, angle, partial, and distortion (Table 1, Fig. 2). The sum of scores for each characteristic was classified for quality as excellent (Q1: score of 6–8), average (Q2: score of 9–12), or poor (Q3: score of >12).

The distinctiveness of individuals was evaluated in each image (Fig. 2), according to the following categories modified from Urian et al. (1999):

- D1(very distinctive): easily detected scars that are evident on all quality levels of image, even poor;
- D2 (average): minimal scarring that is only evident in images of quality Q1 and Q2;
- D3 (not distinctive): lacking marks or with subtle scratches and/or coloration patches that are not long-lasting and do not allow the identification to individual level. Subtle marks are likely to be visible only on Q1 quality images;

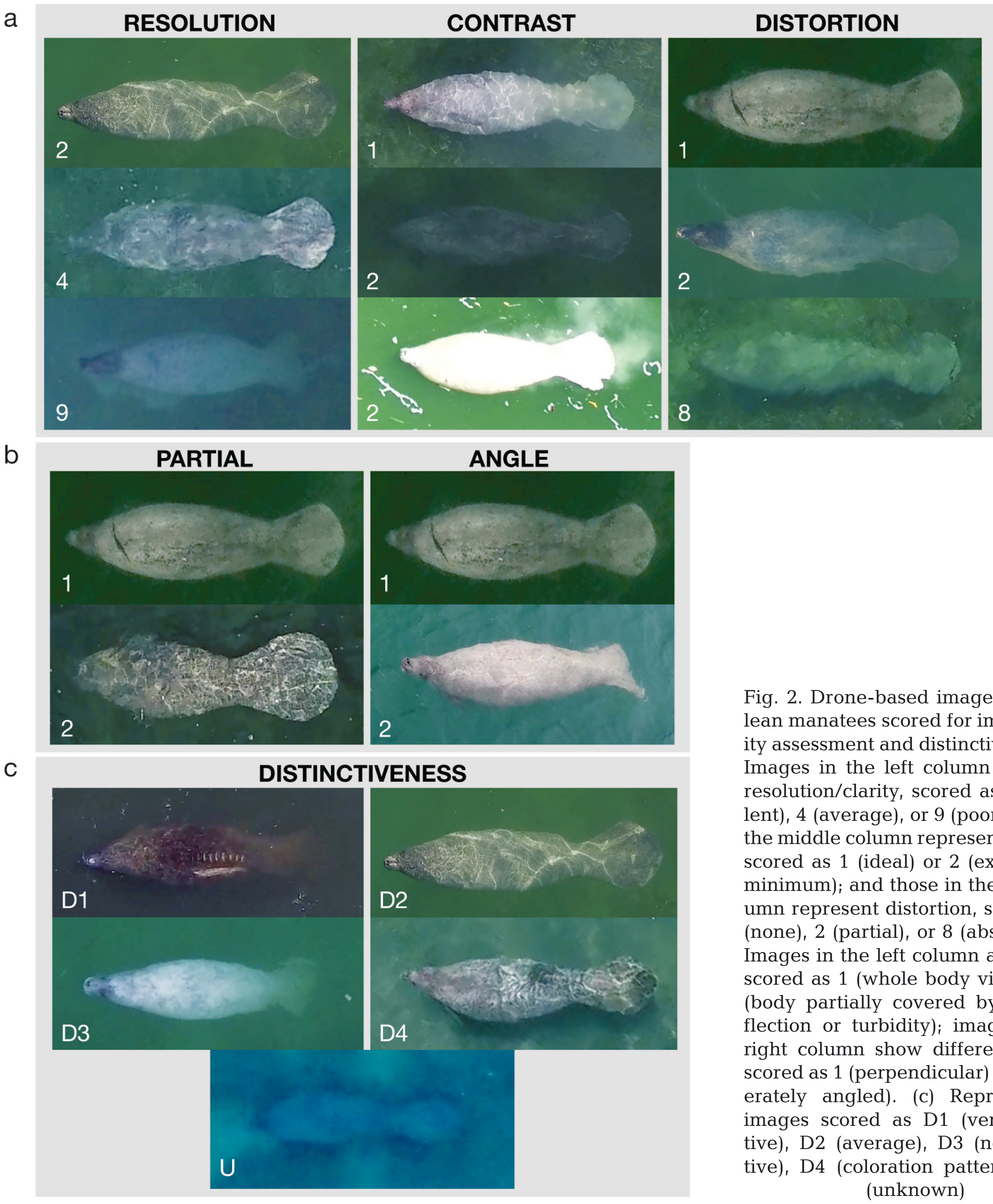

Fig. 2. Drone-based images of Antillean manatees scored for image quality assessment and distinctiveness. (a) Images in the left column represent resolution/clarity, scored as 2 (excellent), 4 (average), or 9 (poor); those in the middle column represent contrast, scored as 1 (ideal) or 2 (excessive or minimum); and those in the right column represent distortion, scored as 1 (none), 2 (partial), or 8 (absolute). (b) Images in the left column are partial, scored as 1 (whole body visible) or 2 (body partially covered by light reflection or turbidity); images in the right column show different angles, scored as 1 (perpendicular) or 2 (moderately angled). (c) Representative images scored as D1 (very distinctive), D2 (average), D3 (not distinctive), D4 (coloration pattern) and U (unknown)

- D4 (pattern): medium to large patterns of coloration, which are not long lasting and therefore do not meet the requirements for photo-dentification. Evident on all image quality levels;
- Unknown (U): a classification cannot be made, generally because of insufficient image quality.

Four authors (S.S.L.-Y., E.A.R., D.N.C.-M., C.A.N.-T.) independently reviewed the images and evaluated them for quality and distinctiveness. For each image, the quality was defined as the average of scores. Distinctiveness, for which categories cannot be scored, was assigned the category selected by most evaluators. Images for which there was no consensus among evaluators were given a D3 or U category. Images classified as sufficiently distinctive for individual identification (D1 and D2) were selected regardless of their quality. From this batch, the best quality images from each identified manatee were entered into the photo-ID catalog. Images not used in the catalog were kept in separate files assigned to each identified manatee. Fig. 3 depicts the data processing workflow from capture of aerial video of manatees from flights with a small drone, through image analysis and the creation of a photo-ID catalog.

2.4. Cataloging and sighting index

We assigned an alpha-numeric code and a name to each photo-identified manatee. Distinctive features were coded following the methods of Beck & Reid (1995), including the position (e.g. left posterior trunk, right peduncle), type (e.g. scar, mutilation), number (e.g. single, 2 to 3), size (e.g. large, medium), and color (e.g. white, dark). All of these traits were represented as a letter or number in the feature code. Notches on the tail were considered mutilations. Manatees were assigned an 'incomplete status' if only a portion of the body was registered or if only poor-quality images (Q3) were available. The photo-ID catalog contained a record of each identified manatee, available information for it, and a set of type specimen images for quick assessment. Each catalog record includes: the individual's code and name; date and place of the first and last sighting; feature code; sex (when possible); age class; identification status (complete or incomplete); and several of the best quality images available depicting recognizable features. A database containing information of photo-identified individuals, i.e. sightings coordinates, time and date, number of manatees encountered, manatee code, and number of images available, was assembled including information from the preliminary sighting database. In this database, we also included the codes of distinctive features, to facilitate the search for individuals by using the letters that compose the code as a filter. Sightings of the same individual on different days were considered re-sightings. Sighting records of the photo-identified manatees were used to construct a cumulative curve of the population at SGC and to calculate the proportion of distinctive manatees within the total sightings (total sightings of photo-identified manatees/total adult manatee sightings).

3. RESULTS

Between 2016 and 2017, 103 flights were conducted in Belize: SGC (n = 94), the mouth of the

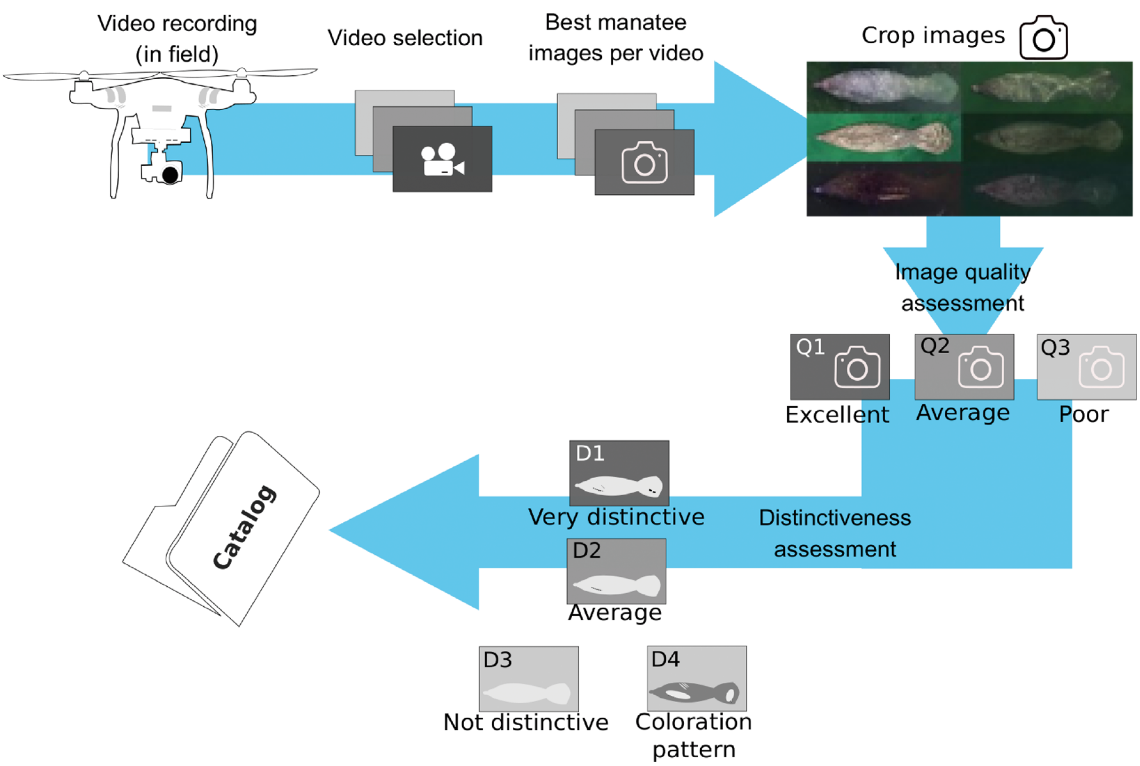

Fig. 3. Workflow for our photo-identification study of Antillean manatees from data acquisition to the creation of a catalog. During video analysis, 1 to 7 images per manatee were selected, rotated, cropped, and coded. All images were scored for image quality and individual distinctiveness. The best available quality images of individuals D1 and D2 were used to create the catalog

Belize River (n = 4), the Drowned Cayes (n = 1), Man O′ War Caye (n = 2), and Placencia Lagoon (n = 2). The review of aerial video (1740 min) resulted in 279 manatee sightings (245 adults, 34 calves). Images extracted from drone videos (n = 466) were evaluated for quality and distinctiveness, resulting in the photo-ID of 17 adult manatees with images that were sufficiently distinctive (D1 and D2, n = 79) to be included in our catalog (Table 1). The altitude at which the selected images were taken ranged between 5 and 24 m, while the best resolution images (resolution score 2 in image quality scoring) were obtained at lower altitudes (5 to 7 m). In general, lower altitudes provided more images of suitable resolution (resolution scores 2 and 4, Fig. 4).

At SGC, sightings of 214 adults and 32 calves were detected in 94 flights (2.6 sightings per flight) from 2016–2017. Photo-identified manatee sightings (n = 45) were reported in 39 flights, and the cumulative curve (Fig. 5) did not show an asymptote as new individuals appeared. Considering all adult sightings, the proportion of photo-identified manatees was 0.21. Fourteen individuals were photo-identified at SGC, 4 of which were re-sighted on different days. The maximum number of these re-sightings was 8 for individual BZ001 (Table 2).

4. DISCUSSION

Small multi-rotor drones, when equipped with adequate sensors and operated at adequate altitudes, can be used for photo-ID of Antillean manatees in shallow-water habitats in the Caribbean Sea. Small commercial drones are low-cost and widely available, offering a versatile tool for sirenian management and conservation projects. These systems are equipped with cameras of sufficient resolution for overhead photo-ID at low altitudes (<30 m, in this study) and can hover in stationary flight, allowing the capture of good quality photographs and videos of surfacing marine mammals (Durban et al. 2015). Therefore, in comparison to other platforms used to collect photographic information of sirenians (from boats, land, or underwater), drones are more effective for remote detection and capture of high-resolution images and videos depicting the entire dorsal view of a sirenian body. Although some features or scars may not be visible from a dorsal view, overhead photos of manatees enable the identification, measurements, and monitoring of most boat-related injuries, which are the most conspicuous features for identification typically found in the manatee dorsum (Beck et al. 1982, Goldsworthy 2018). Other features important for manatee identification are the notches on the tail, generally visible from a dorsal view.

We advise caution in flying drones near manatees for photo-ID. Data for this study were gathered during flights to test the reactions of Antillean manatees to small drones (Ramos et al. 2018). Of all manatees approached with the drone, 24 % reacted strongly to its vertical approach at a wide range of altitudes (6–104 m) by fleeing the area. Unlike most species of marine mammals that show few responses to small drones, typically consisting of short-duration events (Smith et al. 2016, Ramos et al. 2018, Fettermann et al. 2019, Fiori et al. 2020), manatees appear more at risk of disturbance with these systems if approached directly and too closely (Ramos et al. 2018). It is therefore important to evaluate the factors that could trigger a negative behavioral response in manatees, for example, the speed and angle of approach of the drone (Smith et al. 2016). While lower flight altitudes closer to target animals result in higher-resolution imagery (Hodgson et al. 2018), the risk of disturbing animals suggests that careful flight protocols are needed to mitigate disturbances in low-altitude flight (Ramos et al. 2018, Fettermann et al. 2019). Paired with carefully designed protocols, the effects of these systems should be evaluated using behavioral observations of animals exposed to small drone flights at different study sites and in different populations. The impacts on different individuals should be assessed prior to extensive deployment of these systems given that manatees within the same population can vary in their responsivity to small drone flights (Ramos et al. 2018). Additionally, efforts should be made to use

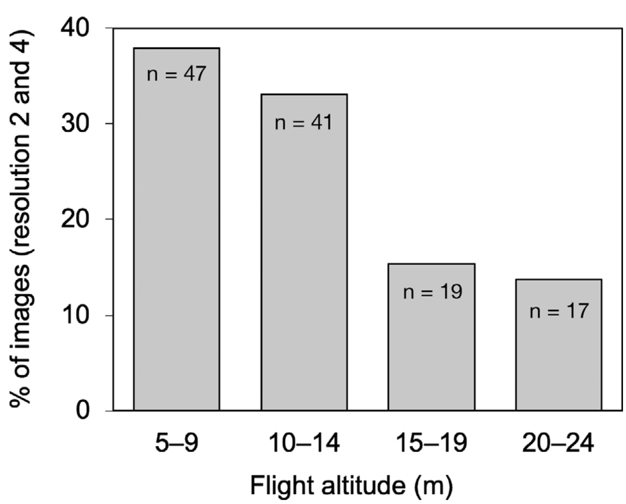

Fig. 4. Percentage of images scored as resolution 2 and 4 (excellent and average, n = 124) during quality image assessment, related to drone flight altitude

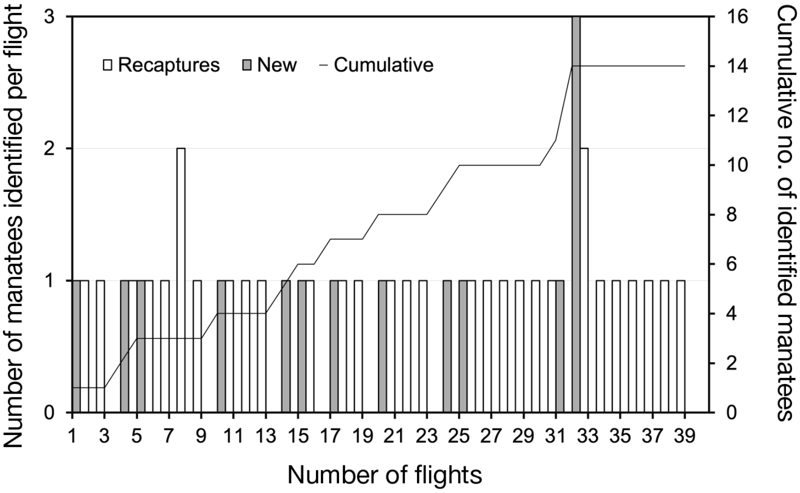

Fig. 5. Cumulative curve of Antillean manatees photo-identified at St. George's Caye, Belize, in 2016 and 2017

systems with reduced noise from the rotors that are equipped with the highest-resolution cameras within budget to minimize the need for lower-altitude flight. Considering that a multirotor produces more noise when changing altitude (Sweeney et al. 2016), we recommend that pilots should minimize aircraft movements when over manatees and advocate for the development of data collection protocols that involve slow aircraft approaches with minimal altitudinal shifts (to reduce noise), reduced movement while animals are at the surface, and maintenance of the highest flight altitudes needed for imagery of sufficient resolution.

Obtaining useful images for photo-ID through this drone-based method can be hindered by environmental factors such as waves or surface ripples, subsurface illumination and shadowing (Mount 2005, Joyce et al. 2018), and minimal ambient light. These factors, individually or combined, reduce the quality of images, causing resolution loss, distortion, and coverage of the manatee body. These environmental factors and their effects are limitations even when using other imaging methods and platforms of observation at the surface. The use of high-resolution aerial video imagery provides more time viewing animals (Ramos et al. 2018), leading to increased opportunities to photograph identifiable features of manatees. However, analyzing images from videos resulted in a loss of some information. For instance, some small marks were better noticed in videos compared to the extracted images because the movement helped the reviewers to differentiate little features from shadows, waves and other visual distortions. Thus, future applications of drone-based photo-identification should evaluate the costs and benefits of capturing aerial video relative to still imagery.

Difficulties associated with photo-identification of manatees such as a lack of distinctive marks on some individuals limit the effectiveness of this method (Beck & Clark 2012). Skin characteristics other than scars include impermanent and subtle features like minor skin abrasions (D3) and coloration patterns (D4). The body of an Antillean manatee is gray, but because of its low mobility during resting or feeding (Violante-Huerta & Suárez-Morales 2016), individuals often appear with brownish, greenish, blackish, or even whitish patches from an accumulation of sediments and/or epibionts on their surfaces (Violante-Huerta et al. 2017). The presence of organisms covering manatees (parasites, algae, commensal associates, etc.) varies substantially between individuals and between habitat types (Stamper & Bonde 2012) and the duration of these kinds of patterning is unknown. Due to the uncertainty of their duration, D3 and D4 features are not suitable for entering into a photo-ID catalog, but those characteristics could be used for a limited range and time using a suitable image quality threshold (Urian et al. 2015). Subtle features (D3) like scratches may only be useful for identification over weeks or

Table 2. Individual adult Antillean manatees identified in this study. Number of sightings of individuals considering different days. Feature codes are described in Section 2.4. F: female; U: unknown; SGC: St. George's Caye; BZR: Belize River

ID	Sex	Area	No. of sightings	Feature type	Feature codes
BZ001	U	SGC	8	Scar	SDA1LGL
BZ002	U	SGC	7	Scar	SDX1SWL
BZ003	U	SGC	1	Scar	SRB1MWL, SDX1MWL
BZ004	F	SGC	1	Scar	SDC3SGL
BZ005	F	SGC	1	Scar	SLX2SGL, SRX1SGL
BZ006	U	SGC	1	Scar	SLD1SGL, SDX3SGL
BZ007	U	SGC	1	Scar	SLC2SWL
BZ008	U	SGC	1	Scar	SRC2SWB
BZ009	U	BZR	1	Scar	SDY2SGL
BZ010	U	BZR	1	Scar	SLC1LWL, SDC3LWL
BZ011	U	BZR	1	Scar	SDC2MWL
BZ012	F	SGC	3	Scar	SDB2SWB
BZ013	U	SGC	3	Scar	SLB1SWL
BZ014	U	SGC	1	Mutilation	MRX1S, MRY1S
BZ015	U	SGC	1	Mutilation	MRY1S
BZ016	U	SGC	1	Scar	SDB1MWB
BZ017	U	SGC	1	Mutilation	MLY1S

months; for example, Anderson (1995) observed that minor skin abrasions persisted for at least 2 wk in dugongs. Similarly, the adult manatee 'George' (BZ001) identified at SGC was distinguished from other manatees using subtle features (D3) for 10 d before it acquired a large distinctive scar (Ramos et al. 2017). Subtle features could be included in a different catalog for monitoring manatees over the short-term (days to weeks) but are unlikely to be useful over months and years due to shifts in skin coverage over seasons and as manatees travel between fresh and salt water habitats.

At SGC, the non-asymptotic nature of the discovery curve (Fig. 5) suggests that the time frame and spatial range of our photo-ID effort were insufficient to identify the entire population (Karczmarski et al. 1999). Estimates of population size by capture-recapture analysis are not yet feasible with small drone flights, and given the large home range of individual Antillean manatees (Castelblanco-Martínez et al. 2012), identifying a large portion of the population would require flights throughout their range. However, drone-based photo-ID of manatees could facilitate studies on individual life history, site fidelity, and habitat use in locations, like SGC, with good visibility and the regular occurrence of manatees. For instance, BZ001 and BZ002 were repeatedly sighted in the SGC area, providing clues on their life history and fine-scale habitat use. Likewise, repeated observation of identified individuals enables the tracking of several aspects of animal health condition, for example, fine-scale temporal patterns in scar acquisition and healing (Ramos et al. 2017, Goldsworthy 2018), and impacts of repeated disturbance (Ramos et al. 2018). Furthermore, obtaining high quality images with reliable aircraft altitude data opens up the possibility for photogrammetric methods of measuring manatee body size (e.g. Flamm et al. 2000) that can be applied to assess health condition and age class of individuals.

Data gathered from drone-based studies of manatees have the potential to provide valuable information on the occurrence of manatees and can facilitate the identification of threats to their well-being. Scars on manatees can be the consequence of vessel collisions, entanglement in fishing gear, fungal infections, and cold lesions (Beck & Clark 2012). Since the current main cause of manatee mortality in Belize is hypothesized to be boat collisions (Castelblanco-Martínez et al. 2018), determining the proportion and severity of scars in manatees from Belize could serve as an important tool to monitor the most significant local threat to this population. For example, the size, shape, and location of scars can be used to determine the type of propeller that caused the wound (Beck et al. 1982), how frequently manatees are struck by boats (Goldsworthy 2018), and which individuals within a population are more vulnerable to boat collisions. Similarly, improved detection of and data collection on manatees in specific areas can be used to advocate for increased animal protection to reduce watercraft collisions with manatees. For example, drone-based detections and identification of manatees at SGC was successfully used to advocate for the establishment of a 'no-wake zone' across the channels along the leeward side of SGC that went into effect in 2017 (Ramos et al. 2017).

Photo-ID of Antillean manatees has great potential in the Caribbean due to its clear waters, facilitating this study and previous photo-ID efforts in Belize (e.g. Self-Sullivan 2007, Arce 2012). Belize has a relative high density of manatees and a high probability of encounters compared to other areas in the subspecies distribution (Quintana-Rizzo & Reynolds 2010). However, manatees often use turbid areas such as the Belize River (one of our study sites), where clear images of manatees were difficult to collect because of the high levels of turbidity and rarity of manatees exposing their bodies at the surface. Overall, compared to other platforms, drones are more effective in these situations because during video recording, they can capture overhead views of the exact moment when a manatee surfaces.

Future studies using drones to identify and track the movements and behavior of manatees across the coast of Belize and Mexico require standardized and rigorous methods for data collection. The novel use of drones for manatee photo-ID will benefit from predictable advancements in technology and rigorous applications of methodology. Improvements to system components like camera resolution and frame rate, increased battery life, reduced rotor noise, and accuracy of sensors (e.g. GPS, altimeter) at lower costs will dramatically enhance the use of drone-based imagery for manatee photo-ID. Such improvements would facilitate photogrammetric measurements of manatee body size while flying at higher altitudes and at lower noise levels, with reduced likelihood of disturbance to target species.

We propose that our method and cataloging system should be considered as another tool for manatee monitoring in order to provide long-term, reliable information on wild populations. For example, the Manatee Individual Photo-identification System has compiled photographs of Florida manatees since the late 1970s and has generated a catalog containing information on more than 2000 individuals (Beck &

Reid 1995, Beck & Clark 2012). This information has enabled studies on the biology, reproduction, conservation status, population structure, and dynamics of Florida manatees, which has been extremely useful in making management decisions. Furthermore, radio-tagging studies of manatees captured in the Caribbean revealed long-distance movements between Mexico, Belize, and Guatemala (Castelblanco-Martínez et al. 2013), suggesting that a regional approach is needed for monitoring the subspecies. Standardizing the coding for individuals and markings across regions will enable comparisons of photo-ID catalogs. Hence, our protocol could be implemented over the long term and expanded to other areas in the Caribbean to improve the Antillean manatee monitoring in the region.

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Evidence of fused vertebrae and spondyloarthritis in Bottlenose dolphin *Tursiops truncatus* (Odontoceti: Cetacea) in Chetumal Bay, Mexico

Mildred Fabiola CORONA-FIGUEROA[1,2] Carlos Alberto NIÑO-TORRES[3,4]* and
Delma Nataly CASTELBLANCO-MARTÍNEZ[3,4,5]

(1) *Programa de monitoreo de Megafauna Acuática del Caribe (PROMMAC), Universidad de Quintana Roo, Bahía s/n esq. Ignacio Comonfort, Col. Del Bosque, Chetumal, Quintana Roo, 77019. México*

(2) *Centro de Datos para la Conservación (CDC), Centro de Estudios Conservacionistas (CECON), Universidad de San Carlos de Guatemala (USAC). Avenida Reforma 0-63 zona 10, Guatemala, 01010. Guatemala*

(3) *Universidad de Quintana Roo. División de Ciencias e Ingeniería. Blvd. Bahía s/n esq. Ignacio Comonfort, Col. Del Bosque, Chetumal, Quintana Roo, 77019. México*

(4) *Fundación Internacional para la Naturaleza y la Sustentabilidad (FINS), Andara, Chetumal, Quintana Roo, 77014. México*

(5) *Consejo Nacional de Ciencia y Tecnología. Ciudad de México, 01020. México*

**Corresponding author: carlosalni@gmail.com*

Abstract: Bone diseases such as deformations, fractures, bone growths and the fusion of some vertebrae processes in dolphins and other marine mammals have been scarcely reported in Mexico. Here, we report a case of spondyloarthritis in several lumbar vertebrae and the fusion of some processes of the lumbar and caudal vertebrae in a Bottlenose dolphin *Tursiops truncatus*. The specimen was an adult female (2.39 m total length) found stranded on the West coast of the Statal Reserve Chetumal Bay Manatee Sanctuary. The anomality in the vertebrae of this individual consisted of the fusion of the neural spines of two lumbar vertebrae, the fusion of the arch sheets of two caudal vertebrae and the presence of spondyloarthrosis in several lumbar vertebrae.

Résumé : *Preuve de vertèbres fusionnées et de spondylarthrite chez le grand dauphin* Tursiops truncatus *(Odontoceti : Cetacea) dans la baie de Chetumal, au Mexique.* Des maladies osseuses telles que déformations, fractures, excroissances osseuses et fusion de certains processus vertébraux chez les dauphins et d'autres mammifères marins ont à peine été signalées au Mexique. Ici, nous rapportons un cas de spondylarthrite dans plusieurs vertèbres lombaires et la fusion de certains processus des vertèbres lombaires et caudales chez un grand dauphin *Tursiops truncatus*. Le spécimen était une femelle adulte (2,39 m de longueur totale) trouvée échouée sur la côte ouest du sanctuaire de lamantins de la réserve nationale de Chetumal Bay. L'anomalie dans les vertèbres de cet individu consistait en la fusion des épines neurales de deux vertèbres lombaires, la fusion des feuilles d'arc de deux vertèbres caudales et la présence de spondyloarthrose dans plusieurs vertèbres lombaires.

Keywords: Developmental malformations ● Degenerative diseases ● Cetacean ● Mexican Caribbean ● Stranding

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Introduction

The Bottlenose dolphin *Tursiops truncatus* (Montagu, 1821) is a cetacean with cosmopolitan distribution (Jefferson et al., 2015), found mainly in tropical and temperate waters, and is the most common cetacean in the Mexican Caribbean (Navarro et al., 1990; Niño-Torres et al., 2015). The Statal Reserve Chetumal Bay Manatee Sanctuary is a shallow coastal lagoon located at the south of Quintana Roo (Mexico), and it is considered a transboundary priority area for the Mesoamerican Barrier Reef System. Bottlenose dolphins are regularly observed in this estuary in groups up to 30 individuals (Ruiz-Hernández et al., 2018), but our knowledge on their connectivity with other populations, health conditions and habitat use is still incipient. The Marine Mammals Stranding Network of the State of Quintana Roo (MMSNSQ) attends between two and four stranded bottlenose dolphins in Chetumal Bay every year, but due to the generally advanced decomposition state of the carcasses, the cause of death remains unknown for most of the events.

According to The International Union for Conservation of Nature (IUCN), *Tursiops truncatus* is categorized as Low Concern (LC) (Hammond et al., 2012), although it has been suggested that some local populations should be classified as Data Deficient (DD). According to the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), the species is found in Appendix II (UNEP-WCMC, 2018), and is enlisted in the Annex II of the SPAW Protocol for the Wider Caribbean Region. The Mexican law considers the Bottlenose dolphin in the category of 'special protection' (Diario Oficial de la Federación, 2010).

In the last decade, the aquatic veterinary diagnosis has shown significant advances, however, this has not yet been extended to marine mammals (Eldin & Abu-Seida, 2015). The cases reported on pathologies in cetaceans usually come from necropsies of individuals stranded and bones deposited in museums and scientific reference collections (Kompanje, 1999, Galatius et al., 2009, Groch et al., 2012; Fettuccia et al., 2013; Costa et al., 2016). In most cases, the pathological changes of vertebrae in cetaceans are easily recognizable, therefore these anomalies are often mentioned in veterinary literature, although in a limited way (Kompanje, 1999). Previous research addressing the description of bones diseases and malformations in Bottlenose dolphins is very scarce, despite the fact of the species being the most studied cetacean in the world. Therefore, the aim of this manuscript is to report the first record of bones lesions in a Bottlenose dolphin *Tursiops truncatus* in the Mexican Caribbean, in order to contribute to the knowledge of the health issues affecting this species in the region.

Methodological section

On March 27th, 2015, an adult female of Bottlenose dolphin stranded dead in Calderitas, Chetumal Bay, Quintana Roo, Mexico (UTM: 368535.5; 2053433.4 Fig. 1). The carcass recovery and necropsy were performed by members of the MMSNSQ. The individual measured 2.39 m in length and was considered a Code 3 (moderately decomposed), according to the decomposition degree categorization of Pugliares et al. (2007). During the gross necropsy, we did not find any evidence suggesting the origin of decease, and therefore the cause of death was classified as 'unknown'. Samples of skin, lung and stomach were collected for further studies, and the carcass was buried in the facilities of the University of Quintana Roo. On February 8th, 2018, the bones were unearthed, carefully cleaned and labeled (UQROO-Tt-27032015), and the skeleton was stored in the Vertebrate Collection of the University of Quintana Roo (SEMARNAT register code: DF-CC-295-15).

Regions of the spine were assigned according to the classical classification system (Rommel, 1990): cervical (C), thoracic (T), lumbar (L) and caudal (Ca), and vertebrae were numbered following the order of the afore mentioned regions. It was observed that the neural spine of the lumbar vertebrae L21 and L22 were fused (Fig. 2 A-B). In addition, osseous protuberances were observed in the intervertebral disc of several lumbar vertebrae and caudal, and the vertebra L32 presented the largest syndesmophyte (Fig. 2 C-D). It was also observed that the arch sheets of caudal vertebrae Ca51 and 52 were fused (Fig. 2 E-F).

Results & Discussion

Fusions of some portions of the vertebrae or symptoms of ankylosis in cetaceans have been previously reported as an anomaly during development, mainly in the cervical vertebrae of bottlenose dolphins and river dolphins (Groch et al., 2012; Fetuccia et al., 2013). This condition has been associated to bone lesions including fractures, discarthrosis and spondyloarthritis (Fettuccia, 2010, as cited by Costa et al., 2016) and in some cases, to the age of the animal (Yoshida et al., 1994; Ferrero &

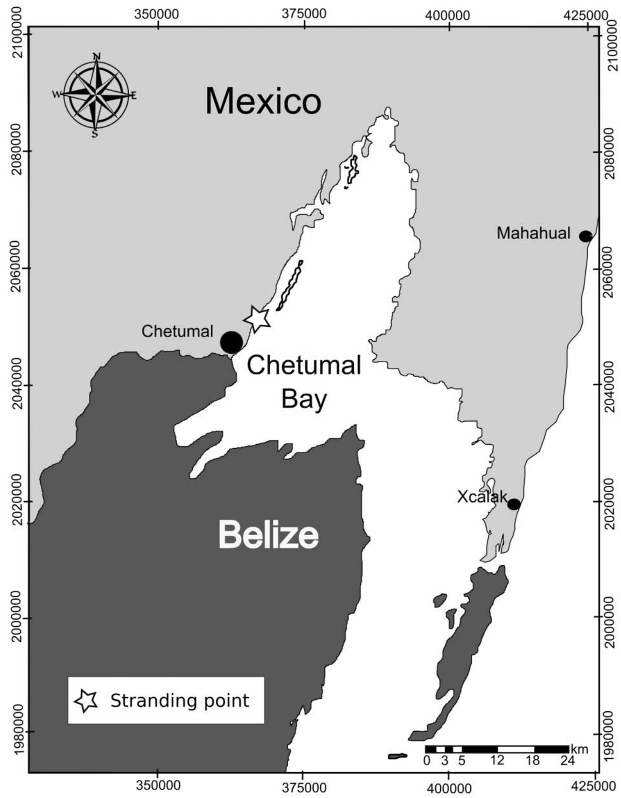

Figure 1. The Chetumal Bay, at the Mexican Caribbean. The white star signals the location where the Bottlenose dolphin stranded.

Walker, 1997; Kompanje, 1999). In the Bottlenose dolphin referred here, the fusion observed in neural spines and vertebrae's arch sheets possibly caused ankylosis and therefore immobilization in these areas (Montes et al., 2004).

The vertebral column in the dolphins is flexible and very variable (Buchholtz & Schur, 2004) and is influenced by the muscles and ligaments (Marchesi et al., 2017), the composition, size and shape of the intervertebral discs, and the morphology of the vertebrae (Gal, 1993; Koob & Long, 2000). The caudal section of the spinal column is a flexible area, and therefore important for the dolphin movement (Costa et al., 2016; Marchesi et al., 2017). Thus, the Ankylosis of vertebrae Ca51 and Ca52 probably caused the immobility of the joint. It has been suggested that this condition is a potential factor contributing to stranding and death in these species (Turnbull & Cowan, 1999; Sweeny et al., 2005; Costa et al., 2016). On the other hand, lumbar vertebrae are one of the most stable areas of the dolphin's spine (Marchesi et al., 2017), therefore the fusion of neural spines reported here may not necessarily cause ankylosis.

The presence of osseous protuberances at the edges of the vertebrae results from deformations of these structures and occurs between the vertebrae or intervertebral discs. In these cases, the protuberance is called syndesmophyte, and its frequently observed in mature individuals of several species of cetaceans (Kompanje, 1999, Galatius et al., 2009, Groch et al., 2012). However, there is ambiguity and confusion between some terms used in the diagnosis of these diseases, so these can be found under different names referring to the same or similar diseases: discarthrosis, zygarthrosis, spondyloarthritis, spondylosis deformans or infectious spondylitis (Kompanje, 1999). In cetaceans, females are more susceptible to suffering from these diseases (Galatius et al., 2009).

Kompajne (1999) describes spondyloarthritis (for cetaceans species) as a kind of join inflammatory disease, characterized by "*smoothly formed paradiscal ossicles, the occurrence of non-marginal syndesmophytes, the absence of signs of degeneration of the intervertebral disc, and the (partial) fusion or erosion of the zygapophyseal joint surfaces*". We suggest that syndesmophytes found in the lumbar and caudal vertebrae, mainly in L32, are cases of spondyloarthritis, which is supported by the following facts: 1) this disease typically involves the formation of osseous protuberances or ossification in the margin of the intervertebral discs; 2) it occurs in several cetacean species; while dyscarthrosis seems to be less common (Kompanje, 1999; Groch et al., 2012); 3) it is likely related to the individual's age and; 4) it is mostly reported in females (Galatius et al., 2009). Our diagnosis was also corroborated by one marine-mammal-specialized veterinarian, member of the MMSNSQ.

Like other systems, the skeletal system reacts to lesions and is susceptible to circulatory, inflammatory, neoplastic, metabolic and developmental disorders (Rosenberg, 2005). Spondyloarthritis (and its different forms, such as Reiter's syndrome and reactive spondylitis) is associated with intestinal or sexually transmitted bacterial infections (Kompanje, 1999). In this case, we did not have the opportunity to gather clinical information and microbiological cultures to support the causes of the disease, due to the high degree of decomposition of the carcass. The general evaluation of the bones contributes, in a certain way, to the understanding of the pathological processes that affect the health of cetaceans; but we highly recommend complementing the diagnosis with microbiological analysis in further opportunities.

This fusion process has been observed in

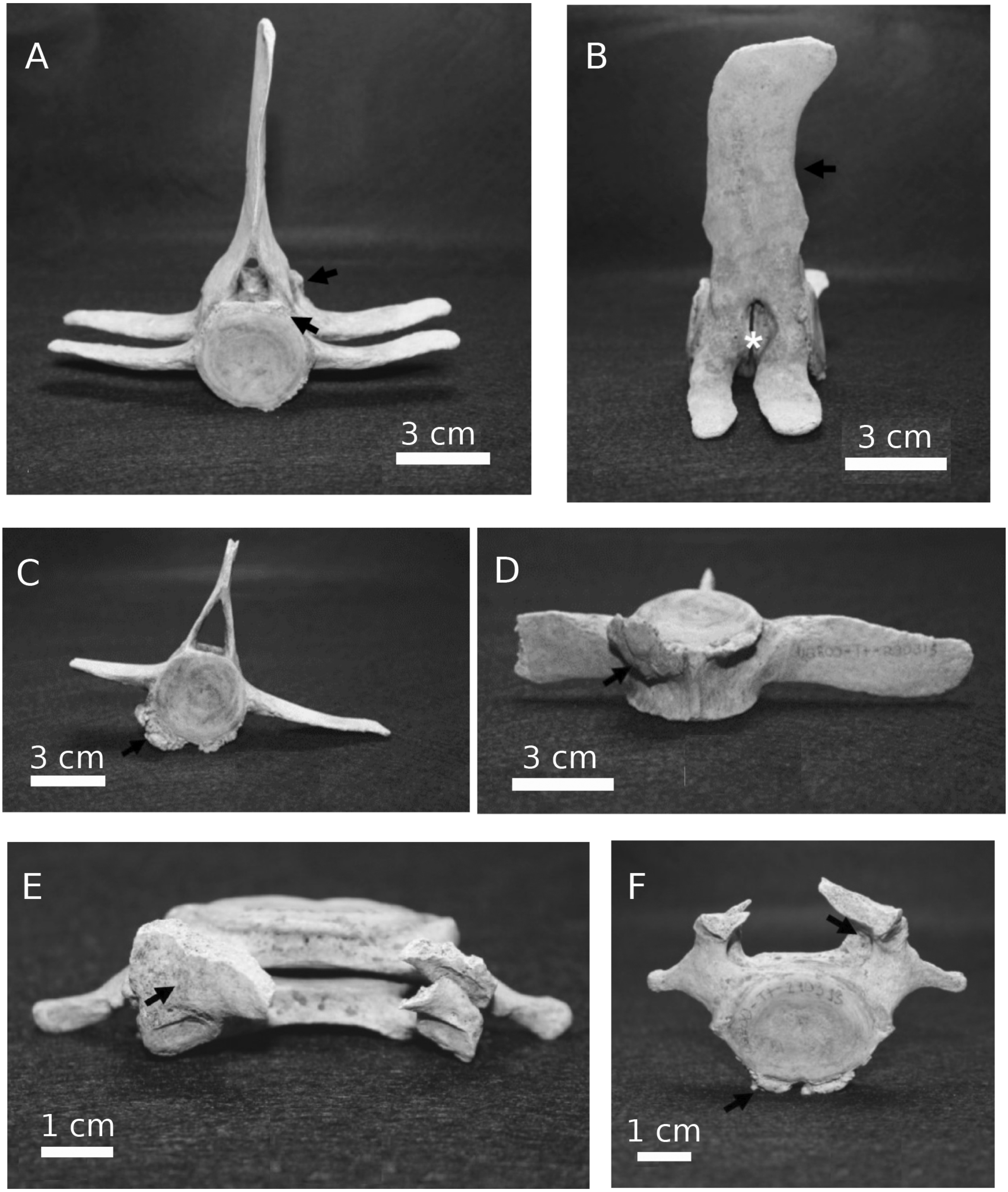

Figure 2. A.-B. *Tursiops truncatus.* Frontal and lateral views of the L21-22 vertebrae of the bottlenose dolphin. The arrows on the figure A indicate syndesmophytes formed on the margin of the body of the vertebrae. On B, the arrow indicates the fusion of the neural spines of both vertebrae; the asterisk notes the intervertebral space that separates the vertebrae. **C.-D.** Different views of the L32 vertebra. The arrows show the syndesmophytes formed in the margin of the lumbar vertebrae. **E.-F.** Dorsal and frontal views of Ca51-52 vertebra. In E, the arrow indicates the fusion of the processes of both vertebrae. On F, the arrow above indicates another view of the merger; the arrow below indicates the syndesmophyte formed in the dorsal part of the caudal vertebrae.

Bottlenose dolphins from the Gulf of Mexico (Turnbull & Cowan, 1999), Brazil (Galatius et al., 2009; Groch et al., 2012; Fetuccia et al., 2013; Costa et al., 2016), Peru, and Ecuador (at both ecotypes, Van Bressem et al., 2007). As well, similar bone conditions have been also reported in White-beaked dolphins *Lagenorhynchus albirostris* (Gray, 1846) from Denmark, Humpback whales *Megaptera novaeangliae* (Borowski, 1781), river dolphins *Sotalia fluviatilis* (Gervais & Deville in Gervais, 1853) from Brazil (Fetuccia et al., 2013; Costa et al., 2016), Pilot whales *Globicephala melas* (Traill, 1809) from the Atlantic Coast of the USA (Sweeny et al., 2005), and false killer whale *Pseudorca crassidens* (Owen, 1846) from Uruguay (Van Bressem et al., 2007). Although Turnbull & Cowan (1999) reported infections of inflammatory arthritis in synovial joints in three specimens of Bottlenose dolphins from the Gulf of Mexico; this study is the first case reported for the Mexican Caribbean.

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Searching for manatees in the dark waters of a transboundary river between Mexico and Belize: a predictive distribution model

M. F. Corona-Figueroa · N. Ríos · D. N. Castelblanco-Martínez · S. Vilchez-Mendoza · D. Delgado-Rodríguez · C. A. Niño-Torres

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Abstract Antillean manatees in the Hondo River have been recorded from aerial and aquatic surveys, and interviews. However, these studies have been conducted only in the lower riverbed, leaving a gap of information about their presence and habitat characteristics in the rest of the river. We characterize and determine the ecohydrological variables influencing the presence and habitat use of manatees in the Hondo River. During 2017 and 2018, 30 based-boat field trips were conducted in five consecutive transects of 15 km each. A mixed methodology was used for manatee detection: side-scan sonar, direct sightings, and feces collection. Ecohydrological variables were measured in all transects and fixed points. The survey effort was 136.5 h. We recorded 123 manatees: 47% were observations during the boat-based surveys, 29% were at fixed points, and 24% were opportunistic. Additionally, 10 manatee feces were found. The first transect of the river showed the highest relative abundance for the two sampled seasons (windy = 0.27 manatees/km, dry = 0.55 manatees/km). According to the Poisson model, the estimated population was equal to 51 manatees. A random forest model suggested high probability of observing manatees in the first transects and decreasing at the upstream. The ecohydrological variables influencing the detection of manatees were conductivity, transparency, depth, and proximity to the Four Mile lagoon. The first two transects have ecohydrological characteristics that makes a benign environment for refuge, rest and feeding of manatees. We recommend carrying out conservation efforts in the first transects, such as protection and the regulation of boat transit.

Keywords Mammalia-sirenia · *Trichechus manatus manatus* · Hondo river · Fluvial ecosystem · Predictions

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M. F. Corona-Figueroa (✉) · N. Ríos · S. Vilchez-Mendoza · D. Delgado-Rodríguez
Centro Agronómico Tropical de Investigación y Enseñanza (CATIE), Turrialba 7170, Costa Rica
e-mail: mildred.corona@catie.ac.cr; fabioco112@gmail.com

D. N. Castelblanco-Martínez
Consejo Nacional de Ciencia y Tecnología (CONACYT), 03940 Ciudad de México, Mexico

D. N. Castelblanco-Martínez · C. A. Niño-Torres
Universidad de Quintana Roo, 77039 Chetumal, Mexico

D. N. Castelblanco-Martínez · C. A. Niño-Torres
Fundación Internacional para la Naturaleza y la Sustentabilidad (FINS), 77014 Chetumal, Mexico

Introduction

The American manatee (*Trichechus manatus*) has two subspecies: The Florida manatee *T. m. latirostris* and

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the Antillean manatee *T. m. manatus*, the latter listed on the IUCN Red List as Endangered (Self-Sullivan and Mignucci-Giannoni 2008). The subspecies is also considered Endangered by the Official Mexican Norm NOM-SEMARNAT-059–2001 (SEMARNAT 2010), and by the Belizean Wildlife Protection Act No. 4 (Government of Belize 2000). The distribution of the subspecies occurs from the Gulf of Mexico, the east coast of Mexico, Central America to the northeast of Brazil, including the Caribbean islands (Lefebvre et al. 2001; Quintana-Rizzo and Reynolds III 2010). The state of Quintana Roo (Mexico) offers relevant habitats for manatees, including among others, the river-lagoon systems, such as the Hondo River.

There are two genetically distinct manatee populations in Mexico: one found in the fluvial-lagoon systems of the Gulf of Mexico and another on the Caribbean coasts (Nourisson et al. 2011). In the case of Belize, manatees found in the Southern Lagoon form a genetically distinct population from the Belize City cays (Hunter et al. 2010), while Chetumal Bay and the Northern Belize coasts share a single population unit (Vianna et al. 2006) estimated to be made up of 90 to 130 individuals (Quintana-Rizzo and Reynolds III 2010; SEMARNAT 2018). Manatees that move along the coastline between Chetumal Bay and the coasts of Belize can cover distances of approximately 240 or 300 km (Castelblanco-Martínez et al. 2013; LaCommare et al. 2008).

Telemetry studies in the Mesoamerican region have demonstrated that manatees occupy marine and estuarine waters, but can also venture into some freshwater rivers such as the Belize and Hondo rivers (Castelblanco-Martínez et al. 2013; Morales-Vela et al. 1999). However, aerial surveys -which are widely accepted as the primary method to estimate the distribution, abundance and critical habitats for manatees in the Caribbean (Castelblanco-Martínez et al. 2019; Morales-Vela et al. 2000; Olivera-Gómez and Mellink 2005)- are typically restricted to coastal, shallow, and transparent waters. Therefore, the distribution, abundance, and habitat selection of manatees in fully freshwater environments of the Mesoamerican region have not been well investigated. In particular, the Hondo River is considered a traditional area for manatees, and the species have been reported in the first 6 km of the main channel of the river and in the Four Mile lagoon (Auil 2004; Colmenero-Rolón and Zárate 1990; Morales-Vela et al. 2000; O'Shea and Salisbury 1991). Also, it is known that manatees move between Chetumal Bay, the mouth of the Hondo River, the Four Mile lagoon and the coasts of Belize (Castelblanco-Martínez et al. 2013; Morales-Vela et al. 1999).

The Hondo River is the natural border between Mexico and Belize, and a critical climatic and hydrological regulator of Chetumal Bay (Magnon-Basnier 2002). Despite its ecological importance, this river is not protected and faces strong environmental pressures including water contamination, land use change, invasive species, among others (Buenfil-Rojas and Flores-Cuevas 2007; Schmitter-Soto et al. 2015; Tun-Canto et al. 2017). Several ecohydrological characteristics influence the presence of manatees such as shallow depths, abundant aquatic vegetation, transparent waters and proximity to confluences (Jiménez-Domínguez and Olivera-Gómez 2014; Jiménez 2005; Morales-Vela et al. 2000; Olivera-Gómez and Mellink 2005; Puc-Carrasco et al. 2016). However, the degree to which these variables influence manatee distribution appears to be context dependent (Favero et al. 2020), and have not being investigated in the Hondo River.

Conservation strategies for an endangered species generally include the protection of the target species and its habitat. Thus, several countries have adopted the use of flag species for the creation of corridors or protected areas (Campbell 2017; Marsh and Morales-Vela 2011). On the other hand, the use of charismatic megafauna has proved to be an effective way to bring attention to broader conservation issues (Armstrong 2002; Lambeck 1997), and manatees in particular may serve as a sentinel species, reflecting the deleterious effects of polluted aquatic ecosystems (Bonde et al. 2004). Whether the long-term objective is to protect an endangered species or to use a charismatic or sentinel species to facilitate the habitat management, it is necessary to gather a basic site-specific understanding of the species distribution and habitat use (Packard and Wetterqvist 1986), including the identification of ecohydrology variables defining the space selection. Here, we investigate the ecohydrological variables influencing manatee presence and spatio-temporal habitat use in the Hondo River system, Quintana Roo (Mexico), to build a predictive model of manatee distribution. Thus, we aimed to compile formative baseline information on manatee habitat selection that will be relevant to guide the decision process for Hondo River management and conservation.

Methods

Study area

The Hondo River is located in the state of Quintana Roo, Mexico, and belongs to the Chetumal Bay and other basins (RH33A) (INEGI 2017a), and to the Hondo River basin, in Belize (Meerman and Clabaugh 2017) (Fig. 1). The source of the river occurs in Guatemala and flows as a natural border between Mexico and Belize, discharging into Chetumal Bay. It is one of the main hydrological components of Chetumal Bay, contributing 1,500 mm^3 of freshwater per year, with a minimum flow of 20 m^3/s in March and a maximum flow of 220 m^3/s in July. Additionally, the Hondo River plays the role of a climatic and hydrological regulator as it is interconnected with wetlands, lagoons, cenotes and other bodies of water in the area (Magnon-Basnier 2002).

On average, the Hondo River is 10 m deep, 50 m wide and has a slope of 5° (Magnon-Basnier 2002). Most of the territory around the river is homogeneous in relief but contains a geological fault on its channel. The climate is warm sub-humid with summer rains, average temperature of 26 °C and average precipitation of 1,550 mm, but it is colder and drier in the upstream region (24 °C, 1,000 mm) and warmer and wetter near the mouth (28 °C, 1,500 mm) (Magnon-Basnier 2002). The riparian vegetation includes various types of rainforests: medium sub-deciduous, low

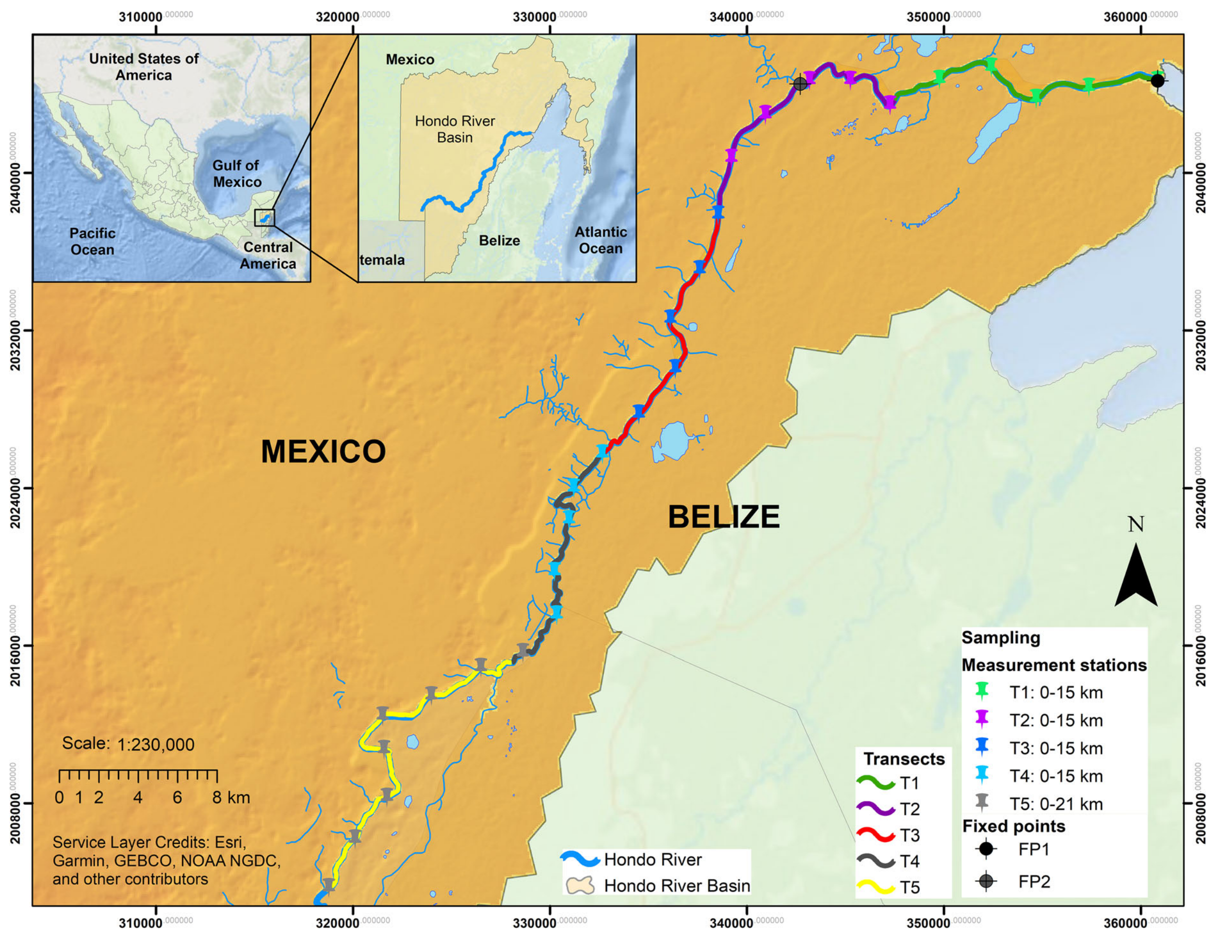

Fig. 1 Transects ($n = 5$) and ecohydrological variables measurement stations (every 3 km) assigned in the first 81 km from the mouth of the Hondo River. Two fixed-points for manatee observation are shown in T1 and T2

evergreen, low flood, savanna, reed beds, cultivated grassland and riparian mangrove (Granados-Sánchez et al. 1998; INEGI 2017b; Meerman and Clabaugh 2017). The dry season occurs from March to April, the rainy season from June to October and the 'Nortes' or windy season from October to February.

Manatee surveys

We used the following methodological approaches in order to record manatee presence: (1) side-scan sonar detections (Fig. 2), (2) sightings during boat-based surveys, at fixed points, or opportunistic and, (3) indirect evidence (feces). The first 84 km of the river was divided in four transects of 15 km long and the last of 21 km long (Fig. 1). Each transect was surveyed in a 7 m-length boat equipped with a

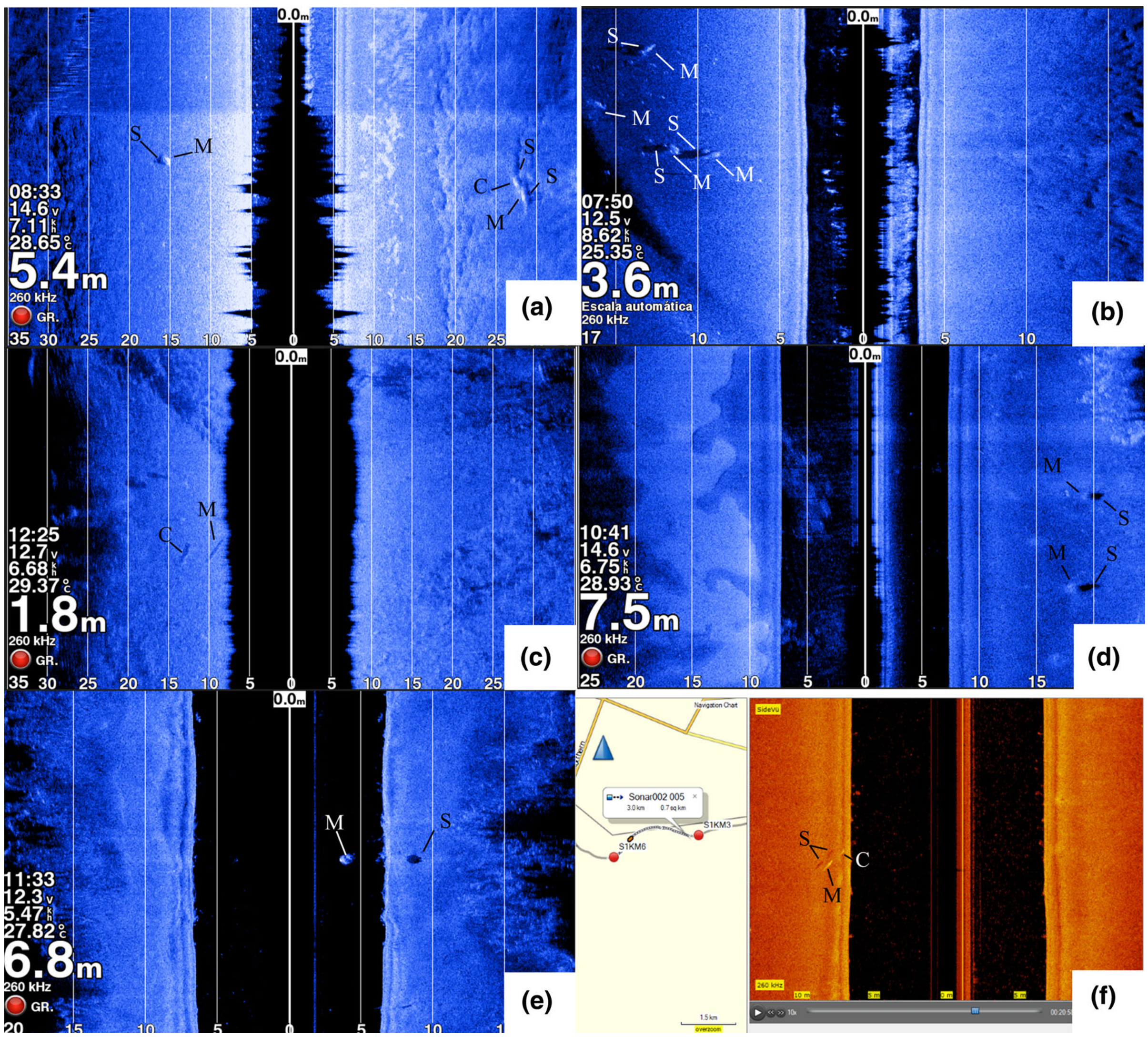

Fig. 2 Manatees detected by side-scan sonar method. Image symbols: manatee (M), calf (C), shadow (S). **a** mother-calf dyad at T1km0 (river mouth) in June 2018, confirmed in the field; **b** group of four manatees at T1km0 in February 2018, confirmed in the field; **c** mother-calf dyad in T2km6 in May 2018, confirmed in the field; **d** two manatees in T3km9-12, in June 2018, according to expert consultation; **e** manatee at T5km15 in April 2018, confirmed in the field, and **f** two manatees detected during the sonar recordings analysis, at T1km3-6, in March 2018

60 HP 4-stroke outboard motor. The navigations were conducted at 7.0–8.0 km/h at the middle and deepest area of the river channel and following the shoreline. Each transect was sampled once a month during November 2017, and from January to June 2018, covering 'Nortes' and dry seasons. The first two transects were sampled in a single day, to reduce the error of double counts of manatees, due to the antecedent that they have been observed mostly at these sites.

The boat was equipped with a side-scan sonar unit (GARMIN echomap 74sv, 500 W power) configured to sweep the area at a 30 m side width and a 260 kHz frequency. The crew consisted of a captain and at least two observers. One observer oversaw the side-scan sonar (SSS), while the other searched for visual clues of manatees or their feces. If a shape suggesting the presence of a large animal was detected in the SSS monitor, we proceeded to pass through the same site twice to confirm if the object corresponded to a manatee (Arriaga-Hernández 2013). After a potential detection in the SSS, the observers tried to visually confirm manatee presence by detecting the exhibition of back, tail or snout above the water; as well as bubbles, sediment and formation of wakes typically left by manatees. From each potential SSS detection -confirmed in situ or not- a screenshot was taken to record ancillary information and the size of the object. The images captured and the recordings of each trip were selected according to the profile, shape, and size of a manatee, using an image viewer and the Home-Port program (Garmin).

Additionally to the boat-based surveys, visual surveys were conducted from fixed points using the anchored vessel as a floating observation platform (Morales-García 2013). The first fixed point (FP1) was located at the Hondo River mouth, and the second (FP2) at the confluence between the Ucum and Hondo rivers. FP2 observations were only made during the dry season. The fixed-point surveys consisted of silent waits of approximately 20 min searching for manatees at 360°. An effective sighting was considered when the manatee's back, tail or snout was observed. When conditions allowed, an overflight was performed with a light drone (Phantom 3 model, with integrated GPS and 12.4 MP camera); flying at less than 50 m height and variable bandwidths, to confirm the sightings and to count individuals (Landeo-Yauri et al. 2020; Ramos et al. 2017). Class ages were assigned when possible: two animals swimming together, one being approx. $\leq 2/3$ the length of the other, was considered a mother-calf dyad. The side-scan sonar images also allowed to distinguish between calves and adults in several opportunities (Fig. 2). The presence of feces was considered indirect evidence of manatee occurrence, and assumed as the record of one indeterminate individual, based on the fact that feces were found at a relative long distance from each other (> 1 km), and on different transects and sampling days. Whenever they were found, fecal samples were collected, conserved in the Laboratory of Biology and Molecular Ecology of the University of Quintana Roo, and used for a parallel study on manatee feeding ecology (see Arévalo-González 2020).

Ecohydrological variables were assessed every three kilometers along each transect, and every time the presence of a manatee was confirmed or suspected. The measured variables were temperature (°C), conductivity (mS/cm), transparency (m), depth (m), distance to the nearest tributary river (m), distance to the Hondo River mouth (m) and distance to land (m). Temperature and conductivity measurements were assessed using a multiparameter probe; the transparency using a Secchi disk; and depth and coordinates using the side-scan sonar. The land use was determined as follows: we created buffer zones of the length of each transect and 400 m width at each margin of the river, and categorized each of them according to the land use type as: mangrove, medium sub-evergreen forest, low sub-perennial forest, tular, savanna, annual and semi-permanent seasonal agriculture, cultivated pastures, and settlements. The land use information layers were obtained in vector format for the year 2017, from the online platforms of the National Institute of Statistics and Geography (INEGI) and Biodiversity & Environmental Resource Data System of Belize.

The main tributary lagoons, rivers, and other hydrogeographic features of the study area were georeferenced. Each selected water body was named in accordance to the nomenclature of the Mexican water network (INEGI 2017a), by the closest locality, or according to the references provided by local people: Boca (mouth), Four Mile, López (Subteniente López), Chac (Estero Chac), Ucum, Diablo (river of the Curva del Diablo) and Román (river of the village of San Román). Other influential variables considered for the analyzes included precipitation (mm), obtained in the databases of the National Meteorological Service of Mexico, and bottom type detected by the side-scan sonar and coded as: mangrove (1), dense grasses (2), scattered grasses (3) and silt substrate (4) (González-Socoloske and Olivera-Gómez 2012; McLarty et al. 2019). For analytical purposes, ecohydrological variables were classified as temporal (temperature, conductivity, and transparency) and non-temporal variables (depth and distances to land uses, river, and mouth), the latter showing little variation among the months of sampling.

Data analysis

A relative abundance index (RAI) was used as an indicator of space use by manatees (Castelblanco-Martínez et al. 2017), and was calculated as the sum of all the evidence types found (SSS detections -those confirmed in the field, corroborated with literature and expert consultation-, direct sightings, opportunistic records, and feces) by kilometer navigated (for boat-based surveys) or by minute (for fixed points). For both cases, the RAI was estimated also by sampling time. To estimate the manatee population size in the Hondo River, we used a modified N-mixture model for spatial replications proposed by Royle (2004), using the transect-specific function of *pcount* from the unmarket library (Fiske and Chandler 2011) in the R program (RCoreTeam 2018). The model was parameterized according to the transect-based sampling design (e.g., transect-specific model), where each 15-km transect was assumed as a sampling location, and the monthly counts were considered as a sampling occasion with a count table of 5×7 dimensions.

The N-mixed model assumes that the study population is closed, that is, no mortality, recruitment, or migration occurs (Royle 2004). Furthermore, the model considers double-counting, i.e., movement between transects or recruitment, as one of the sampling errors. Given that this study is one-off and only considers a short sampling period, we consider it plausible to assume that the manatee population of the study area is closed. In other words, we assume that, at this short scale, processes determining the long-term population dynamic (e.g., mortality, recruitment, migration) are not affecting our results.

Temperature, transparency, and conductivity variables were transformed through a principal component analysis to avoid collinearity in the model. The components were included as explanatory variables to model the probability of detection. Abundance was only modeled on the intercept. Models were adjusted with Poisson distribution and negative binomial that considered the over dispersion. These distributions have been used in previous studies to estimate the population abundance, distribution and habitat use of manatees (Jiménez-Domínguez and Olivera-Gómez 2014; Jiménez 2005). The used integration parameter k is equal to the number of transects multiplied by the temporal samples (k = 35). We estimated the population size as the sum of the estimated abundances by transect. The following ecohydrological variables were used as predictor variables ($p = 21$) for the manatee-presence estimation model: transparency, depth, temperature, conductivity, distance to rivers, distance to the mouth, distance to the use of land, and coordinates. To identify the relative importance of each ecohydrological variable, an ordinal classification model (0–4 manatees) was adjusted with the random forest method, using the *randomForest* function (Liaw and Wiener 2002) of the R program.

The model was generated with 500 classification trees and four variables for each partition selected. The Gini index was used to assess the contribution of the predictor variables and to identify the most important variables, e.g., with the highest predictive power. The quality or robustness of the model was evaluated based on the AUC (Area Under the Curve) statistic or area under the ROC curve (Receiver Operating Characteristics), which assesses the ability of the model to correctly classify the species as present or absent (pseudo-absence) (Hanley and McNeil 1982). The AUC value ranks between 0 and 1 (0–100%), with values between 0.7 and 0.9 corresponding to good models and values greater than 0.90, to very good models (Peterson et al. 2011). The curve was contrasted between absences (value = 0) and presences (values ≥ 1) of manatees. The ROC curve was performed with the *pROC* package (Robin et al. 2011).

We used the following data to estimate the population size and to predict the likelihood of manatee presence: SSS detections (confirmed in the field, corroborated with literature and expert consultation), direct sightings during boat-based surveys, and direct sightings from fixed points. We used the records of manatee absence and sightings in the seven-month sampling period to build the distribution model, considering the observations as independent records.

Since the random forest model could overestimate the population, it was not used to estimate the manatee population size.

A map of probability distribution of manatee presence was created, and continuous raster maps were generated for each temporal and non-temporal ecohydrological variable used in the random forest model. The random forest algorithm was also applied for the construction of continuous maps through interpolation and, in this case, the predictor variables were the geographic coordinates. Coordinates were included for the map of non-temporal variables (depth, land use and bottom type). The maps of the temporal ecohydrological variables (conductivity, temperature, and transparency) included the variable 'month' as a factor and other influential variables such as precipitation, bottom type, depth, land use and distance to tributary rivers.

Results

The sampling effort totaled 136.50 h, and the total operating effort using the side-scan sonar was 528 km. The temperature varied from 24 to 32 °C, with the highest value recorded in May and the lowest in January during the windy ('Nortes') season. The conductivity values ranged from 1.01 to 2.23 mS/cm with highest values recorded mainly in June. The lowest transparency values were recorded in November, February, and March. Although the depth was quite similar in all the months, the lowest depth recorded was 2.30 m and the deepest was 12.70 m. The most represented land use type was the tular (52%), followed by the mangrove forest (17%), low sub-evergreen forest (9%), agriculture (9%), medium sub-evergreen forest (8%) and settlements (4%); the least represented (1%) were cultivated pastures, savanna and others.

A catalog was made of 146 selected side-scan sonar images, which were evaluated by experts in the use of sonar applied to manatee research (Fig. 2). Confidentiality values were assigned to each observation (sensu Castelblanco-Martínez 2014) to discard false positive images. Seventeen images were approved by experts and included for analysis. One-hundred and twenty-three manatees were sighted during boat-based surveys (47%), fixed points (29%), and opportunistically (24%). Additionally, 10 samples of manatee feces were found, mostly (70%) in the first two transects of the river. Of the 133 manatees' records (123 observations plus 10 feces), 45% correspond to adults, 16% to calves and 39% to indeterminate (Table 1). Adult and indeterminate individuals were observed in all transects of the river (except for the fourth), mainly in the dry season; while calves were only observed in the first three transects (Table 1).

The group size was one (51%), two (34%), three (11%) or four (4%) individuals. The highest RAI occurred during the dry season. As for the fixed points, the RAI at the mouth was greater in the dry season. The mean RAI for the transects in the 'Nortes' and dry seasons was 0.07 and 0.16 manatees, respectively (Table 2). The estimated manatee population size for the sampled channel, according to the Akaike Information Criterion (AIC = 151), was 51 individuals (Table 3). The average manatee abundance was greater in the first transect, for the three models compared, and decreased in transects furthest from the mouth. The fourth transect presented the lowest abundance of manatees, according to the three models. There was a positive effect of principal components 1 (B = 0.72, $p < 0.0001$) and 2 (B = 0.70, $p < 0.0001$) in relation to the probability of manatee detection in the model, indicating that as conductivity and transparency increase in component one, the probability of detecting manatees increases. Likewise, as the temperature in component 2 increases, the probability of detection increases (Fig. 3). The probability of manatee detection tended to increase in May and June, with transect 1 having the highest likelihood of manatee detection (Fig. 4).

The random forest model determined an estimated error rate (OOB) of 26%. Of the total number of records ($n = 268$), the model classified 69 records incorrectly (Table 4). That is, the random forest model will be 18% wrong in predicting the presence of manatees. The obtained model showed that the efficiency of predicting manatee presence or absence is within the AUC range (ROC curve), which is set from 0.76 to 0.97. The AUC was 0.9257, indicating a very good precision of the model according to Peterson et al. (2011). According to the Gini index (G), the ecohydrological variables with the highest predictive power were: conductivity (G = 8.5), temperature (G = 8), and depth (G = 7.2), followed by distance to Curva del Diablo (G = 6.5), transparency (G = 6.3), distance to pastures (6.2) and distance to

Table 1 Direct sightings (adults, calves or indeterminate) and indirect evidences (feces) of manatees obtained during boat-based surveys (transects 1–5) and fixed points (FP1 and FP2)

Season	'Nortes'					Dry					Total
Sampling	Adults	Calves	Indet	Feces	Total	Adults	Calves	Indet	Feces	Total	
Transect1	13	3	4	2	22	21	7	19	2	49	71
Transect2	0	0	0	0	0	3	2	3	3	11	11
Transect3	0	0	3	0	3	1	1	2	1	5	8
Transect4	0	0	0	0	0	0	0	0	1	1	1
Transect5	2	0	0	0	2	1	0	2	1	4	6
FP1	4	2	1	0	7	13	6	7	0	26	33
FP2	–	–	–	–	–	2	0	1	0	3	3
Total	19	5	8	2	34	41	16	34	8	99	133

Indet = indeterminate

Table 2 Relative Abundance Index (RAI) of manatees by season and survey type

Season	'Nortes'				Dry			
Sampling	Effort	Sightings	RAI	# Est. Ind	Effort	Sightings	RAI	# Est. Ind
Transect1	45.00	12	0.27	3	60.00	33	0.55	18
Transect2	45.00	0	0.00	0	60.00	8	0.13	1
Transect3	45.00	2	0.04	0	60.00	3	0.05	0
Transect4	30.00	0	0.00	0	60.00	1	0.02	0
Transect5	42.00	2	0.05	0	84.00	3	0.04	0
FP1	220.00	5	0.02	0	340.00	11	0.03	0
FP2	–	–	–	–	260.00	3	0.01	0
Mean (T1–T5)	41.40	3	0.07	1	64.80	10	0.16	2
Mean (FP)	220.00	5	0.02	0	300.00	7	0.02	0

Effort = Sampling effort was defined as distance surveyed (kilometers) for transects, and as waiting time (minutes) for fixed points. # Est. Ind. = Estimated individuals. Dash lines indicate no effort

Table 3 Estimation of population size and average manatee abundance by transect in the Hondo River, according to the Null, Poisson, and Negative Binomial models. Confidence interval is 95%

Transects	Null AIC: 273.68			Poisson AIC: 151.22			N. binomial AIC: 183.26		
	Mean	LL	UL	Mean	LL	UL	Mean	LL	UL
Transect1	34	32	35	34	32	35	34	31	35
Transect2	11	10	13	11	10	12	13	10	16
Transect3	5	3	7	4	3	7	12	7	18
Transect4	1	0	3	0	0	1	6	2	11
Transect5	3	2	6	2	2	4	12	7	18
Total	54			51			77		

savanna, San Román and mouth (G = 6.2). The variables with intermediate importance (G = 3.8–5.0) are coordinates, the distance to the mangrove areas, agriculture, the Four Mile lagoon. The least important ($G \leq 3.7$) were the distance to the Subteniente López and Ucum rivers, and the distance to the sub-evergreen median rainforest. The greatest probability of manatee presence occurs in transect 1 and decreases upstream. However, in transects 2, 3 and 5, conditions in certain areas are potentially predictive for manatee presence. The model predicts the lowest probability of detecting manatees in transect 4 (Fig. 5).

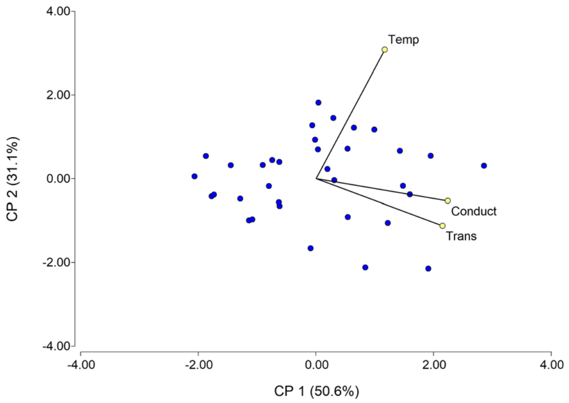

Fig. 3 Principal component analysis for the explanatory variables (temperature, conductivity, and transparency), and the probability of manatee detection

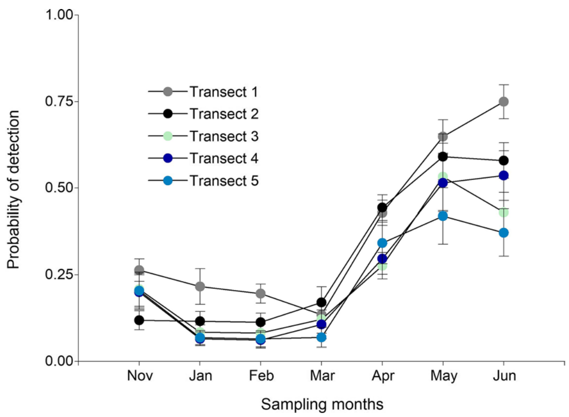

Fig. 4 Inter-monthly temporal variation of manatee detection probability according to the Poisson model

The principal component analysis showed that the first component (CP1) separates conductivity, transparency, distance to the mouth, the Four Mile lagoon and the mangrove forest from the distances to the San Román and Curva del Diablo rivers, the savanna and pastures (Fig. 6). Component 2 separates the variables of depth and distance to agricultural soil. Both components (CP1 and CP2) explain 71% of the total variability of the ecohydrological variables. Thereby, high values of conductivity and transparency and shorter distances to the mouth, the Four Mile lagoon and the mangrove forest are associated with the higher probabilities of manatee presence, while the proximity to the rivers of the Curva del Diablo and San Román and pastures, savanna and agriculture are conditions mostly associated to the absence of manatees. Also, greater water depths were associated with the absence of manatees; the temperature showed no association with the presence or absence of manatees in these first two axes.

Discussion

Observing manatees in continental waters with low transparency as those of the Hondo River is challenging, and the most efficient and productive approach seems to be to use all evidence types (Castelblanco-Martínez et al. 2017). Several studies have used statistical models, such as binary logistic regressions, Bayesian, generalized linear models (GLMs) and multivariate to estimate the presence and population size of manatees based on habitat characteristics (Guzmán and Condit 2017; Jiménez-Domínguez and Olivera-Gómez 2014; Jiménez 2005; LaCommare et al. 2008; Martin et al. 2014). One of the advantages of the random forest model, used in this study, is that it evaluates the contribution of each predictor variable independently, that is, without modifying the others (Cutler et al. 2007).

Solitary individuals were observed in all transects of the river, but only indirect evidences (feces) were found in the fourth transect. Mother-calf observations were more frequent in the first transect, as reported in the studies by Campbell and Gicca (1978), Bengtson and Magor (1979), Fuentes Allen and Aguayo Lobo

Table 4 Confusion matrix of the random forest model that predicts the presence of manatees in the Hondo River

	Counts: absences (0) and presences (1–4 individuals)					Error classes
	0	1	2	3	4	
0	186	6	3	0	0	0.05
1	20	9	4	3	0	0.75
2	12	6	4	3	0	0.84
3	2	3	3	0	1	1.00
4	2	0	1	0	0	1.00

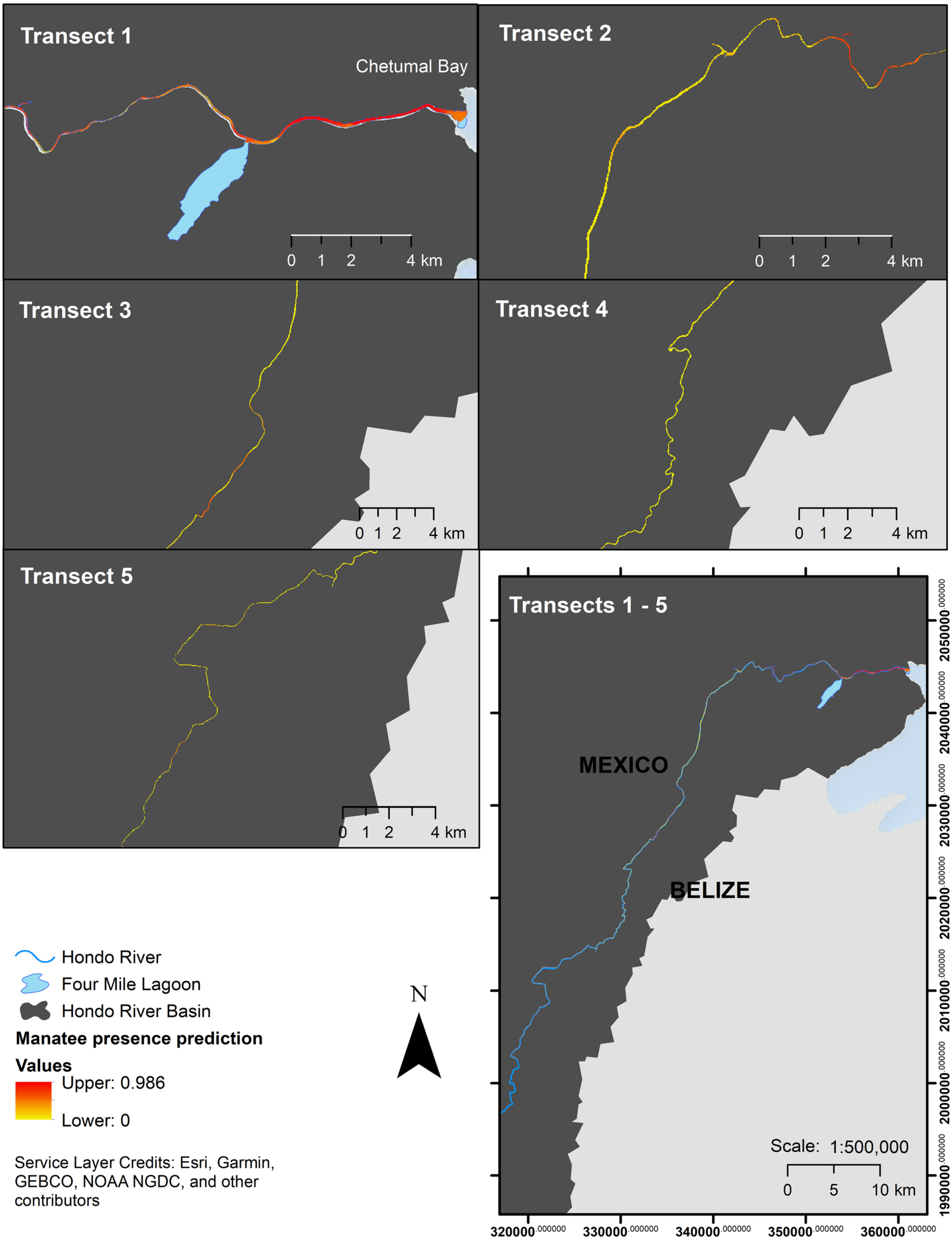

Fig. 5 Predictive models of manatee presence in the Hondo River, Quintana Roo, Mexico

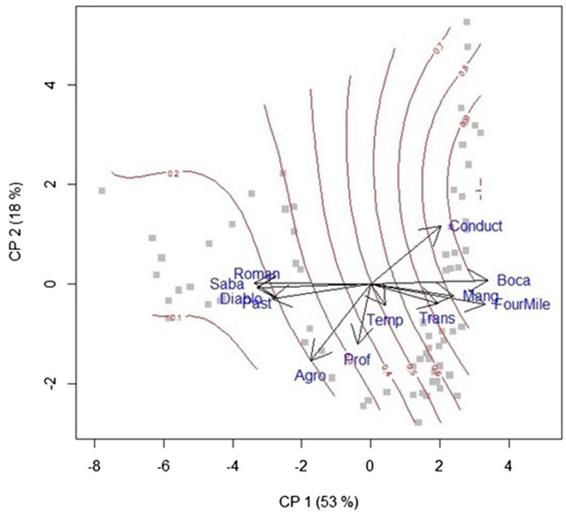

Fig. 6 Biplot for relevant ecohydrological variables, and their relationship with the likelihood of manatee presence (red lines). Gray squares correspond to the 268 manatee records used to estimate the probability of manatee detection in the river

(1989), Colmenero-Rolón and Zárate (1990), O'Shea and Salisbury (1991), Zárate Becerra (1993) and Auil (2004), in the Hondo River. The RAI reflected more records of manatee observations in the transects closest to the mouth. Several studies indicate higher relative abundances in semi-open water systems than on the coasts (Axis-Arroyo et al. 1998) or in sites with more complex or heterogeneous habitats, which have confluences between river-lagoon systems, than in homogeneous sites (Auil 2004; Morales-Vela et al. 2000; Puc-Carrasco et al. 2016).

The estimate of the manatee population size in the river is proportionally similar to other water systems with similar environmental conditions, such as Tabasco, with 28 manatees for a fluvial-lagoon system of around 14 km (Puc-Carrasco et al. 2017) and 18 manatees for an 18 km river in Bocas del Toro, Panama (Guzmán and Condit 2017). In this study, the population size was estimated assuming a closed population; however, this population could be considered open, since there is enough evidence of manatee movements between Chetumal/Corozal Bay and the coasts of Belize (Castelblanco-Martínez et al. 2013; Morales-Vela et al. 1999), with manatees showing a movement rate of 0.13 km/h (Castelblanco-Martínez 2010). Breeding manatee females often have fidelity patterns to specific sites (Deutsch et al. 2003; Gannon et al. 2007; Morales-Vela et al. 1999; Powell and Rathbun 1984; Rathbun et al. 1990). The constant observations of female-calf pairs in the first transects of the Hondo River could indicate that those are important nursing areas for manatees.

The peak of manatee detection occurred during the dry season, mainly in May and June, for all the study area including the furthest ones. Similar results were reported for Belize (Auil 2004) and for French Guyana (Castelblanco-Martínez et al. 2017). In contrast, Morales-Vela et al. (2000) reported low abundance of manatees in rivers and lagoons during the dry season, while in the bay the abundance was higher. Our results suggest that manatees living in saline environments move toward continental waters to cover their need for freshwater. Thus, manatees moving around Chetumal Bay or north of the coastal zone of Belize can supply their freshwater needs in the first transects of the river during the wet season. In the dry season, when the plume of salinity enters the Hondo River, they need to move further upstream where the salinity is lower or null. This strategy of remaining near freshwater sources seems to be advantageous in terms of energy investment (Olivera-Gómez and Mellink 2005).

The Hondo River habitat shows appropriate conditions for manatees. The most important ecohydrological variables explaining the manatee presence were those related to the characteristics of the river (conductivity, temperature, depth, and transparency), while bank characteristics were less important. The Hondo River has several tributary rivers along its channel that differ from each other in length, width, and depth, apart from those referenced in this study. Several of these confluences are found in the classified transects, which is why they offer heterogeneous conditions that favor the presence of manatees (Jiménez-Domínguez and Olivera-Gómez 2014; Puc-Carrasco et al. 2016). Although our results suggest a preference by the transects near the mouth, we present clear evidence that the species also use the upper transects of the Hondo River. In fact, several feces were found upstream, and although we have no evidence of feeding in this study, the area offers riparian vegetation, e.g., submerged grasses, mangrove (*Rhizophora mangle*), water hyacinth (*Eichhornia crassipes*), for the species (this study, Arévalo-González 2020).

The model estimated probabilities, although low, of manatee presence in transects 3–5. The fourth transect was the one that presented the lowest probability values, which may be due to the fact that the environmental conditions in this area are less favorable for manatees (greater depths, low transparencies), in addition to being the most homogeneous (presence of tular as dominant land use), compared to the other transects of the river. The characteristics of the first transects are related to the presence of mangrove forest, dense pastures on the bottom, proximity to the Four Mile lagoon, clear waters with high values of conductivity and relatively low depths, which make an attractive environment for manatees. Indeed, these conditions offer shelter, feeding resources, and resting areas, which has been proven by previous authors as important requirements for manatee space selection (Fuentes Allen and Aguayo Lobo 1989; Jiménez-Domínguez and Olivera-Gómez 2014; Morales-Vela and Olivera-Gómez 1997; Morales-Vela et al. 2000; Olivera-Gómez and Mellink 2005; Puc-Carrasco et al. 2017, 2016).

Water conductivity showed higher values in the lowest section of the river and this may be explained by the proximity to the mouth, i.e., to the most saline environment. Water salinity can influence manatee distribution: The species prefers fresh or brackish waters; thus, manatees are found in areas close to water-bodies that provide fresh water (Hartman 1979; Olivera-Gómez and Mellink 2005). There is evidence of a positive correlation between plant biomass/cover in Mesohaline habitats and the water conductivity/salinity (Olivera-Gómez and Mellink 2005, 2013). Although we did not measure aquatic vegetation biomass, areas associated predominately with mangrove (e.g., *R. mangle*) represent a rich offering of food resources for manatees including dense subaquatic vegetation beds (*Ruppia maritima, Thalassia testudinum, Halodule wrightii, Syringodium filiforme, Najas marina, Chara* sp., *Batophora* sp.) (Arévalo-González 2020; Espinoza-Ávalos 1996; Espinoza-Ávalos et al. 2009; LaCommare et al. 2008; Zárate Becerra 1993), which have been reported as part of the manatee diet (Castelblanco-Martínez et al. 2009; Marsh et al. 2011; Mignucci-Giannoni 1998).

The model also indicated that transparency influences the presence of manatees, mainly because this variable has a positive effect on other variables such as the presence of subaquatic vegetation (Jiménez-Domínguez and Olivera-Gómez 2014; Jiménez 2005). Also, even though other studies indicated that the water transparency itself does not have an important influence on habitat selection (Auil 2004; Hartman 1979), it may have a critical effect on our capacity to visually detect manatees; especially when we are relaying on boat and drone-based surveys. Although the water temperature was one of the important variables for the model, it did not show a clear trend in relation to the probability of observing manatees. This can be explained by the fact that in tropical waters, temperature is not a determining factor in the distribution of manatees, since it is relatively constant and exceeds 20 °C which is critical for manatees (Axis-Arroyo et al. 1998; Irvine 1983; Jiménez 2005).

In the Hondo River, previous research has demonstrated the presence of high levels of heavy metals and organochlorine compounds, derived mainly from the agrochemicals and pesticides used in the cane crops nearby (Buenfil-Rojas and Flores-Cuevas 2007; González Bucio et al. 2013; Tun-Canto et al. 2017). It is necessary to include an ecohydrological approach in the management policies of the banks of the Hondo River and, if possible, at the basin level, to ensure the long-term sustainability of the river-lagoon ecosystem, considering that water resources are part of the functional and interrelated complex systems and all its components and levels (Zalewski 2006). In addition, including a target, charismatic species as the manatee as the core of management plans may facilitate the maintenance of safe ecological conditions, and the biological integrity of the Hondo River fluvial-lagoon ecosystem (Armstrong 2002; Lambeck 1997).

Manatees are completely herbivorous and considered nutrient recyclers (Castelblanco-Martínez et al. 2012), they contribute to the population growth control of aquatic plants and provide nutrients to submerged grass beds through their feces, benefiting the aquatic ecosystem (Etheridge et al. 1985; Reynolds III et al. 2002). The manatee is an endangered marine mammal occupying the Coastal Transverse Corridor of southern Quintana Roo (Hernández-Arana et al. 2015), a critical area for the hydrological regulation of the region. We recommend considering at least the first 30 km of the river as part of the protected areas of the Chetumal Bay Manatee Sanctuary, in Mexico, and the Corozal Bay Wildlife Sanctuary, in Belize. Although the passage of high-speed motorized boats in the river was not evidenced in this study, this is a potential threat to manatees that could increase in the following years. For these reasons, the speed regulation of the boats transiting in the riverbed should be considered within the management actions.

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Author contributions MFC-F, DNC-M, NR, and CAN-T designed the project. MFC-F collected the data; MFC-F, SV-M, DNC-M and NR interpreted data. All authors undertook a first critical revision of the manuscript and approved the final version for publication.

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Data availability We will upload the research database on an online platform, for interested researchers to access all data, prior to the request of the corresponding author.

Complaince with ethical standards:

Conflicts of interest All the authors declare that there is no conflict of interests.

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